CHAPTER 15
THE CHROMOSOMAL BASIS OF INHERITANCE

OUTLINE

I. Relating Mendelism to Chromosomes
   A. Mendelian inheritance has its physical basis in the behavior of chromosomes during sexual life cycles
   B. Morgan traced a gene to a specific chromosome: *science as a process*
   C. Linked genes tend to be inherited together because they are located on the same chromosome
   D. Independent assortment of chromosomes and crossing over produce genetic recombinants
   E. Geneticists can use recombination data to map a chromosome's genetic loci

II. Sex Chromosomes
   A. The chromosomal basis of sex varies with the organism
   B. Sex-linked genes have unique patterns of inheritance

III. Errors and Exceptions to Chromosomal Inheritance
   A. Alterations of chromosome number or structure cause some genetic disorders
   B. The phenotypic effects of some genes depend on whether they were inherited from the mother or father
   C. Extranuclear genes exhibit a non-Mendelian pattern of inheritance

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Explain how the observations of cytologists and geneticists provided the basis for the chromosome theory of inheritance.
2. Describe the contributions that Thomas Hunt Morgan, Walter Sutton, and A.H. Sturtevant made to current understanding of chromosomal inheritance.
3. Explain why *Drosophila melanogaster* is a good experimental organism.
4. Define linkage and explain why linkage interferes with independent assortment.
5. Distinguish between parental and recombinant phenotypes.
6. Explain how crossing over can unlink genes.
7. Map a linear sequence of genes on a chromosome using given recombination frequencies from experimental crosses.
8. Explain what additional information cytological maps provide over crossover maps.
9. Distinguish between a heterogametic sex and a homogametic sex.
10. Describe sex determination in humans.
11. Describe the inheritance of a sex-linked gene such as color-blindness.
12. Explain why a recessive sex-linked gene is always expressed in human males.

13. Explain how an organism compensates for the fact that some individuals have a double dosage of sex-linked genes while others have only one.
14. Distinguish among nondisjunction, aneuploidy, and polyploidy; explain how these major chromosomal changes occur and describe the consequences.
15. Distinguish between trisomy and triploidy.
16. Distinguish among deletions, duplications, translocations, and inversions.
17. Describe the effects of alterations in chromosome structure, and explain the role of position effects in altering the phenotype.
18. Describe the type of chromosomal alterations implicated in the following human disorders: Down syndrome, Klinefelter syndrome, extra Y, triple-X syndrome, Turner syndrome, *cri du chat* syndrome, and chronic myelogenous leukemia.
19. Define genomic imprinting and provide evidence to support this model.
20. Explain how the complex expression of a human genetic disorder, such as fragile-X syndrome, can be influenced by triplet repeats and genomic imprinting.
21. Give some exceptions to the chromosome theory of inheritance, and explain why cytoplasmic genes are not inherited in a Mendelian fashion.

KEY TERMS

chromosome theory of inheritance
wild type
mutant phenotype
sex-linked genes
linked genes
genetic recombination
parental type
recombinants
linkage map
cytological map
Duchenne muscular dystrophy
hemophilia
Barr body
nondisjunction
aneuploidy
trisomic
monosomic
polyploidy
deletion
duplication
inversion
translocation
Down syndrome
fragile *X* syndrome

LECTURE NOTES

I. Relating Mendelism to Chromosomes

A. Mendelian inheritance has its physical basis in the behavior of chromosomes during sexual life cycles

Genetics	Cytology
1860s: Mendel proposed that discrete inherited factors segregate and assort independently during gamete formation	
	1875: Cytologists worked out process of mitosis
	1890: Cytologists worked out process of meiosis
1900: Three botanists (Correns, de Vries, and von Seysenegg) independently rediscovered Mendel's principles of segregation and independent assortment	
1902: Cytology and genetics converged as Walter Sutton, Theodor Boveri, and others noticed parallels between the behavior of Mendel's factors and the behavior of chromosomes. For example: · Chromosomes and genes are both paired in diploid cells. · Homologous chromosomes separate and allele pairs segregate during meiosis. · Fertilization restores the paired condition for both chromosomes and genes.	

Based upon these observations, biologists developed the *chromosome theory of inheritance* (see Campbell, Figure 15.1). According to this theory:

- Mendelian factors or genes are located on chromosomes.
- It is the chromosomes that segregate and independently assort.

B. Morgan traced a gene to a specific chromosome: *science as a process*

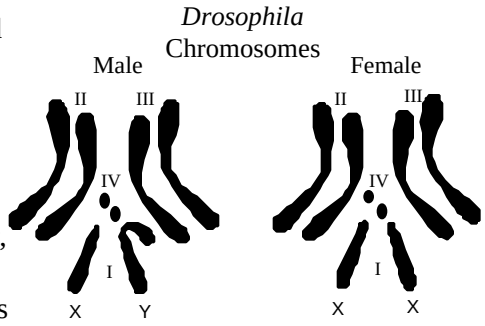
Thomas Hunt Morgan from Columbia University performed experiments in the early 1900s which provided convincing evidence that Mendel's inheritable factors are located on chromosomes.

I. Morgan's choice of an experimental organism

Morgan selected the fruit fly, *Drosophila melanogaster*, as the experimental organism because these flies:

- Are easily cultured in the laboratory
- Are prolific breeders
- Have a short generation time
- Have only four pairs of chromosomes which are easily seen with a microscope

There are three pairs of autosomes (II, III, and IV) and one pair of sex chromosomes. Females have two *X* chromosomes, and males have one *X* and one *Y* chromosome.



Morgan and his colleagues used genetic symbols that are now convention. For a particular character:

- A gene's symbol is based on the first *mutant*, non-wild type discovered.
- If the mutant is recessive, the first letter is lowercase. (e.g., *w* = white eye allele in *Drosophila*.)
- If the mutant is dominant, the first letter is capitalized. (e.g., *Cy* = "curly" allele in *Drosophila* that causes abnormal, curled wings.)
- Wild-type trait is designated by a superscript +. (e.g., Cy^+ = allele for normal, straight wings.)

Wild type = Normal or most frequently observed phenotype (see Campbell, Figure 15.2)

Mutant phenotypes = Phenotypes which are alternatives to the wild type due to mutations in the wild-type gene

2. Discovery of a sex linkage

After a year of breeding *Drosophila* to find variant phenotypes, Morgan discovered a single male fly with white eyes instead of the wild-type red. Morgan mated this mutant white-eyed male with a red-eyed female. The cross is outlined below (see also Campbell, Figure 15.3).

w = white-eye allele
w^+ = red-eye or wild-type allele

Drosophila geneticists symbolize a recessive mutant allele with one or more lower case letters. The corresponding wild-type allele has a superscript plus sign.

P generation:

w^+w^+ red-eyed ♀ × w white-eyed ♂

F_1 generation:

w^+w red-eyed ♀ × w^+ red-eyed ♂

The fact that all the F_1 progeny had red eyes, suggested that the wild-type allele was dominant over the mutant allele.

F_2 generation:

w^+w^+ red-eyed ♀
ww^+ red-eyed ♀
w^+ red-eyed ♂
w white-eyed ♂

White-eyed trait was expressed only in the male, and all the F_2 females had red eyes.

Morgan deduced that eye color is linked to sex and that the gene for eye color is located only on the X chromosome. Premises for his conclusions were:

- If eye color is located only on the X chromosome, then females (XX) carry two copies of the gene, while males (XY) have only one.
- Since the mutant allele is recessive, a white-eyed female must have that allele on both X chromosomes which was impossible for F_2 females in Morgan's experiment.
- A white-eyed male has no wild-type allele to mask the recessive mutant allele, so a single copy of the mutant allele confers white eyes.

Sex-linked genes = Genes located on sex chromosomes. The term is commonly applied only to genes on the X chromosome.

C. Linked genes tend to be inherited together because they are located on the same chromosome

Genes located on the same chromosome tend to be linked in inheritance and do not assort independently.

Linked genes = Genes that are located on the same chromosome and that tend to be inherited together.

- Linked genes do not assort independently, because they are on the same chromosome and move together through meiosis and fertilization.
- Since independent assortment does not occur, a dihybrid cross following two linked genes will not produce an F_2 phenotypic ratio of 9:3:3:1.

T.H. Morgan and his students performed a dihybrid testcross between flies with autosomal recessive mutant alleles for black bodies and vestigial wings and wild-type flies heterozygous for both traits (see Campbell, Figure 15.4).

b	=	black body	*vg*	=	vestigial wings
b^+	=	gray body	vg^+	=	wild-type wings

b^+bvg^+vg	×	*bbvgvg*
gray, normal wings		black, vestigial wings

- Resulting phenotypes of the progeny did not occur in the expected 1:1:1:1 ratio for a dihybrid testcross.
- A disproportionately large number of flies had the phenotypes of the parents: gray with normal wings and black with vestigial wings.
- Morgan proposed that these unusual ratios were due to linkage. The genes for body color and wing size are on the same chromosome and are usually thus inherited together.

D. Independent assortment of chromosomes and crossing over produce genetic recombinants

Genetic recombination = The production of offspring with new combinations of traits different from those combinations found in the parents; results from the events of meiosis and random fertilization.

1. The recombination of unlinked genes: independent assortment of chromosomes

Mendel discovered that some offspring from dihybrid crosses have phenotypes unlike either parent. An example is the following test cross between pea plants:

YY, Yy	=	yellow seeds	RR, Rr	=	round seeds
yy	=	green seeds	rr	=	wrinkled seeds

P generation:

YyRr yellow round	×	yyrr green wrinkled

Testcross progeny:

_ YyRr yellow, round	_ yyrr green, wrinkled	Parental types (50%)
_ yyRr green, round	_ Yyrr yellow, wrinkled	Recombinant types (50%)

Parental types = Progeny that have the same phenotype as one or the other of the parents.

Recombinants = Progeny whose phenotypes differ from either parent.

In this cross, seed shape and seed color are unlinked.

- One-fourth of the progeny have round yellow seeds, and one-fourth have wrinkled green seeds. Therefore, one-half of the progeny are *parental types*.
- The remaining half of the progeny are *recombinants*. One-fourth are round green and one-fourth are wrinkled yellow – phenotypes not found in either parent.
- When half the progeny are recombinants, there is a 50% frequency of recombination.
- A 50% frequency of recombination usually indicates that the two genes are on different chromosomes, because it is the expected result if the two genes assort randomly.
- The genes for seed shape and seed color assort independently of one another because they are located on different chromosomes which randomly align during metaphase of meiosis I.

2. The recombination of linked genes: crossing over

If genes are totally linked, some possible phenotypic combinations should not appear. Sometimes, however, the unexpected recombinant phenotypes do appear.

As described earlier, T.H. Morgan and his students performed the following dihybrid testcross between flies with autosomal recessive mutant alleles for black bodies and vestigial wings and wild-type flies heterozygous for both traits.

b	=	black body	vg	=	vestigial wings
b^+	=	gray body	vg^+	=	wild-type wings

b^+bvg^+vg gray, normal wings	×	$bbvgvg$ black, vestigial wings

Phenotypes	Genotypes	Expected Results If Genes Are Unlinked	Expected Results If Genes Are Totally Linked	Actual Results
Black body, normal wings	$\frac{b\ vg^+}{b\ vg}$	575		206
Gray body, normal wings	$\frac{b^+vg^+}{b\ vg}$	575	1150	965
Black body, vestigial wings	$\frac{b\ vg}{b\ vg}$	575	1150	944
Gray body, vestigial wings	$\frac{b^+vg}{b\ vg}$	575		185

$$\text{Recombination Frequency} = \frac{391 \text{ recombinants}}{2300 \text{ total offspring}} \times 100 = 17\%$$

Morgan's results from this dihybrid testcross showed that the two genes were neither unlinked or totally linked.

- If wing type and body color genes were unlinked, they would assort independently, and the progeny would show a 1:1:1:1 ratio of all possible phenotypic combinations.
- If the genes were completely linked, expected results from the testcross would be a 1:1 phenotypic ratio of *parental types only*.
- Morgan's testcross did not produce results consistent with unlinkage or total linkage. The high proportion of parental phenotypes suggested linkage between the two genes.
- Since 17% of the progeny were recombinants, the linkage must be incomplete. Morgan proposed that there must be some mechanism that occasionally breaks the linkage between the two genes (see Campbell, Figure 15.5).
- It is now known that *crossing over* during meiosis accounts for the recombination of linked genes. The exchange of parts between homologous chromosomes breaks linkages in parental chromosomes and forms recombinants with new allelic combinations.

E. Geneticists can use recombination data to map a chromosome's genetic loci

Scientists used recombination frequencies between genes to *map* the sequence of linked genes on particular chromosomes.

Morgan's *Drosophila* studies showed that some genes are linked more tightly than others.

- For example, the recombination frequency between the *b* and *vg* loci is about 17%.
- The recombination frequency is only 9% between *b* and *cn*, a third locus on the same chromosome. (The cinnabar gene, *cn*, for eye color has a recessive allele causing "cinnabar eyes.")

A.H. Sturtevant, one of Morgan's students, assumed that if crossing over occurs randomly, the probability of crossing over between two genes is directly proportional to the distance between them.

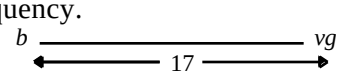
- Sturtevant used recombination frequencies between genes to assign them a linear position on a chromosome *map* (see Campbell, Figure 15.6).

- He defined one *map unit* as 1% recombination frequency. (Map units are now called *centimorgans*, in honor of Morgan.)

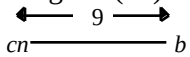
Using crossover data, a map may be constructed as follows:

Loci	Recombination Frequency	Approximate Map Units
b vg	17.0%	18.5*
cn b	9.0%	9.0
cn vg	9.5%	9.5

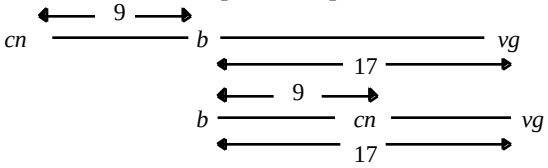
1. Establish the relative distance between those genes farthest apart or with the highest recombination frequency.



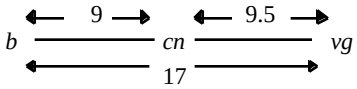
2. Determine the recombination frequency between the third gene (*cn*) and the first (*b*).



3. Consider the two possible placements of the third gene:



4. Determine the recombination frequency between the third gene (*cn*) and the second (*vg)* to eliminate the incorrect sequence.



So, the correct sequence is *b–cn–vg*.

> Note that there are actually 18.5 map units between *b* and *vg*. This is higher than that predicted from the recombination frequency of 17.0%. Because *b* and *vg* are relatively far apart, double crossovers occur between these loci and cancel each other out, leading us to underestimate the actual map distance.

If linked genes are so far apart on a chromosome that the recombination frequency is 50%, they are indistinguishable from unlinked genes that assort independently.

- Linked genes that are far apart can be mapped, if additional recombination frequencies can be determined between intermediate genes and each of the distant genes.

Sturtevant and his coworkers extended this method to map other *Drosophila* genes in linear arrays (see Campbell, Figure 15.7)

- The crossover data allowed them to cluster the known mutations into four major linkage groups.
- Since *Drosophila* has four sets of chromosomes, this clustering of genes into four linkage groups was further evidence that genes are on chromosomes.

Maps based on crossover data only give information about the relative position of linked genes on a chromosome. Another technique, *cytological mapping*, locates genes with respect to chromosomal features, such as stained bands that can be viewed with a microscope.

- The ultimate genetic maps are constructed by sequences, or DNA; in this case, distances between gene loci can be measured in nucleotides.

II. Sex Chromosomes

A. The chromosomal basis of sex varies with the organism

In most species, sex is determined by the presence or absence of special chromosomes. As a result of meiotic segregation, each gamete has one sex chromosome to contribute at fertilization.

Heterogametic sex = The sex that produces two kinds of gametes and determines the sex of the offspring.

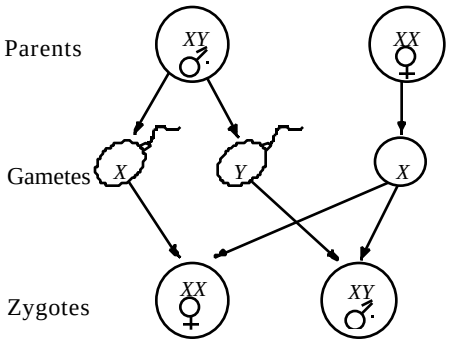
Homogametic sex = The sex that produces one kind of gamete.

Campbell, Figure 15.8 shows four chromosomal systems of sex determination.

1. The chromosomal basis of sex in humans

Mammals, including humans, have an *X*-*Y* mechanism that determines sex at fertilization.

- There are two chromosomes, *X* and *Y*. Each gamete has one sex chromosome, so when sperm cell and ovum unite at fertilization, the zygote receives one of two possible combinations: *XX* or *XY*.
- Males are the heterogametic sex (*XY*). Half the sperm cells contain an *X* chromosome, while the other half contain a *Y* chromosome.



- Females are the homogametic sex (*XX*); all ova carry an *X* chromosome.

Whether an embryo develops into a male or female depends upon the presence of a *Y* chromosome.

- A British research team has identified a gene, *SRY* (sex-determining region of Y), on the *Y* chromosome that is responsible for triggering the complex series of events that lead to normal testicular development. In the absence of SRY, the gonads develop into ovaries.
- *SRY* probably codes for a protein that regulates other genes.

B. Sex-linked genes have unique patterns of inheritance

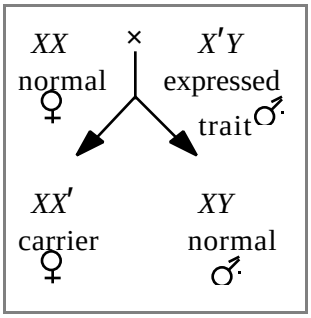
Some genes on sex chromosomes play a role in sex determination, but these chromosomes also contain genes for other traits.

1. Sex-linked disorders in humans

In humans, the term *sex-linked traits* usually refers to *X*-linked traits.

- The human *X*-chromosome is much larger than the *Y*. Thus, there are more *X*-linked than *Y*-linked traits.
- Most *X*-linked genes have no homologous loci on the *Y* chromosome.

- Most genes on the *Y* chromosome not only have no *X* counterparts, but they encode traits found only in males (e.g., testis-determining factor).
- Examples of sex-linked traits in humans are color blindness, *Duchenne muscular dystrophy* and hemophilia.

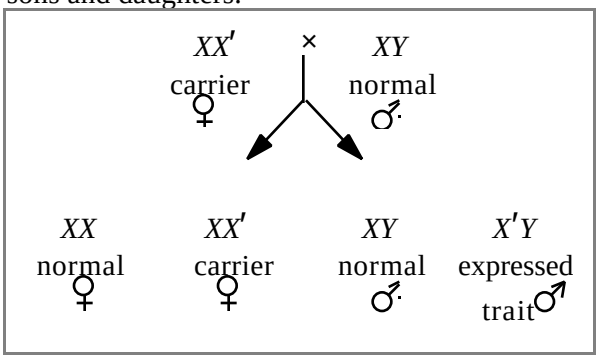


Fathers pass *X*-linked alleles to all their daughters only.

- Males receive their *X* chromosome only from their mothers.
- Fathers cannot pass sex-linked traits to their sons.

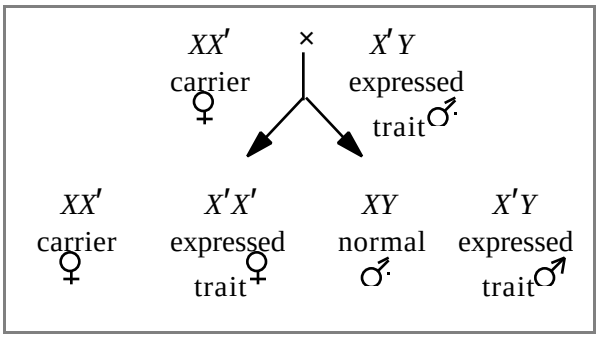
Mothers can pass sex-linked alleles to *both* sons and daughters.

- Females receive two *X* chromosomes, one from each parent.
- Mothers pass on one *X* chromosome (either maternal or paternal homologue) to every daughter and son.



If a sex-linked trait is due to a recessive allele, a female will express the trait only if she is homozygous.

- Females have two *X* chromosomes, therefore they can be either homozygous or heterozygous for sex-linked alleles.
- There are fewer females with sex-linked disorders than males, because even if they have one recessive allele, the other dominant allele is the one that is expressed. A female that is heterozygous for the trait can be a *carrier*, but not show the recessive trait herself.
- A carrier that mates with a normal male will pass the mutation to half her sons and half her daughters.
- If a carrier mates with a male who has the trait, there is a 50% chance that each child born to them will have the trait, regardless of sex.



Campbell, Figure 15.9 depicts the transmission of sex-linked recessive traits.

Because males have only one *X*-linked locus, any male receiving a mutant allele from his mother will express the trait.

- Far more males than females have sex-linked disorders.
- Males are said to be hemizygous.

Hemizygous = A condition where only one copy of a gene is present in a diploid organism.

2. *X*-inactivation in female mammals

How does an organism compensate for the fact that some individuals have a double dosage of sex-linked genes while others have only one?

In female mammals, most diploid cells have only one fully functional *X* chromosome.

- The explanation for this process is known as the *Lyon hypothesis*, proposed by the British geneticist Mary F. Lyon.
- In females, each of the embryonic cells inactivates one of the two *X* chromosomes.
- The inactive *X* chromosome contracts into a dense object called a *Barr body*.

Barr body = Located inside the nuclear envelope, it is a densely staining object that is an inactivated *X* chromosome in female mammalian cells.

- Most Barr body genes are not expressed.
- They are reactivated in gonadal cells that undergo meiosis to form gametes.

Female mammals are a *mosaic* of two types of cellsÑthose with an active maternal *X* and those with an active paternal *X*.

- Which of the two *X*s will be inactivated is determined randomly in embryonic cells.
- After an *X* is inactivated, all mitotic descendants will have the same inactive *X*.
- As a consequence, if a female is heterozygous for a sex-linked trait, about half of her cells will express one allele and the other cells well express the alternate allele.

Examples of this type of mosaicism are coloration in calico cats and normal sweat gland development in humans (see Campbell, Figure 15.10).

X chromosome inactivation is associated with DNA methylation.

- Methyl groups ($-CH_3$) attach to cytosine, one of DNA's nitrogenous bases.
- Barr bodies are highly methylated compared to actively transcribed DNA.

What determines which of the two *X* chromosomes will be methylated?

- A recently discovered gene, *XIST* is active *only* on the Barr body.
- The product of the *XIST* gene, *X-inactive specific transcript*, is an RNA; multiple copies of *XIST* attach to the *X* chromosome inactivating it.

Many questions have yet to be answered.

- How does *XIST* initiate *X*-inactivation?
- What determines which *X* chromosome in each of a female's cells will have an active *XIST* gene and become a Barr body?

III. Errors and Exceptions in Chromosomal Inheritance

A. Alterations of chromosome number or structure cause some genetic disorders

Meiotic errors and mutagens can cause major chromosomal changes such as altered chromosome numbers or altered chromosomal structure.

1. Alterations of chromosome number: aneuploidy and polyploidy

Nondisjunction = Meiotic or mitotic error during which certain homologous chromosomes or sister chromatids fail to separate.

- Meiotic nondisjunction:
  - May occur during meiosis I so that a homologous pair does not separate (see Campbell, Figure 15.11a)
  - May occur during meiosis II when sister chromatids do not separate (see Campbell, Figure 15.11b)

- Results in one gamete receiving two of the same type of chromosome and another gamete receiving no copy. The remaining chromosomes may be distributed normally.

- Mitotic nondisjunction:
  - Also results in abnormal number of certain chromosomes
  - If it occurs in embryonic cells, mitotic division passes this abnormal chromosome number to a large number of cells, and thus, can have a large effect.

Aneuploidy = Condition of having an abnormal number of certain chromosomes

- Aneuploid offspring may result if a normal gamete unites with an aberrant one produced as a result of *nondisjunction.*
- An aneuploid cell that has a chromosome in triplicate is said to be *trisomic* for that chromosome.
- An aneuploid with a missing chromosome is said to be *monosomic* for that chromosome.
- When an aneuploid zygote divides by mitosis, it transmits the chromosomal anomaly to all subsequent embryonic cells.
- Abnormal gene dosage in aneuploids causes characteristic symptoms in survivors. An example is Down's syndrome which results from trisomy of chromosome 21.

Polyploidy = A chromosome number that is more than two complete chromosome sets.

- *Triploidy* is a polyploid chromosome number with three haploid chromosome sets (3N).
- *Tetraploidy* is polyploidy with four haploid chromosome sets (4N).
- Triploids may be produced by fertilization of an abnormal diploid egg produced by nondisjunction of all chromosomes.
- Tetraploidy may result if a diploid zygote undergoes mitosis without cytokinesis. Subsequent normal mitosis would produce a 4N embryo.
- Polyploidy is common in plants and important in plant evolution.
- Polyploids occur rarely among animals, and they are more normal in appearance than aneuploids. Mosaic polyploids, with only patches of polyploid cells, are more common than complete polyploid animals.

2. Alterations of chromosome structure

Chromosome breakage can alter chromosome structure in four ways (see Campbell, Figure 15.12):

- Chromosomes which lose a fragment lacking a centromere will have a deficiency or *deletion.*
- Fragments without centromeres are usually lost when the cell divides, or they may:
  - Join to a homologous chromosome producing a *duplication.*
  - Join to a nonhomologous chromosome (*translocation*).
  - Reattach to the original chromosome in reverse order (*inversion*).

Crossing-over error is another source of deletions and duplications.

- Crossovers are normally reciprocal, but sometimes one sister chromatid gives up more genes than it receives in an unequal crossover.
- A nonreciprocal crossover results in one chromosome with a deletion and one chromosome with a duplication.

Alterations of chromosome structure, can have various effects:

- Homozygous deletions, including a single X in a male, are usually lethal.
- Duplications and translocations tend to have deleterious effects.
- Even if all genes are present in normal dosages, reciprocal translocations between nonhomologous chromosomes and inversions can alter the phenotype because of subtle *position effects*.

Position effect = Influence on a gene's expression because of its location among neighboring genes.

3. Human disorders due to chromosomal alterations

Chromosomal alterations are associated with some serious human disorders.

Aneuploidy, resulting from meiotic nondisjunction during gamete formation, usually prevents normal embryonic development and often results in spontaneous abortion.

- Some types of aneuploidy cause less severe problems, and aneuploid individuals may survive to birth and beyond with a set of characteristic symptoms or *syndrome*.
- Aneuploid conditions can be diagnosed before birth by *fetal testing*.

Down syndrome, an aneuploid condition, affects 1 out of 700 U.S. children (see Campbell, Figure 15.13).

- Is usually the result of trisomy 21
- Includes characteristic facial features, short stature, heart defects, mental retardation, susceptibility to respiratory infections, and a proneness to developing leukemia and Alzheimer's disease
- Though most are sexually underdeveloped and sterile, a few women with Down syndrome have had children.
- The incidence of Down syndrome offspring correlates with maternal age.
    - May be related to the long time lag between the first meiotic division during the mother's fetal life and the completion of meiosis at ovulation.
    - May be that older women have less chance of miscarrying a trisomic embryo.

Other rarer disorders caused by autosomal aneuploidy are:

- *Patau syndrome* (trisomy 13)
- *Edwards syndrome* (trisomy 18)

Sex chromosome aneuploidies result in less severe conditions than those from autosomal aneuploidies. This may be because:

- The *Y* chromosome carries few genes.
- Copies of the *X* chromosome become inactivated as Barr bodies.

The basis of sex determination in humans is illustrated by sex chromosome aneuploidies.

- A single *Y* chromosome is sufficient to produce maleness.
- The absence of *Y* is required for femaleness.

Examples of sex chromosome aneuploidy in males are:

Klinefelter Syndrome

Genotype: Usually *XXY*, but may be associated with *XXYY*, *XXXY*, *XXXXY*, *XXXXXY*.

Phenotype: Male sex organs with abnormally small testes; sterile; feminine body contours and perhaps breast enlargement; usually of normal intelligence.

Extra Y

Genotype: *XYY*.

Phenotype: Normal male; usually taller than average; normal intelligence and fertility.

Abnormalities of sex chromosome number in females include:

Triple-X Syndrome

Genotype: *XXX*.

Phenotype: Usually fertile; can show a normal phenotype.

Turner Syndrome

Genotype: *XO* (only known viable human monosomy).

Phenotype: Short stature; at puberty, secondary sexual characteristics fail to develop; internal sex organs do not mature; sterile.

Structural chromosomal alterations such as deletions and translocations can also cause human disorders.

- Deletions in human chromosomes cause severe defects even in the heterozygous state. For example,
  - *Cri du chat* syndrome is caused by a deletion on chromosome 5. Symptoms are mental retardation, a small head with unusual facial features and a cry that sounds like a mewing cat.
- Translocations associated with human disorders include:
  - Certain cancers such as *chronic myelogenous leukemia* (CML). A portion of chromosome 22 switches places with a small fragment from chromosome 9.
- Some cases of Down syndrome. A third chromosome 21 translocates to chromosome 15, resulting in two normal chromosomes 21 plus the translocation.

B. The phenotypic effects of some genes depend on whether they were inherited from the mother or the father

The expression of some traits may depend upon which parent contributes the alleles for those traits.

- Example: Two genetic disorders, *Prader-Willi syndrome* and *Angelman syndrome*, are caused by the same deletion on chromosome 15. The symptoms differ depending upon whether the gene was inherited from the mother or from the father.
- Prader-Willi syndrome is caused by a deletion from the *paternal* version of chromosome 15. The syndrome is characterized by mental retardation, obesity, short stature, and unusually small hands and feet.
- Angelman syndrome is caused by a deletion from the *maternal* version of chromosome 15. This syndrome is characterized by uncontrollable spontaneous laughter, jerky movements, and other motor and mental symptoms.
- The Prader-Willi/Angelman syndromes imply that the deleted genes normally behave differently in offspring, depending on whether they belong to the maternal or the paternal homologue.

- In other words, homologous chromosomes inherited from males and females are somehow differently *imprinted*, which causes them to be functionally different in the offspring.

Genomic imprinting = Process that induces intrinsic changes in chromosomes inherited from males and females; causes certain genes to be differently expressed in the offspring depending upon whether the alleles were inherited from the ovum or from the sperm cell (see Campbell, Figure 15.14).

- According to this hypothesis, certain genes are imprinted in some way each generation, and the imprint is different depending on whether the genes reside in females or in males.
- The same alleles may have different effects on offspring depending on whether they are inherited from the mother or the father.
- In the new generation, both maternal and paternal imprints can be reversed in gamete-producing cells, and all the chromosomes are re-coded according to the sex of the individual in which they now reside.
- *DNA methylation* may be one mechanism for genomic imprinting

Affecting about one in every 1500 males and one in every 2500 females, *fragile X syndrome* is the most common genetic cause of mental retardation.

- The "fragile *X*" is an abnormal *X* chromosome, the tip of which hangs on the rest of the chromosome by a thin DNA thread.

Fragile X syndrome's complex expression may be a consequence of maternal genomic imprinting.

- The syndrome is more likely to appear if the abnormal *X* chromosome is inherited from the mother rather than the father; this is consistent with the disorder being more common in males.
- Fragile x is unusual in that maternal imprinting (methylation) does not silence the abnormal allele but rather, somehow causes the syndrome.

C. Extranuclear genes exhibit a non-Mendelian pattern of inheritance

There are some exceptions to the chromosome theory of inheritance.

- Extranuclear genes are found in cytoplasmic organelles such as plastids and mitochondria.
- These cytoplasmic genes are not inherited in Mendelian fashion, because they are not distributed by segregating chromosomes during meiosis.

In plants, a zygote receives its plastids from the ovum, not from pollen. Consequently, offspring receive only maternal cytoplasmic genes.

- Cytoplasmic genes in plants were first descaribed by Karl Corens (1909) when he noticed that plant coloration of an ornamental species was determined by the seed bearing plants and not by the pollen producing plants (see Campbell, Figure 15.15).
- It is now known that maternal plastid genes control variegation of leaves.

In mammals, inheritance of mitochondrial DNA is also exclusively maternal.

- Since the ovum contributes most of the cytoplasm to the zygote, the mitochondria are all maternal in origin.

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CHAPTER 16
THE MOLECULAR BASIS OF INHERITANCE

OUTLINE

I. DNA as the Genetic Material
   A. The search for the genetic material led to DNA: *science as a process*
   B. Watson and Crick discovered the double helix by building models to conform to X-ray data: *science as a process*

II. DNA Replication and Repair
   A. During DNA replication, base-pairing enables existing DNA strands to serve as templates for new complementary strands
   B. .A large team of enzymes and other proteins carries out DNA replication
   C. Enzymes proofread DNA during its replication and repair damage to existing DNA
   D. The ends of DNA molecules pose a special function.

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Explain why researchers originally thought protein was the genetic material.
2. Summarize experiments performed by the following scientists, which provided evidence that DNA is the genetic material:
   a. Frederick Griffith
   b. Alfred Hershey and Martha Chase
   c. Erwin Chargaff
3. List the three components of a nucleotide.
4. Distinguish between deoxyribose and ribose.
5. List the nitrogen bases found in DNA, and distinguish between pyrimidine and purine.
6. Explain how Watson and Crick deduced the structure of DNA, and describe what evidence they used.
7. Explain the "base-pairing rule" and describe its significance.
8. Describe the structure of DNA, and explain what kind of chemical bond connects the nucleotides of each strand and what type of bond holds the two strands together.
9. Explain, in their own words, semiconservative replication, and describe the Meselson-Stahl experiment.
10. Describe the process of DNA replication, and explain the role of helicase, single strand binding protein, DNA polymerase, ligase, and primase.
11. Explain what energy source drives endergonic synthesis of DNA.
12. Define antiparallel, and explain why continuous synthesis of both DNA strands is not possible.

13. Distinguish between the leading strand and the lagging strand.
14. Explain how the lagging strand is synthesized when DNA polymerase can add nucleotides only to the 3′ end.
15. Explain the role of DNA polymerase, ligase, and repair enzymes in DNA proofreading and repair.

KEY TERMS

phages	DNA polymerase	primase	nuclease
double helix	leading strand	helicase	excision repair
semiconservative model	lagging strand	single-strand binding protein	telomerase
origins of replication	DNA ligase		
replication fork	primer	mismatch repair	

LECTURE NOTES

Deoxyribonucleic acid, or DNA, is genetic material. DNA is the substance of Mendel's heritable factors and of Morgan's genes on chromosomes. Inheritance has its molecular basis in the precise replication and transmission of DNA from parent to offspring.

I. DNA as the Genetic Material

A. The search for the genetic material led to DNA: *science as a process*

By the 1940s, scientists knew that chromosomes carried hereditary material and consisted of DNA and protein. Most researchers thought protein was the genetic material because:

- Proteins are macromolecules with great heterogeneity and functional specificity.
- Little was known about nucleic acids.
- The physical and chemical properties of DNA seemed too uniform to account for the multitude of inherited traits.

1. Evidence that DNA can transform bacteria

In 1928, Frederick Griffith performed experiments that provided evidence that genetic material is a specific molecule.

Griffith was trying to find a vaccine against *Streptococcus pneumoniae*, a bacterium that causes pneumonia in mammals. He knew that:

- There were two distinguishable strains of the pneumococcus: one produced smooth colonies (S) and the other rough colonies (R).
- Cells of the smooth strain were encapsulated with a polysaccharide coat and cells of the rough strain were not.
- These alternative phenotypes (S and R) were inherited.

Griffith performed four sets of experiments:

Experiment: Griffith injected live S strain of *Streptococcus pneumoniae* into mice.

Results: Mice died of pneumonia.

Conclusions: Encapsulated strain was pathogenic.

Experiment: Mice were injected with live R strain.

Results: Mice survived and were healthy.

Conclusions: The bacterial strain lacking the polysaccharide coat was non-pathogenic.

Experiment: Mice were injected with heat-killed S strain of pneumococcus.

Results: Mice survived and were healthy.

Conclusions: Polysaccharide coat did not cause pneumonia because it was still present in heat-killed bacteria which proved to be non-pathogenic.

Experiment: Heat-killed S cells mixed with live R cells were injected into mice.

Results: Mice developed pneumonia and died. Blood samples from dead mice contained live S cells.

Conclusions: R cells had acquired from the dead S cells the ability to make polysaccharide coats. Griffith cultured S cells from the dead mice. Since the dividing bacteria produced encapsulated daughter cells, he concluded that this newly acquired trait was inheritable. This phenomenon is now called transformation.

Transformation = Change in phenotype due to the assimilation of external genetic material by a cell

What was the chemical nature of the transforming agent?

- Griffith was unable to answer this question, but other scientists continued the search.
- Griffith's experiments hinted that protein is not the genetic material. Heat denatures protein, yet it did not destroy the transforming ability of the genetic material in the heat-killed S cells.
- In 1944, after a decade of research, Oswald Avery, Maclyn McCarty, and Colin MacLeod discovered that the transforming agent had to be DNA.
- The discovery by Avery and his coworkers was met with skepticism by other scientists, because they still believed protein was a better candidate for the genetic material and so little was known about DNA.

2. Evidence that viral DNA can program cells

More evidence that DNA is the genetic material came from studies of bacteriophages.

Bacteriophage (phage) = Virus that infects bacteria

In 1952, Alfred Hershey and Martha Chase discovered that DNA was the genetic material of a phage known as T2. They knew that T2:

- Was one of many phages to infect the enteric bacterium *Escherichia coli* (*E. coli*).
- Like many other viruses, was little more than DNA enclosed by a protein coat.
- Could quickly reprogram an *E. coli* cell to produce T2 phages and release the viruses when the cell lysed.

What Hershey and Chase did not know was which viral component—DNA or protein—was responsible for reprogramming the host bacterial cell. They answered this question by performing the following experiment (see Campbell, Figure 16.1):

Experiment:

Step 1: Viral protein and DNA were tagged with different radioactive isotopes.

- *Protein tagging*: T2 and *E. coli* were grown in media with radioactive sulfur (^{35}S) which was incorporated only into the phage protein.
- *DNA tagging*: T2 and *E. coli* were grown in media containing radioactive phosphorus (^{32}P) which was incorporated only into the phage DNA.

Step 2: Protein-labeled and DNA-labeled T2 phages were allowed to infect separate samples of nonradioactive *E. coli* cells.

Step 3: Cultures were agitated to shake loose phages that remained outside the bacterial cells.

Step 4: Mixtures were centrifuged forcing the heavier bacterial cells into a pellet on the bottom of the tubes. The lighter viruses remained in the supernatant.

Step 5: Radioactivity in the pellet and supernatant was measured and compared.

Results:

1. In tubes with E. coli infected with protein-labeled T2, most of the radioactivity was in the supernatant with viruses.
2. In tubes with E. coli infected with DNA-labeled T2, most of the radioactivity was in the pellet with the bacterial cells.
3. When the bacteria containing DNA-labeled phages were returned to culture medium, the bacteria released phage progeny which contained 32P in their DNA.

Conclusions:

1. Viral proteins remain outside the host cell.
2. Viral DNA is injected into the host cell.
3. Injected DNA molecules cause cells to produce additional viruses with more viral DNA and proteins.
4. These data provided evidence that nucleic acids rather than proteins were the hereditary material.

3. Additional evidence that DNA is the genetic material of cells

Hershey and Chase's experiments provided evidence that DNA is the hereditary material in viruses. Additional evidence pointed to DNA as the genetic material in eukaryotes as well.

Some circumstantial evidence was:

- A eukaryotic cell doubles its DNA content prior to mitosis.
- During mitosis, the doubled DNA is equally divided between two daughter cells.
- An organism's diploid cells have twice the DNA as its haploid gametes.

Experimental evidence for DNA as the hereditary material in eukaryotes came from the laboratory of Erwin Chargaff. In 1947, he analyzed the DNA composition of different organisms. Using paper chromatography to separate nitrogenous bases, Chargaff reported the following:

- DNA composition is species-specific.
- The amount and ratios of nitrogenous bases vary from one species to another.
- This source of molecular diversity made it more credible that DNA is the genetic material.
- In every species he studied, there was a regularity in base ratios.
    - The number of adenine (A) residues approximately equaled the number of thymines (T), and the number of guanines (G) equaled the number of cytosines (C).
    - The A=T and G=C equalities became known later as *Chargaff's rules*. Watson and Crick's structural model for DNA explained these rules.

B. Watson and Crick discovered the double helix by building models to conform to x-ray data: s*cience as a process*

Before this discussion, it may be helpful to review material from Chapter 7, such as: components of nucleotides; nitrogenous bases in DNA; difference between purine and pyrimidine; the kinds of chemical bonds that connect nucleotides of each DNA strand; and the type of bond that holds the two strands together. Because so much time elapses between the lectures on organic molecules and molecular genetics, students may forget crucial information necessary to understand this material. For this reason, you may find it best to defer discussion of nucleic acids (from Chapter 7) until this point in the course.

By the 1950s, DNA was accepted as the genetic material, and the covalent arrangement in a nucleic acid polymer was well established (see Campbell, Figure 16.2). The three-dimensional structure of DNA, however, was yet to be discovered.

Among scientists working on the problem were the following:

Linus Pauling, California Institute of Technology

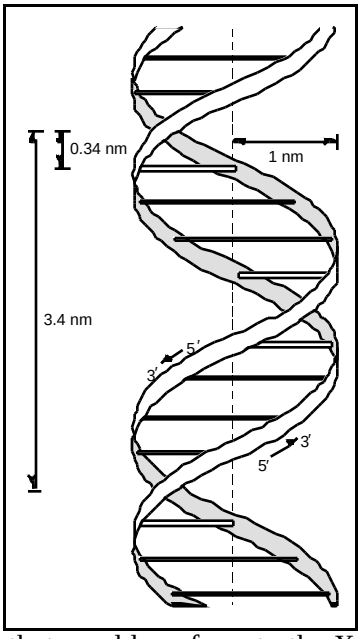
Maurice Wilkins and Rosalind Franklin, King's College in London

James D. Watson (American) and Francis Crick, Cambridge University

James Watson went to Cambridge to work with Francis Crick who was studying protein structure with *X-ray crystallography*.

Watson saw an X-ray photo of DNA produced by Rosalind Franklin at King's College, London (see Campbell, Figure 16.3). Watson and Crick deduced from Franklin's X-ray data that:

a. DNA is a helix with a uniform width of 2 nm. This width suggested that it had two strands.
b. Purine and pyrimidine bases are stacked .34 nm apart.
c. The helix makes one full turn every 3.4 nm along its length.
d. There are ten layers of nitrogenous base pairs in each turn of the helix.



Watson and Crick built scale models of a double helix that would conform to the X-ray data and the known chemistry of DNA.

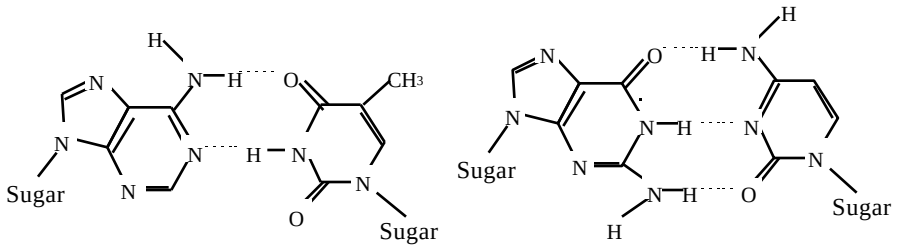
- One of their unsuccessful attempts placed the sugar-phosphate chains inside the molecule.
- Watson next put the sugar-phosphate chains on the outside which allowed the more hydrophobic nitrogenous bases to swivel to the interior away from the aqueous medium (see Campbell, Figure 16.4).
- Their proposed structure was a ladder-like molecule twisted into a spiral, with sugar-phosphate backbones as uprights and pairs of nitrogenous bases as rungs.

- The two sugar-phosphate backbones of the helix were *antiparallel*; that is, they ran in opposite directions.

Watson and Crick finally solved the problem of DNA structure by proposing that there is a specific pairing between nitrogenous bases. After considering several arrangements, they concluded:

- To be consistent with a 2 nm width, a purine on one strand must pair (by hydrogen bonding) with a pyrimidine on the other.
- Base structure dictates which pairs of bases can hydrogen bonds. The base pairing rule is that adenine can only pair with thymine, and guanine with cytosine (see Campbell, Figure 16.5).

Purines	Pyrimidines	Possible Base Pairs	Number of Hydrogen Bonds
Adenine (A)	Thymine (T)	A – T	2
Guanine (G)	Cytosine (C)	G – C	3



ADENINE (A) THYMINE (T) GUANINE (G) CYTOSINE (C)

The base-pairing rule is significant because:

- It explains Chargaff's rules. Since A must pair with T, their amounts in a given DNA molecule will be about the same. Similarly, the amount of G equals the amount of C.
- It suggests the general mechanisms for DNA replication. If bases form specific pairs, the information on one strand complements that along the other.
- It dictates the combination of complementary base pairs, but places no restriction on the linear sequence of nucleotides along the length of a DNA strand. The sequence of bases can be highly variable which makes it suitable for coding genetic information.
- Though hydrogen bonds between paired bases are weak bonds, collectively they stabilize the DNA molecule. Van der Waals forces between stacked bases also help stabilize DNA.

I. DNA Replication and Repair

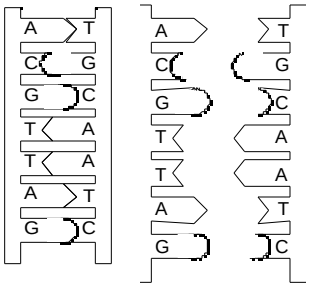
A. During DNA replication, base pairing enables existing DNA strands to serve as templates for new complementary strands

In April 1953, Watson and Crick's new model for DNA structure, the double helix, was published in the British journal *Nature*. This model of DNA structure suggested a *template* mechanism for DNA replication.

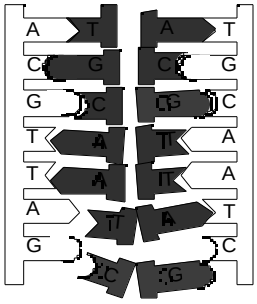
- Watson and Crick proposed that genes on the original DNA strand are copied by a specific pairing of complementary bases, which creates a complementary DNA strand.

- The complementary strand can then function as a template to produce a copy of the original strand.

In a second paper, Watson and Crick proposed that during DNA replication (see also Campbell, Figure 16.6):



1. The two DNA strands separate.
2. Each strand is a template for assembling a complementary strand.



3. Nucleotides line up singly along the template strand in accordance with the base-pairing rules (A-T and G-C).
4. Enzymes link the nucleotides together at their sugar-phosphate groups.

Watson and Crick's model is a *semiconservative model* for DNA replication.

- They predicted that when a double helix replicates, each of the two daughter molecules will have one old or *conserved* strand from the parent molecule and one newly created strand.
- In the late 1950s, Matthew Meselson and Franklin Stahl provided the experimental evidence to support the semiconservative model of DNA replication. A brief description of the experimental steps follows (see Campbell, Figure 16.8.).

Hypotheses:

There were three alternate hypotheses for the pattern of DNA replication (see Campbell, Figure 16.7).:

a. If DNA replication is *conservative*, then the parental double helix should remain intact and the second DNA molecule should be constructed as entirely new DNA.

b. If DNA replication is *semiconservative*, then each of the two resulting DNA molecules should be composed of one original or conserved strand (template) and one newly created strand.

c. If DNA replication is *dispersive*, then both strands of the two newly produced DNA molecules should contain a mixture of old and new DNA.

Experiment:

Step 1: Labeling DNA strands with ^{15}N.

E. coli was grown for several generations in a medium with heavy nitrogen (^{15}N). As *E. coli* cells reproduced, they incorporated the ^{15}N into their nitrogenous bases.

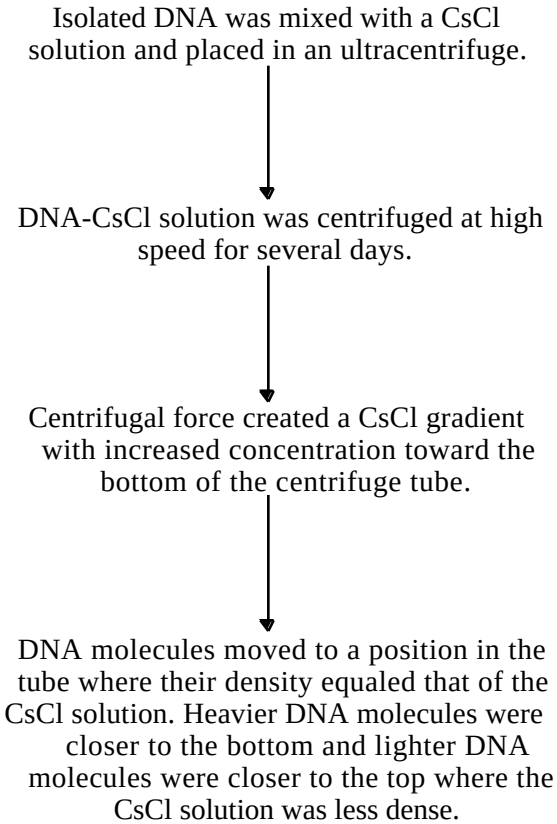
Step 2: Transfer of *E. coli* to a medium with ^{14}N.

E. coli cells grown in heavy medium were transferred to a medium with light nitrogen, ^{14}N. There were three experimental classes of DNA based upon times after the shift from heavy to light medium:

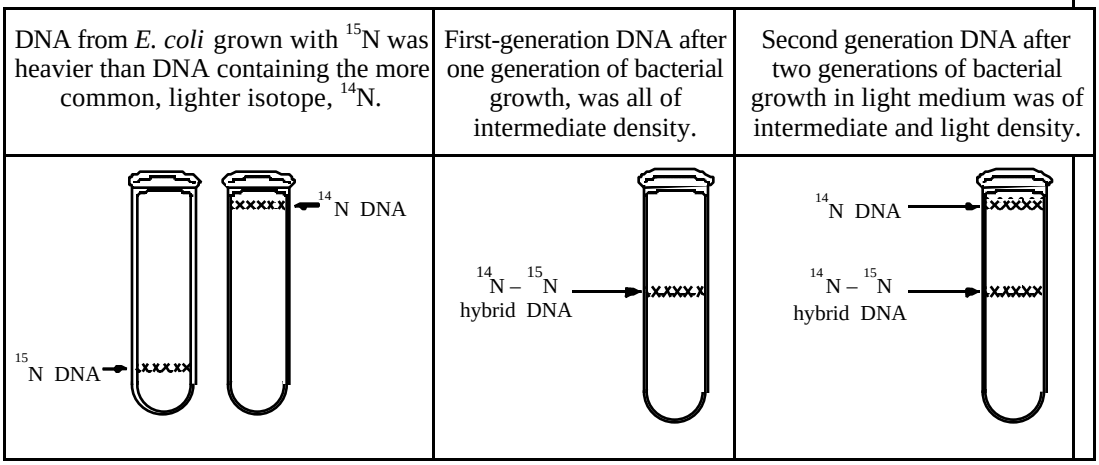
a. Parental DNA from *E. coli* cells grown in heavy medium
b. First-generation DNA extracted from *E. coli* after one generation of growth in the light medium
c. Second-generation DNA extracted from *E. coli* after two replications in the light medium

Step 3: Separation of DNA classes based upon density differences.

Meselson and Stahl used a new technique to separate DNA based on density differences between ^{14}N and ^{15}N.



Results:



Conclusions:

Their results supported the hypothesis of semiconservative replication for DNA.

- The first generation DNA was all hybrid DNA containing one heavy parental strand and one newly synthesized light strand.
- These results were predicted by the semiconservative model. If DNA replication was conservative, two classes of DNA would be produced. The heavy parental DNA would be conserved and newly synthesized DNA would be light. There would be no intermediate density hybrid DNA.
- The results for first-generation DNA eliminated the possibility of conservative replication, but did not eliminate the possibility of dispersive replication.
- Consequently, Meselson and Stahl examined second-generation DNA. The fact that there were two bands, one light and one hybrid band supported the semiconservative model and allowed the investigators to rule out dispersive replication. If DNA replication was dispersive, there would have been only one intermediate density band between light and hybrid DNA.

B. A large team of enzymes and other proteins carries out DNA replication

The general mechanism of DNA replication is conceptually simple, but the actual process is:

- *Complex*. The helical molecule must untwist while it copies its two antiparallel strands simultaneously. This requires the cooperation of over a dozen enzymes and other proteins.
- *Extremely rapid*. In prokaryotes, up to 500 nucleotides are added per second. It takes only a few hours to copy the 6 billion bases of a single human cell.
- *Accurate*. Only about one in a billion nucleotides is incorrectly paired.

Generally similar in prokaryotes and eukaryotes.

1. Getting started: origins of replication

DNA replication begins at special sites called *origins of replication* that have a specific sequence of nucleotides (see Campbell, Figure 16.9).

- Specific proteins required to initiate replication bind to each origin.

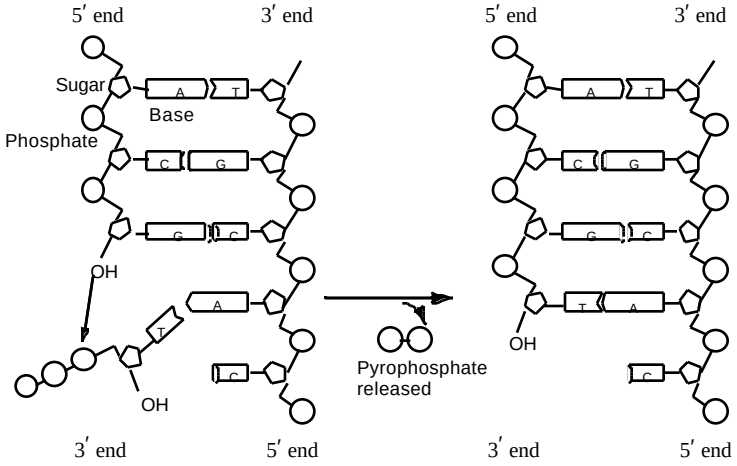
- The DNA double helix opens at the origin and *replication forks* spread in both directions away from the central initiation point creating a *replication bubble*.
- Bacterial or viral DNA molecules have only one replication origin.
- Eukaryotic chromosomes have hundreds or thousands of replication origins. The many replication bubbles formed by this process, eventually merge forming two continuous DNA molecules.

Replication forks = The Y-shaped regions of replicating DNA molecules where new strands are growing.

2. Elongating a new DNA strand

Enzymes called *DNA polymerases* catalyze synthesis of a new DNA strand.

- According to base-pairing rules, new nucleotides align themselves along the templates of the old DNA strands.
- *DNA polymerase* links the nucleotides to the growing strand. These strands grow in the 5′ → 3′ direction since new nucleotides are added only to the 3′ end of the growing strand.



Hydrolysis of *nucleoside triphosphates* provides the energy necessary to synthesize the new DNA strands.

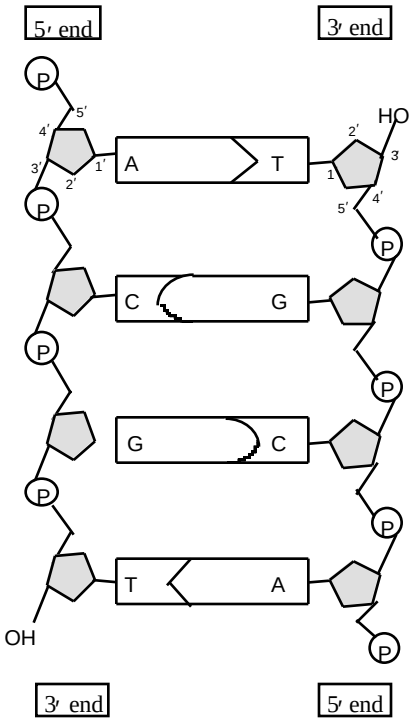
Nucleoside triphosphate = Nucleotides with a triphosphate (three phosphates) covalently linked to the 5′ carbon of the pentose.

- Recall that the pentose in DNA is deoxyribose, and the pentose in RNA is ribose.
- Nucleoside triphosphates that are the building blocks for DNA lose two phosphates (pyrophosphate group) when they form covalent linkages to the growing chain (see Campbell, Figure 16.10).
- Exergonic hydrolysis of this phosphate bond drives the endergonic synthesis of DNA; it provides the required energy to form the new covalent linkages between nucleotides.

3. The problem of antiparallel DNA strands

Continuous synthesis of both DNA strands at a replication fork is not possible, because:

- The sugar phosphate backbones of the two complementary DNA strands run in opposite directions; that is, they are *antiparallel* (see Campbell, Figure 16.11).
- Recall that each DNA strand has a distinct polarity. At one end (3′ end), a hydroxyl group is attached to the 3′ carbon of the terminal deoxyribose; at the other end (5′ end), a phosphate group is attached to the 5′ carbon of the terminal deoxyribose.
- DNA polymerase can only elongate strands in the 5′ to 3′ direction.

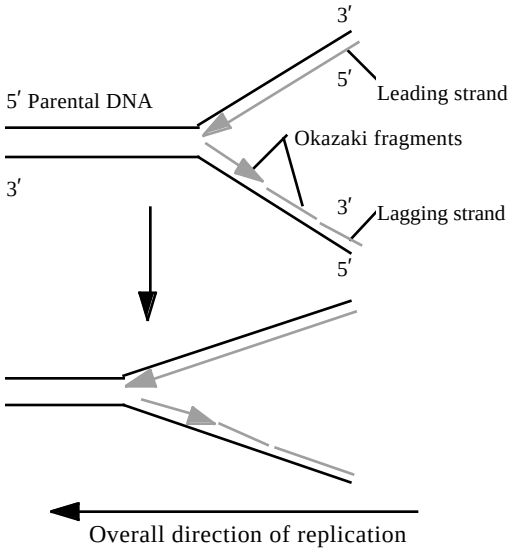


The problem of antiparallel DNA strands is solved by the continuous synthesis of one strand (*leading strand*) and discontinuous synthesis of the complementary strand (*lagging strand*).

Leading strand = The DNA strand which is synthesized as a single polymer in the 5′ → 3′ direction towards the replication fork.

Lagging strand = The DNA strand that is discontinuously synthesized against the overall direction of replication.

- Lagging strand is produced as a series of short segments called *Okazaki fragments* which are each synthesized in the 5′ → 3′ direction.
- Okazaki fragments are 1000 to 2000 nucleotides long in bacteria and 100 to 200 nucleotides long in eukaryotes.
- The many fragments are ligated by *DNA ligase*, a linking enzyme that catalyzes the formation of a covalent bond between the 3′ end of each new Okazaki fragment to the 5′ end of the growing chain.



4. Priming DNA synthesis

Before new DNA strands can form, there must be small preexisting primers to start the addition of new nucleotides.

Primer = Short RNA segment that is complementary to a DNA segment and that is necessary to begin DNA replication.

- Primers are short segments of RNA polymerized by an enzyme called *primase* (see Campbell, Figure 16.13).
- A portion of the parental DNA serves as a template for making the primer with a complementary base sequence that is about ten nucleotides long in eukaryotes.
- Primer formation must precede DNA replication, because DNA polymerase can only add nucleotides to a polynucleotide that is already correctly base-paired with a complementary strand.

Only one primer is necessary for replication of the leading strand, but many primers are required to replicate the lagging strand.

- An RNA primer must initiate the synthesis of each Okazaki fragment.
- The many Okazaki fragments are ligated in two steps to produce a continuous DNA strand:
  - DNA polymerase removes the RNA primer and replaces it with DNA.
  - DNA ligase catalyzes the linkage between the 3′ end of each new Okazaki fragment to the 5′ end of the growing chain.

5. Other proteins assisting DNA replication

There are two types of proteins involved in the separation of parental DNA strands:

a. *Helicases* are enzymes which catalyze unwinding of the parental double helix to expose the template.

b. *Single-strand binding proteins* are proteins which keep the separated strands apart and stabilize the unwound DNA until new complementary strands can be synthesize.

Campbell, Figure 16.14 summarizes the functions of the main proteins that cooperate in DNA replication.

Campbell, Figure 16.15 is a visual summary of DNA replication.

C. Enzymes proofread DNA during its replication and repair damage in existing DNA

DNA replication is highly accurate, but this accuracy is not solely the result of base-pairing specificity.

- Initial pairing errors occur at a frequency of about one in ten thousand, while errors in a complete DNA molecule are only about one in one billion.
- DNA can be repaired as it is being synthesized (e.g., *mismatch repair*) or after accidental changes in existing DNA (e.g., *excision repair*).

Mismatch repair, corrects mistakes when DNA is synthesized.

- In bacteria, DNA polymerase proofreads each newly added nucleotide against its template. If polymerase detects an incorrectly paired nucleotide, the enzyme removes and replaces it before continuing with synthesis.
- In eukaryotes, additional proteins as well as polymerase participate in mismatch repair. A hereditary defect in one of these proteins has been associated with a form of colon cancer. Apparently, DNA errors accumulate in the absence of adequate proofreading.

Excision repair, corrects accidental changes that occur in existing DNA (see Campbell, Figure 16.17).

- Accidental changes in DNA can result from exposure to reactive chemicals, radioactivity, X-rays and ultraviolet light.
- There are more than fifty different types of DNA repair enzymes that repair damage. For example, in *excision repair*:

- The damaged segment is excised by one *repair enzyme* and the remaining gap is filled in by base-pairing nucleotides with the undamaged strand.
- *DNA polymerase* and *DNA ligase* are enzymes that catalyze the filling-in process.

D. The ends of DNA molecules pose a special problem

Because DNA polymerases can only add nucleotides to the 3' end of a preexisting polynucleotide, repeated replication of linear DNA, such as that possessed by all eukaryotes, would result in successively shorter molecules, potentially deleting genes (see Campbell, Figure 16.17).

- Prokaryotes don't have this problem because they possess circular DNA.
- Eukaryotic DNA is flanked by *telomeres*, repeats of short noncoding nucleotide sequences (see Campbell, Figure 16.18).

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CHAPTER 17
FROM GENE TO PROTEIN

OUTLINE

I. The Connection between Genes and Proteins
   A. The study of metabolic defects provided evidence that genes specify proteins: *science as a process*
   B. Transcription and translation are the two main processes linking gene to protein: *an overview*
   C. In the genetic code, nucleotide triplets specify amino acids
   D. The genetic code must have evolved very early in the history of life

II. The Synthesis and Processing of RNA
   A. Transcription is the DNA-directed synthesis of RNA: *a closer look*
   B. Eukaryotic cells modify RNA after transcription

III. The Synthesis of Protein
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   B. Signal peptides target some eukaryotic polypeptides to specific locations in the cell
   C. RNA plays multiple roles in the cell: a review
   D. Comparing protein synthesis in prokaryotes and eukaryotes: *a review*
   E. Point mutations can affect protein structure and function
   F. What is a gene? *revisiting the question*

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Give early experimental evidence that implicated proteins as the links between genotype and phenotype.
2. Describe Beadle and Tatum's experiments with *Neurospora*, and explain the contribution they made to our understanding of how genes control metabolism.
3. Distinguish between "one gene—one enzyme" hypothesis and "one gene—one polypeptide," and explain why the original hypothesis was changed.
4. Explain how RNA differs from DNA.
5. In their own words, briefly explain how information flows from gene to protein.
6. Distinguish between transcription and translation.
7. Describe where transcription and translation occur in prokaryotes and in eukaryotes; explain why it is significant that in eukaryotes, transcription and translation are separated in space and time.
8. Define codon, and explain what relationship exists between the linear sequence of codons on mRNA and the linear sequence of amino acids in a polypeptide.
9. List the three stop codons and the one start codon.
10. Explain in what way the genetic code is redundant and unambiguous.

11. Explain the evolutionary significance of a nearly universal genetic code.
12. Explain the process of transcription including the three major steps of initiation, elongation, and termination.
13. Describe the general role of RNA polymerase in transcription.
14. Explain how RNA polymerase recognizes where transcription should begin.
15. Specifically, describe the primary functions of RNA polymerase II.
16. Distinguish among mRNA, tRNA, and rRNA.
17. Describe the structure of tRNA and explain how the structure is related to function.
18. Given a sequence of bases in DNA, predict the corresponding codons transcribed on mRNA and the corresponding anticodons of tRNA.
19. Describe the wobble effect.
20. Explain how an aminoacyl-tRNA synthetase matches a specific amino acid to its appropriate tRNA; describe the energy source that drives this endergonic process.
21. Describe the structure of a ribosome, and explain how this structure relates to function.
22. Describe the process of translation including initiation, elongation, and termination and explain what enzymes, protein factors, and energy sources are needed for each stage.
23. Explain what determines the primary structure of a protein and describe how a polypeptide must be modified before it becomes fully functional.
24. Describe what determines whether a ribosome will be free in the cytosol or attached to rough ER.
25. Explain how proteins can be targeted for specific sites within the cell.
26. Describe the difference between prokaryotic and eukaryotic mRNA.
27. Explain how eukaryotic mRNA is processed before it leaves the nucleus.
28. Describe some biological functions of introns and gene splicing.
29. Explain why base-pair insertions or deletions usually have a greater effect than base-pair substitutions.
30. Describe how mutagenesis can occur.

KEY TERMS

auxotroph
one gene–one polypeptide
transcription
messenger RNA (mRNA)
translation
RNA processing
primary transcript
triplet code
template strand
codon
reading frame
RNA polymerase
transcription unit
transcription factors
transcription initiation complex
TATA box
terminator
5' cap
poly (A) tail
RNA splicing
intron
exon
spliceosome
domain
transfer RNA (tRNA)
anticodon
wobble
aminoacyl-tRNA synthetases
ribosomal RNA (rRNA)
P site
A site
E site
polyribosome
signal peptide
signal-recognition particle (SRP)
mutation
point mutation
point mutation
base-pair substitution
missense mutation
nonsense mutation
insertion
deletion
frameshift mutation
mutagens
Ames test

LECTURE NOTES

Inherited instructions in DNA direct protein synthesis. Thus, proteins are the links between genotype and phenotype, since proteins are directly involved in the expression of specific phenotypic traits.

I. The Connection between Genes and Proteins

A. The study of metabolic defects provided evidence that genes specify proteins: *science as a process*

Archibald Garrod was the first to propose the relationship between genes and proteins (1909).

- He suggested that genes dictate phenotypes through enzymes that catalyze reactions.
- As a physician, Garrod was familiar with inherited diseases which he called "inborn errors in metabolism." He hypothesized that such diseases reflect the patient's inability to make particular enzymes.
- One example he studied was *alkaptonuria,* which causes the afflicted person's urine to turn black.
  - People with alkaptonuria accumulate alkapton in their urine, causing it to darken on contact with air.
  - Garrod reasoned that alkaptonurics, unlike normal individuals, lack the enzyme that breaks down alkapton.

1. How genes control metabolism

Garrod's hypothesis was confirmed several decades later by research which determined that specific genes direct production of specific enzymes.

- Biochemists found that cells synthesize and degrade organic compounds via metabolic pathways, with each sequential step catalyzed by a specific enzyme.
- Geneticists George Beadle and Boris Ephrussi (1930s) studied eye color in *Drosophila*. They speculated that mutations affecting eye color block pigment synthesis by preventing enzyme production at certain steps in the pigment synthesis pathway.

George Beadle and Edward Tatum were later able to demonstrate the relationship between genes and enzymes by studying mutants of a bread mold, *Neurospora crassa* (see Campbell, Figure 17.1).

- Wild-type *Neurospora* in laboratory colonies can survive on *minimal medium.* All other molecules needed by the mold are produced by its own metabolic pathways from this minimal nutrient source.
- Beadle and Tatum searched for mutants or *auxotrophs* that could not survive on minimal medium because they lacked the ability to synthesize essential molecules.
- Mutants were identified by transferring fragments of growing fungi (in complete medium) to vials containing minimal medium. Fragments that didn't grow were identified as auxotrophic mutants.

Auxotroph = (Auxo = to augment; troph = nourishment); nutritional mutants that can only be grown on *minimal medium* augmented with nutrients not required by the wild type

Minimal medium = Support medium that is mixed only with molecules required for the growth of wild-type organisms

- Minimal medium for *Neurospora* contains inorganic salts, sucrose, and the vitamin biotin.
- Nutritional mutants cannot survive only on minimal medium.

Complete growth medium = Minimal medium supplemented with all 20 amino acids and some other nutrients

- Nutritional mutants can grow on complete growth medium, since all essential nutrients are provided.

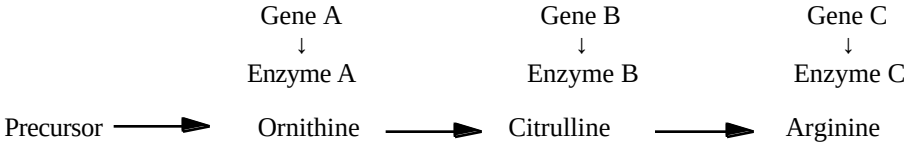
Beadle and Tatum then identified specific metabolic defects (from mutations) by transferring fragments of auxotrophic mutants growing on complete growth medium to vials containing minimal medium each supplemented with only *one* additional nutrient.

- Vials where growth occurred indicated the metabolic defect, since the single supplement provided the necessary component.
- For example, if a mutant grew on minimal medium supplemented with only arginine, it could be concluded that the mutant was defective in the arginine synthesis pathway.

Experiment:

Beadle and Tatum experimented further to more specifically describe the defect in the multistep pathway that synthesizes the amino acid arginine.

- Arginine synthesis requires three steps each catalyzed by a specific enzyme:



- They distinguished between three classes of arginine auxotrophs by adding either arginine, citrulline, or ornithine to the medium and seeing if growth occurred.

Results:

Some mutants required arginine, some either arginine or citrulline, and others could grow when any of the three were added.

	Minimal Medium (MM)	MM plus Ornithine	Mm plus Citrulline	MM plus Arginine
Wild Type	+	+	+	+
Class I Mutants	–	+	+	+
Class II Mutants	–	–	+	+
Class III Mutants	–	–	–	+

+ = growth, – = no growth

Conclusions:

Beadle and Tatum deduced from their data that the three classes of mutants each lacked a different enzyme and were thus blocked at different steps in the arginine synthesis pathway.

- Class I mutants lacked enzyme A; Class II mutants lacked enzyme B; and Class III mutants lacked enzyme C.

- Assuming that each mutant was defective in a single gene, they formulated the *one gene-one enzyme* hypothesis, which states that the function of a gene is to dictate the production of a specific enzyme.

2. One gene—one polypeptide

Beadle and Tatum's one gene-one enzyme hypothesis has been slightly modified:

- While most enzymes are proteins, many proteins are not enzymes. Proteins that are not enzymes are still, nevertheless, gene products.
- Also, many proteins are comprised of two or more polypeptide chains, each chain specified by a different gene (e.g., globulin chains of hemoglobin).

As a result of this new information, Beadle and Tatum's hypothesis has been restated as *one gene-one polypeptide.*

> As we will see later, even this notion is no longer tenable given that a) differential processing of a single RNA transcript can lead to the synthesis of numerous different proteins, and b) not all RNA is translated into protein.

B. Transcription and translation are the two main processes linking gene to protein: *an overview*

Ribonucleic acid (RNA) links DNA's genetic instructions for making proteins to the process of protein synthesis. It copies or transcribes the message from DNA and then translates that message into a protein.

- RNA, like DNA, is a nucleic acid or polymer of nucleotides.
- RNA structure differs from DNA in the following ways:
  - The five-carbon sugar in RNA nucleotides is *ribose* rather than deoxyribose.
  - The nitrogenous base *uracil* is found in place of thymine.

The linear sequence of nucleotides in DNA ultimately determines the linear sequence of amino acids in a protein.

- Nucleic acids are made of four types of nucleotides which differ in their nitrogenous bases. Hundreds or thousands of nucleotides long, each gene has a specific linear sequence of the four possible bases.
- Proteins are made of 20 types of amino acids linked in a particular linear sequence (the protein's primary structure).
- Information flows from gene to protein through two major processes, *transcription* and *translation* (see Campbell, Figure 17.2).

Transcription = The synthesis of RNA using DNA as a template

- A gene's unique nucleotide sequence is transcribed from DNA to a complementary nucleotide sequence in messenger RNA (mRNA).
- The resulting mRNA carries this transcript of protein-building instructions to the cell's protein-synthesizing machinery.

Translation = Synthesis of a polypeptide, which occurs under the direction of messenger RNA (mRNA)

- During this process, the linear sequence of bases in mRNA is translated into the linear sequence of amino acids in a polypeptide.
- Translation occurs on *ribosomes*, complex particles composed of ribosomal RNA (rRNA) and protein that facilitate the orderly linking of amino acids into polypeptide chains.

Prokaryotes and eukaryotes differ in how protein synthesis is organized within their cells.

- Prokaryotes lack nuclei, so DNA is not segregated from ribosomes or the protein-synthesizing machinery. Thus, transcription and translation occur in rapid succession.

- Eukaryotes have nuclear envelopes that segregate transcription in the nucleus from translation in the cytoplasm; mRNA, the intermediary, is modified before it moves from the nucleus to the cytoplasm where translation occurs. This *RNA processing* occurs only in eukaryotes.

C. In the genetic code, nucleotide triplets specify amino acids

There is not a one-to-one correspondence between the nitrogenous bases and the amino acids they specify, since there are only 4 nucleotides and 20 amino acids.

- A two-to-one correspondence of bases to amino acids would only specify 16 (4^2) of the 20 amino acids.
- A three-to-one correspondence of bases to amino acids would specify 64 (4^3) amino acids.

Researchers have verified that the flow of information from a gene to a protein is based on a triplet code (see Campbell, Figure 17.3).

- Triplets of nucleotides are the smallest units of uniform length to allow translation into all 20 amino acids with plenty to spare.
- These three-nucleotide "words" are called *codons*.

Codon = A three-nucleotide sequence in mRNA that specifies which amino acid will be added to a growing polypeptide or that signals termination; the basic unit of the genetic code

Genes are not directly translated into amino acids, but are first transcribed as codons into mRNA.

- For each gene, only one of the two DNA strands (the *template strand*) is transcribed.
- The complementary nontemplate strand is the parental strand for making a new template when DNA replicates.
- The same DNA strand can be the template strand for some genes and the nontemplate strand for others.

An mRNA is complementary to the DNA template from which it is transcribed.

- For example, if the triplet nucleotide sequence on the template DNA strand is CCG; GGC, the codon for glycine, will be the complementary mRNA transcript.
- Recall that according to the base-pairing rules, uracil (U) in RNA is used in place of thymine (T); uracil thus base-pairs with adenine (A).

During translation, the linear sequence of codons along mRNA is translated into the linear sequence of amino acids in a polypeptide.

- Each mRNA codon specifies which one of 20 amino acids will be incorporated into the corresponding position in a polypeptide.
- Because codons are base triplets, the number of nucleotides making up a genetic message is three times the number of amino acids making up the polypeptide product.

1. Cracking the genetic code

The first codon was deciphered in 1961 by Marshall Nirenberg of the National Institutes of Health.

- He synthesized an mRNA by linking only uracil-bearing RNA nucleotides, resulting in UUU codons.
- Nirenberg added this "poly U" to a test-tube mixture containing the components necessary for protein synthesis. The artificial mRNA (poly U) was translated into a polypeptide containing a string of only one amino acid, phenylalanine.
- Nirenberg concluded that the mRNA codon UUU specifies the amino acid phenylalanine.
- These same techniques were used to determine amino acids specified by the codons AAA, GGG, and CCC.

More elaborate techniques allowed investigators to determine all 64 codons by the mid-1960s (see Campbell, Figure 17.4).

- 61 of the 64 triplets code for amino acids.
- The triplet AUG has a *dual* function—it is the start signal for translation and codes for methionine.
- Three codons do not code for amino acids, but signal termination (UAA, UAG, and UGA).

There is redundancy in the genetic code, but no ambiguity.

- *Redundancy* exists since two or more codons differing only in their third base can code for the same amino acid (UUU and UUC both code for phenylalanine).
- *Ambiguity* is absent, since codons code for only one amino acid.

The correct ordering and grouping of nucleotides is important in the molecular language of cells. This ordering is called the *reading frame*.

Reading frame = The correct grouping of adjacent nucleotide triplets into codons that are in the correct sequence on mRNA.

- For example, the sequence of amino acids Trp–Phe–Gly–Arg–Phe can be assembled in the correct order only if the mRNA codons UGGUUUGGCCGUUUU are read in the correct sequence and groups.
- The cell reads the message in the correct frame as a series of *nonoverlapping* three-letter words: UGG–UUU–GGC–CGU–UUU.

D. The genetic code must have evolved very early in the history of life

The genetic code is shared nearly universally among living organisms.

- For example, the RNA codon CCG is translated into proline in all organisms whose genetic codes have been examined.
- The technology exists to transfer genes from one species to another. For example, the human gene for insulin can be inserted into bacteria where it is successfully expressed. Campbell, Figure 17.5 shows the incorporation of a firefly gene into a tobacco plant.

There are some exceptions to this universality:

- Several ciliates (e.g., *Paramecium* and *Tetrahymena*) depart from standard code; codons UAA and UAG are not stop signals, but code for glutamine.
- Mitochondria and chloroplasts have their own DNA that codes for some proteins.
- Mitochondrial genetic codes vary even among organisms; for example, CUA codes for threonine in yeast mitochondria and leucine in mammalian mitochondria.

The fact that the genetic code is shared nearly universally by all organisms indicates that this code was established very early in life's history.

II. The Synthesis and Processing of RNA

A. Transcription is the DNA-directed synthesis of RNA: *a closer look*

Transcription of messenger RNA (mRNA) from template DNA is catalyzed by *RNA polymerases*, which:

- Separate the two DNA strands and link RNA nucleotides as they base-pair along the DNA template.
- Add nucleotides only to the 3′ end; thus, mRNA molecules grow in the 5′ to 3′ direction.

There are several types of RNA polymerase.

- Prokaryotes have only one type of RNA polymerase that synthesizes all types of RNA—mRNA, rRNA, and tRNA.
- Eukaryotes have three RNA polymerases that transcribe genes. *RNA polymerase II* is the polymerase that catalyzes mRNA synthesis; it transcribes genes that will be translated into proteins.

Specific DNA nucleotide sequences mark where transcription of a gene begins (*initiation*) and ends (*termination*). Initiation and termination sequences plus the nucleotides in between are called a transcription unit.

Transcription unit = Nucleotide sequence on the template strand of DNA that is transcribed into a single RNA molecule by RNA polymerase; it includes the initiation and termination sequences, as well as the nucleotides in between

- In eukaryotes, a transcription unit contains a single gene, so the resulting mRNA codes for synthesis of only one polypeptide.
- In prokaryotes, a transcription unit can contain several genes, so the resulting mRNA may code for different, but functionally related, proteins.

Transcription occurs in three stages: a) polymerase binding and initiation; b) elongation; and c) termination (see Campbell, Figure 17.6).

1. RNA polymerase binding and initiation of transcription

RNA polymerases bind to DNA at regions called *promoters*.

Promoter = Region of DNA that includes the site where RNA polymerase binds and where transcription begins (*initiation site*). In eukaryotes, the promoter is about 100 nucleotides long and consists of:

a. The initiation site, where transcription begins (including which DNA strand serves as template).

b. A few nucleotide sequences recognized by specific DNA-binding proteins (transcription factors) that help initiate transcription.

In eukaryotes, RNA polymerases cannot recognize the promoter without the help of *transcription factors* (see Campbell, Figure 17.7).

Transcription factors = DNA-binding proteins which bind to specific DNA nucleotide sequences at the promoter and help RNA polymerase recognize and bind to the promoter region, so transcription can begin.

- RNA polymerase II, the enzyme that synthesizes mRNA in eukaryotes, usually cannot recognize a promoter unless a specific transcription factor binds to a region on the promoter called a TATA box.

TATA box = A short nucleotide sequence at the promoter which is rich in thymine (T) and adenine (A) and located about 25 nucleotides upstream from the initiation site.

- RNA polymerase II recognizes the complex between the bound TATA transcription factor and the DNA binding site.
- Once RNA polymerase recognizes and attaches to the promoter region, it probably associates with other transcription factors before RNA synthesis begins.

When active RNA polymerase binds to a promoter, the enzyme separates the two DNA strands at the initiation site, and transcription begins.

2. Elongation of the RNA strand

Once transcription begins, RNA polymerase II moves along DNA and performs two primary functions:

a. It untwists and opens a short segment of DNA exposing about ten nucleotide bases; one of the exposed DNA strands is the template for base-pairing with RNA nucleotides.

b. It links incoming RNA nucleotides to the 3′ end of the elongating strand; thus, RNA grows one nucleotide at a time in the 5′ to 3′ direction.

During transcription, mRNA grows about 30 to 60 nucleotides per second. As the mRNA strand elongates:

- It peels away from its DNA template.
- The nontemplate strand of DNA re-forms a DNA-DNA double helix by pairing with the template strand.

Following in series, several molecules of RNA polymerase II can simultaneously transcribe the same gene.

- Cells can thus produce particular proteins in large amounts.
- The growing RNA strands hang free from each polymerase. The length of each strand varies and reflects how far the enzyme has traveled from the initiation site on template DNA.

3. Termination of transcription

Transcription proceeds until RNA polymerase transcribes a DNA sequence called a terminator. The transcribed terminator functions as the actual termination signal.

- Additional proteins may cooperate with RNA polymerase in termination.
- In eukaryotes, the most common terminator sequence is AAUAAA.

Prokaryotic mRNA is ready for translation as soon as it leaves the DNA template. Eukaryotic mRNA, however, must be processed before it leaves the nucleus and becomes functional.

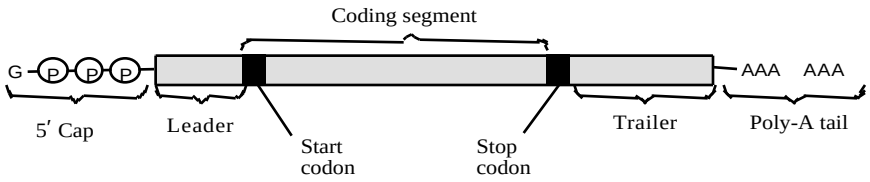
B. Eukaryotic cells modify RNA after transcription

RNA transcripts in eukaryotes are modified, or processed, before leaving the nucleus to yield functional mRNA. Eukaryotic RNA transcripts can be processed in two ways: a) covalent alteration of both the 3’ and 5’ ends and b) removal of intervening sequences.

Primary transcript = General term for initial RNA transcribed from DNA

Pre-mRNA = Primary transcript that will be processed to functional mRNA

1. Alteration of pre-mRNA ends



During pre-mRNA processing, both the 5′ and 3′ ends are covalently modified.

5′ cap = Modified guanine nucleotide (guanosine triphosphate) that is added to the 5′ end of mRNA shortly after transcription begins; has two important functions:

- Protects the growing mRNA from degradation by hydrolytic enzymes.
- Helps small ribosomal subunits recognize the attachment site on mRNA's 5′ end. A *leader* segment of mRNA may also be part of the ribosome recognition signal.

Leader sequence = Noncoding (untranslated) sequence of mRNA from the 5′ end to the start codon.

The 3′ end, which is transcribed last, is modified by enzymatic addition of a *poly-A tail*, before the mRNA exits the nucleus.

Poly (A) tail = Sequence of about 30 to 200 adenine nucleotides added to the 3′ end of mRNA before it exits the nucleus.

- May inhibit degradation of mRNA in the cytoplasm.
- May facilitate attachment to small ribosomal subunit
- May regulate protein synthesis by facilitating mRNA's export from the nucleus to the cytoplasm.
- Is not attached directly to the stop codon, but to an untranslated *trailer* segment of mRNA.

Trailer sequence = Noncoding (untranslated) sequence of mRNA from the stop codon to the poly (A) tail.

2. Split genes and RNA splicing

Genes that code for proteins in eukaryotes may not be continuous sequences.

- Coding sequences of a gene are interrupted by noncoding segments of DNA called intervening sequences, or introns.

Introns = Noncoding sequences in DNA that intervene between coding sequences (exons); are initially transcribed, but not translated, because they are excised from the transcript before mature RNA leaves the nucleus. Not all genes possess introns.

- Coding sequences of a gene are called *exons*, because they are eventually expressed (translated into protein).

Exons = Coding sequences of a gene that are transcribed and expressed

- In 1977, Richard Roberts and Philip Sharp independently found evidence for “split genes”; they received a Nobel Prize in 1993 for their discovery.

Introns and exons are both transcribed to form pre-mRNA, but the introns are subsequently removed and the remaining exons linked together during the process of RNA splicing.

RNA splicing = RNA processing that removes introns and joins exons from eukaryotic pre-mRNA; produces mature mRNA that will move into the cytoplasm from the nucleus (see Campbell, Figure 17.9).

- Enzymes excise introns and splice together exons to form an mRNA with a continuous coding sequence.

- RNA splicing also occurs during post-transcriptional processing of tRNA and rRNA.

Though there is much left to be discovered, some details of RNA splicing are now known.

- Each end of an intron has short boundary sequences that accurately signal the RNA splicing sites.
- Small nuclear ribonucleoproteins (snRNPs), play a key role in RNA splicing.

Small nuclear ribonucleoproteins (snRNPs) = Complexes of proteins and small nuclear RNAs that are found only in the nucleus; some participate in RNA splicing; snRNPs is pronounced "snurps".

- These small nuclear particles are composed of:
  1. *Small nuclear RNA (snRNA).* This small RNA molecule has less than 300 nucleotides—much shorter than mRNA.
  2. *Protein.* Each snRNP possesses several different proteins.
- involved in RNA splicing are part of a larger, more complex assembly called There are various types of snRNPs with different functions; those a spliceosome.

Spliceosome = A large molecular complex that catalyzes RNA splicing reactions; composed of small nuclear ribonucleoproteins (snRNPs) and other proteins (see Campbell, Figure 17.10)

- As the spliceosome is assembled, one type of snRNP base pairs with a complementary sequence at the 5′ end of the intron.
- The spliceosome precisely cuts the RNA transcript at specific splice sites at either end of the intron, which is excised as a lariat-shaped loop.
- The intron is released and the adjacent exons are immediately spliced together by the spliceosome.

3. Ribozymes

Other kinds of RNA primary transcripts, such as those giving rise to tRNA and rRNA, are spliced by mechanisms that do not involve spliceosomes; however, as with mRNA splicing, RNA is often involved in catalyzing the reactions.

Ribozymes = RNA molecules that can catalyze reactions by breaking and forming covalent bonds; called ribozymes to emphasize their catalytic activity.

- Ribozymes were first discovered in *Tetrahymena*, a ciliated protozoan that has self-splicing rRNA. That is, intron rRNA itself catalyzes splicing, which occurs completely without proteins or extra RNA molecules.
- Since RNA is acting as a catalyst, it can no longer be said that "All biological catalysts are proteins."
- It has since been discovered that rRNA also functions as a catalyst during translation.

4. The functional and evolutionary importance of introns

Introns may play a regulatory role in the cell.

- Intron DNA sequences may control gene activity.
- The splicing process itself may help regulate the export of mRNA to the cytoplasm.

Introns may allow a single gene to direct the synthesis of different proteins.

- This can occur if the same RNA transcript is processed differently among various cell types in the same organism.

- For example, all introns may be removed from a particular transcript in one case; but in another, one or more of the introns may be left in place to be translated. Thus, the resulting proteins in each case would be different.

Introns play an important role in the evolution of protein diversity; they increase the probability that recombination of exons will occur between alleles.

- In split genes, coding sequences can be separated by long distances, so they have higher recombination frequencies than continuously coded genes without introns.
- Exons of a "split gene" may code for different *domains* of a protein that have specific functions, such as, an enzyme's active site or a protein's binding site.

Protein domains = Continuous polypeptide sequences that are structural and functional units in proteins with a modular architecture

- Genetic recombination can occur in just one exon resulting in the synthesis of a novel protein with only one altered domain.

Introns also may increase the likelihood of genetic exchange between and among non-allelic genes.

III. The Synthesis of Protein

A. Translation is the RNA-directed synthesis of a polypeptide: *a closer look*

During translation, proteins are synthesized according to a genetic message of sequential codons along mRNA (see Campbell, Figure 17.11).

- *Transfer RNA* (*tRNA*) is the interpreter between the two forms of information—base sequence in mRNA and amino acid sequence in polypeptides.
- tRNA aligns the appropriate amino acids to form a new polypeptide. To perform this function, tRNA must:
  - Transfer amino acids from the cytoplasm's amino acid pool to a ribosome.
  - Recognize the correct codons in mRNA.

Molecules of tRNA are specific for only one particular amino acid. Each type of tRNA associates a distinct mRNA codon with one of the 20 amino acids used to make proteins.

- One end of a tRNA molecule attaches to a specific amino acid.
- The other end attaches to an mRNA codon by base-pairing with its anticodon.

Anticodon = A nucleotide triplet in tRNA that base pairs with a complementary nucleotide triplet (codon) in mRNA.

tRNAs decode the genetic message, codon by codon. For example:

- The mRNA codon UUU is translated as the amino acid phenylalanine (see Campbell, Figure 17.4)
- The tRNA that transfers phenylalanine to the ribosome has an anticodon of AAA.
- When the codon UUU is presented for translation, phenylalanine will be added to the growing polypeptide.
- As tRNAs deposit amino acids in the correct order, ribosomal enzymes link them into a chain.

1. The structure and function of transfer RNA

All types of RNA, including tRNA, are transcribed from template DNA.

- In eukaryotes, tRNA, like mRNA, must travel from the nucleus to the cytoplasm, where translation occurs.
- In prokaryotes and eukaryotes, each tRNA molecule can be used repeatedly.

The ability of tRNA to carry specific amino acids and to recognize the correct codons depends upon its structure; its form fits function (see also Campbell, Figure 17.12).

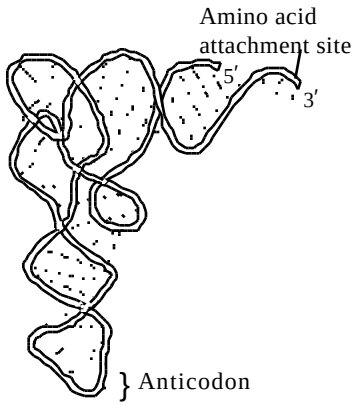
- tRNA is a single-stranded RNA only about 80 nucleotides long.
- The strand is folded, forming several double-stranded regions where short base sequences of hydrogen bond with complementary base sequences.
- A single-plane view reveals a clover leaf shape.

The three-dimensional structure is roughly L-shaped.

- A loop protrudes at one end of the L and has a specialized sequence of three bases called the *anticodon.*
- At the other end of the L protrudes the 3′ end of the tRNA molecule —the attachment site for an amino acid.

There are only about 45 distinct types of tRNA. However, this is enough to translate the 64 codons, since some tRNAs recognize two or three mRNA codons specifying the same amino acid.

- This is possible because the base-pairing rules are relaxed between the third base of an mRNA codon and the corresponding base of a tRNA anticodon.
- This exception to the base-pairing rule is called *wobble.*



Wobble = The ability of one tRNA to recognize two or three different mRNA codons; occurs when the third base (5′ end) of the tRNA anticodon has some play or wobble, so that it can hydrogen bond with more than one kind of base in the third position (3′ end) of the codon.

- For example, the base U in the wobble position of a tRNA anticodon can pair with either A or G in the third position of an mRNA codon.
- Some tRNAs contain a modified base called inosine (I), which is in the anticodon's wobble position and can base pair with U, C, or A in the third position of an mRNA codon.
- Thus, a single tRNA with the anticodon CCI will recognize three mRNA codons: GGU, GGC, or GGA, all of which code for glycine.

2. Aminoacyl-tRNA synthetases

The correct linkage between tRNA and its designated amino acid must occur before the anticodon pairs with its complementary mRNA codon. This process of correctly pairing a tRNA with its appropriate amino acid is catalyzed by an aminoacyl-tRNA synthetase.

Aminoacyl-tRNA synthetase = A type of enzyme that catalyzes the attachment of an amino acid to its tRNA

- Each of the 20 amino acids has a specific aminoacyl-tRNA synthetase.
- In an endergonic reaction driven by the hydrolysis of ATP, the proper synthetase attaches an amino acid to its tRNA in two steps (see Campbell, Figure 17.13):
  1. *Activation of the amino acid with AMP*. The synthetase's active site binds the amino acid and ATP; the ATP loses two phosphate groups and attaches to the amino acid as AMP (adenosine monophosphate).
  2. *Attachment of the amino acid to tRNA*. The appropriate tRNA covalently bonds to the amino acid, displacing AMP from the enzyme's active site.
- The aminoacyl-tRNA complex releases from the enzyme and transfers its amino acid to a growing polypeptide on the ribosome.

3. Ribosomes

Ribosomes coordinate the pairing of tRNA anticodons to mRNA codons.

- Ribosomes have two subunits (small and large) which are separated when not involved in protein synthesis (see Campbell, Figure 17.14a).
- Ribosomes are composed of about 60% *ribosomal RNA* (*rRNA*) and 40% protein.

The large and small subunits of eukaryotic ribosomes are:

- Constructed in the nucleolus
- Dispatched through nuclear pores to the cytoplasm
- Once in the cytoplasm, are assembled into functional ribosomes only when attached to an mRNA

Compared to eukaryotic ribosomes, prokaryotic ribosomes are smaller and have a different molecular composition.

- Selection of effective drug therapies against bacterial pathogens capitalizes on this difference.

- For example, the antibiotics tetracycline and streptomycin can be used to combat bacterial infections, because they inhibit bacterial protein synthesis without affecting the ribosomes of the eukaryotic host.

In addition to an mRNA binding site, each ribosome has three tRNA binding sites (P, A, and E) (see Campbell, Figure 17.14b).

- The *P site* holds the tRNA carrying the growing polypeptide chain.
- The *A site* holds the tRNA carrying the next amino acid to be added.
- Discharged tRNAs exit the ribosome from the *E site*.

As the ribosome holds the tRNA and mRNA molecules together, enzymes transfer the new amino acid from its tRNA to the carboxyl end of the growing polypeptide (see Campbell, Figure 17.14c).

4. Building a polypeptide

The building of a polypeptide, or translation, occurs in three stages: 1) initiation, 2) elongation, and 3) termination.

- All three stages require enzymes and other protein factors.
- Initiation and elongation also require energy provided by GTP (a molecule closely related to ATP).

a. Initiation

Initiation brings together mRNA, a tRNA attached to the first amino acid (initiator tRNA; the first amino acid is always methionine), and the two ribosomal subunits.

The first step of initiation involves the binding of the small ribosomal subunit to mRNA and initiator tRNA (see Campbell, Figure 17.15a).

- In prokaryotes, rRNA in the small subunit base-pairs with specific nucleotides in the leader sequence of the mRNA.
- In eukaryotes, the 5' cap of the mRNA aids in binding of the leader sequence to the small ribosomal subunit.
- With help from the small ribosomal subunit, methionine-bound initiator tRNA finds and base-pairs with the initiation or start codon on mRNA. This start codon, AUG, marks the place where translation will begin and is located just downstream from the leader sequence.
- Assembly of the initiation complex—small ribosomal subunit, initiator tRNA and mRNA—requires:
  - Protein *initiation factors* that are bound to the small ribosomal subunit
  - One GTP molecule that probably stabilizes the binding of initiation factors, and upon hydrolysis, drives the attachment of the large ribosomal subunit.

In the second step, a large ribosomal subunit binds to the small one to form a functional translation complex (see Campbell, Figure 17.15b).

- Initiation factors attached to the small ribosomal subunit are released, allowing the large subunit to bind with the small subunit.
- The initiator tRNA fits into the P site on the ribosome.
- The vacant A site is ready for the next aminoacyl-tRNA.

b. Elongation

Several proteins called *elongation factors* take part in this three-step cycle which adds amino acids one by one to the initial amino acid (see Campbell, Figure 17.16).

1. *Codon recognition.* The mRNA codon in the A site of the ribosome forms hydrogen bonds with the anticodon of an entering tRNA carrying the next amino acid in the chain.
   - An elongation factor directs tRNA into the A site.
   - Hydrolysis of GTP provides energy for this step.
2. *Peptide bond formation.* A peptide bond is formed between the polypeptide in the P site and the new amino acid in the A site by a *peptidyl transferase.*
   - The peptidyl transferase activity appears to be one of the rRNAs in the large ribosomal subunit (ribozyme).
   - The polypeptide separates from its tRNA and is transferred to the new amino acid carried by the tRNA in the A site.
3. *Translocation.* The tRNA in the A site, which is now attached to the growing peptide, is translocated to the P site. Simultaneously, the tRNA that was in the P site is translocated to the E site and from there it exits the ribosome.
   - During this process, the codon and anticodon remain bonded, so the mRNA and the tRNA move as a unit, bringing the next codon to be translated into the A site.
   - The mRNA is moved through the ribosome only in the 5′ to 3′ direction.
   - GTP hydrolysis provides energy for each translocation step.

Some students have trouble visualizing translocation, especially how the tRNA and mRNA move as a unit, exposing a new codon in the A site. Showing the class an animated sequence would no doubt solve the problem. However, if you do not have a monitor or video-projection capability, a paper simulation is just as effective. Paper models can be tacked to a large bulletin board or small cutouts can be moved on an overhead projector. Students may also actively participate by practicing their own simulations.

c. Termination

Each iteration of the elongation cycle takes less than a tenth of a second and is repeated until synthesis is complete and a termination codon reaches the ribosome's A site (see Campbell, Figure 17.7).

Termination codon (stop codon) = Base triplet (codon) on mRNA that signals the end of translation

- Stop codons are UAA, UAG, and UGA.
- Stop codons do not code for amino acids.

Students often confuse terminator sequence (on DNA), which signals the end of transcription, with termination or stop codons (on mRNA), which signal the end of translation.

When a stop codon reaches the ribosome's A site, a protein *release factor* binds to the codon and initiates the following sequence of events:

- Release factor hydrolyzes the bond between the polypeptide and the tRNA in the P site.
- The polypeptide and tRNA are released from the ribosome.
- The remainder of the translation complex dissociates, including separation of the small and a large ribosomal subunits.

5. Polyribosomes

Single ribosomes can make average-sized polypeptides in less than a minute; usually, however, clusters of ribosomes simultaneously translate an mRNA.

Polyribosome = A cluster of ribosomes simultaneously translating an mRNA molecule (see Campbell, Figure 17.8)

- Once a ribosome passes the initiation codon, a second ribosome can attach to the leader sequence of the mRNA.
- Several ribosomes may translate an mRNA at once, making many copies of a polypeptide.
- Polyribosomes are found in both prokaryotes and eukaryotes.

6. From polypeptide to functional protein

The biological activity of proteins depends upon a precise folding of the polypeptide chain into a native three-dimensional conformation.

- Genes determine *primary structure*, the linear sequence of amino acids.
- Primary structure determines how a polypeptide chain will spontaneously coil and fold to form a three-dimensional molecule with *secondary* and *tertiary structure*; chaperone proteins facilitate polypeptide folding

Some proteins must undergo *post-translational modification* before they become fully functional in the cell. Post-translational modification affects function by affecting protein structure.

- Chemical modification
  - Sugars, lipids, phosphate groups, or other additives may be attached to some amino acids.
- Chain-length modification
  - One or more amino acids may be enzymatically cleaved from the leading (amino) end of the polypeptide chain.
  - Single polypeptide chains may be divided into two or more pieces. The translated product of the insulin gene is a large protein precursor (preproinsulin). The precursor is modified by removal of N-terminal fragments and by internal enzymatic cleavage to yield to separate chains held together by disulfide bonds.
  - Two or more polypeptides may join as subunits of a protein that has quaternary structure (e.g., hemoglobin).

B. Signal peptides target some eukaryotic polypeptides to specific destinations in the cell

Eukaryotic ribosomes function either free in the cytosol or bound to endomembranes.

- Bound and free ribosomes are structurally identical and interchangeable.
- Most proteins made by free ribosomes will function in the cytosol.
- Attached to the outside of the endoplasmic reticulum, bound ribosomes generally make proteins that are destined for:
  - Membrane inclusion in membrane component of the endomembrane system (e.g., membrane-bound enzymes of the nuclear envelope, ER, Golgi, lysosomes, vacuoles, and plasma membrane)
  - Partitioning into the lumenal component of the endomembrane system (e.g., lysozymes)
  - Secretion from the cell (e.g., hormones such as insulin)

There is only one type of ribosome, and synthesis of all proteins begins in the cytosol. What determines whether a ribosome will be free in the cytosol or attached to rough ER?

- Messenger RNA for secretory proteins code for an initial *signal sequence* of 16 to 20 hydrophobic amino acids at the amino end of the newly forming polypeptide (see Campbell, Figure 17.19).
- When a ribosome begins to synthesize a protein with a signal sequence, it moves to the ER membrane by a mechanism that involves two other components.
  - *Signal recognition particle (SRP).* SRPs are a complex of protein and RNA (SRP RNA). They serve as an adaptor between the translation complex and the ER. SRPs first attach to the signal sequence of a growing polypeptide and link the translation complex to a receptor protein on the ER membrane (SRP receptor).
  - *SRP receptor.* This receptor protein is built into the ER membrane. The signal recognition particle docks with the receptor, and the ribosome thus becomes bound to the ER membrane.
- The ribosome continues protein synthesis and the leading end of the new polypeptide (N-terminus) threads into the cisternal space.
- The signal sequence is removed by an enzyme.
- Newly formed polypeptide is released from the ribosome and folds into its native conformation.
- If an mRNA does not code for a signal sequence, the ribosome remains free and synthesizes its protein in the cytosol.

Different signal sequences may also dispatch proteins to specific sites other than the ER. For example, newly formed proteins may be targeted for mitochondria or chloroplasts. In these cases, however, translation is completed in the cytoplasm.

C. RNA plays multiple roles in the cell: a review

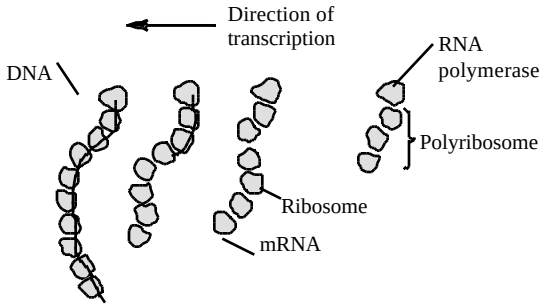
Three-dimensional conformations vary among the types of RNA. These differences in shape give RNA its ability to perform a variety of functions, such as:

1. *Information carrier.* Messenger RNA (mRNA) carries genetic information from DNA to ribosomes; this genetic message specifies a protein's primary structure.
2. *Adaptor molecule.* Transfer RNA (tRNA) acts as an adaptor in protein synthesis by translating information from one form (mRNA nucleotide sequence) into another (protein amino acid sequence). SRP RNA helps direct translation complexes to the ER.
3. *Catalyst and structural molecule.* During translation, ribosomal RNA (rRNA) plays structural and probably enzymatic roles in ribosomes. Small nuclear RNA (snRNA) in snRNP particles also plays structural and enzymatic roles within spliceosomes that catalyze RNA splicing reactions.
4. *Viral genomes.* Some viruses use RNA as their genetic material.

D. Comparing protein synthesis in prokaryotes and eukaryotes: *a review*

While transcription and translation are similar in prokaryotes and eukaryotes, there are some notable differences in the cellular machinery and in some of the details of the processes. The following differ in prokaryotes and eukaryotes:

- RNA polymerases; those of eukaryotes depend on transcription factors
- Termination of transcription
- Ribosomes
- Location (see also Campbell, Figure 17.20)
  - Prokaryotes lack nuclei, so transcription is not segregated from translation; consequently, translation may begin as soon as the 5′ end of mRNA peels away from template DNA, even before transcription is complete.



  - The significance of a eukaryotic cell's compartmental organization is that transcription and translation are segregated by the nuclear envelope. This allows mRNA to be modified before it moves from the nucleus to the cytoplasm. Such *RNA processing* occurs only in eukaryotes.

E. Point mutations can affect protein structure and function

Knowing how genes are translated into proteins, scientists can give a molecular description of heritable changes that occur in organisms.

Mutation = A change in the genetic material of a cell (or virus)

Point mutation = A mutation limited to about one or a few base pairs in a single gene

1. Types of point mutations

There are two categories of point mutations: 1) base-pair substitutions and 2) base-pair insertions or deletions (see Campbell, Figure 17.22).

a. Substitutions

Base-pair substitution = The replacement of one base pair with another; occurs when a nucleotide and its partner in the complementary DNA strand are replaced with another pair of nucleotides according to base-pairing rules.

Depending on how base-pair substitutions are translated, they can result in little or no change in the protein encoded by the mutated gene.

- Redundancy in the genetic code is why some substitution mutations have no effect. A base pair change may simply transform one codon into another that codes for the same amino acid (*silent substitution*).
- Even if the substitution alters an amino acid, the new amino acid may have similar properties to the one it replaces, or it may be in a part of the protein where the exact amino acid sequence is not essential to its activity (*conservative substitution*).

Some base-pair substitutions result in readily detectable changes in proteins.

- Alteration of a single amino acid in a crucial area of a protein will significantly alter protein activity.
- On rare occasions, such a mutation will produce a protein that is improved or has capabilities that enhance success of the mutant organism and its descendants.

- More often, such mutations produce a less active or inactive protein that impairs cell function.

Base-pair substitutions are usually missense mutations or nonsense mutations.

Missense mutation = Base-pair substitution that alters an amino acid codon (sense codon) to a new codon that codes for a different amino acid

- Altered codons make sense (are translated), but not necessarily the right sense.
- Base-pair substitutions are usually missense mutations.

Nonsense mutation = Base-pair substitution that changes an amino acid codon (sense codon) to a chain termination codon, or vice versa

- Nonsense mutations can result in premature termination of translation and the production of a shorter than normal polypeptide.
- Nearly all nonsense mutations lead to nonfunctional proteins.

b. Insertions or deletions

Base-pair insertions or deletions usually have a greater negative effect on proteins than substitutions.

Base-pair insertion = The insertion of one or more nucleotide pairs into a gene

Base-pair deletion = The deletion of one or more nucleotide pairs from a gene

Because mRNA is read as a series of triplets during translation, insertion or deletion of nucleotides may alter the reading frame (triplet grouping) of the genetic message. This type of *frameshift mutation* will occur whenever the number of nucleotides inserted or deleted is not 3 or a multiple of 3.

Frameshift mutation = A base-pair insertion or deletion that causes a shift in the reading frame, so that codons beyond the mutation will be the wrong grouping of triplets and will specify the wrong amino acids

- A frameshift mutation causes the nucleotides following the insertion or deletion to be improperly grouped into codons.
- This results in extensive missense, which will sooner or later end in nonsense (premature termination).
- Frameshift will produce a nonfunctional protein unless the insertion or deletion is very near the end of the gene.

2. Mutagens

Mutagenesis = The creation of mutations

- Mutations can occur as errors in DNA replication, repair, or recombinations that result in base-pair substitutions, insertions, or deletions.
- Mutagenesis may be a naturally occurring event causing *spontaneous mutations* or mutations may be caused by exposure to mutagens.

Mutagen = Physical or chemical agents that interact with genetic material to cause mutations

- Radiation is the most common physical mutagen in nature and has been used in the laboratory to induce mutations.
- Several categories of chemical mutagens are known including *base analogues*, which are chemicals that mimic normal DNA bases, but base-pair incorrectly.
- The Ames test, developed by Bruce Ames, is one of the most widely used tests for measuring the mutagenic strength of various chemicals. Since most mutagens are carcinogenic, this test is also used to screen for chemical carcinogens.

F. What is a gene? ***revisiting the question***

The concept of the gene has emerged as the history of genetics has unfolded.

- The Mendelian concept of a gene was that it served as a discrete unit of inheritance that affected a phenotypic character.
- Morgan and colleagues assigned such units of inheritance (or genes) to specific loci on chromosomes.
- In molecular terms, a gene is a specific sequence of nucleotides at a given location in the genome of an organism. Depending on the gene, the final gene product may be RNA or a specific polypeptide.

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CHAPTER 18

MICROBIAL MODELS: THE GENETICS OF VIRUSES AND BACTERIA

OUTLINE

I. The Genetics of Viruses
   A. Researchers discovered viruses by studying a plant disease: *science as a process*
   B. A virus is a genome enclosed in a protective coat.
   C. Viruses can reproduce only within a host cell: *an overview*
   D. Phages reproduce using lytic or lysogenic cycles
   E. Animal viruses are diverse in their modes of infection and of replication
   F. Plant viruses are serious agricultural pests
   G. Viroids and prions are infectious agents even simpler than viruses
   H. Viruses may have evolved from other mobile genetic elements

II. The Genetics of Bacteria
   A. The short generation span of bacteria facilitates their evolutionary adaptation to changing environments
   B. Genetic recombination produces new bacterial strains
   C. The control of gene expression enables individual bacteria to adjust their metabolism to environmental change

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Recount the history leading up to the discovery of viruses and discuss the contributions of A. Mayer, D. Ivanowsky, Martinus Beijerinck, and Wendell Stanley.
2. List and describe structural components of viruses.
3. Explain why viruses are obligate parasites.
4. Describe three patterns of viral genome replication.
5. Explain the role of reverse transcriptase in retroviruses.
6. Describe how viruses recognize host cells.
7. Distinguish between lytic and lysogenic reproductive cycles using phage T_4 and phage λ as examples.
8. Outline the procedure for measuring phage concentration in a liquid medium.
9. Describe several defenses bacteria have against phage infection.
10. Using viruses with envelopes and RNA viruses as examples, describe variations in replication cycles of animal viruses.
11. Explain how viruses may cause disease symptoms, and describe some medical weapons used to fight viral infections.
12. List some viruses that have been implicated in human cancers, and explain how tumor viruses transform cells.
13. Distinguish between horizontal and vertical routes of viral transmission in plants.

14. List some characteristics that viruses share with living organisms, and explain why viruses do not fit our usual definition of life.
15. Provide evidence that viruses probably evolved from fragments of cellular nucleic acid.
16. Describe the structure of a bacterial chromosome.
17. Describe the process of binary fission in bacteria, and explain why replication of the bacterial chromosome is considered to be semiconservative.
18. List and describe the three natural processes of genetic recombination in bacteria.
19. Distinguish between general transduction and specialized transduction.
20. Explain how the F plasmid controls conjugation in bacteria.
21. Explain how bacterial conjugation differs from sexual reproduction in eukaryotic organisms.
22. For donor and recipient bacterial cells, predict the consequences of conjugation between the following: 1) F^+ and F^- cell, 2) Hfr and F^- cell.
23. Define transposon, and describe two essential types of nucleotide sequences found in transposon DNA.
24. Distinguish between an insertion sequence and a complex transposon.
25. Describe the role of transposase and DNA polymerase in the process of transposition.
26. Explain how transposons can generate genetic diversity.
27. Briefly describe two main strategies cells use to control metabolism.
28. Explain why grouping genes into an operon can be advantageous.
29. Using the *trp* operon as an example, explain the concept of an operon and the function of the operator, repressor, and corepressor.
30. Distinguish between structural and regulatory genes.
31. Describe how the *lac* operon functions and explain the role of the inducer allolactose.
32. Explain how repressible and inducible enzymes differ and how these differences reflect differences in the pathways they control.
33. Distinguish between positive and negative control, and give examples of each from the *lac* operon.
34. Explain how CAP is affected by glucose concentration.
35. Describe how *E. coli* uses the negative and positive controls of the *lac* operon to economize on RNA and protein synthesis.

KEY TERMS

capsid	provirus	transformation	operator
viral envelope	retrovirus	transduction	operon
bacteriophage (phage)	reverse transcriptase	conjugation	repressor
host range	HIV	F factor	regulatory gene
lytic cycle	AIDS	episome	corepressor
virulent virus	vaccine	F plasmid	inducer
lysogenic cycle	virion	R plasmid	cyclic amp (cAMP)
temperate virus	prion	transposon	cAMP receptor protein (CRP)
prophage	nucleoid	insertion sequence	

LECTURE NOTES

Scientists discovered the role of DNA in heredity by studying the simplest of biological systems—viruses and bacteria. Most of the molecular principles discovered through microbe research applies to higher organisms, but viruses and bacteria also have unique genetic features.

- Knowledge of these unique genetic features has helped scientists understand how viruses and bacteria cause disease.
- Techniques for gene manipulation emerged from studying genetic peculiarities of microorganisms.

I. The Genetics of Viruses

A. Researchers discovered viruses by studying a plant disease: *science as a process*

The discovery of viruses resulted from the search for the infectious agent causing tobacco mosaic disease. This disease stunts the growth of tobacco plants and gives their leaves a mosaic coloration (see Campbell, Figure 18.8a).

1883: A. Mayer, a German scientist demonstrated that the disease was contagious and proposed that the infectious agent was an unusually small bacterium that could not be seen with a microscope.

- He successfully transmitted the disease by spraying sap from infected plants onto the healthy ones.
- Using a microscope, he examined the sap and was unable to identify a microbe.

1890s: D. Ivanowsky, a Russian scientist proposed that tobacco mosaic disease was caused by a bacterium that was either too small to be trapped by a filter or that produced a filterable toxin.

- To remove bacteria, he filtered sap from infected leaves.
- Filtered sap still transmitted disease to healthy plants.

1897: Martinus Beijerinck, a Dutch microbiologist proposed that the disease was caused by a reproducing particle much smaller and simpler than a bacterium.

- He ruled out the theory that a filterable toxin caused the disease by demonstrating that the infectious agent in filtered sap could reproduce.

Plants were sprayed with filtered sap from diseased plant.

↓

Sprayed plants developed tobacco mosaic disease.

↓

Sap from newly infected plants was used to infect others.

- This experiment was repeated for several generations. He concluded that the pathogen must be reproducing because its ability to infect was undiluted by transfers from plant to plant.
- He also noted that unlike bacteria, the pathogen:
  - Reproduced only within the host it infected
  - Could not be cultured on media
  - Could not be killed by alcohol

1935: Wendell M. Stanley, an American biologist, crystallized the infectious particle now known as *tobacco mosaic virus* (*TMV*).

B. A virus is a genome enclosed in a protective coat

In the 1950s, TMV and other viruses were finally observed with electron microscopes. Viral structure appeared to be unique from the simplest of cells.

- The smallest viruses are only 20 nm in diameter.
- The virus particle, consists of nucleic acid enclosed by a protein coat and sometimes a membranous envelope.

1. Viral genomes

Depending upon the virus, viral genomes:

- May be double-stranded DNA, single-stranded DNA, double-stranded RNA, or single-stranded RNA
- Are organized as single nucleic acid molecules that are linear or circular
- May have as few as four genes or as many as several hundred

2. Capsids and envelopes

Capsid = Protein coat that encloses the viral genome

- Its structure may be rod-shaped, polyhedral, or complex
- Composed of many *capsomeres,* protein subunits made from only one or a few types of protein.

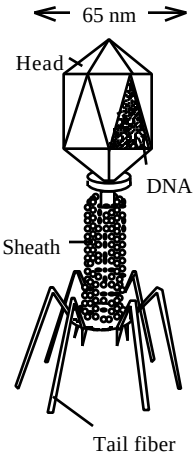
Envelope = Membrane that cloaks some viral capsids

- Helps viruses infect their host
- Derived from host cell membrane which is usually virus-modified and contains proteins and glycoproteins of viral origin

The most complex capsids are found among *bacteriophages* or bacterial viruses.

- Of the first phages studied, seven infected *E. coli.* These were named types 1 – 7 (T1, T2, T3, ... T7).
- The T-even phages – T2, T4, and T6—are structurally very similar.
  - The icosohedral head encloses the genetic material.
  - The protein tailpiece with tail fibers attaches the phage to its bacterial host and injects its DNA into the bacterium.

Campbell, Figure 18.2 shows the structure of various viruses.



Structure of the T-Even Phage

C. Viruses can reproduce only within a host cell: *an overview*

Viral reproduction differs markedly from cellular reproduction, because viruses are *obligate intracellular parasites* which can express their genes and reproduce only within a living cell. Each virus has a specific *host range*.

Host range = Limited number or range of host cells that a parasite can infect

- Viruses recognize host cells by a complementary fit between external viral proteins and specific cell surface *receptor sites*.
- Some viruses have broad host ranges which may include several species (e.g., swine flu and rabies).
- Some viruses have host ranges so narrow that they can:
  - Infect only one species (e.g., phages of *E. coli*)
  - Infect only a single tissue type of one species (e.g., human cold virus infects only cells of the upper respiratory tract; AIDS virus binds only to specific receptors on certain white blood cells)

There are many patterns of viral life cycles, but they all generally involve:

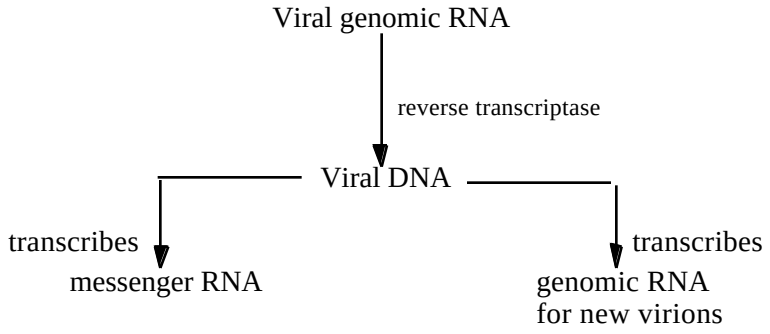
- Infecting the host cell with viral genome
- Co-opting host cell's resources to:
  - Replicate the viral genome
  - Manufacture capsid protein
- Assembling newly produced viral nucleic acid and capsomeres into the next generation of viruses (see Campbell, Figure 18.3)

There are several mechanisms used to infect host cells with viral DNA.

- For example, T-even phages use an elaborate tailpiece to inject DNA into the host cell.
- Once the viral genome is inside its host cell, it commandeers the host's resources and reprograms the cell to copy the viral genes and manufacture capsid protein.

There are three possible patterns of viral genome replication:

1. *DNA → DNA.* If viral DNA is double-stranded, DNA replication resembles that of cellular DNA, and the virus uses DNA polymerase produced by the host.
2. *RNA → RNA*. Since host cells lack the enzyme to copy RNA, most RNA viruses contain a gene that codes for *RNA replicase*, an enzyme that uses viral RNA as a template to produce complementary RNA.
3. *RNA → DNA → RNA*. Some RNA viruses encode *reverse transcriptase*, an enzyme that transcribes DNA from an RNA template.



Regardless of how viral genomes replicate, all viruses divert host cell resources for viral production.

- Viral genes use the host cell's enzymes, ribosomes, tRNAs, amino acids, ATP, and other resources to make copies of the viral genome and produce viral capsid proteins.
- These viral components—nucleic acid and capsids—are assembled into hundreds or thousands of virions, which leave to parasitize new hosts.

Viral nucleic acid and capsid proteins assemble spontaneously into new virus particles, a process called *self-assembly*.

- Since most viral components are held together by weak bonds (e.g., hydrogen bonds and Van der Waals forces), enzymes are not usually necessary for assembly.
- For example, TMV can be disassembled in the laboratory. When mixed together, the RNA and capsids spontaneously reassemble to form complete TMV virions.

D. Phages reproduce using lytic or lysogenic cycles

Bacteriophages are the best understood of all viruses, and many of the important discoveries in molecular biology have come from bacteriophage studies.

- In the 1940s, scientists determined how the T phages reproduce within a bacterium; this research:
- Demonstrated that DNA is the genetic material
- Established the phage-bacterium system as an important experimental tool
- Studies on lambda (λ) phage of *E. coli* showed that some double-stranded DNA viruses reproduce by two alternative mechanisms: the *lytic cycle* and the *lysogenic cycle*.

1. The lytic cycle

Virulent bacteriophages reproduce only by a *lytic* replication cycle.

Virulent phages = Phages that lyse their host cells

Lytic cycle = A viral replication cycle that results in the death or lysis of the host cell

The lytic cycle of phage T4 illustrates this type of replication cycle (see Campbell, Figure 18.4):

1. Phage attaches to cell surface.
   - T4 recognizes a host cell by a complementary fit between proteins on the virion's tail fibers and specific receptor sites on the outer surface of an *E. coli* cell.
2. Phage contracts sheath and injects DNA.
   - ATP stored in the phage tailpiece is the energy source for the phage to: a) pierce the *E. coli* wall and membrane, b) contract its tail sheath, and c) inject its DNA.
   - The genome separates from the capsid leaving a capsid "ghost" outside the cell.
3. Hydrolytic enzymes destroy host cell's DNA.
   - The *E. coli* host cell begins to transcribe and translate the viral genome.
   - One of the first viral proteins produced is an enzyme that degrades host DNA. The phage's own DNA is protected, because it contains modified cytosine not recognized by the enzyme.
4. Phage genome directs the host cell to produce phage components: DNA and capsid proteins.
   - Using nucleotides from its own degraded DNA, the host cell makes many copies of the phage genome.
   - The host cell also produces three sets of capsid proteins and assembles them into phage tails, tail fibers, and polyhedral heads.
   - Phage components spontaneously assemble into virions.
5. Cell lyses and releases phage particles.
   - Lysozymes specified by the viral genome digest the bacterial cell wall.
   - Osmotic swelling lyses the cell which releases hundreds of phages from their host cell.
   - Released virions can infect nearby cells.
   - Lytic cycle takes only 20 to 30 minutes at 37°C. In that period, a T4 population can increase a hundredfold.

Bacteria have several defenses against destruction by phage infection.

- Bacterial mutations can change receptor sites used by phages for recognition, and thus avoid infection.

- Bacterial *restriction nucleases* recognize and cut up foreign DNA, including certain phage DNA. Bacterial DNA is chemically altered, so it is not destroyed by the cell's own restriction enzymes.

Restriction enzymes = Naturally occurring bacterial enzymes that protect bacteria against intruding DNA from other organisms. The enzymes also catalyze restriction, the process of cutting foreign DNA into small segments.

Bacterial hosts and their viral parasites are continually *coevolving*.

- Most successful bacteria have effective mechanisms for preventing phage entry or reproduction.
- Most successful phages have evolved ways around bacterial defenses.
- Many phages check their own destructive tendencies and may coexist with their hosts.

2. The lysogenic cycle

Some viruses can coexist with their hosts by incorporating their genome into the host's genome.

Temperate viruses = Viruses that can integrate their genome into a host chromosome and remain latent until they initiate a lytic cycle

- They have two possible modes of reproduction, the lytic cycle and the lysogenic cycle.
- An example is phage λ, discovered by E. Lederberg in 1951 (see Campbell, Figure 18.5)

Lysogenic cycle = A viral replication cycle that involves the incorporation of the viral genome into the host cell genome

Details of the lysogenic cycle were discovered through studies of phage λ life cycle:

1. Phage λ binds to the surface of an *E. coli* cell.
2. Phage λ injects its DNA into the bacterial host cell.
3. λ DNA forms a circle and either begins a lytic or lysogenic cycle.
4. During a lysogenic cycle, λ DNA inserts by genetic recombination (crossing over) into a specific site on the bacterial chromosome and becomes a prophage.

Prophage = A phage genome that is incorporated into a specific site on the bacterial chromosome

- Most prophage genes are inactive.
- One active prophage gene codes for the production of *repressor protein* which switches off most other prophage genes.
- Prophage genes are copied along with cellular DNA when the host cell reproduces. As the cell divides, both prophage and cellular DNA are passed on to daughter cells.
- A prophage may be carried in the host cell's chromosomes for many generations.

Occasionally, a prophage may leave the bacterial chromosome.

- This may be spontaneous or caused by environmental factors (e.g., radiation).
- The excision process may begin the phage's lytic reproductive cycle.
- Virions produced during the lytic cycle may begin either a lytic or lysogenic cycle in their new host cells.

Lysogenic cell = Host cell carrying a prophage in its chromosome

- It is called lysogenic because it has the potential to lyse.

- Some prophage genes in a lysogenic cell may be expressed and change the cell's phenotype in a process called *lysogenic conversion.*
- Lysogenic conversion occurs in bacteria that cause diphtheria, botulism, and scarlet fever. Pathogenicity results from toxins coded for by prophage genes.

E. Animal viruses are diverse in their modes of infection and replication

1. Reproductive cycles of animal viruses

Replication cycles of animal viruses may show some interesting variations from those of other viruses. Two examples are the replication cycles of: 1) viruses with envelopes, and 2) viruses with RNA genomes that serve as the genetic material. (See Campbell, Table 18.1 for families of animal viruses grouped by type of nucleic acid.)

a. Viral envelopes

Some animal viruses are surrounded by a membranous envelope, which is unique to several groups of animal viruses. This envelope is:

- Outside the capsid and helps the virus enter host cells.
- A lipid bilayer with glycoprotein spikes protruding from the outer surface.

Enveloped viruses have replication cycles characterized by (see Campbell, Figure 18.6):

1. *Attachment*. Glycoprotein spikes protruding from the viral envelope attach to receptor sites on the host's plasma membrane.
2. *Entry*. As the envelope fuses with the plasma membrane, the entire virus (capsid and genome) is transported into the cytoplasm by receptor-mediated endocytosis.
3. *Uncoating*. Cellular enzymes uncoat the genome by removing the protein capsid from viral RNA.
4. *Viral RNA and protein synthesis*. Viral enzymes are required to replicate the RNA genome and to transcribe mRNA.
    - Some viral RNA polymerase is packaged in the virion.
    - Viral RNA polymerase (transcriptase) replicates the viral genome and transcribes viral mRNA. Note that the viral genome is a strand complementary to mRNA.
    - Viral mRNA is translated into viral proteins including:
        - Capsid proteins synthesized in the cytoplasm by free ribosomes
        - Viral-envelope glycoproteins synthesized by ribosomes bound to rough ER. Glycoproteins produced in the host's ER are sent to the Golgi apparatus for further processing. Golgi vesicles transport the glycoproteins to the plasma membrane, where they cluster at exit sites for the virus.
5. *Assembly and release*. New capsids surround viral genomes. Once assembled, the virions envelop with host plasma membrane as they bud off from the cell's surface. The viral envelope is derived from:
    - Host cell's plasma membrane lipid
    - Virus-specific glycoprotein

Some viral envelopes are not derived from host plasma membrane.

For example, herpesviruses are double-stranded DNA viruses which:

- Contain envelopes derived from the host cell's nuclear envelope rather than from the plasma membrane
- Reproduce within the host cell's nucleus
- Use both viral and cellular enzymes to replicate and transcribe their genomic DNA
- May integrate their DNA into the cell's genome as a *provirus*. Evidence comes from the nature of herpes infections, which tend to recur. After a period of latency, physical or emotional stress may cause the proviruses to begin a productive cycle again.

Provirus = Viral DNA that inserts into a host cell chromosome

b. RNA as viral genetic material

All possible types of viral genomes are represented among animal viruses. Since mRNA is common to all types, DNA and RNA viruses are classified according to the relationship of their mRNA to the genome. In this classification:

- mRNA or the strand that corresponds to mRNA is the *plus (+) strand*; it has the nucleotide sequence that codes for proteins.
- The *minus (–) strand* is a template for synthesis of a plus strand; it is complementary to the sense strand or mRNA.

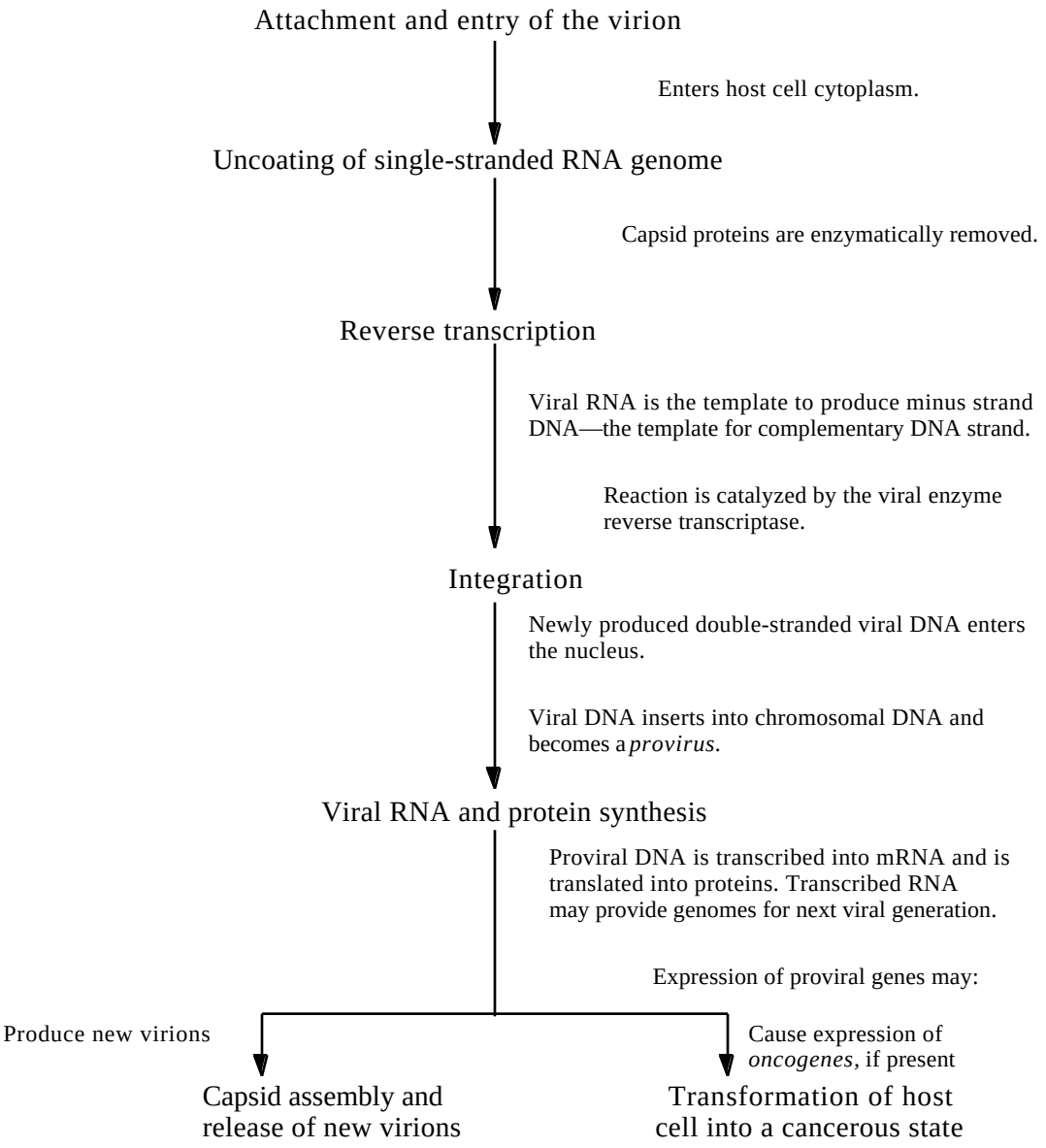
Animal RNA viruses are classified as following:

- *Class III RNA viruses*. Double-stranded RNA genome; the minus strand is the template for mRNA. (Reoviruses)
- *Class IV RNA viruses*. Single plus strand genome; the plus strand can function directly as mRNA, but also is a template for synthesis of minus RNA. (Minus RNA is a template for synthesis of additional plus strands.) Viral enzymes are required for RNA synthesis from RNA templates. (Picornavirus, Togavirus)
- *Class V RNA viruses*. Single minus strand genome; mRNA is transcribed directly from this genomic RNA. (Rhabdovirus, Paramyxovirus, Orthomyxovirus)
- *Class VI RNA viruses*. Single plus strand genome; the plus strand is a template for complementary DNA synthesis. *Reverse transcriptase* catalyzes this reverse transcription from RNA to DNA. mRNA is then transcribed from a DNA template. (*Retroviruses*)

Retrovirus = (Retro = backward) RNA virus that uses *reverse transcriptase* to transcribe DNA from the viral RNA genome.

- *Reverse transcriptase* is a type of DNA polymerase that transcribes DNA from an RNA template.
- *HIV* (*human immunodeficiency virus*), the virus that causes *AIDS* (*acquired immunodeficiency syndrome*) is a retrovirus.

RNA viruses with the most complicated reproductive cycles are the retroviruses, because retroviruses must first carry out *reverse transcription*: (See Campbell, Figure 18.7)



2. Important viral diseases in animals

It is often unclear how certain viruses cause disease symptoms. Viruses may:

- Damage or kill cells. In response to a viral infection, lysosomes may release hydrolytic enzymes.
- Be toxic themselves or cause infected cells to produce toxins.
- Cause varying degrees of cell damage depending upon regenerative ability of the infected cell. We recover from colds because infected cells of the upper respiratory tract can regenerate by cell division. Poliovirus, however, causes permanent cell damage because the virus attacks nerve cells which cannot divide.
- Be indirectly responsible for disease symptoms. Fever, aches and inflammation may result from activities of the immune system.

Medical weapons used to fight viral infections include *vaccines* and *antiviral drugs*.

Vaccines = Harmless variants or derivatives of pathogenic microbes that mobilize a host's immune mechanism against the pathogen

- Edward Jenner developed the first vaccine (against smallpox) in 1796. According to the WHO, a vaccine has almost completely eradicated smallpox.
- Effective vaccines now exist for polio, rubella, measles, mumps, and many other viral diseases.

While vaccines can prevent some viral illnesses, little can be done to cure a viral disease once it occurs. Some *antiviral drugs* have recently been developed.

- Several are analogs of purine nucleosides that interfere with viral nucleic acid synthesis (e.g., adenine arabinoside and acyclovir).

3. Emerging viruses

Emerging viruses are viruses that make an apparent sudden appearance. In reality, they are not likely to be new viruses, but rather existing ones that have expanded their host territory.

Emerging viral diseases can arise if an existing virus:

1. Evolves and thus causes disease in individuals who have immunity only to the ancestral virus (e.g., influenza virus)
2. Spreads from one host species to another
   - For example, the 1993 hantavirus outbreak in New Mexico was the result of a population explosion in deer mice that are the viral reservoirs. Humans became infected by inhaling airborne hantavirus that came from the excreta of deer mice.
3. Disseminates from a small population to become more widespread
   - AIDS, once a rare disease, has become a global epidemic. Technological and social factors influenced the spread of AIDS virus.

Environmental disturbances can increase the viral traffic responsible for emerging diseases. For example:

- Traffic on newly cut roads through remote areas can spread viruses among previously isolated human populations.
- Deforestation activities brings humans into contact with animals that may host viruses capable of infecting humans.

4. Viruses and cancer

Some tumor viruses cause cancer in animals.

- When animal cells grown in tissue culture are infected with tumor viruses, they *transform* to a cancerous state.
- Examples are members of the retrovirus, papovavirus, adenovirus and herpesvirus groups.
- Certain viruses are implicated in human cancers:

Viral Group	Examples/Diseases	Cancer Type
Retrovirus	HTLV-1/adult leukemia	Leukemia
Herpesvirus	Epstein-Barr/infectious mononucleosis	Burkitt's lymphoma
Papovavirus	Papilloma/human warts	Cervical cancer
Hepatitis B virus	Chronic hepatitis	Liver cancer

Tumor viruses transform cells by inserting viral nucleic aids into host cell DNA.

- This insertion is permanent as the provirus never excises.
- Insertion for DNA tumor viruses is straightforward.

Several viral genes have been identified as oncogenes.

Oncogenes = Genes found in viruses or as part of the normal eukaryotic genome, that trigger transformation of a cell to a cancerous state.

- Code for cellular growth factors or for proteins involved in the function of growth factors.
- Are not unique to tumor viruses, but are found in the normal cells of many species. In fact, some tumor viruses transform cells by activating cellular oncogenes.

More than one oncogene must usually be activated to completely transform a cell.

- Indications are that tumor viruses are effective only in combination with other events such as exposure to carcinogens.
- Carcinogens probably also act by turning on cellular oncogenes.

F. Plant viruses are serious agricultural pests

As serious agricultural pests, many of the plant viruses:

- Stunt plant growth and diminish crop yields (see Campbell, Figure 18.8a)
- Are RNA viruses
- Have rod-shaped capsids with capsomeres arranged in a spiral

Capsomere = Complex capsid subunit consisting of several identical or different protein molecules

Plant viruses spread from plant to plant by two major routes: horizontal transmission and vertical transmission.

Horizontal transmission = Route of viral transmission in which an organism receives the virus from an external source

- Plants are more susceptible to viral infection if their protective epidermal layer is damaged.
- Insects may be *vectors* that transmit viruses from plant to plant and can inject the virus directly into the cytoplasm.
- By using contaminated tools, gardeners and farmers may transmit plant viruses.

Vertical transmission = Route of viral transmission in which an organism inherits a viral infection from its parent

- Can occur in asexual propagation of infected plants (e.g., by taking cuttings)
- Can occur in sexual reproduction via infected seeds

Once a plant is infected, viruses reproduce and spread from cell to cell by passing through plasmodesmata (see Campbell, Figure 18.8b).

Most plant viral diseases have no cure, so current efforts focus on reducing viral propagation and breeding resistant plant varieties.

G. Viroids and prions are infectious agents even simpler than viruses

Another class of plant pathogens called *viroids* are smaller and simpler than viruses.

- They are small, naked, circular RNA molecules that do not encode protein, but can replicate in host plant cells.
- It is likely that viroids disrupt normal plant metabolism, development, and growth by causing errors in regulatory systems that control gene expression.
- Viroid diseases affect many commercially important plants such as coconut palms, chrysanthemums, potatoes, and tomatoes.

Some scientists believe that viroids originated as escaped introns.

- Nucleotide sequences of viroid RNA are similar to self-splicing introns found within some normal eukaryotic genes, including rRNA genes.

- An alternative hypothesis is that viroids and self-splicing introns share a common ancestral molecule.

As nucleic acids, viroids self-direct their replication and thus are not diluted during transmission from host to host. Molecules other than nucleic acids can be infectious agents even though they cannot self-replicate.

- *Prions* are pathogens that are proteins, and they appear to cause a number of degenerative brain diseases, such as:
  - Scrapie in sheep
  - "Mad cow" disease
  - Creutzfeldt-Jakob disease in humans
- How can a protein which cannot replicate itself be an infectious pathogen? According to one hypothesis:
  - Prions are defective versions (misfolded) of normally occurring cellular proteins.
  - When prions infect normal cells, they somehow convert the normal protein to the prion version (see Campbell, Figure 18.9).
  - Prions could thus trigger chain reactions that increase their numbers and allow them to spread through a host population without dilution.

H. Viruses may have evolved from other mobile genetic elements

Viruses do not fit our usual definitions of living organisms. They cannot reproduce independently, yet they:

- Have a genome with the same genetic code as living organisms
- Can mutate and evolve

Viruses probably evolved after the first cells, from fragments of cellular nucleic acid that were mobile genetic elements. Evidence to support this includes:

- Genetic material of different viral families is more similar to host genomes than to that of other viral families.
- Some viral genes are identical to cellular genes (e.g., oncogenes in retroviruses).
- Viruses of eukaryotes are more similar in genomic structure to their cellular hosts than to bacterial viruses.
- Viral genomes are similar to certain cellular genetic elements such as plasmids and transposons; they are all *mobile* genetic elements.

II. The Genetics of Bacteria

A. The short generation span of bacteria facilitates their evolutionary adaptation to changing environments

The average bacterial genome is larger than a viral genome, but much smaller than a typical eukaryotic genome.

The major component of the bacterial genome is the *bacterial chromosome* . This structure is:

- Composed of one double-stranded, circular molecule of DNA
- Structurally simpler and has fewer associated proteins than a eukaryotic chromosome
- Found in the *nucleoid* region; since this region is not separated from the rest of the cell (by a membrane), transcription and translation can occur simultaneously.

Many bacteria also contain extrachromosomal DNA in plasmids.

Plasmid = A small double-stranded ring of DNA that carries extrachromosomal genes in some bacteria

Most bacteria can rapidly reproduce by *binary fission*, which is preceded by DNA replication.

- Semi-conservative replication of the bacterial chromosome begins at a single origin of replication.
- The two replication forks move bidirectionally until they meet and replication is complete (see Campbell, Figure 18.10).
- Under optimal conditions, some bacteria can divide in twenty minutes. Because of this rapid reproductive rate, bacteria are useful for genetic studies.

Binary fission is asexual reproduction that produces clones, or daughter cells that are genetically identical to the parent.

- Though mutations are rare events, they can impact genetic diversity in bacteria because of their rapid reproductive rate.
- Though mutation can be a major source of genetic variation in bacteria, it is *not* a major source in more slowly reproducing organisms (e.g., humans). In most higher organisms, genetic recombination from sexual reproduction is responsible for most of the genetic diversity within populations.

B. Genetic recombination produces new bacterial strains

There are three natural processes of genetic recombination in bacteria: *transformation*, *transduction*, and *conjugation*. These mechanisms of gene transfer occur separately from bacterial reproduction, and in addition to mutation, are another major source of genetic variation in bacterial populations.

1. Transformation

Transformation = Process of gene transfer during which a bacterial cell assimilates foreign DNA from the surroundings

- Some bacteria can take up naked DNA from the surroundings. (Refer to Avery's experiments with *Streptococcus pneumoniae* in Chapter 16.)
- Assimilated foreign DNA may be integrated into the bacterial chromosome by recombination (crossing over).
- Progeny of the recipient bacterium will carry a new combination of genes.

Many bacteria have surface proteins that recognize and import naked DNA from closely related bacterial species.

- Though lacking such proteins, *E. coli* can be artificially induced to take up foreign DNA by incubating the bacteria in a culture medium that has a high concentration of calcium ions.
- This technique of artificially inducing transformation is used by the biotechnology industry to introduce foreign genes into bacterial genomes, so that bacterial cells can produce proteins characteristic of other species (e.g., human insulin and human growth hormone).

2. Transduction

Transduction = Gene transfer from one bacterium to another by a bacteriophage (see Campbell, Figure 18.12)

Generalized transduction = Transduction that occurs when random pieces of host cell DNA are packaged within a phage capsid during the lytic cycle of a phage

- This process can transfer almost any host gene and little or no phage genes.
- When the phage particle infects a new host cell, the donor cell DNA can recombine with the recipient cell DNA.

Specialized transduction = Transduction that occurs when a prophage excises from the bacterial chromosome and carries with it only certain host genes adjacent to the excision site. Also known as *restricted transduction*.

- Carried out only by temperate phages
- Differs from general transduction in that:
  - Specific host genes and most phage genes are packed into the same virion.
  - Transduced bacterial genes are restricted to specific genes adjacent to the prophage insertion site. In general transduction, host genes are randomly selected and almost any host gene can be transferred.

3. Conjugation and plasmids

Conjugation = The direct transfer of genes between two cells that are temporarily joined.

- Discovered by Joshua Lederberg and Edward Tatum
- Conjugation in *E. coli* is one of the best-studied examples:

A DNA-donating *E. coli* cell extends external appendages called *sex pili* (see Campbell, Figure 18.13)

↓

Sex pili attach to a DNA-receiving cell

↓

A cytoplasmic bridge forms through which DNA transfer occurs

The ability to form sex pili and to transfer DNA is conferred by genes in a plasmid called the *F plasmid.*

a. General characteristics of plasmids

Plasmid = A small, circular, double-stranded, self-replicating molecule ring of DNA that carries extrachromosomal genes in some bacteria.

- Plasmids have only a few genes, and they are not required for survival and reproduction.
- Plasmid genes can be beneficial in stressful environments. Examples include the F plasmid, which confers ability to conjugate; and the R plasmid, which confers antibiotic resistance.

These small circular DNA molecules replicate independently:

- Some plasmids replicate in synchrony with the bacterial chromosome, so only a few are present in the cell.
- Some plasmids under more relaxed control can replicate on their own schedule, so the number of plasmids in the cell at any one time can vary from only a few to as many as 100.

Some plasmids are episomes that can reversibly incorporate in the cell's chromosome.

Episomes = Genetic elements that can replicate either independently as free molecules in the cytoplasm or as integrated parts of the main bacterial chromosome.

- Examples include some plasmids and temperate viruses such as lambda phage.
- Temperate phage genomes replicate separately in the cytoplasm during a lytic cycle and as an integral part of the host's chromosome during a lysogenic cycle.

While plasmids and viruses can both be episomes, they differ in that:

- Plasmids, unlike viruses, lack an extracellular stage.

- Plasmids are generally beneficial to the cell, while viruses are parasites that usually harm their hosts.

b. The F plasmid and conjugation

The F plasmid (F for fertility) has about 25 genes, most of which are involved in the production of sex pili.

- Bacterial cells that contain the F factor and can donate DNA ("male") are called F^+ *cells*.
- The F factor replicates in synchrony with chromosomal DNA, so the F^+ factor is heritable; that is, division of an F^+ cell results in two F^+ daughter cells.
- Cells without the F factor are designated F^- ("female").

During conjugation between an F^+ and an F^- bacterium:

- The F factor replicates by *rolling circle replication.* The 5′ end of the copy peels off the circular plasmid and is transferred in linear form.
- The F^+ cell transfers a copy of its F factor to the F^- partner, and the F^- cell *becomes* F^+ (see Campbell, Figure 18.14)
- The donor cell remains F^+, with its original DNA intact.

The F factor is an episome and occasionally inserts into the bacterial chromosome.

- Integrated F factor genes are still expressed.
- Cells with integrated F factors are called *Hfr cells* (high frequency of recombination).

Conjugation can occur between an Hfr and an F^- bacterium.

- As the integrated F factor of the Hfr cell transfers to the F^- cell, it pulls the bacterial chromosome behind its leading end.
- The F factor always opens up at the same point for a particular Hfr strain. As rolling circle replication proceeds, the sequence of chromosomal genes behind the leading 5′ end is always the same.
- The conjugation bridge usually breaks before the entire chromosome and tail end of the F factor can be transferred. As a result:
  - Only some bacterial genes are donated.
  - The recipient F^- cell does not become an F^+ cell, because only part of the F factor is transferred.
  - The recipient cell becomes a partial diploid.
  - Recombination occurs between the Hfr chromosomal fragment and the F^- cell. Homologous strand exchange results in a *recombinant* F^- *cell*.
  - Asexual reproduction of the recombinant F^- cell produces a bacterial colony that is genetically different from both original parental cells.

c. R plasmids and antibiotic resistance

One class of nonepisomal plasmids, the *R plasmids* (for resistance), carry genes that confer resistance to certain antibiotics.

- Some carry up to ten genes for resistance to antibiotics.
- During conjugation, some mobilize their own transfer to nonresistant cells.
- Increased antibiotic use has selected for antibiotic resistant bacterial strains carrying the R plasmid.

- Additionally, R plasmids can transfer resistance genes to bacteria of different species including pathogenic strains. As a consequence, resistant strains of pathogens are becoming more common.

4. Transposons

Pieces of DNA called transposons, or transposable genetic elements, can actually move from one location to another in a cell's genome.

Transposons = DNA sequences that can move from one chromosomal site to another.

- Occur as natural agents of genetic change in both prokaryotic and eukaryotic organisms.
- Were first proposed in the 1940s by Barbara McClintock, who deduced their existence in maize. Decades later, the importance of her discovery was recognized; in 1983, at the age of 81, she received the Nobel Prize for her work.

There are two patterns of transposition: a) conservative transposition and b) replicative transposition.

Conservative transposition = Movement of preexisting genes from one genomic location to another; the transposon's genes are not replicated before the move, so the number of gene copies is conserved.

Replicative transposition = Movement of gene copies from their original site of replication to another location in the genome, so the transposon's genes are inserted at some new site without being lost from the original site.

Transposition is fundamentally different from all other mechanisms of genetic recombination, because transposons may scatter certain genes throughout the genome with no apparent single, specific target.

- All other mechanisms of genetic recombination depend upon homologous strand exchange: meiotic crossing over in eukaryotes; and transformation, transduction, and conjugation in prokaryotes.
- Insertion of episomic plasmids into chromosomes is also site specific, even though it does not require an extensive stretch of DNA homologous to the plasmid.

a. Insertion sequences

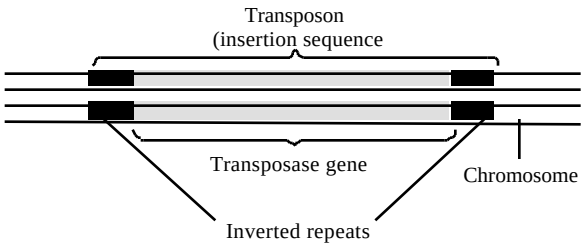
The simplest transposons are insertion sequences (see Campbell, Figure 18.15).

Insertion sequences = The simplest transposons, which contain only the genes necessary for the process of transposition. Insertion sequence DNA includes two essential types of nucleotide sequences:

a. Nucleotide sequence coding for *transposase*
b. Inverted repeats

Transposase = Enzyme that catalyzes insertion of transposons into new chromosomal sites.

- The transposase gene in an insertion sequence is flanked by inverted repeats.



Inverted repeats (IR) = Short noncoding nucleotide sequences of DNA that are repeated in reverse order on opposite ends of a transposon. For example:

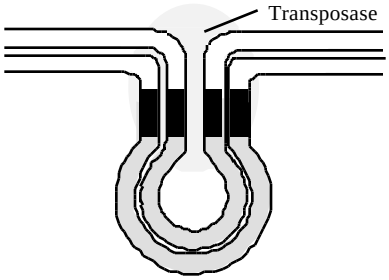
DNA strand #1 ... A T C C G G T... ... A C C G G A T...

DNA strand #2 ... A T C C G G T... ... A C C G G A T...

Note that each base sequence (IR) is repeated in reverse, on the DNA strand *opposite* the inverted repeat at the other end. Inverted repeats:

- Contain only 20 to 40 nucleotide pairs
- Are recognition sites for transposase

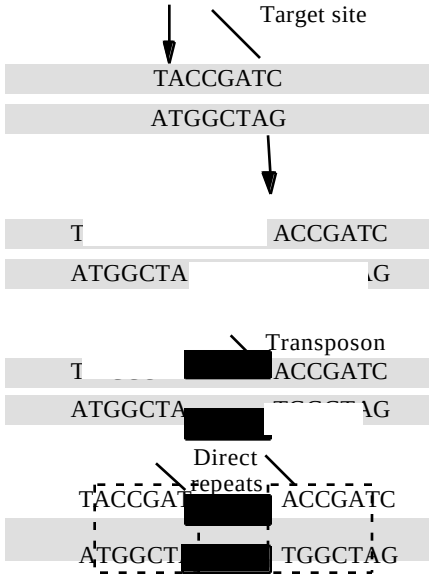
Transposase catalyzes the recombination by:



- Binding to the inverted repeats and holding them close together
- Cutting and resealing DNA required for insertion of the transposon at a new site

Insertion of transposons also requires other enzymes, such as DNA polymerase. For example,

- At the target site, transposase makes staggered cuts in the two DNA strands, leaving short segments of unpaired DNA at each end of the cut.
- Transposase inserts the transposon into the open target site.
- DNA polymerase helps form direct repeats, which flank transposons in their target site. Gaps in the two DNA strands fill in when nucleotides base pair with the exposed single-stranded regions.



Direct repeats = Two or more identical DNA sequences in the same molecule.

- The transposition process creates direct repeats that flank transposons in their target site (see Campbell, Figure 18.16).

Transposed insertion sequences are likely to somehow alter the cell's phenotype; they may:

- Cause mutations by interrupting coding sequences for proteins.
- Increase or decrease a protein's production by inserting within regulatory regions that control transcription rates.

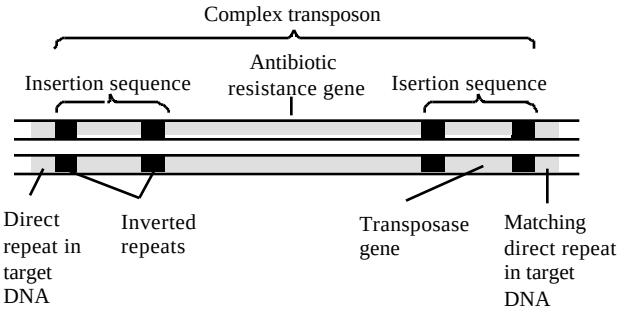
Transposition of insertion sequences probably plays a significant role in bacterial evolution as a source of genetic variation.

- Though insertion sequences only rarely cause mutations (about one in every 10^6 generations), the mutation rate from transpositions is about the same as the mutation rate from extrinsic causes, such as radiation and chemical mutagens.

b. Composite transposons

Composite (complex) transposons = Transposons which include additional genetic material besides that required for transposition; consist of one or more genes flanked by insertion sequences (see Campbell, Figure 18.17).

- The additional DNA may have any nucleotide sequence.
- Can insert into almost any stretch of DNA since their insertion is not dependent upon DNA sequence homology
- Generate genetic diversity in bacteria by moving genes from one chromosome, or even one species, to another. This diversity may help bacteria adapt to new environmental conditions.



An example is a transposon that carries a bacterial gene for antibiotic resistance.

Examples of genetic elements that contain one or more complex transposons include:

- F factor.
- DNA version of the retrovirus genome.

C. The control of gene expression enables individual bacteria to adjust their metabolism to environmental change

This material is difficult to teach. The problem is that there are so many components to track, and the students are not familiar enough with the vocabulary. Figures 18.17 and 18.18 are good teaching aids for the *trp* and *lac* operons. It is also helpful to construct flow charts where there are decision points (e.g., glucose present—glucose absent), so that students can visually follow alternate paths and their respective consequences.

Genes switch on and off as conditions in the intracellular environment change. Bacterial cells have two main ways of controlling metabolism:

1. *Regulation of enzyme activity.* The catalytic activity of many enzymes increases or decreases in response to chemical cues.
   - For example, the end product of an anabolic pathway may turn off its own production by inhibiting activity of an enzyme at the beginning of the pathway (*feedback inhibition*).
   - Useful for immediate short-term response.
2. *Regulation of gene expression.* Enzyme concentrations may rise and fall in response to cellular metabolic changes that switch genes on or off.

- For example, accumulation of product may trigger a mechanism that inhibits transcription of mRNA production by genes that code for an enzyme at the beginning of the pathway (*gene repression*).
- Slower to take effect than feedback inhibition, but is more economical for the cell. It prevents unneeded protein synthesis for enzymes, as well as, unneeded pathway product.

An example illustrating regulation of a metabolic pathway is the tryptophan pathway in *E. coli*. (See Campbell, Figure 18.18) Mechanisms for gene regulation were first discovered for *E. coli*, and current understanding of such regulatory mechanisms at the molecular level is still limited to bacterial systems.

1. Operons: the basic concept

Regulated genes can be switched on or off depending on the cell's metabolic needs. From their research on the control of lactose metabolism in *E. coli*, Francois Jacob and Jacques Monod proposed a mechanism for the control of gene expression, the *operon* concept.

Structural gene = Gene that codes for a polypeptide

Operon = A regulated cluster of adjacent *structural genes* with related functions

- Common in bacteria and phages
- Has a single promoter region, so an RNA polymerase will transcribe all structural genes on an all-or-none basis
- Transcription produces a single *polycistronic* mRNA with coding sequences for all enzymes in a metabolic pathway (e.g., tryptophan pathway in *E. coli*)

Polycistronic mRNA = A large mRNA molecule that is a transcript of several genes

- Is translated into separate polypeptides
- Contains stop and start codons for the translation of each polypeptide

Grouping structural genes into operons is advantageous because:

- Expression of these genes can be coordinated. When a cell needs the product of a metabolic pathway, all the necessary enzymes are synthesized at one time.
- The entire operon can be controlled by a single *operator*.

Operator = A DNA segment located within the promotor or between the promoter and structural genes, which controls access of RNA polymerase to structural genes.

- Sometimes overlaps the transcription starting point for the operon's first structural gene
- Acts as an on/off switch for movement of RNA polymerase and transcription of the operon's structural genes

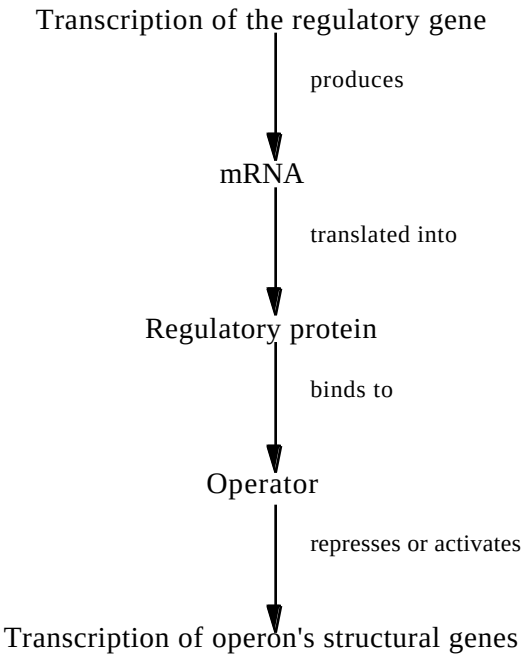
What determines whether an operator is in the "on" or "off" mode? By itself, the operator is on; it is switched off by a protein repressor.

Repressor = Specific protein that binds to an operator and blocks transcription of the operon

- Blocks attachment of RNA polymerase to the promoter
- Is similar to an enzyme, in that it:
  - Has an active site with a specific conformation, which discriminates among operators. Repressor proteins are specific only for operators of certain operons.
  - Binds *reversibly* to DNA
  - May have an allosteric site in addition to its DNA-binding site
- Repressors are encoded by *regulatory genes*.

Regulatory genes = Genes that code for repressor or regulators of other genes

- Are often located some distance away from the operons they control and has its own promotor
- Are involved in switching on or off the transcription of structural genes by the following process:

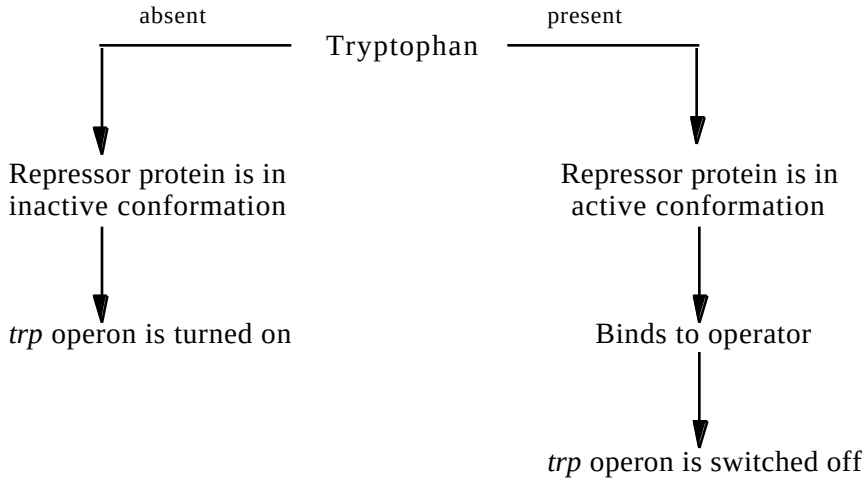


Regulatory genes are continually transcribed, so their activity depends upon how efficient their promoters are in binding RNA polymerase.

- They produce repressor molecules continuously, but slowly.
- Operons are still expressed even though repressor molecules are always present, because repressors are not always capable of blocking transcription; they alternate between inactive and active conformations.

A repressor's activity depends upon the presence of key metabolites in the cell.

- Regulation of the *trp* operon in *E. coli* is an example of how a metabolite cues a repressor (see Campbell, Figure 18.19):
- Repressible enzymes catalyze the anabolic pathway that produces tryptophan, an amino acid.
- Tryptophan accumulation represses synthesis of the enzymes that catalyze its production.



How does tryptophan activate the repressor protein?

- The repressor protein, which normally has a low affinity for the operator, has a DNA binding site plus an allosteric site specific for tryptophan.
- When tryptophan binds to the repressor's allosteric site, it activates the repressor causing it to change its conformation.
- The activated repressor binds to the operator, which switches the *trp* operon off.
- Tryptophan functions in this regulatory system as a *corepressor*.

Corepressor = A molecule, usually a metabolite, that binds to a repressor protein, causing the repressor to change into its active conformation

- Only the *repressor-corepressor complex* can attach to the operator and turn off the operon.
- When tryptophan concentrations drop, it is less likely to be bound to repressor protein. The *trp* operon, once free from repression, begins transcription.
- As concentrations of tryptophan rise, it turns off its own production by activating the repressor.
- Enzymes of the tryptophan pathway are said to be *repressible*.

2. Repressible versus inducible operons: two types of negative gene regulation

Repressible operon = Operons which have their transcription inhibited. Usually associated with anabolic processes, (e.g., tryptophan synthesis via *trp* operon).

Inducible operons = Operons which have their transcription stimulated. Usually associated with catabolic processes.

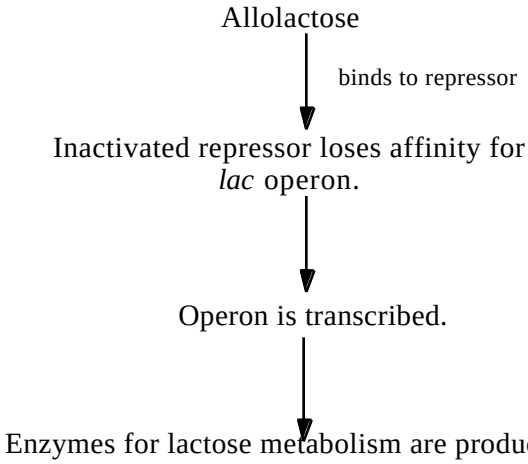
Some operons can be switched on or *induced* by specific metabolites (e.g., *lac* operon in *E. coli*).

- *E. coli* can metabolize the disaccharide lactose. Once lactose is transported into the cell, β-galactosidase cleaves lactose into glucose and galactose:

$$\underset{\text{(disaccharide)}}{\text{lactose}} \xrightarrow{\beta\text{-galactosidase}} \underset{\text{(monosaccharides)}}{\text{glucose + galactose}}$$

- When *E. coli* is in a lactose-free medium, it only contains a few β-galactosidase molecules.

- When lactose is added to the medium, *E. coli* increases the number of mRNA molecules coding for β-galactosidase. These mRNA molecules are quickly translated into thousands of β-galactosidase molecules.
- Lactose metabolism in *E. coli* is programmed by the *lac* operon which has three structural genes:
  1. *lac Z* - Codes for β-galactosidase which hydrolyzes lactose
  2. *lac Y* - Codes for a permease, a membrane protein that transports lactose into the cell
  3. *lac A* - Codes for transacetylase, an enzyme that has no known role in lactose metabolism
- The *lac* operon has a single promoter and operator. The *lac* repressor is innately active, so it attaches to the operon without a corepressor.
- Allolactose, an isomer of lactose, acts as an *inducer* to turn on the *lac* operon (see Campbell, Figure 18.20):



Differences between repressible and inducible operons reflect differences in the pathways they control.

Repressible Operons	Inducible Operons
Their genes are switched on until a specific metabolite activates the repressor.	Their genes are switched off until a specific metabolite inactivates the repressor.
They generally function in anabolic pathways.	They function in catabolic pathways
Pathway end product switches off its own production by repressing enzyme synthesis.	Enzyme synthesis is switched on by the nutrient the pathway uses.

Repressible and inducible operons share similar features of gene regulation. In both cases:

- Specific repressor proteins control gene expression.
- Repressors can assume an active conformation that blocks transcription and an inactive conformation that allows transcription.
- Which form the repressor assumes depends upon cues from a metabolite.

Both systems are thus examples of *negative control*.

- Binding of active repressor to an operator always turns off structural gene expression.
- The *lac* operon is a system with negative control, because allolactose does not interact directly with the genome. The derepression allolactose causes is indirect, by freeing the *lac* operon from the repressor's negative effect.

Positive control of a regulatory system occurs only if an activator molecule interacts directly with the genome to turn on transcription.

3. An example of positive gene regulation

The *lac* operon is under dual regulation which includes negative control by repressor protein and positive control by cAMP receptor protein (CRP).

CRP (cAMP receptor protein) = An allosteric protein that binds cAMP and activates transcription binding to an operon's promoter region (enhances the promoter's affinity for RNA polymerase) (see Campbell, Figure 18.21)

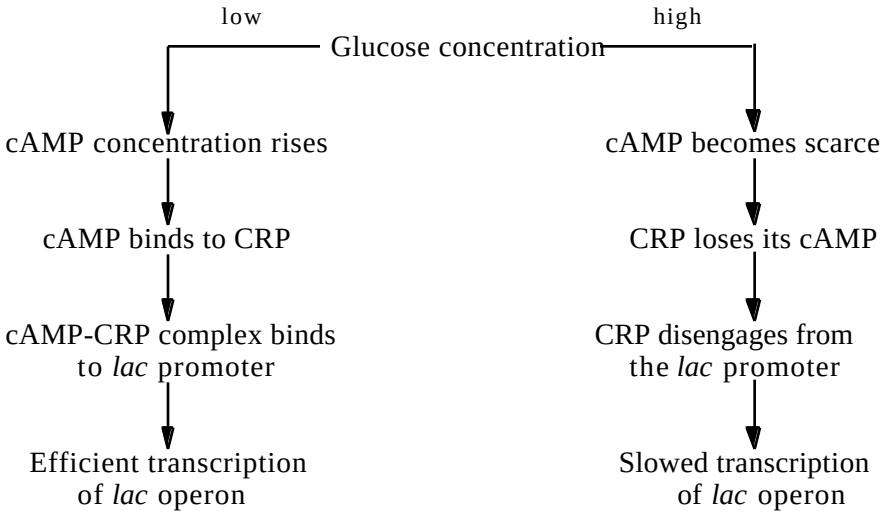
- Exists in two states: inactive (no cAMP bound) and active (cAMP bound). Only the active form of CRP can bind to the promoter to stimulate transcription.
- It is a *positive regulator* because it *directly* interacts with the genome to stimulate gene expression.
- CRP binding to a promoter is dependent on glucose concentration.

E. coli preferentially uses glucose over lactose as a substrate for glycolysis. So normal expression of the lac operon requires:

- Presence of lactose
- *Absence* of glucose

How is CRP affected by the absence or presence of glucose?

- When glucose is missing, the cell accumulates *cyclic AMP* (*cAMP*), a nucleotide derived from ATP. cAMP activates CRP so that it can bind to the *lac* promoter.
- When glucose concentration rises, glucose catabolism decreases the intracellular concentration of cAMP. Thus, cAMP releases CRP.



In this dual regulation of the *lac* operon:

- Negative control by the repressor determines whether or not the operon will transcribe the structural genes.
- Positive control by CRP determines the rate of transcription.

E. coli economizes on RNA and protein synthesis with the help of these negative and positive controls.

- CRP is an activator of several different operons that program catabolic pathways.
- Glucose's presence deactivates CRP. This, in turn, slows synthesis of those enzymes a cell needs to use catabolites other than glucose.
- *E. coli* preferentially uses glucose as its primary carbon and energy source, and the enzymes for glucose catabolism are coded for by unregulated genes that are continuously transcribed (constitutive).
- Consequently, when glucose is present, CRP does not work and the cell's systems for using secondary energy sources are inactive.

When glucose is absent, the cell metabolizes alternate energy sources.

- The cAMP level rises, CRP is activated and transcription begins of operons that program the use of alternate energy sources (e.g., lactose).
- Which operon is actually transcribed depends upon which nutrients are available to the cell. For example, if lactose is present, the *lac* operon will be switched on as allolactose inactivates the repressor.

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CHAPTER 19

THE ORGANIZATION AND CONTROL OF EUKARYOTIC GENOMES

OUTLINE

I. The Structure of Chromatin
   A. Chromatin structure is based on successive levels of DNA packing
II. Genome Organization at the DNA Level
   A. Repetitive DNA and other noncoding sequences account for much of a eukaryotic genome
   B. Gene families have evolved by duplication of ancestral genes
   C. Gene amplification, loss, or rearrangement can alter a cell's genome
III. The Control of Gene Expression
   A. Each cell of a multicellular eukaryote expresses only a small fraction of its genome
   B. The control of gene expression can occur at any step in the pathway from gene to functional protein
   C. Chromatin modifications affect the availability of genes for transcription
   D. Transcription initiation is controlled by proteins that interact with DNA and with each other
   E. Posttranscriptional mechanisms play supporting roles in the control of gene expression
IV. The Molecular Biology of Cancer
   A. Cancer results from genetic changes that affect the cell cycle
   B. Oncogene proteins and faulty tumor-suppressor proteins
   C. Multiple mutations underlie the development of cancer

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Compare the organization of prokaryotic and eukaryotic genomes.
2. Describe the current model for progressive levels of DNA packing.
3. Explain how histones influence folding in eukaryotic DNA.
4. Distinguish between heterochromatin and euchromatin.
5. Using the Barr body as an example, describe the function of heterochromatin in interphase cells.
6. Describe where satellite DNA is found and what role it may play in the cell.
7. Describe the role of telomeres in solving the end-replication problem with the lagging DNA strand.
8. Using the genes for rRNA as an example, explain how multigene families of identical genes can be advantageous for a cell.

9. Using α-globin and β-globin genes as examples, describe how multigene families of nonidentical genes probably evolve, including the role of transposition.
10. Explain the potential role that promoters and enhancers play in transcriptional control.
11. Explain why the nuclear envelope in eukaryotes offers a level of post-transcriptional control beyond that found in prokaryotes.
12. Explain why the ability to rapidly degrade mRNA can be an adaptive advantage for prokaryotes.
13. Describe the importance of mRNA degradation in eukaryotes, describe how it can be prevented.
14. Explain how gene expression may be controlled at the translational and post-translational level.
15. Compare the arrangement of coordinately controlled genes in prokaryotes and eukaryotes.
16. Explain how eukaryotic genes can be coordinately expressed and give some examples of coordinate gene expression in eukaryotes.
17. Provide evidence from studies of polygene chromosomes, that eukaryotic gene expression is controlled at transcription and that gene regulation responds to chemical signals such as steroid hormones.
18. Describe the key steps of steroid hormone action on gene expression in vertebrates.
19. In general terms, explain how genome plasticity can influence gene expression.
20. Describe the effects of gene amplification, selective gene loss and DNA methylation.
21. Explain how rearrangements in the genome can activate or inactivate genes.
22. Explain the genetic basis for antibody diversity.
23. Explain how DNA methylation may be a cellular mechanism for long-term control of gene expression and how it can influence early development.
24. Describe the normal control mechanisms that limit cell growth and division.
25. Briefly describe the four mechanisms that can convert proto-oncogenes to oncogenes.
26. Explain how changes in tumor-suppressor genes can be involved in transforming normal cells into cancerous cells.
27. Explain how oncogenes are involved in virus-induced cancers.

KEY TERMS

histones	multigene family	genomic imprinting	proteasomes
nucleosome	pseudogene	histone acetylation	oncogenes
heterochromatin	gene amplification	control elements	proto-oncogenes
euchromatin	retrotransposons	enhancers	tumor-suppressor genes
repetitive DNA	immunoglobulins	activator	*ras* gene
satellite DNA	differentiation	DNA-binding domain	p53 gene
Alu elements	DNA methylation	alternative splicing	

LECTURE NOTES

Eukaryotic gene regulation is more complex than in prokaryotes, because eukaryotes:

- Have larger, more complex genomes. This requires that eukaryotic DNA be more complexly organized than prokaryotic DNA.
- Require cell specialization or *differentiation.*

I. The Structure of Chromatin

A. Chromatin Structure is Based on Successive Levels of DNA Packing

Prokaryotic and eukaryotic cells both contain double-stranded DNA, but their genomes are organized differently.

Prokaryotic DNA is:

- Usually circular
- Much smaller than eukaryotic DNA; it makes up a small nucleoid region only visible with an electron microscope
- Associated with only a few protein molecules
- Less elaborately structured and folded than eukaryotic DNA; bacterial chromosomes have some additional structure as the DNA-protein fiber forms loops that are anchored to the plasma membrane

Eukaryotic DNA is:

- Complexed with a large amount of protein to form *chromatin*
- Highly extended and tangled during interphase
- Condensed into short, thick, discrete *chromosomes* during mitosis; when stained, chromosomes are clearly visible with a light microscope

Eukaryotic chromosomes contain an enormous amount of DNA, which requires an elaborate system of DNA packing to fit all of the cell's DNA into the nucleus.

B. Nucleosomes, or "beads on a string"

Histone proteins associated with DNA are responsible for the first level of DNA packing in eukaryotes.

Histones = Small proteins that are rich in basic amino acids and that bind to DNA, forming chromatin.

- Contain a high proportion of positively charged amino acids (arginine and lysine), which bind tightly to the negatively charged DNA
- Are present in approximately equal amounts to DNA in eukaryotic cells
- Are similar from one eukaryote to another, suggesting that histone genes have been highly conserved during evolution. There are five types of histones in eukaryotes.

Partially unfolded *chromatin* (DNA and its associated proteins) resembles beads spaced along the DNA string. Each beadlike structure is a nucleosome (see Campbell, Figure 19.1a).

Nucleosome = The basic unit of DNA packing; it is formed from DNA wound around a protein core that consists of two copies each of four types of histone (H2A, H2B, H3, H4). A fifth histone (H1) attaches near the bead when the chromatin undergoes the next level of packing.

- Nucleosomes may control gene expression by controlling access of transcription proteins to DNA.
- Nucleosome heterogeneity may also help control gene expression; nucleosomes may differ in the extent of amino acid modification and in the type of nonhistone proteins present.

C. Higher levels of DNA packing

The *30-nm chromatin fiber* is the next level of DNA packing (see Campbell, Figure 19.1b).

- This structure consists of a tightly wound coil with six nucleosomes per turn.
- Molecules of histone H1 pull the nucleosomes into a cylinder 30nm in diameter.

In the next level of higher-order packing, the 30-nm chromatin fiber forms *looped domains*, which:

- Are attached to a nonhistone protein scaffold
- Contain 20,000 to 100,000 base pairs
- Coil and fold, further compacting the chromatin into a mitotic chromosome characteristic of metaphase

Interphase chromatin is much less condensed than mitotic chromatin, but it still exhibits higher-order packing.

- Its nucleosome string is usually coiled into a 30-nm fiber, which is folded into looped domains.
- Interphase looped domains attach to a scaffolding inside the nuclear envelope (nuclear lamina); this helps organize areas of active transcription.
- Chromatin fibers of different chromosomes do not become entangled as they occupy restricted areas within the nucleus.

Portions of some chromosomes remain highly condensed throughout the cell cycle, even during interphase. Such heterochromatin is not transcribed.

Heterochromatin = Chromatin that remains highly condensed during interphase and that is not actively transcribed

Euchromatin = Chromatin that is less condensed during interphase and is actively transcribed; euchromatin becomes highly condensed during mitosis

What is the function of heterochromatin in interphase cells?

- Since most heterochromatin is not transcribed, it may be a coarse control of gene expression.
- For example, Barr bodies in mammalian cells are *X* chromosomes that are mostly condensed into heterochromatin. In female somatic cells, one *X* chromosome is a Barr body, so the other *X* chromosome is the only one transcribed.

II. Genome Organization at the DNA Level

An organism's genome is plastic, or changeable, in ways that affect the availability of specific genes for expression.

- Genes may be available for expression in some cells and not others, or at some time in the organism's development and not others.
- Genes may, under some conditions, be amplified or made more available than usual.
- Changes in the physical arrangement of DNA, such as levels of DNA packing, affect gene expression. For example, genes in heterochromatin and mitotic chromosomes are not expressed.

The structural organization of an organism's genome is also somewhat plastic; movement of DNA within the genome and chemical modification of DNA influence gene expression.

A. Repetitive DNA and noncoding sequences account for much of a eukaryotic genome

DNA in eukaryotic genomes is organized differently from that in prokaryotes.

- In prokaryotes, most DNA codes for protein (mRNA), tRNA or rRNA, and coding sequences are uninterrupted. Small amounts of noncoding DNA consist mainly of control sequences, such as promoters.
- In eukaryotes, most DNA does *not* encode protein or RNA, and coding sequences may be interrupted by long stretches of noncoding DNA (introns). Certain DNA sequences may be present in multiple copies.

1. Tandemly repetitive DNA

About 10–25% of total DNA in higher eukaryotes is *satellite DNA* that consists of short (five to 10 nucleotides) sequences that are tandemly repeated thousands of times.

Satellite DNA = In eukaryotic chromosomes, highly repetitive DNA consisting of short unusual nucleotide sequences that are tandemly repeated thousands of times.

- Called satellite DNA because its unusual nucleotide ratio gives it a density different from the rest of the cell's DNA. Thus, during ultracentrifugation, satellite DNA separates out in a cesium chloride gradient as a "satellite" band separate from the rest of the DNA.
- Is not transcribed and its function is not known. Since most satellite DNA in chromosomes is located at the tips and the centromere, scientists speculate that it plays a structural role during chromosome replication and chromatid separation in mitosis and meiosis.

It is known that short tandem repeats called telomeres—at the ends of eukaryotic chromosomes—are important in maintaining the integrity of the lagging DNA strand during replication.

Telomere = Series of short tandem repeats at the ends of eukaryotic chromosomes; prevents chromosomes from shortening with each replication cycle

- Before an Okazaki fragment of the lagging DNA strand can be synthesized, RNA primers must be produced on a DNA template ahead of the sequence to be replicated.
- Since such a template is not possible for the end of a linear DNA molecule, there must be a mechanism to prevent DNA strands from becoming shorter with each replication cycle.
- This end-replication problem is solved by the presence of special repeating telomeric sequences on the ends of linear chromosomes.
- To compensate for the loss of telomeric nucleotides that occurs each replication cycle, the enzyme *telomerase* periodically restores this repetitive sequence to the ends of DNA molecules.
- Telomeric sequences are similar among many organisms and contain a block of G nucleotides. For example, human chromosomes have 250–1500 repetitions of the base sequence TTAGGG (AATCCC on the complementary strand).

There are other highly repetitive sequences in eukaryotic genomes. For example,

- Some are transposons; generally regarded as nonfunctional, they are associated with some diseases (e.g., neurofibromatosis-1 or elephant man's disease and some cancers).
- Mutations can extend the repetitive sequences normally found within the boundary of genes and cause them to malfunction. (e.g., fragile X syndrome and Huntington's disease.)

2. Interspersed repetitive DNA

Eukaryotes also possess large amounts (25-40% in mammals) of repeated units, hundreds or thousands of base pairs long, dispersed at random intervals throughout the genome.

B. Gene families have evolved by duplication of ancestral genes

Most eukaryotic genes are *unique sequences* present as single copies in the genome, but some genes are part of a multigene family.

Multigene family = A collection of genes that are similar or identical in sequence and presumably of common ancestral origin; such genes may be clustered or dispersed in the genome.

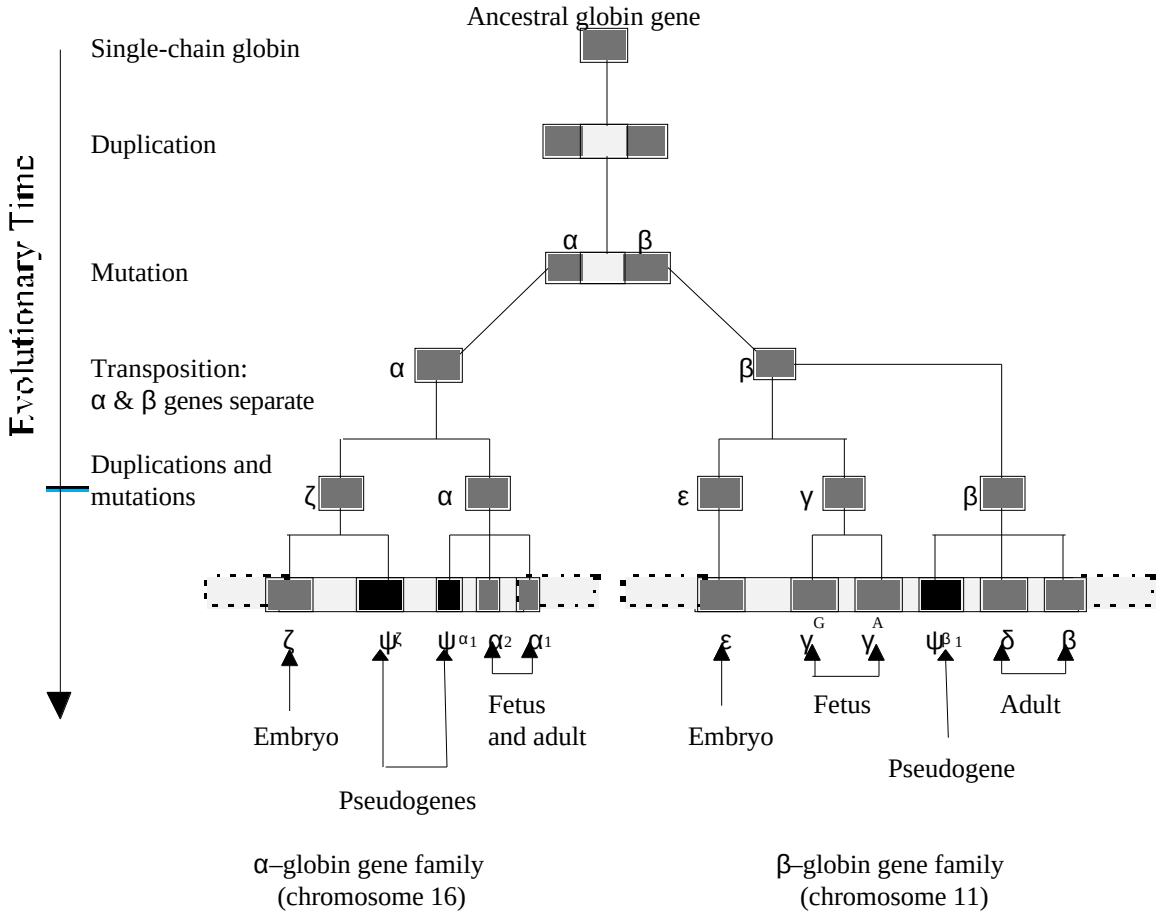
Families of *identical* genes:

- Probably arise from a single ancestral gene that has undergone repeated duplication. Such *tandem gene duplication* results from mistakes made during DNA replication and recombination.
- Are usually clustered and almost exclusively genes for RNA products. (One exception is the gene family coding for histone proteins.)
- Include genes for the major rRNA molecules; huge tandem repeats of these genes enable cells to make millions of ribosomes during active protein synthesis (see Campbell, Figure 19.2).

Families of *nonidentical* genes:

- Arise over time from mutations that accumulate in duplicated genes.
- Can be clustered on the same chromosome or scattered throughout the genome. (Note that for various reasons, gene sequences in tandem arrays on the same chromosome tend to stay very similar to one another. Transposition events that translocate variants of duplicated genes to different chromosomes, help stabilize their differences and thus promote diversity.)
- May include pseudogenes or nonfunctional versions of the duplicated gene.

Pseudogene = Nonfunctional gene that has a DNA sequence similar to a functional gene; but as a consequence of mutation, lacks sites necessary for expression.



A good example of how multigene families can evolve from a single ancestral gene is the globin gene family—actually two related families of genes that encode globins, the α and β polypeptide subunits of hemoglobin.

Based on amino acid homologies, the evolutionary history has been reconstructed as follows:

- The original α and β genes evolved from duplication of a common ancestral globin gene. Gene duplication was followed by mutation.
- Transposition separated the α globin and β globin families, so they exist on different chromosomes.
- Subsequent episodes of gene duplication and mutation resulted in new genes and pseudogenes in each family.

The consequence is that each globin gene family consists of a group of similar, but not identical genes clustered on a chromosome.

- In each gene family, the genes are arranged in order of their expression. During development, genes are turned on or off in response to the organism's changing environment as it develops from an embryo into a fetus, and then into an adult.
- At all times during development, functional hemoglobin consists of two α-like and two β-like polypeptides.
  - During embryogenesis, the ζ and ε forms predominate.
  - About 10 weeks after fertilization, the products of the α-globin genes replace that of the ζ gene, and the γ-chain gene products—γ^G and the γ^A globins—become more prevalent.
  - Prior to birth, the β-gene product begins to replace the γ^G and γ^A globins, so by six months of age, the adult β-like globins, δ and β, are present.
- In humans, embryonic and fetal hemoglobins have a higher affinity for oxygen than the adult forms, allowing efficient oxygen exchange between mother and developing fetus.

C. Gene amplification, loss, or rearrangement can alter a cell's genome

1. Gene amplification and selective gene loss

Gene amplification may temporarily increase the number of gene copies at certain times in development.

Gene amplification = Selective synthesis of DNA, which results in multiple copies of a single gene.,

- For example, amphibian rRNA genes are selectively amplified in the oocyte, which:
  - Results in a million or more additional copies of the rRNA genes that exist as extrachromosomal circles of DNA in the nucleoli.
  - Permits the oocyte to make huge numbers of ribosomes that will produce the vast amounts of proteins needed when the egg is fertilized.
- Gene amplification occurs in cancer cells exposed to high concentrations of chemotherapeutic drugs.
  - Some cancer cells survive chemotherapy, because they contain amplified genes conferring drug resistance.
  - Increased drug resistance can be created experimentally by exposing a cell population to increasing drug doses and artificially selecting for surviving cells that have amplified drug-resistance genes.

Genes may also be selectively lost in certain tissues by elimination of chromosomes.

Chromosome diminution = Elimination of whole chromosomes or parts of chromosomes from certain cells early in embryonic development.

- For example, chromosome diminution occurs in gall midges during early development; all but two cells lose 32 of their 40 chromosomes during the first mitotic division after the 16-cell stage.
- The two cells that retain the complete genome are germ cells that will produce gametes in the adult. The other 14 cells become somatic cells with only eight chromosomes.

2. Rearrangements in the genome

Substantial stretches of DNA can be re-shuffled within the genome; these rearrangements are more common that gene amplification or gene loss.

a. Transposons

All organisms probably have transposons that move DNA from one location to another within the genome (see Campbell, Chapter 17). Transposons can rearrange the genome by:

- Inserting into the middle of a coding sequence of another gene; it can prevent the interrupted gene from functioning normally (see Campbell, Figure 19.4).
- Inserting within a sequence that regulates transcription; the transposition may increase or decrease a protein's production.
- Inserting its own gene just downstream from an active promoter that activates its transcription.

Retrotranposons = Transposable elements that move within a genome by means of an RNA intermediate (see Campbell, Figure 19.5).

Retrotransposons insert at another site by utilizing reverse transcriptase to convert back to DNA.

b. Immunoglobulin genes

During cellular differentiation in mammals, permanent rearrangements of DNA segments occur in those genes that encode antibodies, or *immunoglobulins*.

Immunoglobulins = A class of proteins (antibodies) produced by B lymphocytes that specifically recognize and help combat viruses, bacteria, and other invaders of the body. Immunoglobulin molecules consist of:

- Four polypeptide chains held together by disulfide bridges
- Each chain has two major parts:
  - A *constant region*, which is the same for all antibodies of a particular class
  - A *variable region*, which gives an antibody the ability to recognize and bind to a specific foreign molecule

B lymphocytes, which produce immunoglobulins, are a type of white blood cell found in the mammalian immune system.

- The human immune system contains millions of subpopulations of B lymphocytes that produce different antibodies.
- B lymphocytes are very specialized; each differentiated cell and its descendants produce only one specific antibody.

Antibody specificity and diversity are properties that emerge from the unique organization of the antibody gene, which is formed by a rearrangement of the genome during B cell development (see Campbell, Figure 19.6).

- As an unspecialized cell differentiates into a B lymphocyte, its antibody gene is pieced together randomly from several DNA segments that are physically separated in the genome.

- In the genome of an embryonic cell, there is an intervening DNA sequence between the sequence coding for an antibody's constant region and the site containing hundreds of coding sequences for the variable regions.
- As a B cell differentiates, the intervening DNA is deleted, and the DNA sequence for a variable region connects with the DNA sequence for a constant region, forming a continuous nucleotide sequence that will be transcribed.
- The primary RNA transcript is processed to form mRNA that is translated into one of the polypeptides of an antibody molecule.

- Antibody variation results from:
  - Different combinations of variable and constant regions in the polypeptides
  - Different combinations of polypeptides

III. The Control of Gene Expression

A. Each cell of a multicellular eukaryote expresses only a small fraction of its genes

Cellular differentiation = Divergence in structure and function of different cell types, as they become specialized during an organism's development

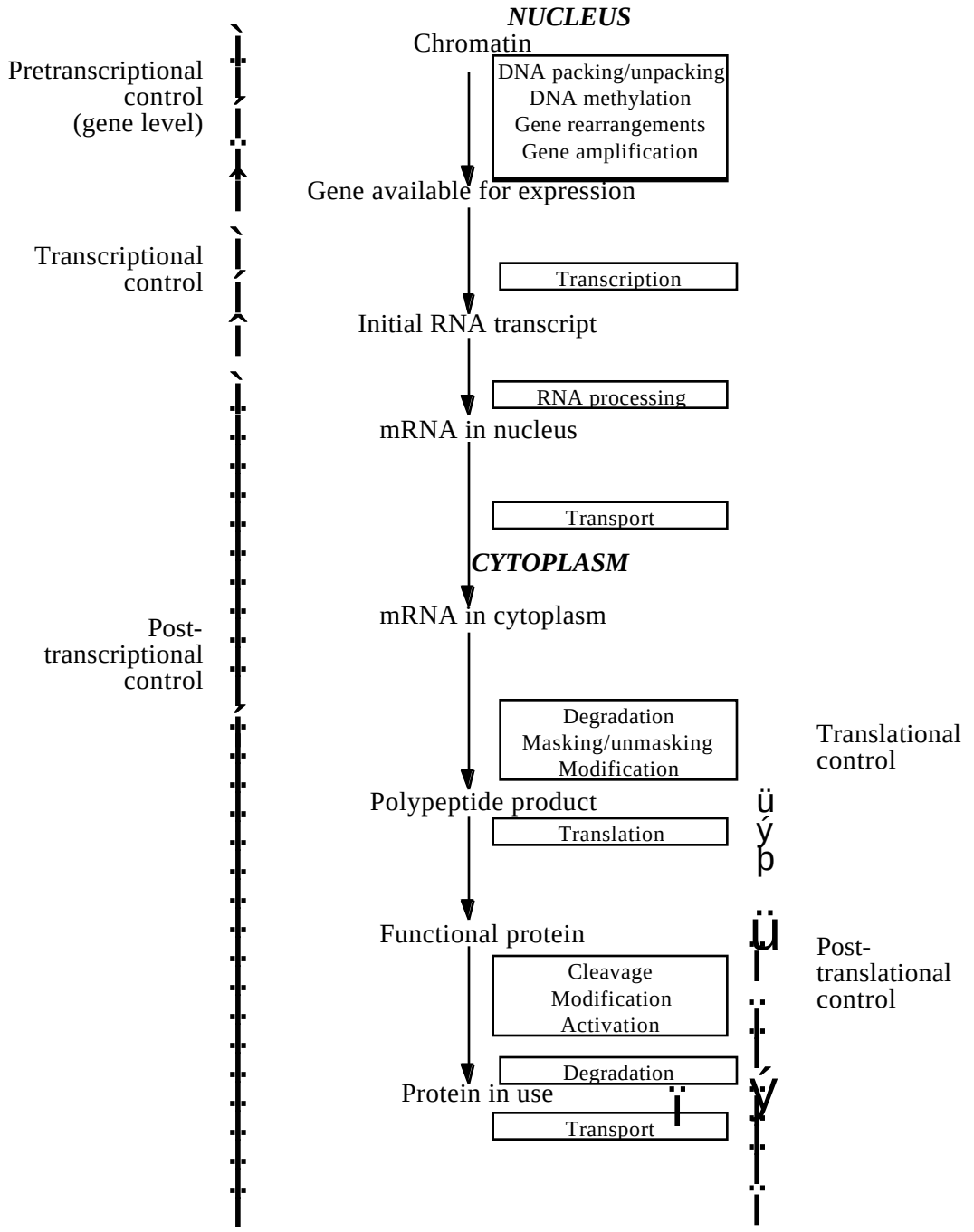
- Cell differentiation requires that gene expression must be regulated on a long-term basis.
- Highly specialized cells, such as muscle or nerve, express only a small percentage of their genes, so transcription enzymes must locate the right genes at the right time.
- Uncontrolled or incorrect gene action can cause serious imbalances and disease, including cancer. Thus, eukaryotic gene regulation is of interest in medical as well as basic research.

DNA-binding proteins regulate gene activity in all organisms—prokaryotes as well as eukaryotes.

- Usually, it is DNA transcription that is controlled.
- Eukaryotes have more complex chromosomal structure, gene organization and cell structure than prokaryotes, which offer added opportunities for controlling gene expression.

B. The control of gene expression can occur at any step in the pathway from gene to functional protein: *an overview*

Complexities in chromosome structure, gene organization and cell structure provide opportunities for the control of gene expression in eukaryotic cells. The steps of gene expression where gene regulation can occur are outlined below (see also Campbell, Figure 19.7).



C. Chromatin modifications affect the availability of genes for transcription

Chromatin organization:

- Packages DNA into a compact form that can be contained by the cell's nucleus.
- Controls which DNA regions are available for transcription.
  - Condensed heterochromatin is not expressed.
  - A gene's location relative to nucleosomes and to scaffold attachment sites influences its expression.

Chemical modifications of chromatin play key roles in both chromatin structure and the regulation of transcription.

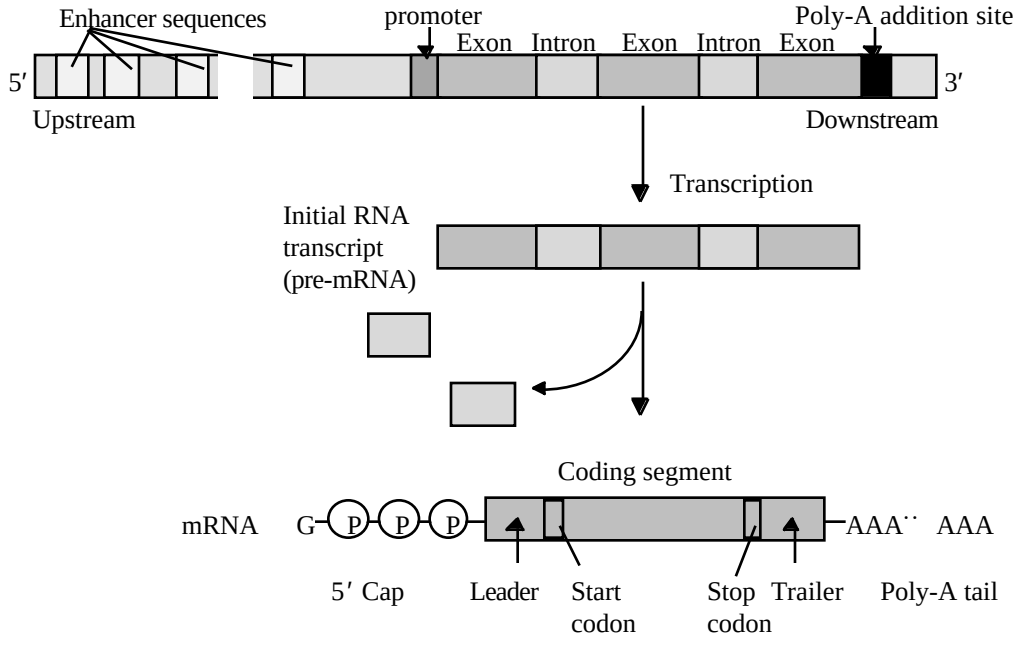
1. DNA methylation

DNA methylation = The addition of methyl groups ($–CH_3$) to bases of DNA, after DNA synthesis

- Most plant and animal DNA contains methylated bases (usually cytosine); about 5% of the cytosine residues are methylated.
- May be a cellular mechanism for long-term control of gene expression. When researchers examine the same genes from different types of cells, they find:
  - Genes that are not expressed (e.g., Barr bodies) are more heavily methylated than those that are expressed.
  - Drugs that inhibit methylation can induce gene reactivation, even in Barr bodies.
- In vertebrates, DNA methylation reinforces earlier developmental decisions made by other mechanisms.
  - For example, genes must be selectively turned on or off for normal cell differentiation to occur. DNA methylation ensures that once a gene is turned off, it stays off.
  - DNA methylation patterns are inherited and thus perpetuated as cells divide; clones of a cell lineage forming specialized tissues have a chemical record of regulatory events that occurred during early development.

2. Histone acetylation

- Acetylation enzymes attach $–COCH_3$ groups to certain amino acids of histone proteins



- Acetylated histone proteins have altered conformation and bind to DNA less tightly; as a result, transcription proteins have easier access to genes in the acetylated region.

D. Transcription initiation is controlled by proteins that interact with DNA and with each other

1. Organization of a typical eukaryotic gene

The following is a brief review of a eukaryotic gene and its transcript (see also Campbell, Figure 19.8).

Eukaryotic genes:

- Contain introns, noncoding sequences that intervene within the coding sequence
- Contain a promoter sequence at the 5′ upstream end; a transcription initiation complex, including RNA polymerase, attaches to a promoter sequence and transcribes introns along with the coding sequences, or exons
- May be regulated by control elements, other noncoding control sequences that can be located thousands of nucleotides away from the promoter

Control element = Segments of noncoding DNA that help regulate the transcription of a gene by binding specific proteins (transcription factors).

The primary RNA transcript (pre-mRNA) is processed into mature mRNA by:

- Removal of introns
- Addition of a modified guanosine triphosphate cap at the 5′ end
- Addition of a poly-A tail at the 3′ end

2. The roles of transcription factors

In both prokaryotes and eukaryotes, transcription requires that RNA polymerase recognize and bind to DNA at the promoter. However, transcription in eukaryotes requires the presence of proteins known as *transcription factors*; transcription factors augment transcription by binding:

- Directly to DNA (protein-DNA interactions)
- To each other and/or to RNA polymerase (protein-protein interactions)

Eukaryotic RNA polymerase cannot recognize the promoter without the help of a specific transcription factor that binds to the TATA box of the promoter.

> Transcription factors and the TATA box were discussed with the material in Chapter 17. This is another point in the course where students, if they compartmentalize their learning, may not relate your discussion of transcriptional control to what they have already learned about transcription. Experiment with organizing the presentation of this material. It may be more prudent to cover transcriptional control of gene expression (both prokaryotic and eukaryotic) with the lectures on protein synthesis. If not, you should probably begin your lecture with a review of transcription, especially RNA polymerase binding and initiation of transcription.

Associations between transcription factors and control elements (specific segments of DNA) are important transcriptional controls in eukaryotes.

- Proximal control elements are close to or within the promoter; distal control elements may be thousands of nucleotides away from the promoter or even downstream from the gene.
- Transcription factors known as activators bind to enhancer control elements to stimulate transcription.
- Transcription factors known as repressors bind to silencer control elements to inhibit transcription

How do activators stimulate transcription?

- One hypothesis is that a hairpin loop forms in DNA, bringing the activator bound to an enhancer into contact with other transcription factors and polymerase at the promoter (see Campbell, Figure 19.9).
- Diverse activators may selectively stimulate gene expression at appropriate stages in cell development.

The involvement of transcription factors in eukaryotes offers additional opportunities for transcriptional control. This control depends on selective binding of specific transcription factors to specific DNA sequences and/or other proteins; the highly selective binding depends on molecular structure.

- There must be a complementary fit between the surfaces of a transcription factor and its specific DNA-binding site.
- Hundreds of transcription factors have been discovered; and though each of these proteins is unique, many recognize their DNA-binding sites with only one of a few possible structural motifs or *domains* containing α helices or β sheets (see Campbell, Figure 19.10).

3. Coordinately controlled genes

Coordinately controlled genes are arranged differently in a eukaryotic chromosome than in prokaryotic genomes.

- Prokaryotic genes that are turned on and off together are often clustered into operons; these adjacent genes share regulatory sites located at one end of the cluster. All genes of the operon are transcribed into one mRNA molecule and are translated together.
- Eukaryotic genes coding for enzymes of a metabolic pathway are often scattered over different chromosomes. Even functionally related genes on the same chromosome have their own promoters and are individually transcribed.

Eukaryotic genes can be coordinately expressed, even though they may be scattered throughout the genome.

- Coordinately controlled genes are each associated with specific regulatory DNA sequences or enhancers. These sequences are recognized by a single type of transcription factor that activates or represses a group of genes in synchrony.
- Examples of coordinate gene expression in eukaryotes include:
  - *Heat shock response.* Exposure to high temperature activates genes coding for heat shock proteins, which help stabilize and repair heat-denatured proteins in the cell.
  - *Steroid hormone action*. Steroids activate protein receptors, and the protein-receptor complex, in turn, activates genes. In a secondary response, proteins produced this way can activate another group of genes (see Campbell Chapter 45).
  - *Cellular differentiation.* During cellular differentiation, coordinately controlled genes producing particular sets of proteins are switched on and off.

E. Posttranscriptional mechanisms play supporting roles in the control of gene expression

Transcription produces a primary transcript, but gene expression—the production of protein, tRNA, or rRNA—may be stopped or enhanced at any posttranscriptional step.

Because eukaryotic cells have a nuclear envelope, translation is segregated from transcription. This offers additional opportunities for controlling gene expression.

1. Regulation of mRNA degradation

Protein synthesis is also controlled by mRNA's lifespan in the cytoplasm.

- Prokaryotic mRNA molecules are degraded by enzymes after only a few minutes. Thus, bacteria can quickly alter patterns of protein synthesis in response to environmental change.
- Eukaryotic mRNA molecules can exist for several hours or even weeks.
- The longevity of a mRNA affects how much protein synthesis it directs. Those that are viable longer can produce more of their protein.
- For example, long-lived mRNAs for hemoglobin are repeatedly translated in developing vertebrate red blood cells.

2. Control of translation

Gene expression can also be regulated by mechanisms that control translation of mRNA into protein. Most of these translational controls repress initiation of protein synthesis; for example.

- Binding of translation repressor protein to the 5′-end of a particular mRNA can prevent ribosome attachment.
- Translation of all mRNAs can be blocked by the inactivation of certain initiation factors. Such global translational control occurs during early embryonic development of many animals.
  - Prior to fertilization, the ovum produces and stores inactive mRNA to be used later during the first embryonic cleavage.
  - The inactive mRNA is stored in the ovum's cytosol until fertilization, when the sudden activation of an initiation factor triggers translation.
  - Delayed translation of stockpiled mRNA allows cells to respond quickly with a burst of protein synthesis when it is needed.

3. Protein processing and degradation

Posttranslational control is the last level of control for regulating gene expression.

- Many eukaryotic polypeptides must be modified or transported before becoming biologically active. Such modifications include:
  - Adding phosphate groups
  - Adding chemical groups, such as sugars
  - Dispatching proteins targeted by signal sequences for specific sites
- Selective degradation of particular proteins and regulation of enzyme activity are also control mechanisms of gene expression.
  - Cells attach ubiquitin to proteins to mark them for destruction
  - Proteasomes recognize the ubiquitin and degrade the tagged protein (see Campbell, Figure 19.11)
  - Mutated cell-cycle proteins that are impervious to proteasome degradation can lead to cancer

IV. The Molecular Biology of Cancer

A. Cancer results from genetic changes that affect the cell cycle

Cancer is a variety of diseases in which cells escape from the normal controls on growth and division—the cell cycle—and it can result from mutations that alter normal gene expression in somatic cells. These mutations:

- Can be random and spontaneous
- Most likely occur as a result of environmental influences, such as:
  - Infection by certain viruses
  - Exposure to carcinogens

Carcinogens = Physical agents such as X-rays and chemical agents that cause cancer by mutating DNA

Whether cancer is caused by physical agents, chemicals or viruses, the mechanism is the same—the activation of *oncogenes* that are either native to the cell or introduced in viral genomes.

Oncogene = Cancer-causing gene

- Discovered during the study of tumors induced by specific viruses
- Harold Varmus and Michael Bishop won a Nobel Prize for their discovery of oncogenes in RNA viruses (retroviruses) that cause uncontrolled growth of infected cells in culture.

Researchers later discovered that some animal genomes, including human, contain genes that closely resemble viral oncogenes. These proto-oncogenes normally regulate growth, division and adhesion in cells.

Proto-oncogenes = Gene that normally codes for regulatory proteins controlling cell growth, division and adhesion, and that can be transformed by mutation into an oncogene.

Three types of mutations can convert proto-oncogenes to oncogenes:

1. *Movement of DNA within the genome.* Malignant cells frequently contain chromosomes that have broken and rejoined, placing pieces of different chromosomes side-by-side and possibly separating the oncogene from its normal control regions. In its new position, an oncogene may be next to active promoters or other control sequences that enhance transcription. Abnormal expression of an oncogene may occur if the oncogene is transposed to a new locus that has a highly active promoter.
2. *Gene amplification.* Sometimes more copies of oncogenes are present in a cell than is normal.
3. *Point mutation.* A slight change in the nucleotide sequence might produce a growth-stimulating protein that is more active or more resistant to degradation than the normal protein.

In addition to mutations affecting growth-stimulating proteins, changes in *tumor-suppressor genes* coding for proteins that normally *inhibit* growth can also promote cancer.

- The protein products of tumor-supressor genes have several functions:
  - Cooperate in DNA repair (helping obviate cancer-causing mutations)
  - Control cell anchorage (cell-cell adhesion; cell interaction with extracellular matrix)
  - Components of cell-signaling pathways that inhibit the cell-cycle

B. Oncogene proteins and faulty tumor-suppressor proteins interfere with normal signaling pathways

Mutation in the *ras* proto-oncogene and the *p*53 tumor suppressor gene are very common in human cancers; the frequency of mutation for *ras* is about 30 % and close to 50% for *p*53.

The *ras* protein is a G protein that relays a growth signal from a growth factor receptor to a cascade of protein kinases. The cellular response is the synthesis of a protein that stimulates the cell cycle (see Campbell Fig 19.13a).

- Under normal conditions, the pathway will not operate unless triggered by the appropriate growth factor.
- A mutated *ras* gene can produce a hyperactive version of the *ras* protein that stimulates the signal transduction cascade on it own, leading to excessive cell division.

The tumor-suppressor protein encoded by the wild-type *p*53 gene is a transcription factor of several genes that promotes the synthesis of growth-inhibiting proteins. Expression of this protective "guardian angel" protein prevents a cell from passing on mutations due to DNA damage in three ways:

- Activates the *p*21 gene, whose product allosterically binds to cyclin-dependent kinases, halting the cell cycle.
- Activates genes directly involved in DNA repair
- When DNA damage is irreparable, activates "suicide" genes, whose products cause cell death (apoptosis)

Mutations in the *p*53 gene can lead to excessive cell growth and cancer.

- For example, mutant tumor-suppressor genes are associated with inherited forms of colorectal cancer, Wilm's tumor, and breast cancer.

C. Multiple mutations underlie the development of cancer

More than one somatic mutation is probably needed to transform normal cells into cancerous cells. One of the best understood examples is colorectal cancer (see Campbell, Figure 19.14).

- Development of metastasizing colorectal cancer is gradual, and the first sign is unusually rapid cell division of apparently normal cells in the colon lining; a benign tumor (polyp) appears, and eventually a malignant tumor may develop.
- During this process, mutations in oncogenes and tumor-suppressor genes gradually accumulate. After a number of genes have changed, the tumor becomes malignant.
- About half a dozen changes must occur at the DNA level for the cell to become fully cancerous: usually, the appearance of at least one active oncogene and the mutation or loss of several tumor-supressor genes.

Viruses play a role in about 15% of human cancer cases worldwide, e.g., some types of leukemia, liver cancer, cervical cancer.

- Viruses might add oncogenes to cells, disrupt tumor-supressor genes DNA, or convert proto-oncogenes to oncogenes.

Breast cancer, the second most common type of cancer in women, is associated with somatic mutations of tumor-suppressor genes.

- Inherited breast cancer accounts for 5–10% of all breast cancer cases.
  - Mutations in either the BRCA1 or BRCA2 (stands for BReast CAncer) gene increase the risk of developing breast cancer. BRCA1 mutations also increase the risk of ovarian cancer.
  - Another locus, accounts for most of the remaining breast cancer cases linked to family history.

The study of genes associated with inherited cancer may lead to early diagnosis and treatment.

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CHAPTER 20
DNA TECHNOLOGY

OUTLINE

I. DNA Cloning
   A. DNA technology makes it possible to clone genes for basic research and commercial applications: *an overview*
   B. Restriction enzymes are used to make recombinant DNA
   C. Genes can be cloned in recombinant DNA vectors: *a closer look*
   D. Cloned genes are stored in DNA libraries
   E. The polymerase chain reaction (PCR) clones DNA entirely *in vitro*

II. Analysis of Cloned DNA
   A. Restriction fragment analysis detects DNA differences that affect restriction sites
   B. Entire genomes can be mapped at the DNA level

III. Practical Applications of DNA Technology
   A. DNA technology is reshaping medicine and the pharmaceutical industry
   B. DNA technology offers forensic, environmental, and agricultural applications
   C. DNA technology raises important safety and ethical questions

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Explain how advances in recombinant DNA technology have helped scientists study the eukaryotic genome.
2. Describe the natural function of restriction enzymes.
3. Describe how restriction enzymes and gel electrophoresis are used to isolate DNA fragments.
4. Explain how the creation of sticky ends by restriction enzymes is useful in producing a recombinant DNA molecule.
5. Outline the procedures for producing plasmid and phage vectors.
6. Explain how vectors are used in recombinant DNA technology.
7. List and describe the two major sources of genes for cloning.
8. Describe the function of reverse transcriptase in retroviruses and explain how they are useful in recombinant DNA technology.
9. Describe how "genes of interest" can be identified with the use of a probe.
10. Explain the importance of DNA synthesis and sequencing to modern studies of eukaryotic genomes.
11. Describe how bacteria can be induced to produce eukaryotic gene products.
12. List some advantages for using yeast in the production of gene products.
13. List and describe four complementary approaches used to map the human genome.

14. Explain how RFLP analysis and PCR can be applied to the Human Genome Project.
15. Describe how recombinant DNA technology can have medical applications such as diagnosis of genetic disease, development of gene therapy, vaccine production, and development of pharmaceutical products.
16. Describe how gene manipulation has practical applications for agriculture.
17. Describe how plant genes can be manipulated using the Ti plasmid carried by *Agrobacterium* as a vector.
18. Explain how foreign DNA may be transferred into monocotyledonous plants.
19. Describe how recombinant DNA studies and the biotechnology industry are regulated with regards to safety and policy matters.

KEY TERMS

genetic engineering
recombinant DNA
biotechnology
nucleic acid probe
gene cloning
restriction enzymes
antisense nucleic acid
restriction fragments length polymorphisms (RFLPs)
cloning vector
nucleic acid hybridization
denaturation
expression vector
restriction site
complementary DNA (cDNA)
sticky ends
DNA ligase
electroporation
genomic library
cDNA library
polymerase chain reaction (PCR)
in vitro mutagenesis
gel electrophoresis
Southern blotting
restriction fragment
artificial chromosomes
in situ hybridization
Ti plasmid
Human Genome Project
chromosome walking
DNA microarray assays
vaccine
DNA fingerprint
simple tandem repeats (STRs)

LECTURE NOTES

Recombinant DNA technology refers to the set of techniques for recombining genes from different sources *in vitro* and transferring this recombinant DNA into a cell where it may be expressed.

- These techniques were first developed around 1975 for basic research in bacterial molecular biology, but this technology has also led to many important discoveries in basic eukaryotic molecular biology.
- Such discoveries resulted in the appearance of the *biotechnology* industry. Biotechnology refers to the use of living organisms or their components to do practical tasks such as:

  The use of microorganisms to make wine and cheese
  Selective breeding of livestock and crops
  Production of antibiotics from microorganisms
  Production of monoclonal antibodies

The use of recombinant DNA techniques allows modern biotechnology to be a more precise and systematic process than earlier research methods.

- It is also a powerful tool since it allows genes to be moved across the species barrier.
- Using these techniques, scientists have advanced our understanding of eukaryotic molecular biology.
- The *Human Genome Project* is an important application of this technology. This project's goal is to transcribe and translate the entire human genome in order to better understand the human organism.
- A variety of applications are possible for this technology, and the practical goal is the improvement of human health and food production.

I. DNA Cloning

A. DNA technology makes it possible to clone genes for basic research and commercial applications: *an overview*

Prior to the discovery of recombinant DNA techniques, procedures for altering the genes of organisms were constrained by the need to find and propagate desirable mutants.

- Geneticists relied on either natural processes, mutagenic radiation, or chemicals to induce mutations.
- In a laborious process, each organism's phenotype was checked to determine the presence of the desired mutation.
- Microbial geneticists developed techniques for screening mutants. For example, bacteria was cultured on media containing an antibiotic to isolate mutants which were antibiotic resistant.

Before 1975, transferring genes between organisms was accomplished by cumbersome and nonspecific breeding procedures. The only exception to this was the use of bacteria and their phages.

- Genes can be transferred from one bacterial strain to another by the natural processes of transformation, conjugation or transduction.
- Geneticists used these processes to carry out detailed molecular studies on the structure and functioning of prokaryotic and phage genes.
- Bacteria and phages are ideal for laboratory experiments because they are relatively small, have simple genomes, and are easily propagated.
- Although the technique was available to grow plant and animal cells in culture, the workings of their genomes could not be examined using existing methods.

Campbell Figure 20.1 provides an overview of how bacterial plasmids are used to clone genes for biotechnology.

Recombinant DNA technology now makes it possible for scientists to examine the structure and function of the eukaryotic genome, because it contains several key components:

- Biochemical tools that allow construction of recombinant DNA
- Methods for purifying DNA molecules and proteins of interest
- Vectors for carrying recombinant DNA into cells and replicating it
- Techniques for determining nucleotide sequences of DNA molecules.

B. Restriction enzymes are used to make recombinant DNA

Restriction enzymes are major tools in recombinant DNA technology.

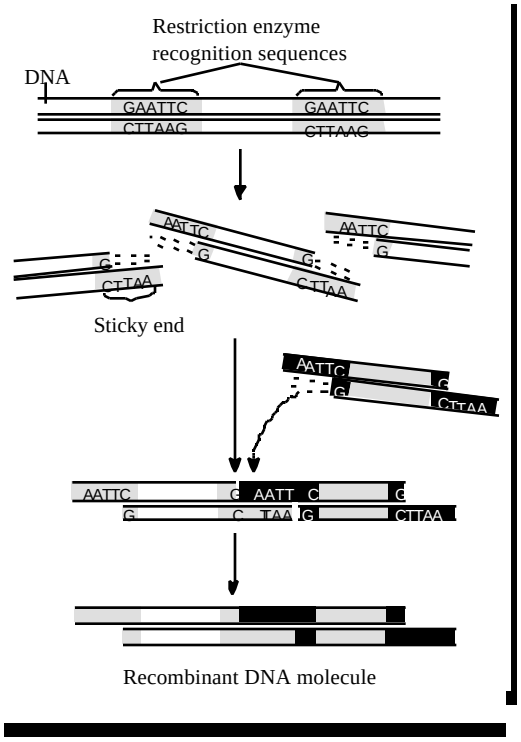
- First discovered in the late 1960s, these enzymes occur naturally in bacteria where they protect the bacterium against intruding DNA from other organisms.
- This protection involves *restriction*, a process in which the foreign DNA is cut into small segments.
- Most restriction enzymes only recognize short, specific nucleotide sequences called *recognition sequences* or restriction sites. They only cut at specific points within those sequences.

Bacterial cells protect their own DNA from restriction through *modification* or methylation of DNA.

- Methyl groups are added to nucleotides within the recognition sequences.
- Modification is catalyzed by separate enzymes that recognize these same DNA sequences.

There are several hundred restriction enzymes and about a hundred different specific recognition sequences.

- Recognition sequences are symmetric in that the same sequence of four to eight nucleotides is found on both strands, but run in opposite directions.
- Restriction enzymes usually cut phosphodiester bonds of both strands in a staggered manner, so that the resulting double-stranded DNA fragments have single-stranded ends, called *sticky ends*.
- The single-stranded short extensions form hydrogen-bonded base pairs with complementary single-stranded stretches on other DNA molecules.



Sticky ends of *restriction fragments* are used in the laboratory to join DNA pieces from different sources (cells or even different organisms).

- These unions are temporary since they are only held by a few hydrogen bonds.
- These unions can be made permanent by adding the enzyme *DNA ligase*, which catalyzes formation of covalent phosphodiester bonds.

The outcome of this process is the same as natural genetic recombination, the production of recombinant DNA – a DNA molecule carrying a new combination of genes (see Campbell, Figure 20.2)..

C. Gene can be cloned in recombinant DNA vectors: *a closer look*

Most DNA technology procedures use carriers or vectors for moving DNA from test tubes back into cells.

Cloning vector = A DNA molecule that can carry foreign DNA into a cell and replicate there

- Two most often used types of vectors are bacterial plasmids and viruses.
- Restriction fragments of foreign DNA can be spliced into a bacterial plasmid without interfering with its ability to replicate within the bacterial cell. Isolated recombinant plasmids can be introduced into bacterial cells by transformation.

Bacteriophages, such as lambda phage, can also be used as vectors.

- The middle of the linear genome, which contains nonessential genes, is deleted by using restriction enzymes.
- Restriction fragments of foreign DNA are then inserted to replace the deleted area.
- The recombinant phage DNA is introduced into an *E. coli* cell.
- The phage replicates itself inside the bacterial cell.
- Each new phage particle carries the foreign DNA "passenger."

Sometimes it is necessary to clone DNA in eukaryotic cells rather than in bacteria. Under the right conditions, yeast and animal cells growing in culture can also take up foreign DNA from the medium.

- If the new DNA becomes incorporated into chromosomal DNA or *can* replicate itself, it can be cloned with the cell.
- Since yeast cells have plasmids, scientists can construct recombinant plasmids that combine yeast and bacterial DNA and that can replicate in either cell type.
- Viruses can also be used as vectors with eukaryotic cells. For example, retroviruses used as vectors in animal cells can integrate DNA directly into the chromosome.

1. Procedure for cloning a eukaryotic gene in a bacterial plasmid

Recombinant DNA molecules are only useful if they can be made to replicate and produce a large number of copies. A typical gene-cloning procedure includes the following steps (see Campbell, Figure 20.3):

Step 1: Isolation of vector and gene-source DNA

- Bacterial plasmids and foreign DNA containing the gene of interest are isolated.
- In this example, the foreign DNA is human, and the plasmid is from *E. coli* and has two genes:
  - amp^R which confers antibiotic resistance to ampicillin
  - *lacZ* which codes for β-galactosidase, the enzyme that catalyzes the hydrolysis of lactose
- Note that the recognition sequence for the restriction enzyme used in this example is *within* the *lacZ* gene.

Step 2: Insertion of gene-source DNA into the vector

a. Digestion
   - The restriction enzyme cuts plasmid DNA at the *restriction site*, disrupting the *lacZ* gene.
   - The foreign DNA is cut into thousands of fragments by the same restriction enzyme; one of the fragments contains the gene of interest.
   - When the restriction enzyme cuts, it produces *sticky ends* on both the foreign DNA fragments and the plasmid.

b. Mixture of foreign DNA fragments with clipped plasmids
   - Sticky ends of the plasmid base pair with complementary sticky ends of foreign DNA fragments.

c. Addition of DNA ligase
   - DNA ligase catalyzes the formation of covalent bonds, joining the two DNA molecules and forming a new plasmid with recombinant DNA.

Step 3: Introduction of cloning vector into bacterial cells

- The naked DNA is added to a bacterial culture.
- Some bacteria will take up the plasmid DNA by transformation.

Step 4: Cloning of cells (and foreign DNA)

- Bacteria with the recombinant plasmid are allowed to reproduce, cloning the inserted gene in the process.
- Recombinant plasmids can be identified by the fact that they are ampicillin resistant and will grow in the presence of ampicillin.

Step 5: Identification of cell clones carrying the gene of interest

- X-gal, a modified sugar added to the culture medium, turns blue when hydrolyzed by β-galactosidase. It is used as an indicator that cells have been transformed by plasmids containing the foreign insert.

- Since the foreign DNA insert disrupts the *lacZ* gene, bacterial colonies that have successfully acquired the foreign DNA fragment will be white. Those bacterial colonies lacking the DNA insert will have a complete *lacZ* gene that produces β-galactosidase and will turn blue in the presence of X-gal.
    - The methods for detecting the DNA of a gene depend directly on base pairing between the gene of interest and a complementary sequence on another nucleic acid molecule, a process called *nucleic acid hybridization.* The complementary molecule, a short piece of RNA or DNA is called a *nucleic acid probe* (see Campbell, Fig. 20.4).

2. Cloning and expressing eukaryotic genes: problems and solutions

Problem: Getting a cloned eukaryotic gene to function in a prokaryotic setting can be difficult because certain details of gene expression are different in the two kinds of cells.

Solution: Expression vectors allow the synthesis of many eukaryotic proteins in bacterial cells.

- Expression vectors contain a prokaryotic promoter just upstream of a restriction site where the eukaryotic gene can be inserted.
- The bacterial host cell recognizes the promoter and proceeds to express the foreign gene that has been linked to it.

Problem: Eukaryotic genes of interest may be too large to clone easily because they contain long noncoding regions (introns), which prevent correct expression of the gene by bacterial cells, which lack RNA-splicing machinery..

Solution: Scientists can make artificial eukaryotic genes that lack introns (see Campbell, Figure 20.5).

Solution: Artificial chromosomes, which combine the essentials of a eukaryotic chromosome with foreign DNA, can carry much more DNA than plasmid vectors, thereby enabling very long pieces of DNA to be cloned.

Bacteria are commonly used hosts in genetic engineering because:

- DNA can be easily isolated from and reintroduced into bacterial cells
- Bacterial cultures grow quickly, rapidly cloning the inserted foreign genes.

Some disadvantages to using bacterial host cells are that bacterial cells:

- May not be able to use the information in a eukaryotic gene, since eukaryotes and prokaryotes use different enzymes and regulatory mechanisms during transcription and translation.
- Cannot make the posttranslational modifications required to produce some eukaryotic proteins (e.g., addition of lipid or carbohydrate groups)

Using eukaryotic cells as hosts can avoid the eukaryotic-prokaryotic incompatibility issue.

- Yeast cells are as easy to grow as bacteria and contain plasmids.
- Some recombinant plasmids combine yeast and bacterial DNA and can replicate in either.
- Posttranslational modifications required to produce some eukaryotic proteins (e.g., addition of lipid or carbohydrate groups) can occur

There are more aggressive techniques for inserting foreign DNA into eukaryotic cells:

- In *electroporation,* a brief electric pulse applied to a cell solution causes temporary holes in the plasma membrane, through which DNA can enter.
- With thin needles, DNA can be injected directly into a eukaryotic cell.

- DNA attached to microscopic metal particles can be fired into plant cells with a gun (see Campbell, Figure 38.13).

Bacteria and yeast are not suitable for every purpose. For certain applications, plant or animal cell cultures must be used.

- Cells of more complex eukaryotes carry out certain biochemical processes not found in yeast (e.g. only animal cells produce antibodies).

D. Cloned genes are stored in DNA libraries

There are two major sources of DNA which can be inserted into vectors and clones:

1. DNA isolated directly from an organism
2. Complementary DNA made in the laboratory from mRNA templates

DNA isolated directly from an organism contains all genes including the gene of interest.

- Restriction enzymes are used to cut this DNA into thousands of pieces which are slightly larger than a gene.
- All of these pieces are then inserted into plasmids or viral DNA.
- These vectors containing the foreign DNA are introduced into bacteria.
- This produces the *genomic library, t*he complete set of thousands of recombinant-plasmid clones, each carrying copies of a particular segment from the initial genome (see Campbell, Figure 20.6).
- Libraries can be saved and used as a source of other genes of interest or for genome mapping.

The cDNA method produces a more limited kind of gene library, a cDNA library. A cDNA library represents only part of the cell's genome because it contains only the genes that were transcribed in the starting cells (recall that cDNA is derived from isolated RNA).

- By using cells from specialized tissues or a cell culture used exclusively for making one gene product, the majority of mRNA produced is for the gene of interest.
- For example, most of the mRNA in precursors of mammalian erythrocytes is for the protein hemoglobin.

E. The polymerase chain reaction (PCR) clones DNA entirely in vitro

PCR is a technique that allows any piece of DNA to be quickly amplified (copied many times) in vitro (see Campbell, Methods Box)

- DNA is incubated under appropriate conditions with special primers and DNA polymerase molecules.
- Billions of copies of the DNA are produced in just a few hours.
- PCR is highly specific; primers determine the sequence to be amplified.
- Only minute amounts of DNA are needed.

PCR is presently being applied in many ways for analysis of DNA from a wide variety of sources:

- Ancient DNA fragments from a woolly mammoth; DNA is a stable molecule and can be amplified by PCR from sources thousands, even millions, of years old.
- DNA from tiny amounts of tissue or semen found at crime scenes
- DNA from single embryonic cells for prenatal diagnosis
- DNA of viral genes from cells infected with difficult to detect viruses such as HIV

Amplification of DNA by PCR is being used in the Human Genome Project to produce linkage maps without the need for large family pedigree analysis.

- DNA from sperm of a single donor can be amplified to analyze the immediate products of meiotic recombination.
- This process eliminates the need to rely on the chance that offspring will be produced with a particular type of recombinant chromosome.
- It makes it possible to study genetic markers that are extremely close together.

II. Analysis of Cloned DNA

Once a gene is cloned, scientists can then analyze the cloned DNA to address numerous questions, such as:

- Does a gene differ in different organisms?
- Are there certain alleles associated with a hereditary disorder?
- Where in the organism is the gene expressed?
- What control the pattern of expression?
- What is the location of the gene within the genome?

A. Restriction fragment analysis detects DNA differences that affect restriction sites

Gel electrophoresis (see Campbell's Methods box) is used to separate either nucleic acids or proteins based upon molecular size, charge, and other physical properties. Using this technique:

- Viral DNA, plasmid DNA, and segments of chromosomal DNA can be identified by their characteristic banding patterns after being cut with various restriction enzymes.
  - Each band corresponds to a DNA restriction fragment of a certain length.
  - DNA segments carrying different alleles of a gene can result in dissimilar banding patterns, since numbers and locations of restriction sites may not be the same in the different nucleotide sequences (see Campbell, Figure 20.7).
  - Similar differences in banding patterns result when *noncoding* segments of DNA are used as starting material.
- DNA fragments containing genes of interest can be isolated, purified, and then recovered from the gel with full biological activity.

The technique of hybridization is used to determine the presence of a specific nucleotide sequence (see Campbell's Methods Box on *Southern blotting* for an explanation of the entire procedure and a demonstration of how it can be used to compare DNA from three individuals).

- Labeled probes complementary to the gene of interest are allowed to bind to DNA from cells being tested (see Campbell, Fig. 20.4).
- Variations of this technique allows researchers to determine whether a:
  - Gene is present in various organisms
  - Sequence is present, how many sequences there are, and the size of the restriction fragments containing these sequences
  - Gene is made into mRNA, how much of that mRNA is present, and whether the amount of that mRNA changes at different stages of development or in response to certain regulatory signals (*Northern blotting*)

Differences in restriction fragment length that reflect variations in homologous DNA sequences are called *restriction fragment length polymorphisms* (*RFLPs*).

- DNA sequence differences on homologous chromosomes that result in RFLPs are scattered abundantly throughout genomes, including the human genome.
- RFLPs are not only abundant, but can easily be detected as to whether they affect the organism's phenotype; they can be located in an exon, intron, or any noncoding part of the genome.
- RFLP are detected and analyzed by Southern blotting.
- Because RFLPs can be readily detected, they are extremely useful as *genetic markers* for making linkage maps.
- A RFLP marker is often found in numerous variants in a population. RFLPs have provided many markers for mapping the human genome since geneticists are no longer limited to genetic variations that lead to phenotypic differences or protein products.

RFLPs are proving useful in several areas.

- Disease genes are being located by examining known RFLPs for linkage to them. RFLP markers inherited at a high frequency with a disease are probably located close to the defective gene on the chromosome.
- An individual's RFLP markers provide a "genetic fingerprint" which can be used in forensics, since there is a very low probability that two people would have the same set of RFLP markers.

B. Entire genomes can be mapped at the DNA level

1. Locating genes by *in situ* hybridization

DNA probes can be used to help map genes on eukaryotic chromosomes.

- *In situ hybridization* uses a radioactive DNA probe that base pairs with complementary sequences in the denatured DNA of intact chromosomes.
- Autoradiography and chromosome staining reveal to which band of which chromosome the probe has attached. Alternatively, the probe is labeled with fluorescent dye.

2. The mapping of entire genomes

The Human Genome Project, begun in 1990, is an international effort to map the entire human genome.

Scientists are also mapping the genomes of species that are particularly useful for genetic research, including *E. coli*, *Saccharomyces cerevisiae* (yeast), *Caenorhabditis elegans* (nematode), *Drosophila melanogaster* (fruit fly), and *Mus musculus* (mouse).

Several complementary approaches are being used to map the precise locations of all of an organism’s genes:

a. Genetic (linkage) mapping

- The first step in mapping a large genome is to construct a linkage map of several thousand genetic markers, which can be genes, RFLPs, or microsatellites.
- Relying primarily on microsatellites, researchers have completed a human genetic map with over 5000 markers.
- This map will enable researchers to locate other markers by testing for genetic linkage to the known markers.

b. Physical mapping: ordering the DNA fragments

- A physical map is made by cutting the DNA of each chromosome into a number of identifiable fragments.

- The key is to make fragments that overlap and to find the overlapping ends
- Campbell, Figure 20.8 shows *chromosome walking*, a method which uses probes to find the overlapping ends.
- Researchers carry out several rounds of DNA cutting, cloning, and mapping in order to prepare supplies of DNA fragments to map large genomes.

- The goal is to find the original order of the fragments in the chromosomal DNA.

c. Sequencing DNA

- The complete nucleotide sequence of a genome is the ultimate map.
- This will be the most time consuming part of the Human Genome Project as each haploid set of human chromosomes contains about 3 *billion* nucleotide pairs.

In addition to chromosome walking, Human Genome Project researchers are using PCR amplification.

- PCR can amplify specific portions of DNA from individual sperm cells—the immediate products of meiotic recombination.
- Researchers can amplify DNA in amounts sufficient for study, and analyze samples as large as thousands of sperm.
- Based on the crossover frequencies between genes, researchers can deduce human linkage maps without having to find large families for pedigree analysis.

These approaches will be used to completely map genomes and provide an understanding of how the human genome compares to those of other organisms. Potential benefits include:

- Identification and mapping of genes responsible for genetic diseases will aid diagnosis, treatment and prevention.
- Detailed knowledge of the genomes of humans and other species will give insight into genome organization, control of gene expression, cellular growth and differentiation, and evolutionary biology.

Achieving the goals of the Human Genome Project in a timely way will come from advances in automation and utilization of the latest electronic technology.

3. Genome analysis

Geneticists are also trying to determine phenotype from genotype, or identify genes within a long DNA sequence and determine their function.

a. Analyzing DNA sequences

Many researchers are studying the structure and organization of genes. This type of genome analysis relies of DNA sequencing (see Campbell's Methods box) and other mapping approaches outlined above.

DNA sequencing techniques have enabled scientists to collect thousands of DNA sequences in computer data banks.

- Using a computer, scientists can scan sequences for protein-coding genes and gene-control sequences.
- A list of nucleotide sequences for putative genes is assembled and compared to sequences of known genes.

- In the sequences compiled to date, many putative genes have been found to be entirely new to science; for example, 38% of the genes of E. Coli, the best studied research organism.

DNA sequences confirm the evolutionary connections between distantly related organisms and the relevance of research on simpler organisms to understanding human biology.

b. Studying gene expression

Other researchers are studying patterns of gene expression and how such patterns act to produce and maintain a functioning organism. This type of genome analysis can be performed without knowledge of the complete DNA sequence of an organism.

- One strategy for evaluating gene expression is to isolate the mRNA made in particular cells, use these molecules as templates for making a cDNA library by reverse transcription, and then compare this cDNA with other collections of DNA by hybridization.
  - This approach reveals which genes are active at different stages of development, in different tissues, or in different physiological conditions (or states of health).

Another approach uses DNA microarray assays to detect and measure the expression of thousands of genes at one time (see Campbell, Figure 20.9).

- This method is being used to compare cancerous and noncancerous tissues.
- Studying the differences in gene expression may lead researchers to new diagnostic techniques and biochemically targeted treatments

c. Determining gene function

Still other researchers are studying the function of genes.

In vitro mutagenesis is a technique that can be used to determine the function of a protein product from cDNA cloning of an mRNA.

- Mutations are induced into the sequence of a cloned gene.
- The mutated gene is returned to the host cell.
- If the mutation alters the function of the protein product, it may be possible to determine the function of the protein by examining what changes occur in cell physiology or developmental pattern.

III. Practical Applications of DNA Technology

A. DNA technology is reshaping medicine and the pharmaceutical industry

Modern biotechnology has resulted in significant advances in many areas of medicine.

1. Diagnosis of diseases

Medical scientists currently use DNA technology to diagnose hundreds of human genetic disorders.

- This allows early disease detection and identification of carriers for potentially harmful recessive mutations – even before the onset of symptoms.
- Genes have been cloned for many genetic disorders including hemophilia, phenylketonuria, cystic fibrosis, and Duchenne muscular dystrophy.

- Gene cloning permits direct detection of gene mutations. A cloned normal gene can be used as a probe to find the corresponding gene in cells being tested; the alleles are compared with normal and mutant standards usually by RFLP analysis.

When the normal gene has not been cloned, a closely linked RFLP marker may indicate the presence of an abnormal allele if the RFLP marker is frequently co-inherited with the disease.

- Blood samples from relatives can be used to determine which RFLP marker is linked to the abnormal allele and which is linked to the normal allele.
- The RFLP markers must be different for the normal and abnormal alleles.
- Under these conditions, the RFLP marker variant found in the person being tested can reveal whether the normal or abnormal allele is likely to be present.
- Alleles for cystic fibrosis and Huntington's disease can be detected in this manner.

2. Human gene therapy

Traceable genetic disorders in individuals may eventually be correctable.

- Theoretically, it should be possible to replace or supplement defective genes with functional normal genes using recombinant DNA techniques.
- Correcting somatic cells of individuals with well-defined, life-threatening genetic defects will be the starting place.

The principle behind human gene therapy is that normal genes are introduced into a patient's own somatic cells.

- For this therapy to be permanent, the cells receiving the normal allele must actively reproduce, so the normal allele will be replicated and continually expressed (see Campbell, Figure 20.11).
- Bone marrow cells are prime candidates.

Attempts at human gene therapy have not yet produced any proven benefits to patients, contrary to claims in the popular media.

- The most promising gene therapy trials under way are ones that involve bone marrow cells but are not necessarily aimed at correcting genetic defects; for example, improving the abilities of immune cells to fight off cancer and resist HIV
- Most experiments to date have been designed to test the safety and feasibility of a procedure rather than attempt a cure.

Many technical questions are posed by gene therapy.

- Can the proper genetic control mechanisms be made to operate on the transferred gene so that cells make appropriate amounts of the gene product at the right time and in the right place?
- How can we be sure that the inserted therapeutic gene does not harm some other necessary cell function?

Gene therapy raises difficult social and ethical questions:

- Is it advisable under any circumstances to alter the genomes of human germ lines (eggs) or embryos in hope of correcting the defect in future generations?
    - Some critics believe tampering with human genes in any way is wrong and may lead to eugenics.
    - Others say that genetic engineering of somatic cells is no different than other conventional medical interventions used to save lives.

Treating germ cells is possible and has been used in mice for some time.

- Mice have been created with sickle cell anemia to further the study of the disease.
- Recipient mice and their descendants contain the active human gene not only in the proper location (erythrocytes) but also during the proper stage of development.

3. Pharmaceutical products

DNA technology has been used to create many useful pharmaceutical products, mostly proteins.

- Highly active promoters and other gene control elements are put into vector DNA to create expression vectors that enable the host cell to make large amounts of the product of a gene inserted into the vector.
- Host cells can be engineered to secrete a protein as it is made, thereby simplifying the task of purifying it.

Human insulin and growth hormone are early applications of gene splicing..

- Two million individuals with diabetes in the United States have benefited from genetically engineered human insulin.
- Insulin produced this way is chemically identical to that made by the human pancreas, and it causes fewer adverse reactions than insulin extracted from pig and cattle pancreas.
- Human growth hormone has been a boon to children with hypopituitarism (pituitary dwarfism).
    - The growth hormone molecule is much larger than insulin (almost 200 amino acids long) and more species specific.
    - Thus, growth hormone from other animals is not an effective growth stimulator in humans.
    - Previously, these individuals were treated with growth hormone obtained from human cadavers.

Another important product produced by genetic engineering is tissue plasminogen activator (TPA).

- This protein helps dissolve blood clots and reduces the risk of later heart attacks if administered very shortly after an initial heart attack
- TPA illustrates a drawback to genetically engineered products. Because the development costs were high and the market relatively limited, the product has been very expensive.

Recent developments include novel ways to fight diseases that don’t respond to traditional drug treatments.

- Antisense nucleic acid is used to base-pair with mRNA molecules and block their translation.
    - This could prevent the spread of diseases by interfering with viral replication or the transformation of cells into a cancerous state.
- Genetically engineered proteins block or mimic surface receptors on cell membranes.
    - For example, an experimental drug mimics a receptor protein that HIV binds to when it attacks white blood cells. The HIV binds to the drug molecules instead and fails to enter the blood cells.

Prevention by vaccine is the only way to fight many viral diseases for which no treatment exists.

Vaccine = A harmless variant or derivative of a pathogen that stimulates the immune system to fight the pathogen

Traditional vaccines for viral diseases are of two types:

- Particles of virulent virus that have been inactivated by chemical or physical means.
- Active virus particles of an attenuated (nonpathogenic) viral strain.

Since the particles in both cases are similar to active virus, both types of vaccine will trigger an animal's immune system to produce antibodies, which react very specifically against invading pathogens.

Biotechnology is being used in several ways to modify current vaccines and to produce new ones. Recombinant DNA techniques can be used to:

- Produce large amounts of specific protein molecules (*subunits*) from the surface of a pathogen. If these protein subunits cause immune responses to the pathogens, they can be used as vaccines.
- Modify genomes of pathogens to directly attenuate them. Vaccination with live, attenuated organisms is more effective than a subunit vaccine. Small amounts of material trigger greater immune response, and pathogens attenuated by gene-splicing may also be safer than using natural mutants.

B. DNA technology offers forensic, environmental, and agricultural applications

1. Forensic uses of DNA technology

Forensic labs can determine blood or tissue type from blood, small fragments of other tissue, or semen left at the scene of violent crime. These tests, however, have limitations:

- They require fresh tissue in sufficient amounts for testing.
- This approach can exclude a suspect but is not evidence of guilt, because many people have the same blood type or tissue type.

DNA testing can identify an individual with a much higher degree of certainty, since everyone's DNA base sequence is unique (except for identical twins).

- RFLP analysis by Southern blotting is a powerful method for the forensic detection of similarities and differences in DNA samples (see Campbell, Figure 20.12).
  - This method is used to compare DNA samples from the suspect, the victim, and a small amount of semen, blood or other tissue found at the scene of the crime.
  - Restriction fragments from the DNA samples are separated by electrophoresis; radioactive probes mark the bands containing RFLP markers.
  - Usually the forensic scientist tests for five markers.
  - Even a small set of RFLP markers from an individual can provide a *DNA fingerprint* that is of forensic use; the probability that two individuals would have the same RFLP markers is quite low.
- Increasingly, variations in the lengths of satellite DNA are used instead of RFLPs in DNA fingerprinting.
  - The most useful satellite sequences for forensic purposes are microsatellites, which are 10 - 100 base pairs long, have repeating units of only 1 - 4 base pairs, and are highly variable from person to person.
  - Individuals have different numbers of repeats at genome loci (*simple tandem repeats (STRs)*)

    - Restriction fragments containing STRs vary in size among individuals because of differences in STR lengths rather than because of different numbers of restriction sites within that region of the genome, as in RFLP analysis.
    - The greater the number of markers examined in a sample, the more likely it is that the DNA fingerprint is unique to one individual.
  - PCR is often used to selectively simplify particular STRs or other markers before electrophoresis. This is especially useful when the DNA is in poor condition or only available in minute quantities (only 20 cells are needed!).

How reliable is DNA fingerprinting?

- Though each individual's DNA fingerprint is unique, most forensic tests do not analyze the entire genome but focus on tiny regions known to be highly variable from one person to another.
- The probability is minute (between on in 100,000 and one in a billion) that two people will have matching DNA fingerprints.
  - The exact figure depends on the number of markers compared and on the frequency of those markers in the population.
  - The frequency of markers varies by ethnic group, which allows forensic scientists to make extremely accurate statistical calculations.
- Problems can arise from insufficient statistical data, human error, or flawed evidence.
- DNA fingerprints are now accepted as compelling evidence by legal and scientific experts.

As with most new technology, forensic applications of DNA fingerprinting raises important ethical questions such as:

- Once collected, what happens to the DNA data?
- Should DNA fingerprints be filed or destroyed? Some states now save DNA data from convicted criminals.

2. Environmental uses of DNA technology

Scientists are engineering metabolic capabilities of organisms so they can transform chemicals and thus help solve environmental problems. For example:

- Some microorganisms can extract heavy metals (e.g., copper, lead, and nickel) from their environments and incorporate them into recoverable compounds such as copper sulfate or lead sulfate.
- As metal reserves are depleted, genetically engineered microbes may perhaps be used in mining and in cleaning up mining waste.

Metabolic diversity of microbes is used in the recycling of wastes and detoxification of toxic chemicals.

- Sewage treatment plants use microorganisms to degrade organic compounds into non-toxic forms.
- Biologically and chemically active compounds that cannot be easily degraded are often released into the environment.
- The intent is to engineer microorganisms that can degrade these compounds and that can be used in waste water treatment plants.
- Such microbes might be incorporated directly into the manufacturing process, preventing toxic chemicals from ever being released as waste in the first place.
- Bacterial strains have been developed to detoxify specific toxic wastes found in spills and waste dumps.

3. Agricultural uses of DNA technology

Recombinant DNA techniques are being used to study plants and animals of agricultural importance to improve their productivity.

a. Animal husbandry

Products produced by recombinant DNA methods, such as vaccines, antibodies and growth hormone, are already used in animal husbandry. For example,

- *Bovine growth hormone* (*bGH*). Made by *E. coli*, bGH is injected into milk cows to enhance milk production and into beef cattle to increase weight gain.
- *Cellulase*. Also produced by *E. coli*, this enzyme hydrolyzes cellulose making all plant parts useable for animal feed.

Transgenic animals, those that contain DNA from other species, have been commercially produced by injecting foreign DNA into egg nuclei or early embryos.

- Transgenic beef and dairy cattle, hogs, sheep and several species of commercially raised fish have been produced for potential agricultural use.

b. Genetic engineering in plants

Plant cells are easier to engineer than animal cells, because an adult plant can be produced from a single cell growing in tissue culture.

- This is important since many types of genetic manipulation are easier to perform and assess on single cells than on whole organisms.
- Asparagus, cabbage, citrus fruits, sunflowers, carrots, alfalfa, millet, tomatoes, potatoes, and tobacco are all commercial plants that can be grown from single somatic cells.

The best developed DNA vector for plant cells is the *Ti plasmid* (tumor inducing), carried by the normally pathogenic bacterium *Agrobacterium tumefaciens*.

- Ti plasmid usually induces tumor formation in infected plants by integrating a segment of its DNA (called T DNA) into the chromosomes of the host plant cell.
- Researchers have turned this plasmid into a useful vector by eliminating its disease-causing ability without interfering with its potential to move genetic material into infected plants.

With recombinant DNA methods, foreign genes can be inserted into Ti plasmid.

- The recombinant plasmid can either be put back into *Agrobacterium tumefaciens*, which is used to infect plant cells in culture, or it can be introduced directly into plant cells.
- Individual modified plant cells are then used to regenerate whole plants that contain, express and pass on to their progeny the foreign gene (see Campbell, Figure 20.13).

Using Ti plasmid as a vector has one major drawback, only dicotyledons are susceptible to infection by *Agrobacterium*; important commercial plants such as corn and wheat are monocotyledons and cannot be infected.

- Newer methods that allow researchers to overcome this limitation are *electroporation* and use of the *DNA particle gun.*
- Electroporation uses high-voltage jolts of electricity to open temporary pores in the cell membrane; foreign DNA can enter the cells through these pores.

- The DNA gun shoots tiny DNA-coated metal pellets through the cell walls into the cytoplasm, where the foreign DNA becomes integrated into the host cell DNA.

Though cloning plant DNA is straightforward, plant molecular geneticists still face several technical problems:

- Identifying genes of interest may be difficult.
- Many important plant traits (such as crop yield) are polygenic.

Genetic engineering of plants has yielded positive results in cases where useful traits are determined by single genes. For example:

- Of the genetically engineered plants now in field trials, over 40% have received genes for herbicide resistance
- A bacterial gene that makes plants resistant to glyphosate (a powerful herbicide) has been successfully introduced into several crop plants; glyphosate-resistant plants makes it easier to grow crops and destroy weeds simultaneously.
- The first gene-spliced fruits approved by the FDA for human consumption were tomatoes engineered with antisense RNA to suppress ripening and retard spoilage. The general process is outlined below:
  1. Researchers clone the tomato gene coding for enzymes responsible for ripening.
  2. The complementary (antisense) gene is cloned.
  3. The antisense gene is spliced into the tomato plant's DNA, where it transcribes mRNA complementary to the ripening genes' mRNA.
  4. When the ripening gene produces a normal mRNA transcript, the antisense mRNA binds to it, blocking synthesis of the ripening enzyme.

Crop plants are being engineered to resist pathogens and pest insects. For example,

- Tomato and tobacco plants can be engineered to carry certain genes of viruses that normally infect and damage plants; expression of these versions of viral genes confer resistance to viral attack.
- Some plants have been engineered to resist insect attack, reducing the need to apply chemical insecticides to crops. Genes for an insecticidal protein have been successfully transferred from the bacterium, *Bacillus thuringiensis*, to corn, cotton and potatoes; field tests show that these engineered plants are insect resistant.

In the near future, crop plants developed with recombinant DNA techniques are likely to:

- Be made more productive by enlarging agriculturally valuable parts - whether they be roots, leaves, flowers, or stems.
- Have an enhanced food value. For example, corn and wheat might be engineered to produce mixes of amino acids optimal for the human diet.

c. The nitrogen-fixation challenge

Nitrogen fixation is the conversion of atmospheric, gaseous nitrogen (N_2) into nitrogen-containing compounds.

- Gaseous nitrogen is useless to plants.
- Nitrogen-fixing bacteria live in the soil or are symbiotic within plant roots.

- The nitrogen-containing compounds produced by nitrogen fixation are taken up from the soil by plants and used to make organic molecules such as amino acids and nucleotides.
- A major limiting factor in plant growth and crop yield is availability of useable nitrogen compounds. This is why nitrogenous fertilizers are used in agriculture.

Recombinant DNA technology can possibly be used to increase biological nitrogen fixation of bacterial species living in the soil or in association with plants.

C. DNA technology raises important safety and ethical questions

When scientists realized the potential power of DNA technology, they also became concerned that recombinant microorganisms could create hazardous new pathogens, which might escape from the laboratory.

- In response to these concerns, scientists developed and agreed upon a self-monitoring approach, which was soon formalized into federal regulatory programs.
- Today, governments and regulatory agencies worldwide are monitoring the biotechnology industry - promoting potential industrial, medical, and agricultural benefits, while ensuring that new products are safe.
- In the U.S., the FDA, National Institutes of Health (NIH) Recombinant DNA Advisory Committee, Department of Agriculture (USDA), and Environmental Protection Agency (EPA) set policies and regulate new developments in genetic engineering.

While genetic engineering holds enormous potential for improving human health and increasing agricultural productivity, new developments in DNA technology raise ethical concerns. For example, mapping the human genome will contribute to significant advances in gene therapy, but:

- Who should have the right to examine someone else's genes?
- Should a person's genome be a factor in their suitability for a job?
- Should insurance companies have the right to examine an applicant's genes?
- How do we weigh the benefits of gene therapy against assurances that the gene vectors are safe?

For environmental problems such as oil spills, genetically engineered organisms may be part of the solution, but what is their potential impact on native species?

For new medical products, what is the potential for harmful side effects, both short-term and long-term?

- New medical products must pass exhaustive tests before the FDA approves it for general marketing.
- Currently awaiting federal approval are hundreds of new genetically engineered diagnostic products, vaccines, and drugs - including some to treat AIDS and cancer.

For agricultural products, what are the potential dangers of introducing new genetically engineered organisms into the environment?

- Some argue that producing transgenic organisms is only an extension of traditional hybridization and should not be treated differently from the production of other hybrid crops or animals. The FDA holds that if products of genetic engineering are not significantly different from products already on the market, testing is not required.

- Others argue that creating transgenic organisms by splicing genes from one species to another is radically different from hybridizing closely related species of plants or animals.
- Some concerns are that genetically altered food products may contain new proteins that are toxic or cause severe allergies; genetically engineered crop plants could become superweeds resistant to herbicides, disease and insect pests; and engineered crop plants may hybridize with native plants and pass their new genes to closely related plants in the wild.

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CHAPTER 21
THE GENETIC BASIS OF DEVELOPMENT

OUTLINE

I. From Single Cell to Multicellular Organism
   A. Embryonic development involves cell division, morphogenesis, and cell differentiation
   B. Researchers study development in model organisms to identify general principles: *science as a process*

II. Differential Gene Expression
   B. Different types of cells in an organism have the same DNA
   C. Different cell types make different proteins, usually as a result of transcriptional regulation
   D. Transcriptional regulation is directed by maternal molecules in the cytoplasm and signals from other cells

III. Genetic and Cellular Mechanisms of Pattern Formation
   A. Genetic analysis of development in *Drosophila* reveals how genes control development: *an overview*
   B. Gradients of maternal molecules in the early embryo control axis formation
   C. A cascade of gene activations sets up the segmentation pattern in *Drosophila*: *a closer look*
   D. Homeotic genes direct the identity of body parts
   E. Homeobox genes have been highly conserved in evolution
   F. Neighboring cells instruct other cells to form particular structures: cell signaling and induction in the nematode
   G. Plant development depends on cell signaling and transcriptional regulation: *science as a process*

OBJECTIVES

After reading the chapter and attending lecture, students should be able to do the following:

1. Distinguish between the patterns of morphogesis in plants and in animals.
2. List the animals used as models for developmental biology research and provide a rationale for their choice.
3. Describe how genomic equivalence was determined for plants and animals.
4. Describe what kinds of changes occur to the genome during differentiation.
5. Describe the general processes by which "Dolly" was cloned.
6. Describe the molecular basis of determination.
7. Describe the two sources of information that instruct a cell to express genes at the appropriate time.
8. Describe how *Drosophila* were used to explain basic aspects of pattern formation (axis formation and segmentation).

9. Describe how homeotic genes serve to identify parts of the developing organism.
10. Provide evidence of the conservation of homeobox sequences.
11. Describe how the study of nematodes contributed to the general understanding of embryonic induction.
12. Describe how apoptosis functions in normal and abnormal development.
13. Describe how the study of tomatoes has contributed to the understanding of flower development.
14. Describe how the study of *Arabidopsis* has contributed to the understanding of organ identity in plants.

KEY TERMS

differentiation	determination	maternal effect genes	segment-polarity genes
morphogenesis	cytoplasmic determinants	egg-polarity genes	homeotic genes
apical meristems	pattern formation	morphogens	homeobox
model organism	induction	segmentation genes	apoptosis
cell lineage	positional information	gap genes	chimeras
totipotent	embryonic lethals	pair-rule genes	organ-identity genes

LECTURE NOTES

The study of how a single cell develops into a multicellular organism and the functional maintenance of the developed structures is one of the most intriguing aspects of biology. The complete instructions to execute the developmental program of an organism are encoded in its genes. This chapter discusses how control of the spatial and temporal expression of genes contributes to the development of a multicellular organism.

I. From Single Cell to Multicellular Organism

A. Embryonic development involves cell division, morphogenesis, and cell differentiation

A multicellular organism develops from a fertilized egg through three processes: cell division, cell differentiation, and morphogenesis (see Campbell, Figure 21.1).

- Cell division increases cell number.
- During cell *differentiation*, the cells become specialized in structure and function.
- Through a host of processes, collectively referred to as *morphogenesis*, the overall shape of the organism is established.

During development, these three processes overlap in time.

- Initial aspects of morphogenesis during early development establish the basic body plan (e.g., which end of an animal will give rise to the head).
- Cell division and differentiation as well as selective cell death are important components of morphogenesis.

Animals and plants differ in their developmental programs (see Campbell, Figure 21.2).

- In animals, movement of cells and tissues are involved in the development of physical form.
- Growth in plants is not limited to embryonic and juvenile periods as it is in animals. The root and shoot tips of plants possess perpetual embryonic tissues, known as *apical meristems*, that are responsible for the continuous growth of new organs.

B. Researchers study development in model organisms to identify general principles: *science as a process*

Researchers use *model organisms* to facilitate to discovery of fundamental developmental processes. Model organisms are chosen because they possess features that make the study easier to conduct; the criteria used to select an organism include the following:

- Large eggs (easy to manipulate and observe)
- Readily observable embryos
- Short generation times
- Small genomes
- Preexisting knowledge of organism's genes and life history

Frogs were used widely as models in early studies of development, but they actually have relatively complex genetics. As a result, most current research is conducted on the following organisms because of their unique characteristics (see Campbell, Figure 21.3):

- The fruit fly, *Drosophila melanogaster:* easily grown in the lab, short generation time, embryos grow outside of the mother's body
- The nematode, *Caenorhabditis elegans:* easily grown in lab, transparent body composed of only a few cell types that always arise in the same way, short generation time, hermaphroditic

  Researchers have been able to construct the complete *cell lineage* of *C. elegans*, or the ancestry of every cell in the adult body (see Campbell, Figure 21.4).
- The zebrafish, *Danio rerio*: small and easy to breed in the lab, transparent embryo, rapid embryonic development, smaller genome size than that of mice
- The mouse, *Mus musculus*: long used as a vertebrate model, much is known about its genes; gene manipulations and gene "knock out" technologies are available; however, complex genetics and large genome
- The plant, *Arabidopsis thaliana:* easily grown in culture, small genome, cells take up foreign DNA

II. Differential Gene Expression

Differences among the cells of a multicellular organism arise from different patterns of gene expression and not from differences in the genomes of the cells.

A. Different types of cells in an organism have the same DNA

Nearly all of the cells of an organism have the same genes (genomic equivalence). What happens to these genes as the cells differentiate?

1. Totipotency in plants

Genomic equivalence among the cells of plants was demonstrated by experiments in which entire individuals developed from differentiated somatic cells (see Campbell, Figure 21.5).

- The observation that somatic cells can dedifferentiate and then give rise to all of the various cells of a new individual demonstrates that differentiation does involve irreversible changes to the genome.
- Cells that retain the ability of the zygote to give rise to all the specialized cells of a mature organism are called *totipotent*.

2. Nuclear transplantation in animals

Because the cells of animals will not often divide in culture, scientists have adopted alternative approaches to examine genomic equivalence in animals (see Campbell, Figure 21.6).

- By transplanting the nuclei of differentiated cells into enucleated egg cells of frogs, Briggs and King determined that the genome within the transplanted nuclei could support development; however, normal development was inversely related to the age of donor embryos.

Such transplantation studies lead to the following conclusions:

- Nuclei do change in some ways during differentiation.
- Changes do not occur to the sequence of DNA, but rather, in chromatin structure.

  The age-related relationship in developmental potential of frog nuclei is related to development-related changes in chromatin structure.

Mammals have been successfully cloned form nuclei and cells of early embryos.

- An adult sheep, "Dolly," was cloned by Ian Wilmut and colleagues in Scotland by transplanting the nucleus of a dedifferentiated mammary cell from one sheep into an unfertilized, enucleated egg of another sheep.

B. Different cell types make different proteins, usually as a result of transcription regulation

As cells differentiate, they become obviously different in structure and function.

- The earliest changes are subtle, manifested only at the molecular level; such changes, known as *determination*, irreversibly commit the cell to its final fate.
- The result of determination is the presence of tissue-specific proteins (e.g., crystallins of the vertebrate lens, muscle-specific forms of actin and myosin) characteristic of a cell's structure and function.

The complement of proteins that a cell makes results from the pattern of gene expression in the differentiating cell; a pattern that is, for the most part, regulated at the level of transcription.

Transcription regulation of gene expression during development is exemplified in muscle cell determination (see Campbell, Figure 21.8).

Researchers tested the hypothesis that certain muscle-specific regulatory genes were active in myoblasts in the following way:

- By using reverse transcriptase, a *cDNA* library of genes was generated from RNA isolated from cultured myoblasts. (These cDNAs were intron-lacking versions of the genes that normally occur in myoblasts.)
- The cDNAs were ligated into bioengineered plasmids that contained a promoter that would turn on any kind of gene.
- The plasmids were then inserted into embryonic precursor cells to determine if differentiation into myoblasts and muscle cells would occur.

Researchers determined that the molecular basis of muscle cell determination is the transcription (and translation) of critical muscle-determination genes (a type of "master regulatory gene"). One of these muscle-determination genes is called *myoD*.

- The protein product of *myoD*, called MyoD, is a transcription factor that binds to control elements of DNA, and in turn, enhances the expression of other muscle-specific transcription factors.
- The secondary transcription factors activate genes encoding muscle-protein.

C. Transcriptional regulation is directed by maternal molecules in the cytoplasm and signals from other cells

Explaining the molecular basis of determination of a single cell type, such as the role of *myoD* in muscle cell differentiation, is only part of how a multicellular organism arises.

Lingering questions about how such master regulatory genes themselves are turned on remain. The answers to such questions rest again on understanding control of differential gene expression during early development.

Two sources of information instruct a cell on which genes to express at a given time:

- Information in the cytoplasm of the unfertilized egg, in the form of RNA and protein, that is of maternal origin (*cytoplasmic determinants*) (see Campbell, Figure 21.9).
- Chemical signals produced by neighboring embryonic cells; such signals, through a process known as *induction*, influence the growth and differentiation of adjacent cells.

III. Genetic and Cellular Mechanisms of Pattern Formation

Cytoplasmic determinants and inductive signals contribute to morphogenesis by modeling *pattern formation*, the spatial organization of tissues and organs characteristic of a mature organism. In plants, pattern formation occurs continuously; in animals, pattern formation is usually restricted to embryos and juveniles.

A. Genetic analysis of *Drosophila* reveals how genes control development: *an overview*

By studying *Drosophila*, researchers have identified how specific molecules influence position and direct differentiation.

1. The life cycle of *Drosophila*

Fruit flies and other arthropods are segmented into three major body parts: head, thorax, and abdomen.

The cytoplasmic determinants provide positional information.

- In unfertilized eggs, the placement of the anterior-posterior and dorsal-ventral axes is determined
- After fertilization, orientation of body segments and development of associated structures is initiated.

The developmental stages of *Drosophila* are shown in Campbell, Figure 21.10. Note that by division 13, the basic body plan, including body axes and segmentation, has been determined.

2. Genetic analysis of early development in *Drosophila*: *science as a process*

By using mutants, E.B. Lewis in the 1940s demonstrated that genes somehow direct development.

In the 1970s, Nusslein-Volhard and Wieschaus (who were awarded a Nobel prize in 1995), studied pattern formation, specifically, the basis of segmentation at the molecular level.

- Their research was fraught with many challenges (see Campbell Methods box on *Drosophila* development):

  Segmentation may be influenced a large number of genes (out of a possible 12,000).

  Mutations affecting segmentation would be lethal to embryos (*embryonic lethals*).

  Because maternally-derived cytoplasmic determinants affected segmentation, the scope of their analysis would have to include maternal genes as well as embryonic genes.
- Eventually, they identified some 1200 genes that were essential for development, of which 120 played a role in segmentation.
- Various cytoplasmic determinants were found to control the expression of segmentation genes.

B. Gradients of maternal molecules in the early embryo control axis formation

Cytoplasmic determinants are encoded by maternal genes called *maternal effect genes* (or sometimes *egg-polarity genes* because of the effects of their products on orientation/polarity).

- One set of genes helps establish the anterior-posterior axis of the embryo.
- A second set of genes is involved with the dorsal-ventral axis of the embryo.

The means by which maternal effect genes influence pattern formation is exemplified by the *bicoid* gene.

- A mother missing the *bicoid* gene gives rise to an embryo that lacks the front half of its body.

  The phenotype of the offspring suggests that the *bicoid* gene is essential for development of the anterior end of the fly, possibly because the gene product, a cytoplasmic determinant, is required at the anterior end.

  The requirement for the appropriate distribution of cytoplasmic determinants is a special version of the gradient hypothesis developed over 100 years ago. It maintained that gradients of substances, or *morphogens*, were required to establish the axes of the embryo.

Recent research indicates that the *bicoid* product is a morphogen that affects head-end development.

- *Bicoid* mRNA is concentrated at the anterior end of unfertilized eggs produced by wild-type mothers. After fertilization, the mRNA is translated and forms a gradient of *bicoid* protein within the embryo.
- Injection of *bicoid* mRNA into early embryos results in the development of anterior structures at the injection sites.

The factors involved with posterior end development, as well as with the development of anterior and posterior surfaces, also have been identified.

C. A cascade of gene activations sets up the segmentation pattern of *Drosophila*: a closer look

The *bicoid* protein and other morphogens are transcription factors that regulate the transcription of selected genes of the embryo.

- The gradients of the morphogens are responsible for the pattern of regional differences in the expression of *segmentation genes* (the genes that control segmentation following the establishment of the major body axes).

The sequential activation of three sets of segmentation genes are responsible for refinement of the body plan; in order of activation, the gene sets are as follows (se Campbell, Figure 21.12):

- Products of *map genes* influence basic subdivision along the anterior-posterior axis.
- *Pair-rule* genes control the pairing of segments.
- *Segment-polarity genes* serve to direct anterior-posterior orientation within each segment.

The products of the segmentation genes operate in numerous ways:

- Many are transcription factors that enhance the expression of the segmentation gene next in the sequence.
- Others are components of signaling pathways, including signal molecules used in the cell-cell communication and the membrane receptors that recognize them.

D. Homeotic genes direct the identity of body parts

Continued morphogenesis, including the appropriate placement of appendages, requires identification of specific regions of the body. The identity of segments is conveyed through master regulatory genes called *homeotic genes*.

- Homeotic genes encode for transcription factors that influence the genes responsible for specific structures.

  For example, homeotic proteins produced in cells of a particular thoracic segment lead to leg development.

  Homeotic mutations replace structures characteristic of one part of an animal with structures normally found at some other location (see Campbell, Figure 21.13).
- Scientists are in the process of identifying the genes activated by homeotic genes.

E. Homeobox genes have been highly conserved in evolution

The homeotic genes of Drosophila all contain a 180-nucleotide sequence called the *homeobox*. (For this reason, all genes that contain the homeobox are referred to as *Hox* genes.)

Sequences identical or very similar to the homeobox of *Drosophila* have been discovered in other invertebrates and vertebrates, as well as yeast and prokaryotes.

- Such sequence similarity suggests that the homeobox sequence emerged early during the evolution of life.
- Animal genes homologous to the homeotic genes of fruit flies have even kept their chromosomal arrangement (see Campbell, Figure 21.14)

Not all homeobox genes serve as homeotic genes, however, most homeobox genes are associated with some aspect of development. For example, in Drosophila, homeoboxes are present in homeotic genes, the bicoid gene, several of the segmentation genes, and in the master regulatory gene for eye development.

What is the role of the protein segment encoded by the homeobox sequence?

- The homeobox encodes for a 60-amino-acid-long homeodomain. Proteins containing homeodomains serve as transcription factors.
- The homeodomain influences protein-protein interactions critical to transcriptional regulation.

F. Neighboring cells instruct other cells to form particular structures: cell signaling and induction in the nematode

Communication between and among cells of the embryo is critical to the development of the organism. The signaling process helps to coordinate the appropriate spatial and temporal expression of genes.

1. Induction in vulval development

Research on the development of the opening (*vulva*) through which nematodes lay their eggs has provided much insight into cell signaling and induction of development. By studying mutants, scientists have identified a number of genes involved in vulval development (see Campbell, Figure 21.15).

The anchor cell releases an inducer that binds to vulval precursor cells. (This inducer is a growth factor that binds to a tyrosine kinase receptor; see Campbell, Figure 19.13a.)

- Initially, all precursor cells are the same. The cell that gives rise to the inner part of the vulva receives a higher concentration of inducer.
- The high concentration of inducer stimulates:

  Division and differentiation that lead to inner vulva formation

  The production of a second inducer
- The second inducer binds to the other precursor cells, stimulating them to form the outer vulva.

Vulva development illustrates several important developmental concepts not only in nematodes, but in animals generally.

- Sequential inductions control organ formation.
- The effect of an inducer can depend on its concentration.
- Inducers operate through signal systems similar to those in adult organisms.
- Induction results in the selective activation or inactivation of specific genes within the target cell.
- Genetics is useful to our understanding of the mechanisms that underlie development.

2. Programmed cell death (apoptosis)

The study of *C. elegans* also has revealed that normal pattern formation depends on selective, programmed cell death (*apoptosis*).

- Selective cell death occurs 131 times during normal development.
- Chemical signals initiate the activation of a cascade "suicide genes."

  Two key suicide genes are *ced-3* and *ced-4*; the protein products of these genes are continuously present in the cell in an inactive form.

  Control of apoptosis, then, depends not on transcription or translation, but on regulating protein activity (see Campbell, Figure 21.16)
- The cell is killed when enzymes are activated to hydrolyze DNA and protein.

Certain degenerative diseases and cancers may have their basis in faulty apoptotic mechanisms.

G. Plant development depends on cell signaling and transcriptional regulation.

Because the last common ancestor of plants and animals was a single-celled organism living millions of years ago, the developmental processes in these two phyla most likely evolved independently.

Despite the differences between plants and animals, some of the basic molecular, cellular, and genetic mechanisms of development are similar.

Clues to the details associated with plant development come from DNA technology, insights from animal research, and the study of the model plant *Arabidopsis*.

1. Cell signaling in flower development

Environmental cues (e.g., day length) initiate processes that convert ordinary shoot meristems to floral meristems. Such induction is exemplified with the development of tomato flowers (see Campbell, Figure 21.17).

- Tomato plants homozygous for a mutant allele, called fasciated (*f*), produce flowers with an abnormally large number of organs.
- Stems from mutant plants grafted onto wild-type plants resulted in new plants that were *chimeras*, organisms with a mix of genetically different cells.
- Some of the chimeras possessed floral meristems in which the three cell layers did not all arise from the same "parent."
- By tracing the sources of the meristem layers, it was determined that the number of organs per flower depended on genes in the L3 (innermost) cell layer.

2. Organ-identity genes in plants

Organ-identity genes determine the type of structure (e.g., petal) that will grow from a meristem. Most of the information on organ-identity genes comes from studies of *Arabidopsis*.

- Organ-identity genes are analogous to homeotic genes.
- Organ-identity genes are divided into three classes: A, B, and C.

- The simple model in Campbell, Figure 21.16 shows how three kinds of genes direct the formation of four type of organs.
- Organ-identity genes appear to be acting like master regulatory genes that control the transcription of other genes directly involved in plant morphogenesis.

    The organ-identity genes of plants do not contain the homeobox sequence

    A different sequence of about the same length is present; this sequence is also present in some transcription-factor genes of yeast and animals

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CHAPTER 22
DESCENT WITH MODIFICATION: A DARWINIAN VIEW OF LIFE

OUTLINE

I. Historical Context for Evolutionary Theory
   A. Western culture resisted evolutionary views of life
   B. Theories of geological gradualism helped clear the path for evolutionary biologists
   C. Lamarck placed fossils in an evolutionary context

II. The Darwinian Revolution
   A. Field research helped Darwin frame his view of life: *science as a process*
   B. *The Origin of Species* developed two main points: the occurrence of evolution and natural selection as its mechanism

III. Evidence of Evolution
   A. Evidence of evolution pervades biology
   B. What is theoretical about the Darwinian view of life?

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. State the two major points Darwin made in *The Origin of Species* concerning the Earth's biota.
2. Compare and contrast Plato's philosophy of idealism and Aristotle's *scala naturae*.
3. Describe Carolus Linnaeus' contribution to Darwin's theory of evolution.
4. Describe Georges Cuvier's contribution to paleontology.
5. Explain how Cuvier and his followers used the concept of catastrophism to oppose evolution.
6. Explain how the principle of gradualism and Charles Lyell's theory of uniformitarianism influenced Darwin's ideas about evolution.
7. Describe Jean Baptiste Lamarck's model for how adaptations evolve.
8. Describe how Charles Darwin used his observations from the voyage of the HMS *Beagle* to formulate and support his theory of evolution.
9. Describe how Alfred Russel Wallace influenced Charles Darwin.
10. Explain what Darwin meant by the principle of common descent and "descent with modification".
11. Explain what evidence convinced Darwin that species change over time.
12. State, in their own words, three inferences Darwin made from his observations, which led him to propose natural selection as mechanism for evolutionary change.
13. Explain why variation was so important to Darwin's theory.
14. Explain how Reverend Thomas Malthus' essay influenced Charles Darwin.
15. Distinguish between artificial selection and natural selection.
16. Explain why the population is the smallest unit that can evolve.

17. Using some contemporary examples, explain how natural selection results in evolutionary change.
18. Explain why the emergence of population genetics was an important turning point for evolutionary theory.
19. Describe the lines of evidence Charles Darwin used to support the principle of common descent.
20. Describe how molecular biology can be used to study the evolutionary relationships among organisms.
21. Explain the problem with the statement that Darwinism is "just a theory".
22. Distinguish between the scientific and colloquial use of the word "theory".

KEY TERMS

evolution	fossils	descent with modification	vestigial organs
natural selection	sedimentary rocks	artificial selection	ontogeny
evolutionary adaptations	paleontology	biogeography	phylogeny
natural theology	gradualism	homology	
taxonomy	uniformitarianism	homologous structures	

LECTURE NOTES

Evolution, the unifying theme woven throughout the text and course, refers to the processes that have transformed life on earth from its earliest forms to the enormous diversity that characterizes it today.

The first convincing case for evolution was published in a book by Charles Darwin on November 24, 1859. In this book, *On the Origin of Species by Means of Natural Selection*, Darwin:

- Synthesized seemingly unrelated facts into a conceptual framework that accounts for both the unity and diversity of life.
- Discussed important biological issues about organisms, such as why there are so many kinds of organisms, their origins and relationships, similarities and differences, geographic distribution, and adaptations to their environment.
- Made two major points:
    1. Species evolved from ancestral species and were not specially created.
    2. *Natural selection* is a mechanism that could result in this evolutionary change.

I. Historical Context for Evolutionary Theory

A. Western culture resisted evolutionary views of life

The impact of Darwin's ideas partially depended upon historical and social context (see Campbell, Figure 22.1).

- Darwin's view of life contrasted sharply with the accepted viewpoint: the Earth was only a few thousand years old and was populated by unchanging life forms made by the Creator during a single week.
- Thus, *On the Origin of Species by Means of Natural Selection* not only challenged prevailing scientific views, but also challenged the roots of Western culture.

1. The scale of nature and natural theology

Many Greek philosophers believed in the gradual evolution of life. However, the two that influenced Western culture most, Plato (427 – 347 B.C.) and his student Aristotle (384 – 322 B.C.), held opinions which were inconsistent with a concept of evolution.

- Plato, whose philosophy is known as *idealism* (*essentialism*), believed that there were two coexisting worlds: an ideal, eternal, real world and an illusionary imperfect world that humans perceive with their senses. To Plato,

  Variations in plant and animal populations were merely imperfect representatives of ideal forms; only the perfect ideal forms were real.

  Evolution would be counterproductive in a world where ideal organisms were already perfectly adapted to their environments.

- Aristotle questioned the Platonic philosophy of dual worlds, but his beliefs also excluded evolution.

  Recognizing that organisms vary from simple to complex, he believed that they could be placed on a scale of increasing complexity (*scala naturae*); on this ladder of life, each form had its allotted rung and each rung was occupied.

  In this view of life, species were fixed and did not evolve.

  The *scala naturae* view of life prevailed for over 2000 years.

The *creationist–essentialist* dogma that species were individually created and fixed became embedded in Western thought as the Old Testament account of creation from the Judeo–Christian culture fortified prejudice against evolution.

- *Natural Theology*, a philosophy that the Creator's plan could be revealed by studying nature, dominated European and American biology even as Darwinism emerged.
- For natural theologians, adaptations of organisms were evidence that the Creator had designed every species for a particular purpose.
- Natural theology's major objective was to classify species revealing God's created steps on the ladder of life.

Carolus Linnaeus (1707 – 1778), a Swedish physician and botanist, sought order in the diversity of life *ad majorem Dei gloriam* (for the greater glory of God).

- Known as the father of *taxonomy*—the naming and classifying of organisms—he developed the system of *binomial nomenclature* still used today.
- He adopted a system for grouping species into categories and ranking the categories into a hierarchy. For example, similar species are grouped into a genus; similar genera are grouped into the same order.

Linnaeus found order in the diversity of life with his hierarchy of taxonomic categories.

- The clustering of species in taxonomic groups did not imply evolutionary relationships to Linnaeus, since he believed that species were permanent creations.
- Linnaeus, a natural theologian, developed his classification scheme only to reveal God's plan and even stated *Deus creavit, Linnaeus disposuit* ("God creates, Linnaeus arranges").

2. Cuvier, fossils, and catastrophism

Fossils = Relics or impressions of organisms from the past preserved in rock

- Most fossils are found in *sedimentary rocks*, which:

  Form when new layers of sand and mud settle to the bottom of seas, lakes, and marshes, covering and compressing older layers into rock (e.g. sandstone and shale)

  May be deposited in many layers (*strata*) in places where shorelines repeatedly advance and retreat. Later erosion can wear away the upper (younger) strata, revealing older strata which had been buried.
- The fossil record thus provides evidence that Earth has had a succession of flora and fauna (see Campbell, Figure 22.2).

The study of fossils, *paleontology*, was founded by the French anatomist Georges Cuvier (1769-1832) who:

- Realized life's history was recorded in fossil-containing strata and documented the succession of fossil species in the Paris Basin
- Noted each stratum was characterized by a unique set of fossil species and that the older (deeper) the stratum, the more dissimilar the flora and fauna from modern life forms
- Understood that extinction had been a common occurrence in the history of life since, from stratum to stratum, new species appeared and others disappeared

Even with paleontological evidence, Cuvier was an effective opponent to the evolutionists of his day.

- He reconciled the fossil evidence with his belief in the fixity of species by speculating that boundaries between fossil strata corresponded in time to catastrophic events, such as floods or droughts.
- This view of Earth's history is known as *catastrophism*.

Catastrophism = Theory that major changes in the Earth's crust are the result of catastrophic events rather than from gradual processes of change

Cuvier explained the appearance of new species in younger rock that were absent from older rock by proposing that:

- Periodic localized catastrophes resulted in mass extinctions.
- After the local flora and fauna had become extinct, the region would be repopulated by foreign species immigrating from other areas.

B. Theories of geological gradualism helped clear the path for evolutionary biologists

In the late 18th century, a new theory of geological *gradualism* gained popularity among geologists that would greatly influence Darwin.

Gradualism = Principle that profound change is the cumulative product of slow, continuous processes

- Competed with Cuvier's theory of catastrophism
- Proposed by James Hutton (1975), a Scottish geologist. He proposed that it was possible to explain the various land forms by looking at mechanisms currently operating in the world.

  Example: Canyons form by erosion from rivers, and fossil-bearing sedimentary rocks form from particles eroded from the land and carried by rivers to the sea.

Charles Lyell, a leading geologist of Darwin's time, expanded Hutton's gradualism into the theory known as uniformitarianism.

Uniformitarianism = Theory that geological processes are uniform and have operated from the origin of the Earth to the present

- It was Lyell's extreme idea that geological processes are so uniform that their rates and effects must balance out through time.
- Example: Processes that build mountains are eventually balanced by the erosion of mountains.

Darwin rejected uniformitarianism, but was greatly influenced by conclusions that followed directly from the observations of Hutton and Lyell:

- The Earth must be ancient. If geological change results from slow, gradual processes rather than sudden events, then the Earth must be much older than the 6000 years indicated by many theologians on the basis of biblical inference.
- Very slow and subtle processes persisting over a great length of time can cause substantial change.

C. Lamarck placed fossils in an evolutionary context

Several 18th century naturalists suggested that life had evolved along with Earth's changes. Only Jean Baptiste Lamarck (1744-1829) developed and published (1809) a comprehensive model which attempted to explain how life evolved.

Lamarck was in charge of the invertebrate collection at the Natural History Museum in Paris, which allowed him to:

- Compare modern species to fossil forms, and in the process, identify several lines of descent composed of a chronological series of older fossils to younger fossils to modern species.
- Envision many ladders of life which organisms could climb (as opposed to Aristotle's single ladder without movement).

    The bottom rungs were occupied by microscopic organisms which were continually generated spontaneously from nonliving material.

    At the tops of the ladders were the most complex plants and animals.

Lamarck believed that evolution was driven by an innate tendency toward increasing complexity, which he equated with perfection.

- As organisms attained perfection, they became better and better adapted to their environments.
- Thus, Lamarck believed that evolution responded to organisms' *sentiments interieurs* ("felt needs").

Lamarck proposed a mechanism by which specific adaptations evolve, which included two related principles:

1. *Use and disuse*. Those body organs used extensively to cope with the environment become larger and stronger while those not used deteriorate.
2. *Inheritance of acquired characteristics*. The modifications an organism acquired during its lifetime could be passed along to its offspring.

Although his mechanism of evolution was in error, Lamarck deserves credit for proposing that:

- Evolution is the best explanation for both the fossil record and the extant diversity of life.
- The Earth is ancient.

- Adaptation to the environment is a primary product of evolution.

II. The Darwinian Revolution

At the beginning of the 19th century, natural theology still dominated the European and American intellectual climate. In 1809, the same year Lamarck published his theory of evolution, Charles Darwin was born in Shrewsbury, England.

- Though interested in nature, Charles (at 16) was sent by his physician father to the University of Edinburgh to study medicine, which he found boring and distasteful.
- He left Edinburgh without a degree and enrolled at Christ College, Cambridge University to prepare for the clergy.

  Nearly all naturalists and other scientists were clergymen, and a majority held to the philosophy of natural theology.

  Charles studied under the Reverend John Henslow, a botany professor at Cambridge, and received his B.A. degree in 1831.

  Professor Henslow recommended him to Captain Robert FitzRoy who was preparing the survey ship HMS *Beagle* for an around the world voyage.

A. Field research helped Darwin frame his view of life: *science as a process*

1. The voyage of the Beagle

The HMS *Beagle*, with Darwin aboard, sailed from England in December 1831 (see Campbell Figure 22.3).

- The voyage's mission was to chart the poorly known South American coastline.
- While the ship's crew surveyed the coast, Darwin spent most of his time ashore collecting specimens of the exotic and diverse flora and fauna.

While the ship worked its way around the continent, Darwin observed the various adaptations of plants and animals that inhabited the diverse environments of South America: Brazilian jungles, grasslands of the Argentine pampas, desolate islands of Tierra del Fuego, and the Andes Mountains. Darwin noted the following:

- The South American flora and fauna from different regions were distinct from the flora and fauna of Europe.
- Temperate species were taxonomically closer to species living in tropical regions of South America than to temperate species of Europe.
- The South American fossils he found (while differing from modern species) were distinctly South American in their resemblance to the living plants and animals of that continent.

Geographical distribution was particularly confusing in the case of the fauna of the Galapagos, recently formed volcanic islands which lie on the equator about 900 km west of South America.

- Most animal species on the Galapagos are unique to those islands, but resemble species living on the South American mainland.
- Darwin collected 13 types of finches from the Galapagos, and although they were similar, they seemed to be different species.

  Some were unique to individual islands

  Others were found on two or more islands that were close together

By the time the *Beagle* left the Galapagos, Darwin had read Lyell's *Principles of Geology*, and was influenced by Lyell's ideas.

- Darwin had begun to doubt the church's position that the Earth was static and had been created only a few thousand years before.
- When Darwin acknowledged that the Earth was ancient and constantly changing, he had taken an important step toward recognizing that life on Earth had also evolved.

2. Darwin focuses on adaptation

Darwin was not sure whether the 13 types of finches he collected on the Galapagos were different species or varieties of the same species.

- After he returned to England in 1836, an ornithologist indicated that they were actually different species.
- He reassessed observations made during the voyage and in 1837 began the first notebook on the origin of species.

Darwin perceived the origin of new species and adaptation as closely related processes; new species could arise from an ancestral population by gradually accumulating adaptations to a different environment. For example,

- Two populations of a species could be isolated in different environments and diverge as each adapted to local conditions.
- Over many generations, the two populations could become dissimilar enough to be designated separate species.
- This is apparently what happened to the Galapagos finches; their different beaks are adaptations to specific foods available on their home islands. (See Campbell, Figure 22.4)

By the early 1840s, Darwin had formed his theory of natural selection as the mechanism of adaptive evolution, but delayed publishing it.

- Reclusive and in poor health, Darwin was well known as a naturalist from the specimens and letters he had sent to Britain from the voyage on the *Beagle*.
- He frequently corresponded and met with Lyell, Henslow, and other scientists.

In 1844, Darwin wrote a long essay on the origin of species and natural selection.

- He realized the importance and subversive nature of his work, but did not publish the information because he wished to gather more evidence in support of his theory.
- Evolutionary thinking was emerging at this time, and Lyell admonished Darwin to publish on the subject before someone else published it first.

In June 1858, Darwin received a letter from Alfred Wallace, who was working as a specimen collector in the East Indies.

- Accompanying the letter was a manuscript detailing Wallace's own theory of natural selection which was almost identical to Darwin's.
- The letter asked Darwin to evaluate the theory and forward the manuscript to Lyell if it was thought worthy of publication.
- Darwin did so, although he felt that his own originality would be "smashed."
- Lyell and a colleague presented Wallace's paper along with excerpts from Darwin's unpublished 1844 essay to the Linnaean Society of London on July 1, 1858.

Darwin finished *The Origin of Species* and published it the next year.

- Darwin is considered the main author of the idea since he developed and supported natural selection much more extensively than Wallace.
- Darwin's book and its proponents quickly convinced the majority of biologists that biodiversity is a product of evolution.
- Darwin succeeded where previous evolutionists had failed not only because science was moving away from natural theology, but because he convinced his readers with logic and evidence.

B. *The Origin of Species* developed two main points: the occurrence of evolution and natural selection as its mechanism

1. Descent with modification

Darwin used the phrase "descent with modification," not evolution, in the first edition of *The Origin of Species*.

- He perceived a unity in life with all organisms related through descent from some unknown ancestral population that lived in the remote past.
- Diverse modifications (*adaptations*) accumulated over millions of years, as descendants from this common ancestor moved into various habitats.

Darwin's metaphor for the history of life was a branching tree with multiple branching from a common trunk to the tips of living twigs, symbolic of the diversity of contemporary organisms.

- At each fork or branch point is an ancestral population common to all evolutionary lines of descent branching from that fork.
- Species that are very similar share a common ancestor at a recent branch point on the phylogenetic tree.
- Less closely related organisms share a more ancient common ancestor at an earlier branch point.
- Most branches of evolution are dead ends since about 99% of all species that ever lived are extinct.

To Darwin, Linnaeus' taxonomic scheme reflected the branching genealogy of the tree of life.

- It recognized that the diversity of organisms could be ordered into "groups subordinate to groups", with organisms at the different taxonomic levels related through descent from common ancestors.
- Classification alone does not confirm the principle of common descent, but when combined with other lines of evidence, the relationships are clear.
- For example, genetic analysis of species that are thought to be closely related on the basis of anatomical features and other criteria reveals a common hereditary background.

2. Natural selection and adaptation

Darwin's book focused on the role of natural selection in adaptation (see Campbell, Figure 22.5). Ernst Mayr of Harvard University dissected the logic of Darwin's theory into three inferences based on five observations:

- *Observation 1*: All species have such great potential fertility that their population size would increase exponentially if all individuals that are born reproduced successfully.
- *Observation 2*: Populations tend to remain stable in size, except for seasonal fluctuations.

- *Observation 3*: Environmental resources are limited.
  - ***Inference 1***: Production of more individuals than the environment can support leads to a struggle for existence among individuals of a population, with only a fraction of offspring surviving each generation.
- *Observation 4*: Individuals of a population vary extensively in their characteristics; no two individuals are exactly alike.
- *Observation 5*: Much of this variation is heritable.
  - ***Inference 2***: Survival in the struggle for existence is not random, but depends in part on the hereditary constitution of the surviving individuals. Those individuals whose inherited characteristics fit them best to their environment are likely to leave more offspring than less fit individuals.
  - ***Inference 3***: This unequal ability of individuals to survive and reproduce will lead to a gradual change in a population, with favorable characteristics accumulating over the generations.

Summarizing Darwin's ideas:

- Natural selection is this differential success in reproduction, and its product is adaptation of organisms to their environment.
- Natural selection occurs from the interaction between the environment and the inherent variability in a population.
- Variations in a population arise by chance, but natural selection is not a chance phenomenon, since environmental factors set definite criteria for reproductive success.

Darwin was already aware of the struggle for existence caused by overproduction, when he read an essay on human population written by the Reverend Thomas Malthus (1798).

- In this essay, Malthus held that much of human suffering was a consequence of human populations growing faster than the food supply.
- This capacity for overproduction is common to all species, and only a fraction of new individuals complete development and leave offspring of their own; the rest die or are unable to reproduce.

Variation and overproduction in populations make natural selection possible.

- On the average, the most fit individuals pass their genes on to more offspring than less fit individuals.
- This results from environmental editing, which favors some variations over others.

From his experiences with *artificial selection*, Darwin inferred that natural selection could cause substantial change in populations.

- Through the breeding of domesticated plants and animals, humans have modified species over many generations by selecting individuals with desired traits as breeding stock.
- The plants and animals we grow for food show little resemblance to their wild ancestors (see Campbell, Figure 22.6).
- Darwin reasoned that if such change could be achieved by artificial selection in a relatively short period of time, then natural selection should be capable of considerable modifications of species over hundreds of thousands of generations.

- Even if the advantages of some heritable traits over others are slight, they will accumulate in the population after many generations of natural selection eliminating less favorable variations.

Gradualism is fundamental to the Darwinian view of evolution. Darwin reasoned that:

- Life did not evolve suddenly by quantum leaps, but instead by a gradual accumulation of small changes.
- Natural selection operating in differing contexts over vast spans of time could account for the diversity of life.

Summarizing Darwin's view of evolution:

- The diverse forms of life have arisen by descent with modification from ancestral species.
- The mechanism of modification has been natural selection working gradually over long periods of time.

a. Some subtleties of natural selection

Populations are important in evolutionary theory, since a population is the smallest unit that can evolve.

Population = A group of interbreeding individuals belonging to a particular species and sharing a common geographic area

Natural selection is a consequence of interactions between individual organisms and their environment, but individuals do not evolve.

- Evolution can only be measured as change in relative proportions of variations in a population over several generations.
- Natural selection can only amplify or diminish heritable variations.
- Organisms can adapt to changes in their immediate environment and can be otherwise modified by life experiences, but these acquired characteristics cannot be inherited.
- Evolutionists must distinguish between adaptations an organism acquires during its lifetime and those inherited adaptations that evolve in a population over many generations as a result of natural selection.

Specifics of natural selection are situational.

- Environmental factors vary from area to area and from time to time.
- An adaptation under one set of conditions may be useless or detrimental in different circumstances.

b. Examples of natural selection in action

In an effort to test Darwin’s hypothesis that the beaks of Galapagos finches are evolutionary adaptations to different food sources, Peter and Rosemary Grant of Princeton University have been conducting a long-term study on medium ground finches (*Geospiza fortis*) on Daphne Major, a tiny Galapagos island. They have discovered that:

- Average beak depth (an inherited trait) oscillates with rainfall (see Campbell, Figure 22.7).

  In wet years, birds preferentially feed on small seeds, and average beak depth decreases.

  In dry years, small seeds are less plentiful, so survival depends on the finches being able to crack the less preferred larger seeds. Average beak depth increases during dry years.

- It can be inferred that the change in beak depth is an adaptive response to the relative availability of small seeds from year to year.

This study illustrates some important points about adaptive change:

- *Natural selection is situational.* What works in one environmental context may not work in another.
- *Beak evolution on Daphne Major does not result from inheritance of acquired characteristics*. The environment did not *create* beaks specialized for large or small seeds, but only acted on inherited variations already present in the population. The proportion of thicker-beaked finches increased during dry periods because, on average, thicker-beaked birds transmitted their genes to more offspring than did thinner-beaked birds.

Michael Singer and Camille Parmesan of the University of Texas, have documented rapid evolutionary adaptation in a butterfly population (Edith's checkerspot) living in a meadow near Carson City, Nevada.

- In only a decade, this butterfly population apparently adapted to changing vegetation by inherited changes in reproductive behavior.
- Females lay eggs preferentially on certain plants which provide food for the larvae after they hatch. In 1983, checkerspots laid about 80% of their eggs on a native plant, *Collinsia parviflora.*
- By 1993, the butterflies were laying about 70% of their eggs on *Plantago lanceolata,* an invading weed from surrounding cattle ranches.
- The researchers demonstrated that the switch in plant preference is genetic; daughters of butterflies that deposited eggs on *Plantago* inherited the taste for that plant, choosing it over *Collinsia* when they laid their eggs.

There are hundreds of examples of natural selection in laboratory populations of such organisms as *Drosophila*. Other examples of natural selection in action include:

- Antibiotic resistance in bacteria (see Campbell, Chapter 18)
- Body size of guppies exposed to different predators (see Campbell, Chapter 1)

III. Evidence of Evolution

A. Evidence of evolution pervades biology

Darwin used several lines of evidence to support his principle of common descent, an evolutionary change. Recent discoveries, including those from molecular biology, lend support to his evolutionary view of life.

1. Biogeography

It was biogeographical evidence that first suggested common descent to Darwin, because the biogeographical patterns he observed only made sense in the light of evolution.

Biogeography = The geographical distribution of species

Islands have many endemic species which are closely related to species on the nearest mainland or neighboring island. Some logical questions follow:

- Why are two islands with similar environments in different parts of the world not populated by closely related species, but rather by species more closely related to those from the nearest mainland even when that environment is quite different?
- Why are South American tropical animals more closely related to South American desert animals than to African tropical animals?
- Why does Australia have such a diversity of marsupial animals and very few placental animals even though the environment can easily support placentals?

2. The fossil record

Darwin was troubled by the absence of transitional fossils linking modern life to ancestral forms.

- Even though the fossil record is still incomplete, paleontologists continue to find important new fossils, and many key links are no longer missing.
- For example, fossilized whales link these aquatic mammals to their terrestrial predecessors (see Campbell, Figure 22.8).

Although still incomplete, the fossil record provides information that supports other types of evidence about the major branches of the phylogenetic tree. For example:

- Prokaryotes are placed as the ancestors of all life by evidence from cell biology, biochemistry, and molecular biology.
- Fossil evidence shows the chronological appearance of the vertebrates as being sequential with fishes first, followed by amphibians, reptiles and then birds and mammals. This sequence is also supported by many other types of evidence.

3. Comparative anatomy

Anatomical similarities among species grouped in the same taxonomic category are a reflection of their common descent.

- The skeletal components of mammalian forelimbs are a good example (see Campbell, Figure 22.9)

  Although the limbs are used for different functions, it is obvious that the same skeletal elements are present.

  It is logical that whether the forelimb is a foreleg, wing, flipper, or arm, the basic similarity is the consequence of descent from a common ancestor and that the limbs have been modified for different functions. They are homologous structures.

Homologous structures = Structures that are similar because of common ancestry

- Other evidence from comparative anatomy supports that evolution is a remodeling process in which ancestral structures that functioned in one capacity have become modified as they take on new functions.
- Some homologous structures are *vestigial organs*.

Vestigial organs = Rudimentary structures of marginal or no use to an organism

- Vestigial organs are remnants of structures that had important functions in ancestral forms but are no longer essential.
- Example: The remnants of pelvic and leg bones in snakes show descent from a walking ancestor, but have no function in the snake.

- Because it would be wasteful to continue providing blood, nutrients, and space to structures that no longer have a major function, vestigial organs serve evidence of evolution by natural selection.

4. Comparative embryology

Closely related organisms go through similar stages in their embryonic development.

- Vertebrate embryos (fishes, amphibians, reptiles, birds, mammals) go through an embryonic stage in which they possess gill slits on the sides of their throats (see Campbell, Figure 22.10).
- As development progresses, the gill slits develop into divergent structures characteristic of each vertebrate class.
- In fish, the gill slits form gills; in humans, they form the eustachian tubes that connect the middle ear with the throat.

Comparative embryology often establishes homology among structures, such as gill pouches, that become so altered in later development that their common origin is not apparent by comparing their fully developed forms.

In the late nineteenth century, embryologists developed the view that "ontogeny recapitulates phylogeny."

- This view held that the embryonic development of an individual organism (*ontogeny*) is a replay of the evolutionary history of the species (*phylogeny*).
- This is an extreme view; what does occur is a series of similar *embryonic* stages that exhibit the same characteristics, not a sequence of adult-like stages.
- Ontogeny can provide clues to phylogeny, but all stages of development may become modified over the course of evolution.

5. Molecular biology

An organism's hereditary background is reflected in its genes and their protein products.

- Siblings have greater similarity in their DNA and proteins than do two unrelated organisms of the same species.
- Likewise, two species considered to be closely related by other criteria should have a greater proportion of their DNA and proteins in common than more distantly related species.

Molecular taxonomists use a variety of modern techniques to measure the degree of similarity among DNA nucleotide sequences of different species.

- The closer two species are taxonomically, the higher the percentage of common DNA; this evidence supports common descent.
- Common descent is also supported by the fact that closely related species also have proteins of similar amino acid sequence (resulting from inherited genes).
- If two species have many genes and proteins with sequences of monomers that match closely, the sequences must have been copied from a common ancestor.

Molecular biology has also substantiated Darwin's idea that all forms of life are related to some extent through branching descent from the earliest organisms (see Campbell, Figure 22.11).

- Even taxonomically distant organisms (bacteria and mammals) have some proteins in common.
- For example, cytochrome *c* (a respiratory protein) is found in all aerobic species. Cytochrome *c* molecules of all species are very similar in structure and function, even though mutations have substituted amino acids in some areas of the protein during the course of evolution.
- Additional evidence for the unity of life is the common genetic code. This mechanism has been passed through all branches of evolution since its beginning in an early form of life.

B. What is theoretical about the Darwinian view of life?

Dismissing Darwinism as "just a theory" is flawed because:

- Darwin made *two* claims:
  1. Modern species evolved from ancestral forms.
  2. The mechanism for evolution is natural selection.
- The conclusion that species change or evolve is based on historical fact.

What, then, is theoretical about evolution?

- Theories are conceptual frameworks with great explanatory power used to interpret facts.
- That species can evolve is fact, but the *mechanism* Darwin proposed for that change—natural selection—is a theory. Darwin used this theory of natural selection to explain facts of evolution documented by fossils, biogeography, and other historical evidence.

In science, "theory" is very different from the colloquial use of the word, which comes closer to what scientists mean by a hypothesis, or educated guess.

- Unifying concepts do not become scientific theories, unless their predictions stand up to thorough and continuous testing by experiment and observation.
- Good scientists, however, do not allow theories to become dogma; many evolutionary biologists now question whether natural selection alone can account for evolutionary history observed in the fossil record.

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Mayr, E. *The Growth of Biological Thought: Diversity, Evolution and Inheritance*. Cambridge, Massachusetts: Harvard University Press, 1982. This is an excellent reference for historical perspective and could be a useful companion to the text for supplementing lecture material on many topics.

CHAPTER 23
THE EVOLUTION OF POPULATIONS

OUTLINE

I. Population Genetics
   - A. The modern evolutionary synthesis integrated Darwinian selection and Mendelian inheritance
   - B. The genetic structure of a population is defined by its allele and genotype frequencies
   - C. The Hardy-Weinberg theorem describes a nonevolving population

II. Causes of Microevolution
   - A. Microevolution is a generation–to–generation change in a population's allele or genotype frequencies
   - B. The five causes of microevolution are genetic drift, gene flow, mutation, nonrandom mating, and natural selection

III. Genetic Variation, the Substrate for Natural Selection
   - A. Genetic variation occurs within and between populations
   - B. Mutation and sexual recombination generate genetic variation
   - C. Diploidy and balanced polymorphism preserve variation

IV. Natural Selection as the Mechanisms of Adaptive Evolution
   - A. Evolutionary fitness is the relative contribution an individual makes to the gene pool of the next generation
   - B. The effect of selection on a varying characteristic can be stabilizing, directional, or diversifying
   - C. Sexual selection may lead to pronounced secondary differences between the sexes
   - D. Natural selection cannot fashion perfect organisms

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Explain what is meant by the "modern synthesis".
2. Explain how microevolutionary change can affect a gene pool.
3. In their own words, state the Hardy-Weinberg theorem.
4. Write the general Hardy-Weinberg equation and use it to calculate allele and genotype frequencies.
5. Explain the consequences of Hardy-Weinberg equilibrium.
6. Demonstrate, with a simple example, that a disequilibrium population requires only one generation of random mating to establish Hardy-Weinberg equilibrium.
7. Describe the usefulness of the Hardy-Weinberg model to population geneticists.

8. List the conditions a population must meet in order to maintain Hardy-Weinberg equilibrium.
9. Explain how genetic drift, gene flow, mutation, nonrandom mating and natural selection can cause microevolution.
10. Explain the role of population size in genetic drift.
11. Distinguish between the bottleneck effect and the founder effect.
12. Explain why mutation has little quantitative effect on a large population.
13. Describe how inbreeding and assortative mating affect a population's allele frequencies and genotype frequencies.
14. Explain, in their own words, what is meant by the statement that natural selection is the only agent of microevolution which is adaptive.
15. Describe the technique of electrophoresis and explain how it has been used to measure genetic variation within and between populations.
16. List some factors that can produce geographical variation among closely related populations.
17. Explain why even though mutation can be a source of genetic variability, it contributes a negligible amount to genetic variation in a population.
18. Give the cause of nearly all genetic variation in a population.
19. Explain how genetic variation may be preserved in a natural population.
20. In their own words, briefly describe the neutral theory of molecular evolution and explain how changes in gene frequency may be nonadaptive.
21. Explain what is meant by "selfish" DNA.
22. Explain the concept of relative fitness and its role in adaptive evolution.
23. Explain why the rate of decline for a deleterious allele depends upon whether the allele is dominant or recessive to the more successful allele.
24. Describe what selection acts on and what factors contribute to the overall fitness of a genotype.
25. Give examples of how an organism's phenotype may be influenced by the environment.
26. Distinguish among stabilizing selection, directional selection and diversifying selection.
27. Define sexual dimorphism and explain how it can influence evolutionary change.
28. Give at least four reasons why natural selection cannot breed perfect organisms.

KEY TERMS

population genetics
modern synthesis
population
species
gene pool
genetic structure
Hardy-Weinberg theorem, equilibrium, and equation
microevolution
bottleneck effect
founder effect
gene flow
mutation
inbreeding
assortative mating
natural selection
polymorphism
geographical variation
cline
balanced polymorphism
heterozygote advantage
hybrid vigor
frequency-dependent selection
neutral variation
Darwinian fitness
relative fitness
stabilizing selection
directional selection
diversifying selection
sexual dimorphism
sexual selection

LECTURE NOTES

Natural selection works on individuals, but it is the *population* that evolves (see Campbell Figure 23.1). Darwin understood this, but was unable to determine its genetic basis.

I. Population Genetics

A. The modern evolutionary synthesis integrated Darwinian selection and Mendelian inheritance

Shortly after the publication of *The Origin of Species*, most biologists were convinced that species evolved. Darwin was less successful in convincing them that natural selection was the mechanism for evolution, because little was known about inheritance.

- An understanding about inheritance was necessary to explain how:
  - Chance variations arise in populations
  - These variations are precisely transmitted from parents to offspring
- Though Gregor Mendel was a contemporary of Darwin's, Mendel's principles of inheritance went unnoticed until the early 1900s.

For Darwin, the raw material for natural selection was variation in quantitative characters that vary along a continuum in a population.

- We now know that continuous variation is usually determined by many segregating loci (polygenic inheritance).
- Mendel and geneticists in the early 1900s recognized only discrete characters inherited on an either-or basis. Thus, for them, there appeared to be no genetic basis for the subtle variations that were central to Darwin's theory.

In the 1930s, the science of *population genetics* emerged, which:

- Emphasized genetic variation within populations and recognized the importance of quantitative characters
- Was an important turning point for evolutionary theory, because it reconciled Mendelian genetics with Darwinian evolution

In the 1940s, the genetic basis of variation and natural selection was worked out, and the *modern synthesis* was formulated. This comprehensive theory:

- Integrated discoveries from different fields (e.g., paleontology, taxonomy, biogeography, and population genetics)
- Was collectively developed by many scientists including:
  - Theodosius Dobzhansky – geneticist
  - Ernst Mayr – biogeographer and systematist
  - George Gaylord Simpson – paleontologist
  - G. Ledyard Stebbins – botanist
- Emphasized the following:
  - Importance of populations as units of evolution
  - The central role of natural selection as the primary mechanism of evolutionary change
  - Gradualism as the explanation of how large changes can result from an accumulation of small changes occurring over long periods of time

Most of Darwin's ideas persisted in the modern synthesis, although many evolutionary biologists are challenging some generalizations of the modern synthesis.

- This debate focuses on the rate of evolution and on the relative importance of evolutionary mechanisms other than natural selection.
- These debates do not question the fact of evolution, only what mechanisms are most important in the process.
- Such disagreements indicate that the study of evolution is very lively and that it continues to develop as a science.

B. The genetic structure of a population is defined by its allele and genotype frequencies

Population = Localized group of organisms which belong to the same species

Species =A group of populations whose individuals have the potential to interbreed and produce fertile offspring in nature

Most species are not evenly distributed over a geographical range, but are concentrated in several localized population centers.

- Each population center is isolated to some extent from other population centers with only occasional gene flow among these groups.
- Obvious examples are isolated populations found on widely separated islands or in unconnected lakes.
- Some populations are not separated by such sharp boundaries.

  For example, a species with two population centers may be connected by an intermediate sparsely populated range.

  Even though these two populations are not absolutely isolated, individuals are more likely to interbreed with others from their population center (see Campbell, Figure 23.2). Gene flow between the two population centers is thus reduced by the intermediate range.

Gene pool = The total aggregate of genes in a population at any one time

- Consists of all the alleles at all gene loci in all individuals of a population. Alleles from this pool will be combined to produce the next generation.
- In a diploid species, an individual may be homozygous or heterozygous for a locus since each locus is represented twice.
- An allele is said to be *fixed* in the gene pool if all members of the population are homozygous for that allele.
- Normally there will be two or more alleles for a gene, each having a relative frequency in the gene pool.

C. The Hardy-Weinberg theorem describes a nonevolving population

> The Hardy- Weinberg model is much easier to teach if the students calculate gene frequencies along with the instructor. This means that you must pause frequently to allow plenty of time for students to actively process the information and practice the calculations.

In the absence of other factors, the segregation and recombination of alleles during meiosis and fertilization will not alter the overall genetic makeup of a population.

- The frequencies of alleles in the gene pool will remain constant unless acted upon by other agents; this is known as the *Hardy-Weinberg theorem*.
- The Hardy-Weinberg model describes the genetic structure of nonevolving populations. This theorem can be tested with theoretical population models.

To test the Hardy-Weinberg theorem, imagine an isolated population of wildflowers with the following characteristics (see Campbell, Figure 23.3):

- It is a diploid species with both pink and white flowers.
- The population size is 500 plants: 480 plants have pink flowers, 20 plants have white flowers.
- Pink flower color is coded for by the dominant allele "A," white flower color is coded for by the recessive allele "a."
- Of the 480 pink-flowered plants, 320 are homozygous (AA) and 160 are heterozygous (Aa). Since white color is recessive, all white flowered plants are homozygous aa.

- There are 1000 genes for flower color in this population, since each of the 500 individuals has two genes (this is a diploid species).
- A total of 320 genes are present in the 160 heterozygotes (Aa): half are dominant (160 A) and half are recessive (160 a).
- 800 of the 1000 total genes are dominant.
- The frequency of the A allele is 80% or 0.8 (800/1000).

Genotypes	# of plants	# of A alleles per individual		Total # A alleles
AA plants	320	×	2	640
Aa plants	160	= ×	1	160
		=		800

- 200 of the 1000 total genes are recessive.
- The frequency of the a allele is 20% or 0.2 (200/1000).

Genotypes	# of plants	# of A alleles per individual		Total # A alleles
aa plants	20	×	2	40
Aa plants	160	= ×	1	160
		=		200

Assuming that mating in the population is completely random (all male-female mating combinations have equal chances), the frequencies of A and a will remain the same in the next generation.

- Each gamete will carry one gene for flower color, either A or a.
- Since mating is random, there is an 80% chance that any particular gamete will carry the A allele and a 20% chance that any particular gamete will carry the a allele.

The frequencies of the three possible genotypes of the next generation can be calculated using the rule of multiplication (see Campbell, Chapter 14):

- The probability of two A alleles joining is $0.8 \times 0.8 = 0.64$; thus, 64% of the next generation will be AA.
- The probability of two a alleles joining is $0.2 \times 0.2 = 0.04$; thus, 4% of the next generation will be aa.
- Heterozygotes can be produced in two ways, depending upon whether the sperm or ovum contains the dominant allele (Aa or aA). The probability of a heterozygote being produced is thus $(0.8 \times 0.2) + (0.2 \times 0.8) = 0.16 + 0.16 = 0.32$.

The frequencies of possible genotypes in the next generation are 64% AA, 32% Aa and 4% aa.

- The frequency of the A allele in the new generation is 0.64 + (0.32/2) = 0.8, and the frequency of the a allele is 0.04 + (0.32/2) = 0.2. Note that the alleles are present in the gene pool of the new population at the *same* frequencies they were in the original gene pool.
- Continued sexual reproduction with segregation, recombination and random mating would *not alter* the frequencies of these two alleles: the gene pool of this population would be in a state of equilibrium referred to as *Hardy-Weinberg equilibrium.*
- If our original population had not been in equilibrium, only one generation would have been necessary for equilibrium to become established.

From this theoretical wildflower population, a general formula, called the *Hardy-Weinberg equation,* can be derived to calculate allele and genotype frequencies.

- The Hardy-Weinberg equation can be used to consider loci with three or more alleles.
- By way of example, consider the simplest case with only two alleles with one dominant to the other.
- In our wildflower population, let p represent allele A and q represent allele a, thus p = 0.8 and q = 0.2.
- The sum of frequencies from all alleles must equal 100% of the genes for that locus in the population: p + q = 1.
- Where only two alleles exist, only the frequency of one must be known since the other can be derived:

$$1 - p = q \quad \text{or} \quad 1 - q = p$$

When gametes fuse to form a zygote, the probability of producing the AA genotype is p^2; the probability of producing aa is q^2; and the probability of producing an Aa heterozygote is 2pq (remember heterozygotes may be formed in two ways).

- The sum of these frequencies must equal 100%, thus:

$$\underset{\text{Frequency of AA}}{p^2} + \underset{\text{Frequency of Aa}}{2pq} + \underset{\text{Frequency of aa}}{q^2} = 1$$

The Hardy-Weinberg equation permits the calculation of allelic frequencies in a gene pool, if the genotype frequencies are known. Conversely, the genotype can be calculated from known allelic frequencies.

For example, the Hardy-Weinberg equation can be used to calculate the frequency of inherited diseases in humans (e.g., phenylketonuria):

- 1 of every 10,000 babies in the United States is born with phenylketonuria (PKU), a metabolic disorder that, if left untreated, can result in mental retardation.
- The allele for PKU is recessive, so babies with this disorder are homozygous recessive = q^2.
- Thus $q^2 = 0.0001$, with q = 0.01 (the square root of 0.0001).
- The frequency of p can be determined since p = 1 − q:

$$p = 1 - 0.01 = 0.99$$

- The frequency of carriers (heterozygotes) in the population is 2pq.

$$2pq = 2(0.99)(0.01) = 0.0198$$

- Thus, about 2% of the U.S. population are carriers for PKU.

II. Causes of Microevolution

A. Microevolution is a generation–to–generation change in a population's allele or genotype frequencies

The Hardy-Weinberg equilibrium is important to the study of evolution since it tells us what will happen in a *nonevolving* population.

- This equilibrium model provides a base line from which evolutionary departures take place.
- It provides a reference point with which to compare the frequencies of alleles and genotypes of natural populations whose gene pools may be changing.

If the frequencies of alleles or genotypes deviate from values expected from the Hardy-Weinberg equilibrium, then the population is evolving

- Therefore, a refined definition of evolution at the population level is a generation-to-generation change in a population's frequencies of alleles or genotypes.
- Because such change in a gene pool is evolving on the smallest scale, it is referred to as microevolution.

B. The five causes of microevolution are genetic drift, gene flow, mutation, nonrandom mating, and natural selection

For Hardy-Weinberg equilibrium to be maintained, five conditions *must* be met:

1. Very large population size
2. Isolation from other populations. There is no migration of individuals into or out of the population.
3. No net mutations
4. Random mating
5. No natural selection. All genotypes are equal in survival and reproductive success. Differential reproductive success can alter gene frequencies.

In real populations, several factors can upset Hardy-Weinberg equilibrium and cause *microevolutionary* change.

Microevolution = Small scale evolutionary change represented by a generational shift in a population's relative allelic frequencies.

- Microevolution can be caused by *genetic drift, gene flow, mutation, nonrandom mating,* and *natural selection*; each of these conditions is a deviation from the criteria for Hardy-Weinberg equilibrium.
- Of these five possible agents for microevolution, only natural selection generally leads to an accumulation of favorable adaptations in a population.
- The other four are nonadaptive and are usually called non-Darwinian changes.

1. Genetic drift

Genetic drift = Changes in the gene pool of a small population due to chance

- If a population is small, its existing gene pool may not be accurately represented in the next generation because of sampling error.
- Chance events may cause the frequencies of alleles to drift randomly from generation to generation, since the existing gene pool may not be accurately represented in the next generation.

For example, assume our theoretical wildflower population contains only 25 plants, and the genotypes for flower color occur in the following numbers: 16 AA, 8 Aa and 1 aa. In this case, a chance event could easily change the frequencies of the two alleles for flower color (see Campbell, Figure 23.4).

- A rock slide or passing herbivore which destroys three AA plants would immediately change the frequencies of the alleles from A = 0.8 and a = 0.2, to A = 0.77 and a = 0.23.
- Although this change does not seem very drastic, the frequencies of the two alleles were changed by a chance event.

The larger the population, the less important is the effect of genetic drift.

- Even though natural populations are not infinitely large (in which case genetic drift could be completely eliminated as a cause of microevolution), most are so large that the effect of genetic drift is negligible.
- However, some populations are small enough that genetic drift can play a major role in microevolution, especially when the population has less than 100 individuals.

Two situations which result in populations small enough for genetic drift to be important are the *bottleneck effect* and the *founder effect*.

a. The bottleneck effect

The size of a population may be reduced drastically by such natural disasters as volcanic eruptions, earthquakes, fires, floods, etc. which kill organisms nonselectively.

- The small surviving population is unlikely to represent the genetic makeup of the original population.
- Genetic drift which results from drastic reduction in population size is referred to as the *bottleneck effect*.
- By chance some individuals survive. In the small remaining population, some alleles may be overrepresented, some underrepresented, and some alleles may be totally absent (see Campbell, Figure 23.5).
- Genetic drift which has occurred may continue to affect the population for many generations, until it is large enough for random drift to be insignificant.

The bottleneck effect reduces overall genetic variability in a population since some alleles may be entirely absent.

- For example, a population of northern elephant seals was reduced to just 20 individuals by hunters in the 1890's.

  Since this time, these animals have been protected and the population has increased to about 30,000 animals.

  Researchers have found that *no* variation exists in the 24 loci examined from the present population. A single allele has been fixed at each of the 24 loci due to genetic drift by the bottleneck effect.

  This contrasts sharply with the large amount of genetic variation found in southern elephant seal populations which did not undergo the bottleneck effect.
- A lack of genetic variation in South African cheetahs may also have resulted from genetic drift, since the large population was severely reduced during the last ice age and again by hunting to near extinction.

b. The founder effect

When a few individuals colonize a new habitat, genetic drift is also likely to occur. Genetic drift in a new colony is called the *founder effect*.

- The smaller the founding population, the less likely its gene pool will be representative of the original population's genetic makeup.
- The most extreme example would be when a single seed or pregnant female moves into a new habitat.

- If the new colony survives, random drift will continue to affect allele frequencies until the population reaches a large enough size for its influence to be negligible.
- No doubt, the founder effect was instrumental in the evolutionary divergence of the Galapagos finches.

The founder effect probably resulted in the high frequency of *retinitis pigmentosa* (a progressive form of blindness that affects humans homozygous for this recessive allele) in the human population of Tristan da Cunha, a group of small Atlantic islands.

- This area was colonized by 15 people in 1814, and one must have been a carrier.
- The frequency of this allele is much higher on this island than in the populations from which the colonists came.

Although inherited diseases are obvious examples of the founder effect, this form of genetic drift can alter the frequencies of any alleles in the gene pool.

2. Gene flow

Gene flow = The migration of fertile individuals, or the transfer of gametes, between populations

- Natural populations may gain or lose alleles by gene flow, since they do not have gene pools which are closed systems required for Hardy-Weinberg equilibrium.
- Gene flow tends to reduce between-population differences which have accumulated by natural selection or genetic drift.
- An example of gene flow would be if our theoretical wildflower population was to begin receiving wind blown pollen from an all white-flower population in a neighboring field. This new pollen could greatly increase the frequency of the white flower allele, thus also altering the frequency of the red flower allele.
- Extensive gene flow can eventually group neighboring populations into a single population.

3. Mutations

A new mutation that is transmitted in gametes immediately changes the gene pool of a population by substituting one allele for another.

In our theoretical wildflower population, if a mutation in a white flowered plant caused that plant to begin producing gametes which carried a red flower allele, the frequency of the white flower allele is reduced and the frequency of the red flower allele is increased.

Mutation itself has little quantitative effect on large populations in a single generation, since mutation at any given locus is very rare.

- Mutation rates of one mutation per 10^5 to 10^6 gametes are typical, but vary depending on the species and locus.
- An allele with a 0.50 frequency in the gene pool that mutates to another allele at a rate of 10^{-5} mutations per generation would take 2000 generations to reduce the frequency of the original allele from 0.50 to 0.49.
- The gene pool is effected even less, since most mutations are reversible.
- If a new mutation increases in frequency, it is because individuals carrying this allele are producing a larger percentage of offspring in the population due to genetic drift or natural selection, not because mutation is producing the allele in abundance.

Mutation is important to evolution since it is the original source of genetic variation, which is the raw material for natural selection.

4. Nonrandom mating

Nonrandom mating increases the number of homozygous loci in a population, but does not in itself alter frequencies of alleles in a population's gene pool. There are two kinds of nonrandom mating: *inbreeding* and *assortative mating*.

a. Inbreeding

Individuals of a population usually mate with close neighbors rather than with more distant members of a population, especially if the members of the population do not disperse widely.

- This violates the Hardy-Weinberg criteria that an individual must choose its mate at random from the population.
- Since neighboring individuals of a large population tend to be closely related, inbreeding is promoted.
- Self-fertilization, which is common in plants, is the most extreme example of inbreeding.

Inbreeding results in relative genotypic frequencies (p^2, 2pq, q^2) that deviate from the frequencies predicted for Hardy-Weinberg equilibrium, but does not in itself alter frequencies of alleles (p and q) in the gene pool.

Self-fertilization in our theoretical wildflower population would increase the frequencies of homozygous individuals and reduce the frequency of heterozygotes.

- Selfing of AA and aa individuals would produce homozygous plants.
- Selfing of Aa plants would produce half homozygotes and half heterozygotes.
- Each new generation would see the proportion of heterozygotes decrease, while the proportions of homozygous dominant and homozygous recessive plants would increase.
- Inbreeding without selfing would also result in a reduction of heterozygotes, although it would take much longer.

One effect of inbreeding is that the frequency of homozygous recessive phenotypes increases.

An interesting thing to note is that even if the phenotypic and genotypic ratios change, the values of p and q do not change in these situations, only the way they are combined. A smaller proportion of recessive alleles are masked by the heterozygous state.

b. Assortative mating

Assortative mating is another type of nonrandom mating which results when individuals mate with partners that are like themselves in certain phenotypic characters.

Examples:

- Some toads (*Bufo*) most commonly mate with individuals of the same size (see Campbell, Figure 23.6).
- Snow geese occur in a blue variety and a white variety, with the blue color allele being dominant. Birds prefer to mate with those of their own color, blue with blue and white with white; this results in a lower frequency of heterozygotes than predicted by Hardy-Weinberg.
- Blister beetles (*Lytla magister*) in the Sonoran Desert usually mate with a same-size individual.

5. Natural selection

The Hardy-Weinberg equilibrium condition that all individuals in a population have equal ability to produce viable, fertile offspring is probably never met.

- In any sexually reproducing population, variation among individuals exists and some variants leave more offspring than others.
- *Natural selection* is this differential success in reproduction.

Due to selection, alleles are passed on to the next generation in disproportionate numbers relative to their frequencies in the present generation.

- If in our theoretical wildflower population, white flowers are more visible to herbivores than pink flowers, plants with pink flowers (both AA and Aa) would leave more offspring on the average.
- Genetic equilibrium would be disturbed and the frequency of allele A would increase and the frequency of the a allele would decrease.

Natural selection is the only agent of microevolution which is adaptive, since it accumulates and maintains favorable genotypes.

- Environmental change would result in selection favoring genotypes present in the population which can survive the new conditions.
- Variability in the population makes it possible for natural selection to occur.

III. Genetic Variation, the Substrate for Natural Selection

Members of a population may vary in subtle or obvious ways. It is the genetic basis of this variation that makes natural selection possible.

A. Genetic variation occurs within and between populations

Darwin considered the slight differences between individuals of a population as raw material for natural selection.

While we are more conscious of the variation among humans, an equal if not greater amount of variation exists among the many plant and animals species.

- Phenotypic variation is a product of inherited genotype and numerous environmental influences.
- Only the genetic or inheritable component of variation can have adaptive impact as a result of natural selection.

1. Variation within populations

Both quantitative and discrete characters contribute to variation within a population.

- Polygenic characters, which vary quantitatively within a population, are responsible for much of the inheritable variation.
  - For example, the height of the individuals in our theoretical wildflower population may vary from very short to very tall with all sorts of intermediate heights.
- Discrete characters, such as pink vs. white flowers, can be classified on an either-or basis, usually because they are determined by only one locus with different alleles that produce distinct phenotypes.

a. Polymorphism

In our wildflower population, the red and white flowers would be referred to as different *morphs* (contrasting forms of a Mendelian character).

A population is referred to as *polymorphic* for a character if two or more morphs are present in noticeable frequencies (see Campbell, Figure 23.7).

Polymorphism is found in human populations not only in physical characters (e.g., presence or absence of freckles) but also in biochemical characters (e.g., ABO blood group).

b. Measuring genetic variation

Darwin did not realize the extent of genetic variation in populations, since much of the genetic variation can only be determined with biochemical methods.

- Electrophoresis has been used to determine genetic variation among individuals of a population. This technique allows researchers to identify variations in protein products of specific gene loci.
- Electrophoretic studies show that in *Drosophila* populations the gene pool has two or more alleles for about 30% of the loci examined, and each fly is heterozygous at about 12% of its loci.
- Thus, there are about 700 – 1200 heterozygous loci in each fly. Any two flies in a *Drosophila* population will differ in genotype at about 25% of their loci.
- Electrophoretic studies also show comparable variation in the human population.

- Note that electrophoresis underestimates genetic variation:
  - Proteins produced by different alleles may vary in amino acid composition and still have the same overall charge, which makes them indistinguishable by electrophoresis.
  - Also, DNA variation not expressed as protein is not detected by electrophoresis.

2. Variation between populations

Geographical variation in allele frequencies exists among populations of most species.

- Natural selection can contribute to geographical variation, since at least some environmental factors are different between two locales. For example, one population of our wildflowers may have a higher frequency of white flowers because of the prevalence in that area of pollinators that prefer white flowers.
- Genetic drift may cause chance variations among different populations.
- Also, subpopulations may appear within a population due to localized inbreeding resulting from a "patchy" environment.

Cline = One type of geographical variation that is a graded change in some trait along a geographic transect

- Clines may result from a gradation in some environmental variable.
- It may be a graded region of overlap where individuals of neighboring populations interbreed.
- For example, the average body size of many North American mammal species gradually increases with increasing latitude. It is presumed that the reduced surface area to volume ratio associated with larger size helps animals in cold environments conserve body heat.
- Studies of geographical variation confirm that genetic variation affects spatial differences of phenotypes in some clines. For example, yarrow plants are shorter at higher elevations, and some of this phenotypic variation has a genetic basis (see Campbell, Figure 23.8)

B. Mutation and sexual recombination generate genetic variation

Two random processes, mutation and sexual recombination (see Chapter 15), create variation in the genetic composition of a population.

1. Mutation

Mutations produce new alleles. They are rare and random events which usually occur in somatic cells and are thus not inheritable.

- Only mutations that occur in cell lines which will produce gametes can be passed to the next generation.
- Geneticists estimate that only an average of one or two mutations occur in each human gamete-producing cell line.

Point mutation = Mutation affecting a single base in DNA

- Much of the DNA in eukaryotes does not code for proteins, and it is uncertain how a point mutation in these regions affect an organism.
- Point mutations in structural genes may cause little effect, partly due to the redundancy of the genetic code.

Mutations that alter a protein enough to affect the function are more often harmful than beneficial, since organisms are evolved products shaped by selection and a chance change is unlikely to improve the genome.

- Occasionally, a mutant allele is beneficial, which is more probable when environmental conditions are changing.
- The mutation which allowed house flies to be resistant to DDT was present in the population and under normal conditions resulted in reduced growth rate. It became beneficial to the house fly population only after a new environmental factor (DDT) was introduced and tipped the balance in favor of the mutant alleles.

Chromosomal mutations usually affect many gene loci and tend to disrupt an organism's development.

- On rare occasions, chromosomal rearrangement may be beneficial. These instances (usually by translocation) may produce a cluster of genes with cooperative functions when inherited together.

Duplication of chromosome segments is nearly always deleterious.

- If the repeated segment does not severely disrupt genetic balance, it may persist for several generations and provide an expanded genome with extra loci.
- These extra loci may take on new functions by mutation while the original genes continue to function.
- Shuffling of exons within the genome (single locus or between loci) may also produce new genes.

Mutation can produce adequate genetic variation in bacteria and other microorganisms which have short generation times.

- Some bacteria reproduce asexually by dividing every 20 minutes, and a single cell can produce a billion descendants in only 10 hours.
- With this type of reproduction, a beneficial mutation can increase in frequency in a bacterial population very rapidly.
- A bacterial cell with a mutant allele which makes it antibiotic resistant could produce an extremely large population of clones in a short period, while other cells without that allele are eliminated.
- Although bacteria reproduce primarily by asexual means, most increase genetic variation by occasionally exchanging and recombining genes through processes such as conjugation, transduction and transformation.

2. Sexual recombination

The contribution of mutations to genetic variation is negligible.

- Mutations are so infrequent at a single locus that they have little effect on genetic variation in a large gene pool.
- Although mutations produce new alleles, nearly all genetic variation in a population results from new combinations of alleles produced by sexual recombination.

Gametes from each individual vary extensively due to crossing over and random segregation during meiosis.

- Thus, each zygote produced by a mating pair possesses a unique genetic makeup.
- Sexual reproduction produces new combinations of old alleles each generation.

Plants and animals depend almost entirely on sexual recombination for genetic variation which makes adaptation possible.

C. Diploidy and balanced polymorphism preserve variation

Natural selection tends to produce genetic uniformity in a population by eliminating unfavorable genotypes. This tendency is opposed by several mechanisms that preserve or restore variation.

1. Diploidy

Diploidy hides much genetic variation from selection by the presence of recessive alleles in heterozygotes.

- Since recessive alleles are not expressed in heterozygotes, less favorable or harmful alleles may persist in a population.
- This variation is only exposed to selection when two heterozygotes mate and produce offspring homozygous for the recessive allele.
- If a recessive allele has a frequency of 0.01 and its dominant counterpart 0.99, then 99% of the recessive allele copies will be protected in heterozygotes. Only 1% of the recessive alleles will be present in homozygotes and exposed to selection.
- The more rare the recessive allele, the greater its protection by heterozygosity. That is, a greater proportion are hidden in heterozygotes by the dominant allele.
- This type of protection maintains a large pool of alleles which may be beneficial if conditions change.

2. Balanced polymorphism

Selection may also preserve variation at some gene loci.

Balanced polymorphism = The ability of natural selection to maintain diversity in a population

One mechanism by which selection preserves variation is *heterozygote advantage.*

- Natural selection will maintain two or more alleles at a locus if heterozygous individuals have a greater reproductive success than any type of homozygote.
- An example is the recessive allele that causes sickle-cell anemia in homozygotes. The locus involved codes for one chain of hemoglobin.
- Homozygotes for this recessive allele develop sickle-cell anemia which is often fatal.

- Heterozygotes are resistant to malaria. Heterozygotes thus have an advantage in tropical areas where malaria is prevalent, since homozygotes for the dominant allele are susceptible to malaria and homozygous recessive individuals are incapacitated by the sickle-cell condition.
- In some African tribes from areas where malaria is common, 20% of the hemoglobin loci in the gene pool is occupied by the recessive allele.

Other examples of heterozygote advantage are found in crop plants (e.g., corn) where inbred lines become homozygous at more loci and show stunted growth and sensitivity to diseases.

- Crossbreeding different inbred varieties often produces hybrids which are more vigorous than the parent stocks.
- This *hybrid vigor* is probably due to:
  1. Segregation of harmful recessives that were homozygous in the inbred varieties.
  2. Heterozygote advantage at many loci in the hybrids.

Balanced polymorphism can also result from patchy environments where different phenotypes are favored in different subregions of a populations geographic range (see Campbell, Figure 23.9).

Frequency-dependent selection also causes balanced polymorphism (see Campbell, Figure 23.10).

- In this situation the reproductive success of any one morph declines if that phenotype becomes too common in the population.
- For example, in *Papilio dardanus,* an African swallowtail butterfly, males have similar coloration but females occur in several morphs.
- The female morphs resemble other butterfly species which are noxious to bird predators. *Papilio* females are not noxious, but birds avoid them because they look like distasteful species.
- This type of protective coloration (*Batesian mimicry*) would be less effective if all the females looked like the same noxious species, because birds would encounter good-tasting mimics as often as noxious butterflies and would not associate a particular color pattern with bad taste.

3. Neutral variation

Some genetic variations found in populations confer no selective advantage or disadvantage. They have little or no impact on reproductive success. This type of variation is called *neutral variation.*

- Much of the protein variation found by electrophoresis is adaptively neutral.
- For example, 99 known mutations affect 71 of 146 amino acids in the beta hemoglobin chain in humans. Some, like the sickle-cell anemia allele, affect the reproductive potential of an individual, while others have no obvious effect.
- The *neutral theory* of molecular evolution states than many variant alleles at a locus may confer no selective advantage or disadvantage.
- Natural selection would not affect the relative frequencies of neutral variations. Frequency of some neutral alleles will increase in the gene pool and others will decrease *due the chance effects of genetic drift.*

Variation in DNA which does not code for proteins may also be nonadaptive.

- Most eukaryotes contain large amounts of DNA in their genomes which have no known function. Such noncoding DNA can be found in varying amounts in closely related species.

- Some scientists speculate that noncoding DNA has resulted from the inherent capacity of DNA to replicate itself and has expanded to the tolerance limits of the each species. The entire genome could exist as a consequence of being self-replicating rather than by providing an adaptive advantage to the organism.
- Transposons might fit this definition of "selfish DNA," although the degree of influence these sequences have on the evolution of genomes is not known.

Evolutionary biologists continue to debate how much variation, or even whether variation, is neutral.

- It is easy to show that an allele is deleterious to an organism.
- It is not easily shown that an allele provides no benefits, since such benefits may occur in immeasurable ways.
- Also, a variation may appear to be neutral under one set of environmental conditions and not neutral under other conditions.

We cannot know how much genetic variation is neutral, but if even a small portion of a population's genetic variation significantly affects the organisms, there is still a tremendous amount of raw material for natural selection and adaptive evolution.

IV. Natural Selection as the Mechanisms of Adaptive Evolution

Adaptive evolution results from a combination of:

- Chance events that produce new genetic variation (e.g., mutation and sexual recombination)
- Natural selection that favors propagation of some variations over others

A. Evolutionary fitness is the relative contribution an individual makes to the gene pool of the next generation

Darwinian fitness is measured by the relative contribution an individual makes to the gene pool of the next generation.

- It is not a measure of physical and direct confrontation, but of the success of an organism in producing progeny.
- Organisms may produce more progeny because they are more efficient feeders, attract more pollinators (as in our wildflowers), or avoid predators.

Survival does not guarantee reproductive success, since a sterile organism may outlive fertile members of the population.

- A long life span may increase fitness if the organism reproduces over a longer period of time (thus leaving more offspring) than other members of the population.
- Even if all members of a population have the same life span, those that mature early and thus have a longer reproductive time span, have increased their fitness.
- Every aspect of survival and fecundity are components of fitness.

Relative fitness = The contribution of a genotype to the next generation compared to the contributions of alternative genotypes for the same locus

- For example, if pink flower plants (AA and Aa) in our wildflower population produce more offspring than white flower plants (aa), then AA and Aa genotypes have a higher relative fitness.

Statistical estimates of fitness can be produced by the relative measure of selection *against* an inferior genotype. This measure is called the *selection coefficient*.

- For comparison, relative fitness of the most fecund variant (AA or Aa in our wildflower population) is set at 1.0.

- If white flower plants produce 80% as many progeny on average, then the white variant relative fitness is 0.8.
- The selection coefficient is the difference between these two values (1.0 − 0.8 = 0.2).
- The more disadvantageous the allele, the greater the selection coefficient.
- Selection coefficients can range up to 1.0 for a lethal allele.

The rate of decline in relative frequencies of deleterious alleles in a population depends on the magnitude of the selection coefficient working against it and whether the allele is dominant or recessive to the more successful allele.

- Deleterious recessives are normally protected from elimination by heterozygote protection.
- Selection against harmful dominant alleles is faster since they are expressed in heterozygotes.

The rate of increase in relative frequencies of beneficial alleles is also affected by whether it is a dominant or recessive.

- New recessive mutations spread slowly in a population (even if beneficial) because selection can not act in its favor until the mutation is common enough for homozygotes to be produced.
- New dominant mutations that are beneficial increase in frequency faster since even heterozygotes benefit from the allele's presence (for example, the mutant dark color producing allele in peppered moths).

Most new mutations, whether harmful or beneficial, probably disappear from the gene pool early due to genetic drift.

Selection acts on phenotypes, indirectly adapting a population to its environment by increasing or maintaining favorable genotypes in the gene pool.

- Since it is the phenotype (physical traits, metabolism, physiology, and behavior) which is exposed to the environment, selection can only act indirectly on genotypes.

The connection between genotype and phenotype may not be as simple and definite as with our wildflower population where pink was dominant to white.

- *Pleiotropy* (the ability of a gene to have multiple effects) often clouds this connection. The overall fitness of a genotype depends on whether its beneficial effects exceed any harmful effects on the organism's reproductive success.
- Polygenic traits also make it difficult to distinguish the phenotype-genotype connection. Whenever several loci influence the same characteristic, the members of the population will not fit into definite categories, but represent a continuum along a range.

An organism is an integrated composite of many phenotypic features, and the fitness of a genotype at any one locus depends upon the entire genetic context. A number of genes may work cooperatively to produce related phenotypic traits.

B. The effect of selection on a varying characteristic can be stabilizing, directional, or diversifying

The frequency of a heritable characteristic in a population may be affected in one of three different ways by natural selection, depending on which phenotypes are favored (see Campbell, Figure 23.11)

1. Stabilizing selection

Stabilizing selection favors intermediate variants by selecting against extreme phenotypes.

- The trend is toward reduced phenotypic variation and greater prevalence of phenotypes best suited to relatively stable environments.

- For example, human birth weights are in the 3 – 4 kg range. Much smaller and much higher birth weight babies have a greater infant mortality.

2. Directional selection

Directional selection favors variants of one extreme. It shifts the frequency curve for phenotypic variations in one direction toward rare variants which deviate from the average of that trait.

- This is most common when members of a species migrate to a new habitat with different environmental conditions or during periods of environmental change.
- For example, fossils show the average size of European black bears increased after periods of glaciation, only to decrease during warmer interglacial periods.

3. Diversifying selection

In diversifying selection, opposite phenotypic extremes are favored over intermediate phenotypes.

- This occurs when environmental conditions are variable in such a way that extreme phenotypes are favored.
- For example, balanced polymorphism of *Papilio* where butterflies with characteristics between two noxious model species (thus not favoring either) gain no advantage from their mimicry.

C. Sexual selection may lead to pronounced secondary differences between the sexes

Sexual dimorphism = Distinction between the secondary sexual characteristics of males and females

- Often seen as differences in size, plumage, lion manes, deer antlers, or other adornments in males.
- In vertebrates it is usually the male that is the "showier" sex.
- In some species, males use their secondary sexual characteristics in direct competition with other males to obtain female mates (especially where harem building is common). These males may defeat other males in actual combat, but more often they use ritualized displays to discourage male competitors.

Darwin viewed sexual selection as a separate selection process leading to sexual dimorphism.

- These enhanced secondary sexual characteristics usually have no adaptive advantage other than attracting mates.
- However, if these adornments increase a males ability to attract more mates, his reproductive success is increased and he contributes more to the gene pool of the next generation.

The evolutionary outcome is usually a compromise between natural selection and sexual selection.

- In some cases the line between these two types of selection is not distinct, as in male deer.
- A stag may use his antlers to defend himself from predators and also to attract females.

Every time a female chooses a mate based particular phenotypic traits, she perpetuates the alleles that caused her to make that choice and allows the male with a particular phenotype to perpetuate his alleles (see Campbell, Figure 23.12).

D. Natural selection cannot fashion perfect organisms

Natural selection *cannot* breed perfect organisms because:

1. *Organisms are locked into historical constraints.* Each species has a history of descent with modification from ancestral forms.
   - Natural selection modifies existing structures and adapts them to new situations, it does not rebuild organisms.
   - For example, back problems suffered by some humans are in part due to the modification of a skeleton and musculature from the anatomy of four-legged ancestors which are not fully compatible to upright posture.
2. *Adaptations are often compromises.*
   - Each organism must be versatile enough to do many different things.
   - For example, seals spend time in the water and on rocks; they could walk better with legs, but swim much better with flippers.
   - Prehensile hands and flexible limbs allow humans to be very versatile and athletic, but they also make us prone to sprains, torn ligaments, and dislocations. Structural reinforcement would prevent many of these disabling occurrences but would limit agility.
3. *Not all evolution is adaptive.*
   - Genetic drift probably affects the gene pool of populations to a large extent.
   - Alleles which become fixed in small populations formed by the founder effect may not be better suited for the environment than alleles that are eliminated.
   - Similarly, small surviving populations produced by bottleneck effect may be no better adapted to the environment or even less well adapted than the original population.
4. Selection can only edit variations that exist.
   - These variations may not represent ideal characteristics.
   - New genes are not formed by mutation on demand.

These limitations allow natural selection to operate on a "better than" basis and subtle imperfections are the best evidence for evolution.

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CHAPTER 24
THE ORIGIN OF SPECIES

OUTLINE

I. What Is a Species?
   A. The biological species concept emphasizes reproductive isolation
   B. Prezygotic and postzygotic barriers isolate the gene pools of biological species
   C. The biological species concept does not work in all situations
   D. Other species concepts emphasize features and processes that identify and unite species members

II. Modes of Speciation
   A. Geographical isolation can lead to the origin of species: allopatric speciation
   B. A new species can originate in the geographical midst of the parent species: sympatric speciation
   C. Genetic change in populations can account for speciation
   D. The punctuated equilibrium model has stimulated research on the tempo of speciation

The Origin of Evolutionary Novelty
   A. Most evolutionary novelties are modified versions of older structures
   B. Genes that control development play a major role in evolutionary novelty
   C. An evolutionary trend does not mean that evolution is goal oriented

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Distinguish between anagenesis and cladogenesis.
2. Define morphospecies and explain how this concept can be useful to biologists.
3. Define biological species (E. Mayr).
4. Describe some limitations of the biological species concept.
5. Explain how gene flow between closely related species can be prevented.
6. Distinguish between prezygotic and postzygotic isolating mechanisms.
7. Describe five prezygotic isolating mechanisms and give an example of each.
8. Explain why many hybrids are sterile.
9. Explain, in their own words, how hybrid breakdown maintains separate species even if gene flow occurs.
10. Distinguish between allopatric and sympatric speciation.
11. Explain, in their own words, the allopatric speciation model and describe the role of intraspecific variation and geographical isolation.

12. Explain why peripheral isolates are susceptible if geographic barriers arise.
13. Describe the adaptive radiation model and use it to describe how it might be possible to have many sympatric closely related species even if geographic isolation is necessary for them to evolve.
14. Define sympatric speciation and explain how polyploidy can cause reproductive isolation.
15. Distinguish between autopolyploidy and allopolyploidy.
16. List some points of agreement and disagreement between the two schools of thought about the tempo of speciation (gradualism vs. punctuated equilibrium).
17. Describe the origins of evolutionary novelty.

KEY TERMS

macroevolution	postzygotic barriers	adaptive radiation	allometric growth
speciation	morphological species concept	polyploidy	heterochrony
anagenesis	recognition species concept	autopolyploid	homeosis
phyletic evolution	cohesion species concept	allopolyploid	
cladogenesis	ecological species concept	hybrid zone	
branching evolution	evolutionary species concept	punctuated equilibrium	
species	allopatric speciation	exaptation	
prezygotic barriers	sympatric speciation	paedomorphosis	

LECTURE NOTES

Evolutionary theory must explain *macroevolution*, the origin of new taxonomic groups (e.g., new species, new genera, new families). *Speciation*, or the origin of new species, is a central process of macroevolution because any genus, family, or higher taxon originates with a new species novel enough to be the first member of the higher taxon

The fossil record provides evidence for two patterns of speciation: *anagenesis* and *cladogenesis* (see Campbell, Figure 24.1).

- *Anagenesis (phyletic evolution)* = The transformation of an unbranched lineage of organisms, sometimes to a state different enough from the ancestral population to justify renaming it as a new species.
- *Cladogenesis (branching evolution)* = The budding of one or more new species from a parent species that continues to exist; is more important than anagenesis in life's history, because it is more common and can promote biological diversity.

I. What Is a Species?

Species = Latin term meaning "kind" or "appearance

Linnaeus (founder of modern taxonomy) described species in terms of their physical form (morphology). Morphology is still the most common method used for describing species.

Modern taxonomists also consider genetic makeup and functional and behavior features when describing species.

A. The biological species concept emphasizes reproductive isolation

In 1942, Ernst Mayr proposed the *biological species concept.*

Biological species = A population or group of populations whose members have the potential to interbreed with one another in nature and to produce viable, fertile offspring, but cannot produce viable, fertile offspring with members of other species (see Campbell, Figure 24.2).

- Is the largest unit of population in which gene flow is possible
- Is defined by reproductive isolation from other species in *natural* environments (hybrids may be possible between two species in the laboratory or in zoos)

B. Prezygotic and postzygotic barriers isolate the gene pools of biological species

Any factor that impedes two species from producing viable, fertile hybrids contributes to reproductive isolation.

- Most species are genetically sequestered from other species by more than one type of reproductive barrier.
- Only intrinsic biological barriers to reproduction will be considered here. Geographic segregation (even though it prevents interbreeding) will not be considered.
- Reproductive barriers prevent interbreeding between closely related species.

The various reproductive barriers which isolate the gene pools of species are classified as either prezygotic or postzygotic, depending on whether they function before or after the formation of zygotes.

- *Prezygotic barriers* impede mating between species or hinder fertilization of the ova should members of different species attempt to mate.
- In the event fertilization does occur, *postzygotic barriers* prevent the hybrid zygote from developing into a viable, fertile adult.

1. Prezygotic barriers

a. Habitat isolation

Two species living in different habitats within the same area may encounter each other rarely, if at all, even though they are not technically geographically isolated.

- For example, two species of garter snakes (*Thamnophis*) occur in the same areas but for intrinsic reasons, one species lives mainly in water and the other is mainly terrestrial.
- Since these two species live primarily in separate habitats, they seldom come into contact as they are ecologically isolated.

c. Behavioral isolation

Species-specific signals and elaborate behavior to attract mates are important reproductive barriers among closely related species.

- Male fireflies of different species signal to females of the same species by blinking their lights in a characteristic pattern; females discriminate among the different signals and respond only to flashes of their own species by flashing back and attracting the males.

Many animals recognize mates by sensing pheromones (distinctive chemical signals).

- Female Gypsy moths attract males by emitting a volatile compound to which the olfactory organs of male Gypsy moths are specifically tuned: when a male detects this pheromone, it follows the scent to the female.
- Males of other moth species do not recognize this chemical as a sexual attractant.

Other factors may also act as behavioral isolating mechanisms:

- Eastern and western meadowlarks are almost identical in shape, coloration, and habitat, and their ranges overlap in the central United States (see Campbell, Figure 24.2a).

- They retain their biological species integrity partly because of the difference in their songs, which enables them to recognize potential mates as members of their own kind.

Another form of behavioral isolation is courtship ritual specific to a species (see Campbell, Figure 24.3).

c. Temporal isolation

Two species that breed at different times of the day, seasons, or years cannot mix their gametes.

- For example, brown trout and rainbow trout cohabit the same streams, but brown trout breed in the fall and rainbow trout breed in the spring.
- Since they breed at different times of the year, their gametes have no opportunity to contact each other and reproductive isolation is maintained.

d. Mechanical isolation

Anatomical incompatibility may prevent sperm transfer when closely related species attempt to mate.

- For example, male dragonflies use a pair of special appendages to clasp females during copulation. When a male tries to mount a female of a different species, he is unsuccessful because his clasping appendages do not fit the female's form well enough to grip securely.
- In plants that are pollinated by insects or other animals, the floral anatomy is often adapted to a specific pollinator that transfers pollen only among plants of the same species.

e. Gametic isolation

Gametes of different species that meet rarely fuse to form a zygote.

- For animals that use internal fertilization, the sperm of one species may not be able to survive the internal environment of the female reproductive tract of a different species.
- Cross-specific fertilization is also uncommon for animals that utilize external fertilization due to a lack of gamete recognition.

Gamete recognition may be based on the presence of specific molecules on the coats around the egg which adhere only to complementary molecules on sperm cells of the same species.

- Similar mechanisms of molecular recognition enables a flower to discriminate between pollen of the same species and pollen of different species.

2. Postzygotic barriers

When prezygotic barriers are crossed and a hybrid zygote forms, one of several postzygotic barriers may prevent development of a viable, fertile hybrid.

a. Reduced hybrid viability

Genetic incompatibility between the two species may abort development of the hybrid at some embryonic stage.

- For example, several species of frogs in the genus *Rana* live in the same regions and habitats.
- They occasionally hybridize but the hybrids generally do not complete development, and those that do are frail and soon die.

b. Reduced hybrid fertility

If two species mate and produce hybrid offspring that are viable, reproductive isolation is intact if the hybrids are sterile because genes cannot flow from one species' gene pool to the other.

- One cause of this barrier is that if chromosomes of the two parent species differ in number or structure, meiosis cannot produce normal gametes in the hybrid.
- The most familiar case is the mule which is produced by crossing a donkey and a horse; very rarely are mules able to backbreed with either parent species (see Campbell, Figure 24.4).

c. Hybrid breakdown

When some species cross-mate, the first generation hybrids are viable and fertile, but when these hybrids mate with one another or with either parent species, offspring of the next generation are feeble or sterile.

- For example, different cotton species can produce fertile hybrids, breakdown occurs in the next generation when progeny of the hybrids die in their seeds or grow into weak defective plants.

Campbell, Figure 24.5 summarizes the reproductive barriers between closely related species.

C. The biological species concept does not work in all situations

The biological species concept cannot be applied to:

- Organisms that are completely asexual in their reproduction. Some protists and fungi, some commercial plants (bananas), and many bacteria are exclusively asexual.

  Asexual reproduction effectively produces a series of clones, which genetically speaking, represent a single organism.

  Asexual organisms can be assigned to species only by grouping clones with the same morphology and biochemical characteristics.
- Extinct organisms represented only by fossils. These must be classified by the morphospecies concept.

In some cases, unambiguous determination of species is not possible, even though the populations are sexual, contemporaneous, and contiguous.

- Four phenotypically distinct populations of the deer mouse (*Peromyscus maniculatus*) found in the Rocky Mountains are geographically isolated and referred to as *subspecies*. (see Campbell, Figure 24.6)
- These populations overlap at certain locations and some interbreeding occurs in these areas of cohabitation, which indicates they are the same species by the biological species criteria.
- Two subspecies (*P. m. artemisiae* and *P. m. nebrascensis*) are an exception, since they do not interbreed in the area of cohabitation. However, their gene pools are not completely isolated since they freely interbreed with other neighboring populations.
- This circuitous route could only produce a very limited gene flow, but the route is open and possible between the populations of *P. m. artemisiae* and *P. m. nebrascensis* through the other populations.

- If this route was closed by extinction or geographic isolation of the neighboring populations, then *P. m. artemisiae* and *P. m. nebrascensis* could be named separate species without reservation.

More examples are being discovered where there is a blurry distinction between populations with limited gene flow and full biological species with segregated gene pools.

- If two populations cannot interbreed when in contact, they are clearly distinct species.
- When there is gene flow (even very limited) between two populations that are in contact, it is difficult to apply the biological species concept.
- This is equivalent to finding two populations at different stages in their evolutionary descent from a common ancestor, which is to be expected if new species arise by gradual divergence of populations.

Other species concepts have been developed in an effort to accommodate the dynamic, quantitative aspects of speciation; however, the species problem may never be completely resolved as it is unlikely that a single definition of species will apply to all cases.

D. Other species concepts emphasize features and processes that identify and unite species members

The *morphological species concept* characterizes species on the basis of measurable physical features.

- Can be useful in the field
- Sometimes difficult to apply (e.g., Do physical differences between a set of organisms represent species differences or phenotypic variation within a species?)

In the *recognition species concept*, a species is defined by a unique set of characteristics that maximize successful mating.

- Characteristics may be molecular, morphological, or behavioral in nature
- Characteristics are subject to natural selection

The *cohesion species concept* relies on the mechanisms that maintain species as discrete phenotypic entities.

- Mechanisms may include reproductive barriers, stabilizing selection, and linkages among sets of genes that make a zygote develop into a an adult organism with species-specific characteristics (e.g., sexual reproduction)
- This concept acknowledges that interbreeding between some species produces fertile hybrids (e.g., corn)

The *ecological species concept* defines a species on the basis of where they live and what they do.

The *evolutionary species concept* defines a species as a sequence of ancestral and descendent populations that are evolving independently of other such groups

- Each evolutionary species has its own unique role in the environment; roles are influenced by natural selection.

Campbell, Table 24.1 reviews the species concepts.

II. Modes of Speciation

Reproductive barriers form boundaries around species, and the evolution of these barriers is the key biological event in the origin of new species.

- An essential episode in the origin of a species occurs when the gene pool of a population is separated from other populations of the parent species.

- This genetically isolated splinter group can then follow its own evolutionary course, as changes in allele frequencies caused by selection, genetic drift, and mutations occur undiluted by gene flow from other populations.

There are two general modes of speciation: allopatric speciation and sympatric speciation.

A. Geographical isolation can lead to the origin of species: allopatric speciation

1. Geographic barriers

Allopatric speciation = Speciation that occurs when the initial block to gene flow is a geographical barrier that physically isolates the population

- Geological processes can fragment a population into two or more allopatric populations (having separate ranges).

  Such occurrences include emergence of mountain ranges, movement of glaciers, formation of land bridges, subsidence of large lakes.

  Also small populations may become geographically isolated when individuals from the parent population travel to a new location.

- The extent of development of a geographical barrier necessary to isolate two populations depends on the ability of the organisms to disperse due to the mobility of animals or the dispersibility of spores, pollen and seeds of plants.

  For example, the Grand Canyon is an impassable barrier to small rodents, but is easily crossed by birds. As a result, the same bird species populate both rims of the canyon, but each rim has several unique species of rodents (see Campbell, Figure 24.7).

An example of how geographic isolation can result in allopatric speciation is the pupfish.

- About 50,000 years ago, during an ice age, the Death Valley region of California and Nevada had a rainy climate and a system of interconnecting lakes and rivers.
- A drying trend began about 10,000 years ago, and by 4000 years ago, the region had become a desert.
- Presently, isolated springs in deep clefts between rocky walls are the only remnants of the lake and river networks. Living in many of these isolated springs are small pupfishes (*Cyprinodon* spp.).
- Each inhabited spring contains its own species of pupfish which is adapted to that pool and found nowhere else in the world.
- The endemic pupfish species probably descended from a single ancestral species whose range was fragmented when the region became arid, thus isolating several small populations that diverged in their evolution as they adapted to their spring's environment.

2. Conditions favoring allopatric speciation

When populations become allopatric, speciation can potentially occur as the isolated gene pools accumulate differences by microevolution that may cause the populations to diverge in phenotype.

- A small isolated population is more likely to change substantially enough to become a new species than is a large isolated population.
- The geographic isolation (peripheral isolate) of a small population usually occurs at the fringe of the parent population's range.
- As long as the gene pools are isolated from the parental population, *peripheral isolates* are good candidates for speciation for three reasons:

1. *The gene pool of the peripheral isolate probably differs from that of the parent population from the outset.* Since fringe inhabiters usually represent the extremes of any genotypic and phenotypic clines in an original sympatric population. With a small peripheral isolate, there will be a founder effect with chance resulting in a gene pool that is not representative of the gene pool of the parental population.
2. *Genetic drift will continue to cause chance changes in the gene pool of the small peripheral isolate until a large population is formed.* New mutations or combinations of alleles that are neutral in adaptive value may become fixed in the population by chance alone, causing phenotypic divergence from the parent population.
3. *Evolution caused by selection is likely to take a different direction in the peripheral isolate than in the parental population.* Since the peripheral isolate inhabits a frontier with a somewhat different environment, it will probably be exposed to different selection pressures than those encountered by the parental population.

- Due to the severity of a fringe environment, most peripheral isolates do not survive long enough to speciate.

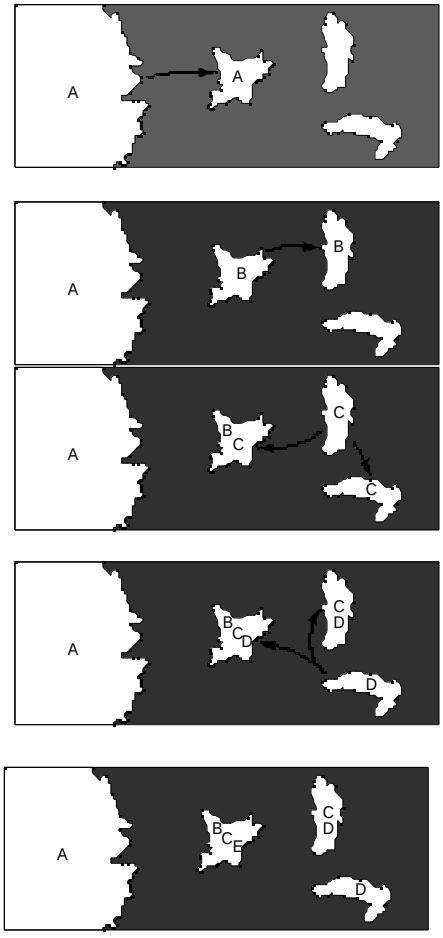
Although most peripheral isolates become extinct, evolutionary biologists agree that a small population can accumulate enough genetic change to become a new species in only hundreds to thousands of generations.

3. Adaptive radiation on island chains

Allopatric speciation occurs on island chains where new populations, which stray or are passively dispersed from their ancestral populations, evolve in isolation.

Adaptive radiation = The evolution of many diversely adapted species from a common ancestor.

Examples of adaptive radiation are the endemic species of the Galapagos Islands which descended from small populations which floated, flew, or were blown from South America to the islands. Darwin's finches can be used to illustrate a model for such adaptive radiation on island chains (see also, Campbell, Figure 24.8).



- A single dispersal event may have seeded one island with a peripheral isolate of the ancestral finch which diverged as it underwent allopatric speciation.
- A few individuals of this new species may have reached neighboring islands, forming new peripheral isolates which also speciated (see Campbell, Figure 24.9).
- After diverging on the island it invaded, a new species could re-colonize the island from which its founding population emigrated and coexist with the ancestral species or form still another species.
- Multiple invasions of islands could eventually lead to coexistence of several species on each island since the islands are distant enough from each other to permit geographic isolation, but near enough for occasional dispersal.

Similar evolutionary events have occurred on the Hawaiian Archipelago. These volcanic islands are 3500 km from the nearest continent.

- Hawaii is the youngest (<one million years old), largest island and has active volcanoes.
- The islands grow progressively older in a northwesterly direction away from Hawaii.
- As each island was formed and cooled, flora and fauna carried by ocean and wind currents from other islands and continents became established.
- The physical diversity of each island provided many environmental opportunities for evolutionary divergence by natural selection.
- Multiple invasions and allopatric speciations have permitted such a degree of adaptive radiation that there are thousands of endemic species on the archipelago which are found no where else on Earth.

In contrast to the Hawaiian Archipelago, islands such as the Florida Keys are close enough to a mainland to allow free movement from the island to the mainland.

- Such islands are not characterized by endemic species since there is no long-term isolation of founding populations.
- Intrinsic reproductive barriers that block gene flow do not develop due to a steady influx of immigrants from the mainland parental populations.

B. A new species can originate in the geographical midst of the parent species: sympatric speciation

Sympatric speciation = Formation of new species within the range of parent populations

- Reproductive isolation evolves without geographical isolation.
- This can occur quickly (in one generation) if a genetic change results in a reproductive barrier between the mutants and the parent population.

Many plant species have originated from improper cell division that results in extra sets of chromosomes—a mutant condition called *polyploidy* (see Campbell, Figure 24.10)

Depending on the origin of the extra set of chromosomes, polyploids are classified in two forms: autopolyploids and allopolyploids.

Autopolyploid = An organism that has more than two chromosome sets, all derived from a single species. For example,

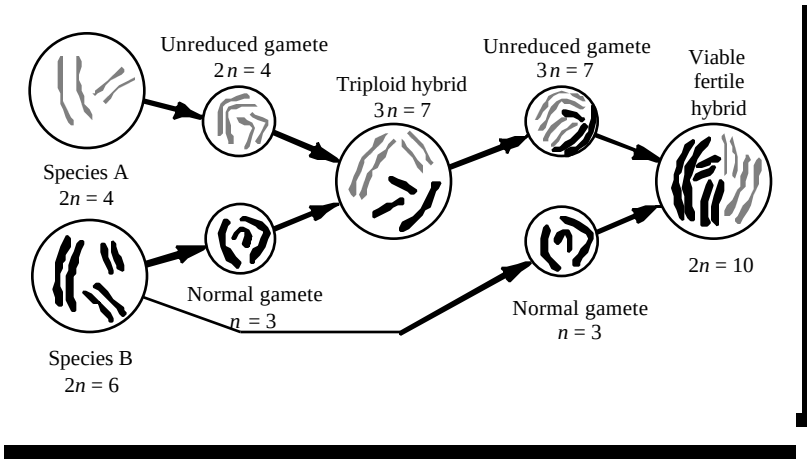
- Nondisjunction in the germ cell line (in either mitosis or meiosis) results in diploid gametes.
- Self-fertilization would double the chromosome number to the tetraploid state.
- Tetraploids can self-pollinate or mate with other tetraploids.
- The mutants cannot interbreed with diploids of the parent population because hybrids would be triploid (3*n*) and sterile due to impaired meiosis from unpaired chromosomes.
- An instantaneous special genetic event would thus produce a postzygotic barrier which isolates the gene pool of the mutant in just one generation.
- Sympatric speciation by autopolyploidy was first discovered by Hugo De Vries in the early 20th century while working with *Oenothera*, the evening primrose.

Allopolyploid = A polyploid hybrid resulting from contributions by two different species.

- More common than autopolyploidy.
- Potential evolution of an allopolyploid begins when two different species interbreed and a hybrid is produced (see Campbell, Figure 24.10b).
- Such interspecific hybrids are usually sterile, because the haploid set of chromosomes from one species cannot pair during meiosis with the haploid set of chromosomes from the second species.
- These sterile hybrids may actually be more vigorous than the parent species and propagate asexually.

At least two mechanisms can transform sterile allopolyploid hybrids into fertile polyploids:

1. During the history of the hybrid clone, mitotic nondisjunction in the reproductive tissue may double the chromosome number (see Campbell, Figure 24.10b).
   - The hybrid clone will then be able to produce gametes since each chromosome will have a homologue to synapse with during meiosis.
   - Gametes from this fertile tetraploid could unite and produce a new species of interbreeding individuals, reproductively isolated from both parent species.
2. Meiotic nondisjunction in one species produces an unreduced (diploid) gamete.



- This abnormal gamete fuses with a normal haploid gamete of a second species and produces a triploid hybrid.
- The triploid hybrid will be sterile, but may propagate asexually.
- During the history of this sterile triploid clone, meiotic nondisjunction again produces an unreduced gamete (triploid).
- Combination of this triploid gamete with a normal haploid gamete from the second parent species would result in a fertile hybrid with homologous pairs of chromosomes.
- his allopolyploid would have a chromosome number equal to the sum of the chromosome numbers of the two ancestral species (as in 1 above).

Speciation of polyploids (especially allopolyploids) has been very important in plant evolution.

- Some allopolyploids are very vigorous because they contain the best qualities of both parent species.
- The accidents required to produce these new plant species (interspecific hybridization coupled with nondisjunction) have occurred often enough that between 25% and 50% of all plant species are polyploids.

Some of these species have originated and spread in relatively recent times and many others are of importance to humans.

- Spartina angelica is a species of salt-marsh grass which evolved as an allopolyploid in the 1870s.

  It is derived from a European species (*Spartina maritima*) and an American species (*Spartina alternaflora*).

  In addition to being morphologically distinct and reproductively isolated from its parent species, *S. angelica* has a chromosome number ($2n = 122$) indicative of its mechanism of speciation (*S. maritima*, $2n =$, *S. alternaflora*, $2n = 62$).
- *Triticum aestivum*, bread wheat, is a 42-chromosome allopolyploid that is believed to have originated about 8000 years ago as a hybrid of a 28 chromosome cultivated wheat and a 14 chromosome wild grass (see Campbell, Figure 24.11).
- Other important polyploid species include oats, cotton, potatoes, and tobacco.
- Plant geneticists are presently inducing these genetic accidents to produce new polyploids which will combine high yield and disease resistance.

Sympatric speciation may also occur in animal evolution through different mechanisms.

- A group of animals may become isolated within the range of a parent population if genetic factors cause them to become fixed on resources not used by the parent population as a whole. For example,

  A particular species of wasp pollinates each species of figs. The wasps mate and lay their eggs in the figs.

  A genetic change causing wasps to select a different fig species would segregate mating individuals of the new phenotype from the parental population.

  Divergence could then occur after such an isolation.

  The great diversity of cichlid fishes in Lake Victoria may have arisen from isolation due to exploitation of different food sources and other resources in the lake.

- Sympatric speciation could also result from a balanced polymorphism combined with assortative mating.

  For example, if birds in a population that is dimorphic for beak size began to selectively mate with birds of the same morph, speciation could occur over time.

While both allopatric speciation and sympatric speciation have important roles in plant evolution, allopatric speciation is far more common in animals.

C. Genetic change in populations can account for speciation

Classifying modes of speciation as allopatric or sympatric emphasizes biogeographical factors but does not emphasize the actual genetic mechanisms. An alternative method which takes genetic mechanism into account, groups speciation into two categories: *speciation by adaptive divergence* and *speciation by shifts in adaptive peaks.*

1. Adaptive divergence

Two populations which adapt to different environments accumulate differences in the frequencies of alleles and genotypes.

- During this gradual adaptive divergence of the two gene pools, reproductive barriers may evolve between the two populations.
- Evolution of reproductive barriers would differentiate these populations into two species.

A key point in evolution by divergence is that reproductive barriers can arise without being favored directly by natural selection.

- Divergence of two populations is due to their adaptation to separate environments, with reproductive isolation being a secondary development.
- Postzygotic barriers may be pleiotropic effects of interspecific differences in those genes that control development.

  Hybrids may not be viable if both sets of genes for rRNA synthesis are not active (e.g., hybrids between *D. melanogaster* and *D. simulans*).

- Gradual genetic divergence of two populations may also result in the evolution of prezygotic barriers.

  For instance, an ecological barrier to inbreeding may secondarily result from the adaptation of an insect population to a new host plant different from the original population's host.

In some isolated populations, reproductive isolation has evolved more directly from sexual selection. For example,

- In *Drosophila heteroneura,* the male's wide head enhances reproductive success with females of the same species while reducing the probability that a male *D. heteroneura* will mate with females of other species.
- Sexual selection, in this case, probably evolved as an adaptation for enhanced reproductive success. A secondary consequence is that it prevents interbreeding with other *Drosophila* species.
- Since reproductive barriers usually evolve when populations are allopatric, they do not function directly to isolate the gene pools of populations.

  For this reason, the emphasis on reproductive isolating mechanisms is one criticism of the biological species concept.

2. Hybrid zones and the cohesion concept of species

Three possible outcomes are possible when two closely related populations that have been allopatric for some time come back into contact:

- The two populations may interbreed freely.

  The gene pools would become incorporated into a single pool indicating that speciation had not occurred during their time of geographical isolation.
- The two populations may not interbreed due to reproductive barriers.

  The gene pools would remain separate due to the evolutionary divergence which occurred during the time of geographical isolation. Speciation has taken place.
- A hybrid zone may be established.

Hybrid zone = A region where two related populations that diverged after becoming geographically isolated make secondary contact and interbreed where their geographical ranges overlap

- For example, the red-shafted flicker of western North America and the yellow-shafted flicker of central North America are two phenotypically distinct populations of woodpeckers that interbreed in a hybrid zone stretching from southern Alaska to the Texas panhandle.
- The two populations came into renewed contact a few centuries ago after being separated during the ice ages.
- The hybrid zone is relatively stable and not expanding.

  The introgression of alleles between the populations has not penetrated far beyond the hybrid zone, although the two populations have been interbreeding for at least two hundred years.

  The genotypic and phenotypic frequencies that distinguish the two populations form steep clines into the hybrid zone.
- Away from the hybrid zone, the two populations remain distinct.

Should two populations which form a hybrid zone be considered subspecies or separate species?

- Some researchers who support species status for such populations, recognize that the presence of stable hybrid zones creates a problem for the biological species concept.

  If the taxonomic identity of two species is maintained, even though they hybridize, there must be cohesive forces other than reproductive isolation that maintain the species and prevent their merging into a single species.

- These researchers favor an alternative known as the *cohesion concept of species*.

The cohesion concept of species holds that the cohesion may involve a distinctive, integrated set of adaptations that has been refined during the evolutionary history of a population.

- Phenotypic variation would be restricted by stabilizing selection to a range narrow enough to define the species as separate from other species.
- In the adaptive landscape view:

  The red-shafted and yellow-shafted flickers are clustered around different adaptive peaks.

  Specific combinations of alleles and specific linkages between gene loci on chromosomes may form a genetic basis for the cohesion of phenotypes.

  The clinal change of genetic structure and phenotype noted in the hybrid zone may be correlated with transitions in environmental factors that help shape the two distinct populations.

3. How much genetic change is required for speciation?

No generalizations can be made about genetic distance between closely related species. Reproductive isolation may result from changes in many loci or only in a few.

- Two species of Hawaiian *Drosophila* (*D. silvestris* and *D. heteroneura*) differ at only one locus which determines head shape, an important factor in mate recognition (see Campbell, Figure 24.12).

  The phenotypic effect of different alleles at this locus is multiplied by epistasis involving at least ten other loci.

  Thus, no more than one mutation was necessary to differentiate the two species.
- Changes in one gene in a coadapted gene complex can substantially impact the development of an organism.

D. The punctuated equilibrium model has stimulated research on the tempo of speciation

Traditional evolutionary trees diagram the descent of species from ancestral forms as branches that gradually diverge with each new species evolving continuously over long spans of time (see Campbell, Figure 24.13a).

- The theory behind such a tree is the extrapolation of microevolutionary processes (allele frequency changes in the gene pool) to the divergence of species.
- Big changes thus occur due to the accumulation of many small changes.

Paleontologists rarely find gradual transitions of fossil forms but often observe species appearing as new forms suddenly in the rock layers.

- These species persist virtually unchanged and then disappear as suddenly as they appeared.
- Even Darwin, who believed species from a common ancestral stock evolve differences gradually, was perplexed by the lack of transitional forms in the fossil record.

Advocates of *punctuated equilibrium* have redrawn the evolutionary tree to represent fossil evidence for evolution occurring in spurts of relatively rapid change instead of gradual divergence (see Campbell, Figure 24.13b).

- This theory was proposed by Niles Eldredge and Stephen Jay Gould in 1972.

- It depicts species undergoing most of their morphological modification as they first separate from the parent species then showing little change as they produce additional species.
- In this theory gradual change is replaced with long periods of stasis punctuated with episodes of speciation.
- The origin of new polyploid plants through genome changes is one mechanism of sudden speciation.
- Allopatric speciation of a splinter population separated from its parent population by geographical barriers may also be rapid.

    For a population facing new environmental conditions, genetic drift and natural selection can cause significant change in only a few hundred or thousand generations.

A few thousand generations is considered rapid in reference to the geologic time scale.

- The fossil record indicates that successful species survive for a few million years on average.
- If a species survives for five million years and most of its morphological changes occur in the first 50,000 years, then the speciation episode occurred in just 1% of the species' lifetime.
- With this time scale, a species will appear suddenly in rocks of a certain age, linger relatively unchanged for millions of years, then become extinct.
- While forming, the species may have gradually accumulated modifications, but with reference to its overall history, its formation was sudden.
- An evolutionary spurt preceding a longer period of morphological stasis would explain why paleontologists find so few transitions in the fossils record of a species.

Because "sudden" can refer to thousands of years on the geological time scale, differing opinions of punctuationalists and gradualists about the rate of speciation may be more a function of time perspective than conceptual difference. There is clear disagreement, however, over how much a species changes after its origin.

- In a species adapted to an environment that stays the same, natural selection would counter changes in the gene pool.

    Once selection during speciation produces new complexes of coadapted genes, mutations are likely to impose disharmony on the genome and disrupt the development of the organism.
- Stabilizing selection would thus hold a population at one adaptive peak to produce long periods of stasis.

Some gradualists feel that stasis is an illusion since many species may continue to change, after they have diverged from the parent population, in ways undetectable in fossils.

- Changes in internal anatomy, physiology and behavior would go unnoticed by paleontologists as fossils only show external anatomy and skeletons.
- Population geneticists also point out that many microevolutionary changes occur at the molecular level without affecting morphology.

It is obvious that additional extensive studies of fossil morphology where specific lineages are preserved should be carried out to assess the relative importance of gradual and punctuated tempos in the origin of new species.

III. The Origin of Evolutionary Novelty

What processes cause the evolutionary changes that can be traced through the fossil record? How do the novel features that define taxonomic groups above the species level arise? The following concepts address the processes relevant to these questions.

A. Most evolutionary novelties are modified versions of older structures

Higher taxa such as families and classes are defined by evolutionary novelties. For example:

- Birds evolved from dinosaurs, and their wings are homologous to the forelimbs of modern reptiles.
- Birds are adapted to flight, yet their ancestors were earthbound.

How could these new designs evolve?

- One mechanism is the gradual refinement of existing structures for new functions.
- Most biological structures have an evolutionary plasticity that makes alternative functions possible.

Exaptation is a term applied to a structure that evolves in one context and becomes co-opted for another function.

- Natural selection cannot anticipate the future, but it can improve an existing structure in context of its current utility. For example,

  The honeycombed bones and feathers of birds did not evolve as adaptations for flight.

  They must have been beneficial to the bipedal reptilian ancestors of birds (reduction of weight, gathering food, courtship), and later through modification, became functional for flying.
- Exaptation cannot be proven, but provides an explanation for how novel designs can arise gradually through a series of intermediate stages, each having some function in the organism.
- The evolution of novelties by remodeling of old structures for new functions reflects the Darwinian tradition of large changes being crafted by natural selection through an accumulation of many small changes.

B. Genes that control development play a major role in evolutionary novelty

The evolution of complex structures (e.g., wings) requires such large modifications that changes at many gene loci are probably involved.

- In other cases, relatively few changes in the genome can cause major modifications in morphology (e.g., humans vs. chimpanzees).
- Thus, slight genetic divergence can become magnified into major differences.

In animal development, a system of regulatory genes coordinates activities of structural genes to guide the rate and pattern of development from zygote to adult.

- A slight alteration of development becomes compounded in its effect on adult *allometric growth* (differences in relative rates of growth of various parts of the body) which helps to shape an organism.
- A slight change in these relative rates of growth will result in a substantial change in the adult (see Campbell, Figure 24.14a).
- Thus, altering the parameters of allometric growth is one way relatively small genetic differences can have major morphological impact (see Campbell, Figure 24.14b).

Changes in developmental dynamics, both *temporal* and *spatial*, have played a major role in macroevolution.

Temporal changes in development that create evolutionary novelties are called *heterochrony*.

Heterochrony = Evolutionary changes in the timing or rate of development

- Genetic changes that alter the timing of development can also produce novel organisms.

Paedomorphosis = Retention of ancestral juvenile structures in a sexually mature adult organism

Campbell, Figure 24.15 shows the effect of developmental timing on zebra stripes. Figure 24.16 shows paedomorphosis in salamanders.

A slight change in timing that retards the development of some organs in comparison to others produces a different kind of animal.

- Changes in developmental chronology may have contributed to human evolution.

Humans and chimpanzees are closely related through descent from a common ancestor.

They are much more similar as fetuses than as adults.

Different allometric properties and variations result in the human brain being proportionally larger than that in chimpanzees.

The human brain continues to grow several years longer than the chimpanzee brain.

Thus, the genetic changes responsible for humans are not great, but have profound effects.

Equally important in evolution is the alteration of the spatial pattern of development or homeosis.

Homeosis = Alteration in the placement of different body parts (for example, to the arrangement of different kinds of appendages in animals or the placement of flower parts on a plant)

Since each regulatory gene may influence hundreds of structural genes, there is a potential for evolutionary novelties that define higher taxa to arise much faster than would occur by the accumulation of changes in only structural genes.

C. An evolutionary trend does not mean that evolution is goal oriented

Extracting a single evolutionary progression from the fossil record that is likely to be incomplete is misleading.

- For example, by selecting certain species from available fossils, it is possible to arrange a succession of animals between *Hyracotherium* and modern horses that shows a trend toward increased size, reduced number of toes, and modification of teeth for grazing (see Campbell, Figure 24.17 yellow line). Consideration of all known fossil horses negates this trend, and reveals that the line to modern horses is one of a series of species episodes.

Branching evolution (cladogenesis) can produce a trend even if some new species counter the trend.

- There was an overall trend in reptilian evolution toward large size during the Mesozoic era which eventually produced the dinosaurs.
- This trend was sustained even though some new species were smaller than their parental species.

One view of macroevolution, forwarded by Steven Stanley of Johns Hopkins, holds that species are analogous to individuals.

- In this analogy, speciation is birth and extinction is death.
- An evolutionary trend is produced by *species selection*, which is analogous to the production of a trend within a population by natural selection.
- The species that endure the longest and generate the greatest number of new species determine the direction of major evolutionary trends.
- Differential speciation plays a role in macroevolution similar to the way differential reproduction plays a role in microevolution.

Qualities unrelated to the success of organisms in a specific environment may be equally important in species selection.

- The ability of a species to disperse to new habitats may result in development of new "daughter species" as organisms adapt to new conditions.
- A criticism of species selection is the argument that gradual modification of populations in response to environmental change is the most common stimulus to evolutionary trends.

No intrinsic drive toward a preordained state of being is indicated by the presence of an evolutionary trend.

- Evolution is a response to interactions between organisms and their current environments.
- An evolutionary trend may cease or reverse itself under changing conditions. For example, conditions of the Mesozoic era favored giant reptiles, but by the end of that era, smaller species prevailed.

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CHAPTER 25
TRACING PHYLOGENY

OUTLINE

I. The Fossil Record and Geologic Time
- A. Sedimentary rocks are the richest source of fossils
- B. Paleontologists use a variety of methods to date fossils
- C. The fossil record is a substantial, albeit incomplete, chronicle of evolutionary history
- D. Phylogeny has a biogeographical basis in continental drift
- E. The history of life is punctuated by mass extinctions followed by adaptive radiations of the survivors

II. Phylogeny and Systematics
- A. Taxonomy employs a hierarchical system of classification
- B. The branching pattern of a phylogenetic tree represents the taxonomic hierarchy
- C. Determining monophyletic taxa is a key to classifying organisms according to their evolutionary history
- D. Molecular biology provides powerful new tools for systematics
- E. The search for fossilized DNA continues despite recent setbacks: *science as a process*

III. The Science of Phylogenetic Systematics
- A. Phenetics increased the objectivity of systematic analysis
- B. Cladistic analysis uses novel homologies to define branchpoints on phylogenetic trees
- C. Phylogenetic systematics relies on both morphology and molecules

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Explain the importance of the fossil record to the study of evolution.
2. Describe how fossils form.
3. Distinguish between relative dating and absolute dating.
4. Explain how isotopes can be used in absolute dating.
5. Explain how continental drift may have played a role in history of life.
6. Describe how radiation into new adaptive zones could result in macroevolutionary change.
7. Explain how mass extinctions could occur and affect evolution of surviving forms.
8. List the major taxonomic categories from the most to least inclusive.

9. Explain why it is important when constructing a phylogeny to distinguish between homologous and analogous character traits.
10. Distinguish between homologous and analogous structures.
11. Describe three techniques used in molecular systematics and explain what information each provides.
12. Distinguish between a monophyletic and a polyphyletic group, and explain what is meant by a "natural taxon."
13. Describe the contributions of phenetics and cladistics to phylogenetic systematics.
14. Describe how cladistic analysis uses novel homologies to define branch points on phylogenetic trees.

KEY TERMS

phylogeny	binomial	monophyletic	DNA sequence analysis
systematics	genus (genera, pl.)	polyphyletic	phenetics
fossil record	specific epithet	paraphyletic	cladistic analysis
geological time scale	family	homology	clade
radiometric dating	order	convergent evolution	outgroup
half-life	class	analogy	synapomorphies
Pangaea	phylum (phyla, pl.)	DNA-DNA	parsimony
adaptive zone	kingdom	hybridization	phylogenetic biology
phylogenetic trees	taxon (taxa, pl.)	restriction maps	

LECTURE NOTES

Biologists reconstruct evolutionary history by studying the succession of organisms in the fossil record. Fossils are collected and interpreted by *paleontologists*.

Phylogeny = The evolutionary history of a species or group of related species

- Phylogeny is usually diagrammed with *phylogenetic trees* that trace inferred evolutionary relationships.

Systematics = The study of biological diversity in an evolutionary context; reconstructing phylogeny is part of the scope of systematics

- Biological diversity reflects past episodes of speciation and macroevolution.
- Encompasses the identification and classification of species (*taxonomy*) in its search for evolutionary relationships.

Fossil record = The ordered array in which fossils(any preserved remnant or impression left by an organism that lived in the past) appear within layers of rock that mark the passing of geological time

I. The Fossil Record and Geological Time

A. Sedimentary rocks are the richest sources of fossils

Sedimentary rocks are the richest sources of fossils.

- These rocks form from deposits of sand and silt that have weathered or eroded from the land and are carried by rivers to seas and swamps.
- Aquatic organisms and some terrestrial forms were swept into seas and swamps and became trapped in sediment when they died.
- New deposits pile on and compress older sediments below into rock. Sand is compressed into sandstone and mud into shale.
- A small proportion of the organisms left a fossil record.

Fossils usually form from mineral-rich hard parts of organisms (bones, teeth, shells of invertebrates) since most organic substances usually decay rapidly (see Campbell, Figure 25.1 a and b).

- Paleontologists usually find parts of skulls, bone fragments, or teeth; although nearly complete skeletons of dinosaurs and other forms have been found.
- Many of the parts found have been hardened by *petrification*, which occurs when minerals dissolved in groundwater seep into the tissues of dead organisms and replace organic matter (see Campbell, Figure 25.1c).

Some fossils, found as thin films pressed between layers of sandstone or shale, retain organic material.

- Paleontologists have found leaves millions of years old that are still green with chlorophyll and preserved well enough that their organic composition and ultrastructure could be analyzed (see Campbell, Figure 25.1d).
- One research team was even able to clone a very small sample of DNA from an ancient magnolia leaf.

Other fossils found by paleontologists are replicas formed in molds left when corpses were covered by mud or sand (see Campbell, Figure 25.1e).

- Minerals from the water which filled the mold eventually crystallized in the shape of the organism.

Trace fossils form in footprints, animal burrows, and other impressions left in sediments by animal activity. These can provide a great deal of information.

- Dinosaur tracks can provide information about the animals gait, stride length, and speed.

Rarely has an entire organism been fossilized. This only happens if the organism was buried in a medium that prevented bacteria and fungi from decomposing the body (see Campbell, Figure 25.1g).

B. Paleontologists use a variety of methods to date fossils

Several methods are used to determine the age of fossils, which makes them useful in studies of macroevolution.

1. Relative dating

Sedimentation may occur when the sea-level changes or lakes and swamps dry and refill.

- The rate of sedimentation and the types of particles that sediment vary with time when a region is submerged.
- The different periods of sedimentation resulted in formation of rock layers called *strata*.
- Younger strata are superimposed on top of older ones.
- The succession of fossil species chronicles phylogeny, since fossils in each layer represent organisms present at the time of sedimentation.

Strata from different locations can often be correlated by the presence of similar fossils, known as index fossils.

- The shells of widespread marine organisms are the best index fossils for correlating strata from different areas.
- Gaps in the sequence may appear in an area if it was above sea level (which prevents sedimentation) or if it was subjected to subsequent erosion.

Geologists have formulated a sequence of geological periods by comparing many different sites. This sequence is known as the *geological time scale* (see Campbell, Table 25.1).

- These periods are grouped into four eras with boundaries between the eras marking major transitions in the life forms fossilized in the rocks.
- Periods within each era are subdivided into shorter intervals called *epochs*.
- The divisions are not arbitrary, but are associated with boundaries that correspond to times of change.

This record of the rocks presents a chronicle of the relative ages of fossils, showing the order in which species groups evolved.

2. Absolute dating

Absolute dating is not errorless, but it does give the age in years rather than in relative terms (e.g., before, after).

The most common method for determining the age of rocks and fossils on an absolute time scale is *radiometric dating.*

- Fossils contain isotopes of elements that accumulated in the living organisms.
- Since each radioactive isotope has a fixed half-life, it can be used to date fossils by comparing the ratio of certain isotopes (e.g., ^{14}C and ^{12}C) in a living organism to the ratio of the same isotopes in the fossil.
- *Half-life* = The number of years it takes for 50% of the original sample to decay
- The half-life of an isotope is not affected by temperature, pressure or other environmental variables.

Carbon-14 has a half-life of 5600 years, meaning that one-half of the carbon-14 in a specimen will be gone in 5600 years; half of the remaining carbon-14 would disappear from the specimen in the next 5600 years; this would continue until all of the carbon-14 had disappeared (see Campbell, Figure 25.2).

- Thus a sample beginning with 8g of carbon-14 would have 4g left after 5600 years and 2g after 11,200 years.
- Carbon-14 is useful in dating fossils less than 50,000 years old due to its relatively short half-life.

Paleontologists use other radioactive isotopes with longer half-lives to date older fossils.

- Uranium-238 has a half-life of 4.5 billion years and is reliable for dating rocks (and fossils within those rocks) hundreds of millions of years old.
- This isotope was used to place the oldest fossil-containing rocks in the Cambrian period.
- An error of < 10% is present with radioactive dating.

Other methods may also be used to date some fossils.

- Amino acids can have either left-handed (L-form) or right-handed (D-form) symmetry.
- Living organisms only synthesize L-form amino acids to incorporate into proteins.
- After an organism dies, L-form amino acids are slowly converted to D-form.
- The ratio of L-form to D-form amino acids can be measured in fossils.
- Knowing the rate of chemical conversion (*racemization*) allows this ratio to be used in determining how long the organism has been dead.
- This method is most reliable in environments where the climate has not changed significantly, since the conversion is temperature sensitive.

The dating of rocks and fossils they contain has enabled researchers to determine the geological periods (see Campbell, Table 25.1)

C. The fossil record is a substantial, albeit incomplete, chronicle of evolutionary history

A fossil represents a sequence of improbable events:

- An organism had to die in the right place and at the proper time for burial conditions favoring fossilization.
    - The rock layer containing the fossil had to escape geologic events (erosion, pressure, extreme heat) which would have distorted or destroyed the rock.
    - The fossil had to be exposed and not destroyed.
- Someone who knew what they were doing had to find the fossil.

The fossil record is slanted in favor of species that existed for a long time, were abundant and widespread, and had shells or hard skeletons

Paleontologists thus work with an incomplete record for many reasons.

- A large fraction of species that have lived probably left no fossils.
- Most fossils that were formed have probably been destroyed.
- Only a small number of existing fossils have been discovered.

Even though it is incomplete, the fossil record provides the outline of macroevolution, but the evolutionary relationships between modern organisms must be studied to provide the details.

D. Phylogeny has a biogeographical basis in continental drift

Evolution has dimension in space as well as time.

- Biogeography was a major influence on Darwin and Wallace in developing their views on evolution.
- Drifting of continents is the major geographical factor correlated with the spatial distribution of life (see Campbell, Figure 25.3a).

Continental drift results from the movement of great plates of crust and upper mantle that float on the Earth's molten core.

- The relative positions of two land masses to each other changes unless they are embedded on the same plate.
- North America and Europe are drifting apart at a rate of 2 cm per year.
- Where two plates meet (boundaries), many important geological phenomena occur: mountain building, volcanism, and earthquakes (see Campbell, Figure 25.3b).

    Volcanism, in turn, forms volcanic islands (e.g., Galapagos), which opens new environments for founders and adaptive radiation.

Plate movements continually rearrange geography, however, two occurrences had important impacts on life: the formation of *Pangaea* and the subsequent breakup of Pangaea (see Campbell, Figure 25.4).

At the end of the Paleozoic era (250 million years ago), plate movements brought all land masses together into a super-continent called Pangaea.

- Species evolving in isolation where brought together and competition increased.
- Total shoreline was reduced and the ocean basins became deeper (draining much of the remaining shallow coastal seas).
- Marine species (which inhabit primarily the shallow coastal areas) were greatly affected by reduction of habitat.
- Terrestrial organisms were affected as continental interior habitats (and their harsher environments) increased in size.

- Changes in ocean currents would have affected both terrestrial and marine organisms.
- Overall diversity was thus impacted by extinctions and increased opportunities for surviving species.

During the early Mesozoic era (about 180 million years ago) Pangaea began to breakup due to continuing continental drift.

- This isolated the fauna and flora occupying different plates.
- The biogeographical realms were formed and divergence of organisms in the different realms continued.

Many biogeographical puzzles are explained by the pattern of continental separations. For example,

- Matching fossils recovered from widely separated areas.

  Although Ghana and Brazil are separated by 3000 km of ocean, matching fossils of Triassic reptiles have been recovered from both areas.
- Australia has unique fauna and flora.

  Australian marsupials are very diverse and occupy the same ecological roles as placental mammals on other continents.

  Marsupials probably evolved on the portion of Pangaea that is now North America and migrated into the area that would become Australia.

  The breakup of Pangaea isolated Australia (and its marsupial populations) 50 million years ago, while placental mammals evolved and diversified on the other continents.

E. The history of life is punctuated by mass extinctions followed by adaptive radiations of survivors

The evolution of modern life has included long, relatively quiescent periods punctuated by briefer intervals of more extensive turnover in species composition.

- These intervals of extensive turnover included explosive adaptive radiations of major taxa as well as mass extinctions.

1. Examples of major adaptive radiations

The evolution of some novel characteristics opened the way to new *adaptive zones* allowing many taxa to diversify greatly during their early history. For example,

- Evolution of wings allowed insects to enter an adaptive zone with abundant new food sources and adaptive radiation resulted in hundreds of thousands of variations on the basic insect body plan.
- A large increase in the diversity of sea animals occurred at the boundary between the Precambrian and Paleozoic eras. This was a result, in part, of the origin of shells and skeletons in a few key taxa.

  Precambrian rock contains the oldest animals (700 million years old) which were shell-less invertebrates that differed significantly from their successors found in Paleozoic rock.

  Nearly all the extant animal phyla and many extinct phyla evolved in less than 10 million years during the mid-Cambrian (early Paleozoic era).

  Shells and skeletons opened a new adaptive zone by making many new complex body designs possible and altering the basis of predator-prey relationships.

  It is possible that genes controlling development evolved during this time, resulting in a potential for increased morphological complexity and diversity.

An empty adaptive zone can be exploited only if the appropriate evolutionary novelties arise. For example,

- Flying insects existed for 100 million years before the appearance of flying reptiles and birds that fed on them.

Conversely, an evolutionary novelty cannot enable organisms to exploit adaptive zones that are occupied or that do not exist.

- Mass extinctions have often opened adaptive zones and allowed new adaptive radiations.
- For example, mammals existed 75 million years before their first large adaptive radiation in the early Cenozoic. This may have resulted from the ecological void created with the extinction of the dinosaurs.

2. Examples of mass extinctions

Extinction is inevitable in a changing world. The average rate of extinction has been between 2.0 and 4.6 families (each family may include many species) per million years.

Extinctions may be caused by habitat destruction or by unfavorable environmental changes.

- Many very well adapted marine species would become extinct if the ocean's temperature fell only a few degrees.
- Changes in biological factors may cause extinctions even if physical factors remain stable.
- Since many species coexist in each community, an evolutionary change in one species will probably impact other species. For example,

  The evolution of shells by some Cambrian animals may have contributed to the extinction of some shell-less forms.

There have been periods of global environmental change which greatly disrupted life and resulted in mass extinctions.

- During these periods, the rate of extinction escalated to as high 19.3 families per million years.
- These mass extinctions are recognized primarily from the decimation of hard-bodied animals in shallow seas which have the most complete fossil record.
- Two (of about a dozen) mass extinction episodes have been studied extensively by paleontologists.

The Permian extinctions (the boundary between the Paleozoic and Mesozoic eras) eliminated over 90% of the species of marine animals about 250 million years ago (see Campbell, Figure 25.5).

- Terrestrial life was probably also affected greatly. For example, eight of the 27 orders of Permian insects did not survive into the Triassic.
- This mass extinction took place in less than five million years and probably resulted from several factors.

  Occurred about the time Pangaea was formed by the merging of continents which disturbed many habitats and altered the climate.

  A period of extreme vulcanism and resulting volcanic debris (including carbon dioxide) in the atmosphere may have altered the global temperature.

The Cretaceous extinction (the boundary between the Mesozoic and Cenozoic eras) occurred about 65 million years ago.

- More than 50% of the marine species and many terrestrial plants and animals (including dinosaurs) were eliminated.

- During this time the climate was cooling and many shallow seas receded from continental lowlands.
- Increased volcanic activity during this time may have contributed to the cooling by releasing materials into the atmosphere and blocking the sunlight.

Evidence also indicates that an asteroid or comet struck the Earth (*impact hypothesis*) while the Cretaceous extinctions were in progress.

- Iridium, an element rare on earth but common in meteorites, is found in large quantities in the clay layer separating Mesozoic and Cenozoic sediments.
- Walter and Luis Alvarez (and colleagues), after studying this clay layer, proposed that it is fallout from a huge cloud of dust ejected into the atmosphere when an asteroid collided with the Earth.
- This cloud would have both blocked the sunlight and severely disturbed the climate for several months.
- Although the asteroid hit the earth during this time, some researchers feel it did not cause the mass extinction of this period.

The impact hypothesis consisted of two parts: a large asteroid or comet collided with the Earth and the collision caused the Cretaceous extinctions.

- Many forms of evidence support the idea that a large comet or small asteroid collided with Earth 65 million years ago.

  Many craters have been found and indicate that a large number of objects have fallen to the Earth's surface.

  A large crater (≈ 180 km in diameter) located beneath sediments on the Yucatan coast of Mexico has been located.
- Questions about the impact hypothesis are now concentrated on the second part: the collision caused the Cretaceous extinctions.
- Advocates of the impact hypothesis point to several items in their support (see Campbell, Figure 25.6):

  The large size of the impact would darken the Earth for several years and the reduction in photosynthesis output would be sufficient to cause food chains to collapse.

  Severe acid precipitation would result from the increased mineral content of the atmosphere.

  The content of sediments at the upper Cretaceous boundary indicated global fires were occurring and smoke from these fires would increase the atmospheric effects of the impact.
- Opponents of the impact hypothesis hold that the impact occurred within the period of mass extinction, but that the two occurrences are not a cause and effect event.

  Many paleontologists and geologists believe that the climatic changes which occurred were due to continental drift, increased vulcanism, and other processes.

  They also feel these events were sufficient to cause the mass extinction.

Many paleontologists are now trying to determine how sudden and uniform the Cretaceous extinctions were (on a geological time scale).

- Disappearance of diverse groups (from microscopic marine plankton to dinosaurs) during a short time span would support the impact hypothesis.
- A gradual decline with different groups disappearing at different rates, would support hypotheses emphasizing terrestrial causes.

- It is possible that the impact was a final, sudden event in environmental changes that were affecting the biota of the late Cretaceous.

Mass extinctions, whatever the cause, profoundly affect biological diversity.

- Not only are many species eliminated, but those that survive are able to undergo new adaptive radiations into the vacated adaptive zones and produce new diversity.

II. Phylogeny and Systematics

One of the main goals of systematics is to make biological classification reflect phylogeny.

A. Taxonomy employs a hierarchical system of classification

The taxonomic system used today was developed by Linnaeus in the eighteenth century. This system has two main features: the assignment of a binomial to each species and a filing system for grouping species (see Campbell, Figure 25.7).

The *binomial* (two part Latin name) assigned to each species is unique to that species.

- The first word of the binomial is the *genus* (pl. genera); the second word is the *specific epithet* of the species.
- The scientific name of a species combines the genus and specific epithet.
- Each genus can include many species of related organisms. For example, *Felis silvestris* is the domestic cat; *Felis lynx* is the lynx.
- Use of the scientific name defines the organism referred to and removes ambiguity.

The filing system for grouping species into a hierarchy of increasingly general categories formalizes the grouping of organisms.

- Binomial nomenclature is the first step in grouping: similar species are grouped in the same genus.
- The system then progresses into broader categories:

  Similar genera are grouped into the same family.

  Families are grouped into orders.

  Orders are grouped into classes.

  Classes are grouped into phyla.

  Phyla are grouped into kingdoms.
- Each taxonomic level is more inclusive than the one below. The more closely related two species are, the more levels they share:

Category	Domestic Cat	Bobcat	Lion	Dog
specific epithet	silvestris	rufus	leo	familiaris
genus	Felis	Felis	Panthera	Canis
family	Felidae	Felidae	Felidae	Canidae
order	Carnivora	Carnivora	Carnivora	Carnivora
class	Mammalia	Mammalia	Mammalia	Mammalia
phylum	Chordata	Chordata	Chordata	Chordata
kingdom	Animalia	Animalia	Animalia	Animalia

The two main objectives of taxonomy are to sort out and identify closely related species and to order species into the broader taxonomic categories.

- In sorting, closely related organisms are assigned to separate species (with the proper binomial) and described using the diagnostic characteristics which distinguish the species from one another.
- In categorizing, the species are grouped into broader categories from genera to kingdoms.
- In some cases, intermediate categories (e.g., subclasses; between orders and classes) are also used.
- The named taxonomic unit at any level is called a *taxon* (pl. taxa).
- Rules of nomenclature have been established: the genus name and specific epithet are italicized, all taxa from the genus level and higher are capitalized.

B. The branching pattern of a phylogenetic tree represents the taxonomic hierarchy

The goal of systematics is to have classification reflect the evolutionary affinities of species. The taxonomic hierarchy is set up to fit evolutionary history (see Campbell, Table 25.2).

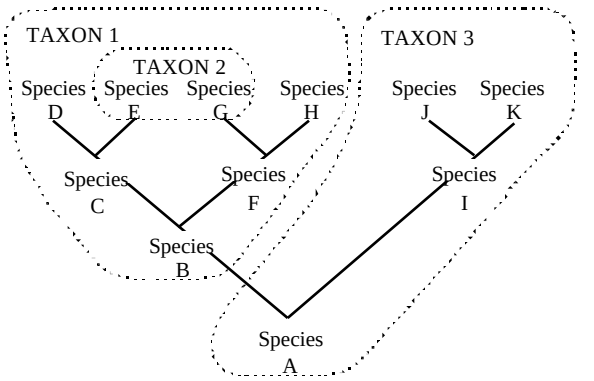
- In general, groups subordinate to other groups in the taxonomic hierarchy should represent finer and finer branching of phylogenetic trees (see Campbell, Figure 25.8)

Classification schemes and phylogenetic trees are hypotheses of history based on current data. Like all hypotheses, they may be refined with further study.

C. Determining monophyletic taxa is key to classifying organisms according to their evolutionary history

In order for a classification scheme to reflect the evolutionary history of an organism, the species must be grouped into taxa that are monophyletic.

- A *monophyletic taxon* is one where a single ancestor gave rise to all species in that taxon and to no species placed in any other taxon. For example, Family Ursidae evolved from a common ancestor (Taxon 1) (see also Campbell, Figure 25.9).



By contrast, other kinds of taxa do not accurately reflect evolutionary history

- A *polyphyletic taxon* is one whose members are derived from two or more ancestral forms not common to all members. For example, Kingdom Plantae includes both vascular plants and mosses which evolved from different algal ancestors (Taxon 2).
- A *paraphyletic taxon* is one that excludes species that share a common ancestor that gave rise to the species included in the taxon. For example, Class Reptilia excludes the Class Aves although a reptilian ancestor common to all reptiles is shared (Taxon 3).

1. Sorting homology from analogy

Systematists classify species into higher, preferably monophyletic, taxa based on the extent of similarities in morphology and other characteristics.

Homology = Likeness attributed to shared ancestry

- The forelimbs of mammals are homologous, they share a similarity in the skeletal support that has a genealogical basis.
- Homology must be distinguished from analogy in evolutionary trees.

Analogy = Similarities due to *convergent evolution,* not common ancestry

Convergent evolution = Acquisition of similar characteristics in species from different evolutionary branches due to sharing similar ecological roles with natural selection shaping analogous adaptations (see Campbell, Figure 25.10)

The distinction between homology and analogy is sometimes relative.

Example: The wings of birds and bats are modifications of the vertebrate forelimb.

The appendages are thus homologous.

As wings they are analogous since they evolved independently from the forelimbs of different flightless ancestors.

- Insect wings and bird wings are analogous.

  They evolved independently and are constructed from entirely different structures.
- Convergent evolution has produced analogous similarities between Australian marsupials and placental mammals on other continents.

Homology must be sorted from analogy to reconstruct phylogenetic trees on the basis of homologous similarities.

- Generally, the greater the amount of homology, the more closely related the species and this should be reflected in their classification.
- Adaptation and convergence often obscure homologies, although studies of embryonic development can expose homology that is not apparent in mature structures.
- Additionally, the more complex two similar structures are, the less likely it is that they have evolved independently.

Example: The skulls of humans and chimpanzees are composed of many bones which are fused together and match almost perfectly. It is unlikely such a complex structure would have evolved independently in separate groups.

D. Molecular biology provides powerful new tools for systematics

Molecular comparisons of proteins and DNA have added another useful method for studying the evolutionary relationships between species.

- Inherited nucleotide sequences in DNA program the corresponding sequences of amino acids in proteins.
- Examination of these macromolecules provide much information about evolutionary relationships.

Molecular comparisons are:

- Objective and quantitative
- Used to assess relationships between species so distantly related that no morphological similarities exist

1. Protein comparisons

The primary structure of proteins is genetically programmed and a similarity in the amino acid sequence of two proteins from different species indicates that the genes for those proteins evolved from a common gene present in a shared ancestor.

Studies of cytochrome *c* (an ancient protein common to all aerobic organisms) have been used to compare many diverse species.

- The amino acid sequence has been determined for species ranging from bacteria to complex plants and animals.
- The sequence in cytochrome *c* is identical in chimpanzees and humans.
- The sequence in humans and chimpanzees differs at only one of the 104 amino acid positions in the rhesus monkeys.
- Chimpanzees, humans, and rhesus monkeys belong to the Order Primates.

  Comparing these sequences with nonprimate species shows greater differences (13 with the dog and 20 with the rattlesnake).
- Phylogenetic trees based on cytochrome *c* are consistent with evidence from comparative anatomy and the fossil record.

One disadvantage to the use of amino acid data for phylogenetic studies is that such sequences provide information only about those genes that code for protein; this can be a small fraction of the genome (e.g., 2% in humans)

2. DNA and RNA comparisons

The most direct measure of common inheritance from shared ancestors is a comparison of the genes or genomes of two species. Comparison can be made by three methods: DNA-DNA hybridization, restriction maps, and DNA sequencing.

DNA-DNA hybridization can compare whole genomes by measuring the degree of hydrogen bonding between single-stranded DNA obtained from two sources.

- DNA is extracted from different species and the complementary strands separated by heating.
- The single-stranded DNA from the two species is mixed and cooled to allow double-stranded DNA reformation which results from hydrogen bonding.
- The hybrid DNA is then reheated to separate the double strands.
- The temperature necessary to separate the hybrid DNA is indicative of the similarity in the DNA from the two species.

  The temperature correlation is based on the degree of bonding between the strands of the two species with more bonding occurring with greater similarity.

  The more extensive the pairing, the more heat is needed to separate the hybrid strand.
- Evolutionary trees constructed through DNA-DNA hybridization usually agree with those based on other methods, however, this technique is very beneficial in settling taxonomic debates that have not been finalized by other methods.

Restriction maps provide precise information about the match-up of specific DNA nucleotide sequences.

- Restriction enzymes are used to cut DNA into fragments which can be separated by electrophoresis and compared to restriction fragments of other species.
- Two samples of DNA with similar maps for the locations of restriction sites will produce similar collections of fragments.
- The greater the divergence of two species from the common ancestor, the greater the differences in restriction sites and less similarity of the restriction fragments.
- This method works best when comparing small fragments of DNA.

- Mitochondrial DNA (mtDNA) is best suited for this type of comparison since it is smaller than nuclear DNA (produces smaller fragments) and mutates about ten times faster than nuclear DNA.
- The faster mutation rate of mtDNA allows it to be used to determine phylogenetic relationships between not only closely related species, but also populations of the same species.

  mtDNA was used to establish the close relationship among the Pima, Mayan, and Yanomami groups of Native Americans.

  The results supported linguistic evidence that these groups descended from the first wave of immigrants to cross the Bering land bridge from Asia during the late Pleistocene.

DNA sequence analysis is the most precise method of comparing DNA as it determines the actual nucleotide sequence of a DNA segment.

- Uses polymerase chain reaction (PCR) technology to clone traces of DNA.
- PCR is coupled with automated sequencing to provide a simpler and faster method of collecting sequence data.
- DNA sequencing and comparisons show exactly how much divergence there has been in the evolution of two genes derived from the same ancestral gene.
- Ribosomal RNA (rRNA) sequencing is a similar technique which can provide information about some of the earliest branching in phylogenetic relationships since DNA coding for rRNA changes very slowly.
- rRNA sequencing has been very useful in examining the relationships among bacteria.

3. Identifying and comparing homologous DNA sequences

Comparing nucleotide sequences between corresponding DNA segments from different species has the potential of telling us how much divergence there has been in the evolution of two genes derived from the same gene.

To measure differences between two species, it is necessary to identify homologous nucleotide sequences Following sequencing, the sequences are aligned and evaluated.

- Common ancestry is clear when two sequences of the same gene from two species that have diverged very recently are the same or differ by only a few bases.
- Mutations tend to accumulate as species diverge; the number of differences is a measure of evolutionary distance.
- Once a match is achieved, usually with the aid of computer programs, the investigator can develop a phylogenetic hypothesis (see Campbell, Figure 25.12).

4. Molecular clocks

Different proteins and nucleic acids evolve at different rates, although each type of molecule evolves at a relatively constant rate over time.

When comparing homologous proteins and nucleotide sequences from taxa that are known to have diverged from common ancestors, the number of amino acid substitutions is proportional to the elapsed time since divergence.

> Example: Homologous proteins of bats and dolphins are more similar than those of sharks and tuna.

- This is consistent with fossil evidence showing that tuna and sharks have been separated much longer than bats and dolphins.

DNA comparisons may be even more reliable than protein comparisons.

- Phylogenetic branching based on nucleotide substitutions in DNA generally approximates dates determined from the fossil record.
- The difference in DNA between two taxa is more closely correlated with the time since divergence than is morphological difference.

Molecular clocks (DNA and protein) are calibrated by graphing the number of nucleotide or amino acid differences against the times for a series of evolutionary branch points known from the fossil record.

- The graph can then be used to determine the time of divergence between taxa for which no substantial fossil record is available.

The assumption that mutation rates for genes (and their protein products) are relatively constant is the basis for using molecular clocks in evolutionary biology.

- This assumption is relatively solid when comparing groups of closely related species.
- Molecular clocks are less reliable when comparing more distantly related groups since differences in generation times and metabolic rates affect mutation rates.

Among closely related species, the constant mutation rate for specific genes implies that the accumulation of selectively neutral mutations changes the genome more than adaptive mutations.

- Many evolutionary biologists doubt the prevalence of neutral variation, so they also question the use of molecular clocks to accurately date the time of divergence.
- There is less skepticism about the value of molecular clocks for determining the relative sequence of branch points in phylogeny.
- Modern systematists use available molecular data along with all other evidence to reconstruct phylogeny.

E. The search for fossilized DNA continues despite recent setbacks: *science as a process*

Science is a dynamic process that continuous tests hypotheses; sometime the hypotheses are supported and sometimes they are rejected

The nucleotide sequences in DNA traces recovered from fossils that retain organic material can be analyzed by using PCR.

- Fossilized DNA from 17 million-year-old magnolia leaves was first reported in 1990 .
- Since 1990, DNA fragments have been sequenced from a frozen mammoth (40,000 years old), an insect fossilized in amber (40 million years old), a 65-million-year-old *Tyrannosaurus rex* from Montana, a 30,000-year old fossil arm bone from an extinct member of the human family, and a frozen Stone Age man (5000 years old).

It now appears that most of the DNA first reported to be ancient is actually DNA from contaminating fungus or other organisms.

III. The Science of Phylogenetic Systematics

The two significant features of a phylogenetic tree are the location of branch points along the tree and the degree of divergence between branches.

- The locations of branch points along the tree symbolize the relative times of origin for different taxa.
- The degree of divergence between branches represents how different two taxa have become since branching from a common ancestor.

Initially, phylogenies were devised largely on morphology and were considered by many to be too subjective. In the 1960s, new computational technology helped usher in two new, more objective analytical approaches: phenetics and cladistics.

A. Phenetics increased the objectivity of systematic analysis

Phenetics makes no evolutionary assumptions and decides taxonomic affinities entirely on the basis of measurable similarities and differences.

- A comparison is made of as many characters (anatomical characteristics) as possible without attempting to sort homology from analogy.
- Pheneticists feel that the contribution of analogy to overall similarity will be overridden by the degree of homology if enough characters are compared.
- Critics of phenetics argue that overall phenotypic similarity is not a reliable index of phylogenetic proximity.
- While supported by few systematists, the emphasis of phenetics on multiple quantitative comparisons has made important contributions to systematics.

    Especially useful for analyzing DNA sequence data and other molecular comparisons between species.

B. Cladistic analysis uses novel homologies to define branchpoints on phylogenetic trees

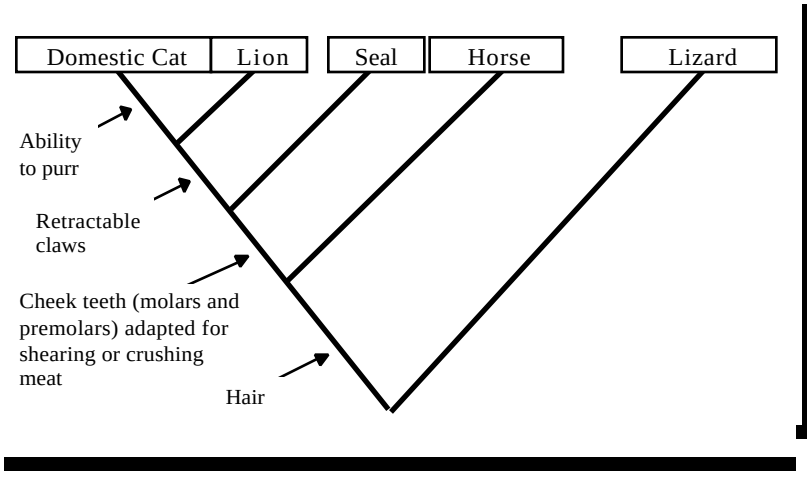
Cladistic analysis, which has become synonymous with phylogenetic systematics, classifies organisms according to the order in time that branches arise along a phylogenetic tree, without considering the degree of divergence.

- This produces a cladogram, a dichotomous tree that branches repeatedly.

    Each branch point is defined by novel homologies unique to the various species on that branch or *clade*.

1. Outgroup comparison

Each taxon has a mixture of primitive characters that existed in the common ancestor and characters that evolved more recently.



- The sharing of primitive characters indicates nothing about the pattern of evolutionary branching from a common ancestor.

Cladistic analysis uses a concept called outgroup comparison as an objective means of defining the roots of a phylogenetic tree and thereby distinguishing among the shared characters that are more primitive (see Campbell, Figures 25.13 and 25.14).

Outgroup = A species or group of species that is relatively closely related to a group of species being studied, but clearly not as closely related as any study-group member is to any other study-group member

- All members of the study group are compared as a whole to the outgroup

- Characters common to the outgroup and group being studied are likely to have been present in a common ancestor and are considered shared primitive characters

2. Use of synapomorphies (shared derived characters) and parsimony

A major difficulty in cladistic analysis is finding characters that are appropriate for identifying each branch point.

- Branch points of a phylogenetic tree can be identified by finding shared derived characters, or *synapomorphies*.
- These are homologies that evolved in an ancestor common to all species on one branch of a fork of the tree, but not common to the other branch.

A guiding principle of cladistic analysis is parsimony, the quest for the simplest, and probably most likely, explanation for observed phenomena.

- In systematics, parsimony means that a phylogenetic tree using the minimum number of changes to illustrate evolutionary relationships has the greatest likelihood of being correct.

3. Acceptance of only monophyletic taxa

By focusing on phylogenetic branching, cladistic analysis accepts only monophyletic taxa. As a result, some taxonomic surprises are produced by cladistic systematics:

- The branch point between crocodiles and birds is more recent than between crocodiles and other reptiles (a fact also supported by the fossil record).

  Crocodiles and birds have synapomorphies not present in lizards and snakes.
- In a strict cladistic analysis, the Class Aves and the Class Reptilia, as we know them now, would be eliminated.

  The birds would be included in a cladogram of the animals we know as reptiles.
- Birds are deemed superficially different because of the morphological changes associated with flight which have developed since their divergence from reptilian ancestors.
- Continued debate over how life evolved is indicative that evolutionary biology is an active science.

C. Phylogenetic systematics relies on both morphology and molecules

Modern systematics depends on cladistic analysis to formulate hypotheses about the history of life.

Cladistic analysis now relies upon both morphology and on molecular data; the strongest support for any phylogenetic hypothesis is agreement between data derived from both of these sources.

- Scientists have been collecting phenotypic information about living and extinct organisms for centuries. The amount of this morphological information exceeds by far the current molecular data.
- Molecular databases are expanding rapidly. While no means complete, the genome contains more information about an organism's evolutionary history than its anatomical features.

Phylogenetic biology, or the application of cladistic analysis in the study of evolutionary history and its relations to all aspects of life, pervades virtually every field of biology.

- Physiology - evolution of body temperature regulation
- Plant development - evolution of genes regulating flower development

- Behavior - evolutionary history of animal social systems
- Conservation biology - evaluates genetic differences among populations of endangered species

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CHAPTER 26

EARLY EARTH AND THE ORIGIN OF LIFE

OUTLINE

I. Introduction to the History of Life
   A. Life on Earth originated between 3.5 and 4.0 billion years ago
   B. Major episodes in the history of life: *a preview*

II. Prebiotic Chemical Evolution and the Origin of Life
   A. The first cells may have originated by chemical evolution on a young Earth: *an overview*
   B. Abiotic synthesis of organic monomers is a testable hypothesis: *science as a process*
   C. Laboratory simulations of early Earth conditions have produced organic polymers
   D. Protobionts can form by self-assembly
   E. RNA was probably the first genetic material
   F. The origin of hereditary information made Darwinian evolution possible
   G. Debate about the origin of life abounds

III. The Major Lineages of Life
   A. Arranging the diversity of life into the highest taxa is a work in progress

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Provide at least two lines of evidence for the antiquity of life.
2. Describe the contributions that A.I. Oparin, J.B.S. Haldane, Stanley Miller and Harold Urey made towards developing a model for abiotic synthesis of organic molecules.
3. Provide plausible evidence to support the hypothesis that chemical evolution resulting in life's origin occurred in four stages:
   a. Abiotic synthesis of organic monomers
   b. Abiotic synthesis of polymers
   c. Formation of protobionts
   d. Origin of genetic information
4. Describe the basis for Whittaker's five-kingdom system.
5. Describe three alternatives to the five-kingdom system and explain the rationale for each.

KEY TERMS

stromatolites protobionts ribozyme

LECTURE NOTES

The history of living organisms and the history of Earth are inextricably linked.

Examples:

- Formation and subsequent breakup of Pangaea affected biotic diversity.
- The first photosynthetic organisms released oxygen into the air and altered Earth's atmosphere.
- *Homo sapiens* has changed the land, water and air on a scale and at a rate unprecedented for a single species.

In order to reconstruct life's history, scientists use evidence from:

- The fossil record, which is less complete the older the strata studied. In fact, there is no fossil record for the seminal episode of the origin of Earth's life.
- Contemporary organisms which, in their molecules and anatomy, carry traces of their evolutionary histories.

I. Introduction to the History of Life

A. Life on Earth originated between 3.5 and 4.0 billion years ago

Life probably appeared relatively early in the Earth's history. Scientists have found isotopes of carbon in 3.8 billion year old rocks in Greenland.

Because of the relatively simple structure of prokaryotes, it is reasonable to assume that the earliest organisms were prokaryotes. The fossil record supports this notion.

- Fossils similar to spherical and filamentous prokaryotes have been recovered from stromatolites 3.5 billion years old in western Australia and southern Africa (see Campbell, Figure 26.2).

Stromatolites = Banded domes of sediment similar to the layered mats constructed by colonies of bacteria and cyanobacteria currently living in salty marshes (see Campbell, Figure 26.1)

- The western Australian fossils appear to be of photosynthetic organisms, which indicates life evolved before these organisms lived—perhaps some 4 billion years ago.
- Other fossils similar to the prokaryotes have been recovered from the Fig Tree Chert rock formation in southern Africa which date to 3.4 billion years.

B. Major episodes in the history of life: *a preview*

Campbell, Figure 26.3, is a diagram of some major episodes in the history of life.

Fossil evidence suggests that prokaryotes appeared at least 2 billion years before the oldest eukaryotes

- Two distinct groups of prokaryotes, Bacteria and Archaea, diverged early, between 2 to 3 billion years ago.
- Photosynthetic bacteria started the production of oxygen about 2.5 billion years ago, setting the stage for aerobic life.

Eukaryotes emerged some 2 billion years ago

- Strong evidence supports the hypothesis that eukaryotic cells evolved from a symbiotic community of prokaryotes

Plants, fungi, and animals arose from distinct groups of unicellular eukaryotes during the Precambrian.

- Plants evolved from green algae.

- Fungi and animals arose from different groups of heterotrophic unicells. Based on molecular evidence, fungi are more closely related to animals than they are to plants.

The oldest fossils of animals are those of soft-bodied invertebrates from about 700 million years ago. The basic body plans of most of the modern animal phyla probably arose in the late Precambrian.

The transition from the aquatic environment to land was a pivotal point in the history of life.

- The first terrestrial colonization was by plants and fungi some 475 million years ago; the move may have depended upon a beneficial association between the two groups.
- The transformation of the landscape by plants created new opportunities for all forms of life.

II. Prebiotic Chemical Evolution and the Origins of Life

A. The first cells may have originated by chemical evolution on a young Earth: *an overview*

Life originated between 3.5 and 4.0 billion years ago. During this timespan the Earth's crust began to solidify (4.1 billion) and bacteria advanced enough to build stromatolites (3.5 billion).

The origin of life was possible in Earth's ancient environment, which was different from today:

- There was little atmospheric oxygen.
- Lightning, volcanic activity, meteorite bombardment, and ultraviolet radiation were more intense.

One hypothesis about the first living organisms is that they were the products of a chemical evolution that occurred in four stages:

1. Abiotic synthesis and accumulation of monomers, or small organic molecules, that are the building blocks for more complex molecules
2. Joining of monomers into polymers (e.g., proteins and nucleic acids)
3. Formation of *protobionts*, droplets which formed from aggregates of abiotically produced molecules and which differed chemically from their surroundings
4. Origin of heredity during or before protobiont appearance.

B. Abiotic synthesis of organic monomers is a testable hypothesis: *science as a process*

In the 1920', A.I. Oparin and J.B.S. Haldane independently postulated that the reducing atmosphere and greater UV radiation on primitive Earth favored reactions that built complex organic molecules from simple monomers as building blocks. This is not possible today because:

- Oxygen in Earth's oxidizing environment attacks chemical bonds, removing electrons. An important characteristic of the early atmosphere must have been the rarity of oxygen.
- The modern atmosphere has a layer of ozone that screens UV radiation, so the energy required to abiotically synthesize organic molecules is not available. On primitive Earth, energy was available from frequent lightning and intense UV. radiation that penetrated the atmosphere.

Stanley Miller and Harold Urey tested the Oparin/Haldane hypothesis (see Campbell, Figure 26.4). They simulated conditions on early Earth by constructing an apparatus containing H_2O, H_2, CH_4 and NH_3.

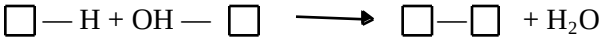
- Their simulated environment produced some amino acids and other organic molecules.
- Now we know the atmosphere of early Earth probably included CO, CO_2, and N_2, and was less reducing than the Miller-Urey model, and thus, less favorable to formation of organic compounds.
- Additional experiments have produced all 20 amino acids, ATP, some sugars, lipids and purine and pyrimidine bases of RNA and DNA.

C. Laboratory simulations of early Earth conditions have produced organic polymers

The forming of complex organic molecules, or polymers, from simpler building-block molecules may have been inevitable on the primitive Earth.

Polymers = Chains of similar building blocks or monomers

Polymers are synthesized by dehydration (condensation) reactions. For example:



- H and OH groups are removed from the monomers.
- H_2O is produced as a by-product.

Abiotic polymerization reactions in early-Earth conditions must have occurred:

- Without the help of enzymes
- With dilute solutions of monomers (spontaneous dehydration reactions that produce water would be unlikely in already dilute solutions)

Abiotic polymerization does occur with dilute solutions of monomers under certain laboratory conditions:

- Dilute solutions of organic monomers are dripped onto hot sand, clay, or rock. Water vaporizes and concentrates the monomers on the substrate.
- Sidney Fox (University of Miami) used this method to abiotically produce polypeptides called proteinoids.

Clay may have been an important substrate for abiotic synthesis of polymers since:

- Monomers bind to charged sites in clay, concentrating amino acids and other monomers.
- Metal ions (e.g., iron and zinc) could catalyze dehydration reactions.
- The binding sites on clay could have brought many monomers close together and assisted in forming polymers.
- Pyrite (iron and sulfur) may also have been an important substrate. It has a charged surface and electrons freed during its formation could support bonding between molecules.

D. Protobionts can form by self-assembly

Living cells may have been preceded by protobionts.

Protobionts = Aggregates of abiotically produced molecules able to maintain an internal environment different from their surroundings and exhibiting some life properties such as metabolism and excitability

There is experimental evidence for the spontaneous formation of protobionts:

- When mixed with cool water, proteinoids self-assemble into microspheres (see Campbell, Figure 26.5a) surrounded by a selectively permeable protein membrane. These microspheres:

  Undergo osmotic swelling and shrinking

  Have potential energy in the form of a membrane potential
- Liposomes can form spontaneously when phospholipids form a bilayered membrane similar to those of living cells.

- Coacervates (colloidal drops of polypeptides, nucleic acids, and polysaccharides) self-assemble.

E. RNA was probably the first genetic material

Today's cells transcribe DNA into RNA, which is then translated into proteins. This chain of command must have evolved from a simpler mechanism of heritable control.

- One hypothesis is that before DNA, there existed a primitive mechanism for aligning amino acids along RNA molecules, which were the first genes. Evidence to support this hypothesis includes:

  RNA molecules may have been able to self-replicate. Short polymers of ribonucleotides that can base pair (5 – 10 bases without enzyme, up to 40 bases with zinc added as catalyst) have been produced abiotically in test tubes (see Campbell, Figure 26.6).

  RNA is autocatalytic, as indicated by *ribozymes* (RNA that acts as a catalyst to remove introns, or catalyze synthesis of mRNA, tRNA or rRNA).
- RNA folds uniquely depending on sequence (unlike DNA), thereby providing raw materials for natural selection—different molecular shapes (phenotypes) varying in stability and catalytic properties. Replication errors (mutations) probably created additional variation within families of closely related sequences.
- In addition to molecular competition, molecular cooperation probably evolved as RNA-directed protein synthesis produced short polypeptides that catalyzed RNA replication.
- Once this simple machinery for replication and translation of genetic information became sequestered into membrane-bound protobionts, molecular cooperation could be refined as natural selection acted on the level of the entire protobiont.

F. The origin of hereditary information made Darwinian evolution possible

Perhaps this hypothetical membrane-bound protobiont:

- Incorporated genetic information
- Selectively accumulated monomers from its surroundings
- Used enzymes programmed by genes to make polymers and carry out other chemical reactions
- Grew and split, distributing copies of its genes to offspring

If these cell precursors could also grow, divide, and distribute genes to offspring, the descendant protobionts would vary because of errors in the copying of RNA (mutations).

- The variation among related protobionts would be subject to natural selection.
- Evolution in the Darwinian sense—differential reproductive success—presumably accumulated refinements to primitive metabolism and inheritance, including the appearance of DNA as the hereditary material.

  Initially, RNA could have provided the template to produce DNA.

  Because it is more stable, DNA would have replaced RNA as the store of genetic information.

  RNA's role would change as it became an intermediate in translation.

G. Debate about the origin of life abounds

No one knows how life actually began on Earth. The chemical evolution described and supporting lab simulations indicate key steps that could have occurred.

Several alternatives have been proposed.

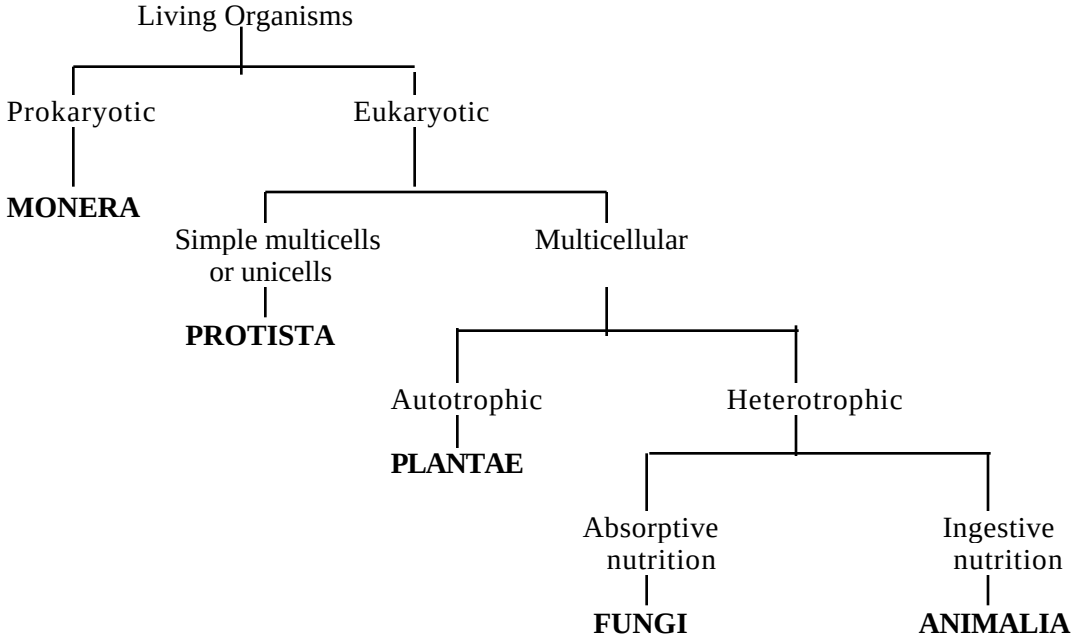
- Panspermia - Some organic compounds may have reached Earth by way of meteorites and comets. Organic compounds (e.g., amino acids) have been recovered from modern meteorites. These extraterrestrial organic compounds may have contributed to the pool of molecules which formed early life.
- Most researchers believe life first appeared in shallow water or moist sediments. Some now feel the first organisms developed on the sea floor due to the harsh conditions on the surface during that time. This position was strengthened in the 1970s by discovery of the deep sea vents. Hot water and minerals emitted from such vents may have provided the energy and chemicals necessary for early protobionts.
- Simpler hereditary systems may have preceded nucleic acid genes. Julius Rebek synthesized a simple organic molecule in 1991. The importance of this molecule was that it served as a template for self-replication (see Campbell, Figure 26.8). This discovery supported the idea held by some biologists that RNA strands are too complicated to be the first self-replicating molecules.

III. The Major Lineages of Life

A. Arranging the diversity of life into the highest taxa is a work in progress

Systematists have traditionally considered the kingdom to be the highest, most inclusive taxonomic category.

- The two kingdom system (animals and plants) long prevailed, but was not suitable as biologists learned more about the structures and life histories of different organisms.
- The five kingdom system was proposed by Robert H. Whittaker (1969) and modified by Lynn Margulis (see also Campbell, Figure 26.9).



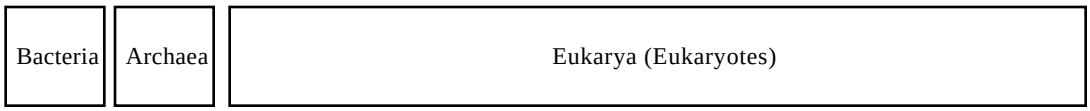
Classifying living systems is a work in progress that reflects our increased understanding of the phylogeny of living organisms.

- Using the tools of molecular systematics, biologists have gathered new data that leads them to challenge the traditional five-kingdom system.

- This new information has reopened issues of biological diversity at the highest taxonomic levels. Three alternative classification systems are outlined below (see also Campbell, Figure 26.10):

Six-kingdom system. The prokaryotes are split into two kingdoms based on molecular evidence for an early evolutionary divergence between eubacteria and archaebacteria.

Three-domain system. This scheme assigns more significance to the ancient evolutionary split between eubacteria and archaebacteria by using a superkingdom taxon called the *domain.* The domain Eukarya includes four kingdoms of eukaryotic organisms.



Eight-kingdom system. In addition to two separate prokaryotic kingdoms, this system also splits the protists into three kingdoms.

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CHAPTER 27

PROKARYOTES AND THE ORIGINS OF METABOLIC DIVERSITY

OUTLINE

I. The World of Prokaryotes
   A. They're (almost) everywhere! *an overview of prokaryotic life*
   B. Bacteria and Archaea are the two main branches of prokaryotic evolution

II. Structure, Function, and Reproduction
   A. Nearly all prokaryotes have cell walls external to their plasma membranes
   B. Many prokaryotes are motile
   C. The cellular and genomic organization of prokaryotes is fundamentally different from that of eukaryotes
   D. Populations of prokaryotes grow and adapt rapidly

III. Nutritional and Metabolic Diversity
   A. Prokaryotes can be grouped into four categories according to how they obtain energy and carbon
   B. The evolution of prokaryotic metabolism was both cause and effect of changing environments on Earth

IV. Phylogeny of Prokaryotes
   A. Molecular systematics is leading to a phylogenetic classification of prokaryotes

V. Ecological Impact of Prokaryotes
   A. Prokaryotes are indispensable links in the recycling of chemical elements in the ecosystem
   B. Many prokaryotes are symbiotic
   C. Humans use prokaryotes in research and technology

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. List unique characteristics that distinguish archaea from bacteria.
2. Describe the three-domain system of classification and explain how it differs from previous systems.
3. Using a diagram or micrograph, distinguish among the three most common shapes of prokaryotes.
4. Describe the structure and functions of prokaryotic cell walls.
5. Distinguish between the structure and staining properties of gram-positive and gram-negative bacteria.
6. Explain why disease-causing gram-negative bacterial species are generally more pathogenic than disease-causing gram-positive bacteria.

7. Describe three mechanisms motile bacteria use to move.
8. Explain how prokaryotic flagella work and why they are not considered to be homologous to eukaryotic flagella.
9. Indicate where photosynthesis and cellular respiration take place in prokaryotic cells.
10. Explain how organization of the prokaryotic genome differs from that in eukaryotic cells.
11. Explain what is meant by geometric growth.
12. List the sources of genetic variation in prokaryotes and indicate which one is the major source.
13. Distinguish between autotrophs and heterotrophs.
14. Describe four modes of bacterial nutrition and give examples of each.
15. Distinguish among obligate aerobes, facultative anaerobes and obligate anaerobes.
16. Describe, with supporting evidence, plausible scenarios for the evolution of metabolic diversity of prokaryotes
17. Explain how molecular systematics has been used in developing a classification of prokaryotes.
18. List the three main groups of archaea, describe distinguishing features among the groups and give examples of each.
19. List the major groups of bacteria, describe their mode of nutrition, some characteristic features and representative examples.
20. Explain how endospores are formed and why endospore-forming bacteria are important to the food-canning industry.
21. Explain how the presence of *E. coli* in public water supplies can be used as an indicator of water quality.
22. State which organism is responsible for the most common sexually transmitted disease in the United States.
23. Describe how mycoplasmas are unique from other prokaryotes.
24. Explain why all life on earth depends upon the metabolic diversity of prokaryotes.
25. Distinguish among mutualism, commensalism and parasitism.
26. List Koch's postulates that are used to substantiate a specific pathogen as the cause of a disease.
27. Distinguish between exotoxins and endotoxins.
28. Describe how humans exploit the metabolic diversity of prokaryotes for scientific and commercial purposes.
29. Describe how *Streptomyces* can be used commercially.

KEY TERMS

bacteria
archaea
domains
domain Archaea
domain Bacteria
peptidoglycan
Gram stain
gram-positive
gram-negative
capsule
nucleoid region
binary fission
transformation
conjugation
transduction
endospores
antibiotics
photoautotrophs
chemoautotrophs
photoheterotrophs
parasites
nitrogen fixation
obligate aerobes
facultative anaerobes
obligate anaerobes
anaerobic respiration
bacteriorhodopsin
cyanobacteria
signature sequences
methanogens
decomposers
symbiosis
symbionts
host
mutualism
commensalism
parasitism
parasite
Koch's postulates
exotoxins

pili (sing., pilus)	chemoheterotrophs	extreme halophiles	endotoxins
taxi	saprobes	extreme thermophiles	

LECTURE NOTES

Appearing about 3.5 billion years ago, prokaryotes were the earliest living organisms and the only forms of life for 2 billion years.

I. The World of Prokaryotes

A. They're (almost) everywhere! *an overview of prokaryotic life*

Prokaryotes dominate the biosphere; they are the most numerous organisms and can be found in all habitats.

- Approximately 4000 species are currently recognized, however, estimates of the actual diversity range from 400,000 – 4 million species
- They are structurally and metabolically diverse.

Prokaryotic cells differ from eukaryotic cells in several ways:

- Prokaryotes are smaller and lack membrane-bound organelles.
- Prokaryotes have cell walls but the composition and structure differ from those found in plants, fungi and protists.
- Prokaryotes have simpler genomes. They also differ in genetic replication, protein synthesis, and recombination.

Prokaryotes, while very small, have a tremendous impact on the Earth.

- A small percentage cause disease.
- Some are decomposers, key organisms in life-sustaining chemical cycles.
- Many form symbiotic relationships with other prokaryotes and eukaryotes. Mitochondria and chloroplasts may have evolved from such symbioses.

B. Bacteria and Archaea are the two main branches of prokaryotic evolution

The traditional five-kingdom system recognizes one kingdom of prokaryotes (Monera) and four kingdoms of eukaryotes (Protista, Plantae, Fungi, and Animalia).

- This system emphasizes the structural differences between prokaryotic and eukaryotic cells.

Recent research in systematics has resulted in questions about the placement of a group as diverse as the prokaryotes in a single kingdom. Two major branches of prokaryotic evolution have been indicated by comparing ribosomal RNA and other genetic products:

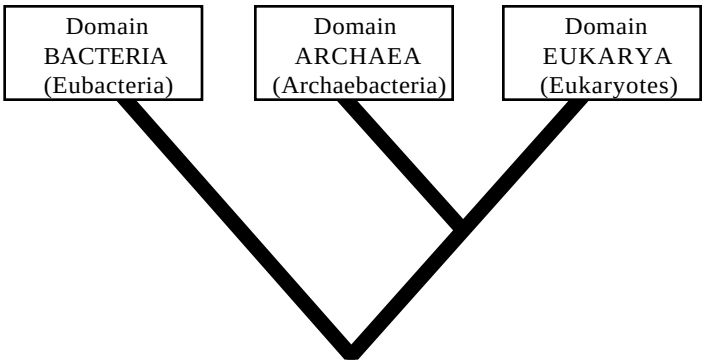
- One branch is called the Archaea (formerly archaebacteria).
  - Believed to have evolved from the earliest cells
  - Inhabit extreme environments which may resemble the Earth's early habitats (hot springs and salt ponds)
- The second branch is called Bacteria (formerly eubacteria).
  - Considered the more "modern" prokaryotes, having evolved later in Earth's history
  - More numerous than Archaea
  - Differ from Archaea in structural, biochemical, and physiological characteristics

This recently acquired molecular data has led to new proposals for the systematic relationships of organisms.

- Initially, researchers including Carl Woese recognized the distinction between Archaea and Bacteria and proposed a six-kingdom system.

- Because the Archaea and Bacteria diverged so early in the history of life, many systematists now favor organizing life into three *domains*, a taxonomic level higher than kingdom (see Campbell, Figure 27.1).

  Prokaryotes comprise two of these domains: *domain Archaea* and *domain Bacteria*.



II. Structure, Function, and Reproduction

A majority of prokaryotes are unicellular, although some aggregate into two-celled to several-celled groups. Others form true, permanent aggregates and some bacterial species have a simple multicellular form with a division of labor between specialized cells.

- Cells have a diversity of shapes, the most common being spheres (cocci), rods (bacilli), and helices (spirilla and spirochetes).
- One rod-shaped species measures 0.5 mm in length, and is the largest prokaryotic cell known (see Campbell, Figure 27.3).
- Most have diameters of 1–5μm (compared to eukaryotic diameters of 10–100μm).

A. Nearly all prokaryotes have cell walls external to their plasma membranes

A majority of prokaryotes have external cell walls that:

- Maintain the cell shape
- Protect the cell
- Prevent the cell from bursting in a hypotonic environment
- Differ in chemical composition and construction from the cell walls of protists, fungi, and plants

In bacteria, the major cell material is peptidoglycan (cellulose is the main component of plant cell walls); archaea lack peptidoglycan in their cell walls.

- *Peptidoglycan* = Modified sugar polymers cross-linked by short polypeptides
  - Exact composition varies among species.
  - Some antibiotics work by preventing formation of the cross-links in peptidoglycan, thus preventing the formation of a functional cell wall.

Gram stain = A stain used to distinguish two groups of bacteria by virtue of a structural difference in their cell walls

Gram-positive bacteria

- Have simple cell walls with large amounts of peptidoglycan
- Stain blue

Gram-negative bacteria:

- Have more complex cell walls with smaller amounts of peptidoglycan
- Have an outer lipopolysaccharide-containing membrane that covers the cell wall
- Stain pink
- Are more often disease-causing (pathogenic) than gram-positive bacteria
- Lipopolysaccharides:
  - Are often toxic and the outer membrane helps protect these bacteria from host defense systems
  - Impede entry of drugs into the cells, making gram-negative bacteria more resistant to antibiotics

Many prokaryotes also secrete sticky, gelatinous substances that form a layer outside the cell well called a *capsule*. Capsules also aid in adhesion to other cells (to form prokaryotic aggregates or facilitate attachment to host cells).

Some prokaryotes adhere to one another and/or to a substrate by means of a surface appendage called a *pili.* Some pili are specialized for transferring DNA when bacteria conjugate (see Campbell, Figure 27.4).

B. Many prokaryotes are motile

Motile bacteria (≈ 50% of known species) use one of three mechanisms to move:

1. Flagella
   - Prokaryotic flagella differ from eukaryotic flagella in that they are:
     - Unique in structure and function; prokaryotic flagella lack the "9 + 2" microtubular structure and rotate rather than whip back and forth like eukaryotic flagella
     - Not covered by an extension of the plasma membrane
     - One-tenth the width of eukaryotic flagella (see Campbell, Figure 27.5)
   - Filaments, composed of chains of the protein *flagellin*, are attached to another protein hook which is inserted into the basal apparatus.
   - The basal apparatus consist of 35 different proteins arranged in a system of rings which sit in the various cell wall layers.
   - Their rotation is powered by the diffusion of protons into the cell. The proton gradient is maintained by an ATP-driven proton pump.
2. Filaments, which are characteristic of spirochetes, or helical-shaped bacteria
   - Several filaments spiral around the cell inside the cell wall.
   - Similar to prokaryotic flagella in structure, axial filaments are attached to basal motors at either end of the cell. Filaments attached at opposite ends move relative to each other, rotating the cell like a corkscrew.
3. Gliding
   - Some bacteria move by gliding through a layer of slimy chemicals secreted by the organism.
   - The movement may result from flagellar motors that lack flagellar filaments.

Prokaryotic movement is fairly random in homogenous environments but may become directional in a heterogenous environment.

Taxis = Movement to or away from a stimulus

- The stimulus can be light (phototaxis), a chemical (chemotaxis), or a magnetic field (magnetotaxis).

- Movement toward a stimulus is a positive taxis (e.g., positive phototaxis = toward light) while movement away from a stimulus is a negative taxis (e.g., negative phototaxis = away from light).

During taxis (directed movement), bacteria move by running and tumbling movements.

- Enabled by rotation of flagella either counterclockwise or clockwise
- Caused by flagella moving coordinately about each other (for a run), or in separate and randomized movements (for a tumble)

C. The cellular and genomic organization of prokaryotes is fundamentally different from that of eukaryotes

Prokaryotes lack the diverse internal membranes characteristic of eukaryotes. Some prokaryotes, however, do have specialized membranes, formed by invaginations of the plasma membranes (see Campbell, Figure 27.6).

- Infoldings of the plasma membrane function in the cellular respiration of aerobic bacteria.
- Cyanobacteria have thylakoid membranes that contain chlorophyll and that function in photosynthesis.

The prokaryotic genome has only 1/1000 as much DNA as the eukaryotic genome.

Genophore = The bacterial chromosome, usually one double-stranded, circular DNA molecule

- This DNA is concentrated in the *nucleoid region*, and is not surrounded by a membrane; therefore, there is no true nucleus.
- Has very little protein associated with the DNA

Many bacteria also have plasmids.

- *Plasmid* = Smaller rings of DNA having supplemental (usually not essential) genes for functions such as antibiotic resistance or metabolism of unusual nutrients
  - Replicate independently of the genophore
  - Can be transferred between partners during conjugation

While prokaryotic and eukaryotic DNA replication and translation are similar, there are some differences. For example,

- Bacterial ribosomes are smaller and have different protein and RNA content than eukaryotic ribosomes.

  This difference permits some antibiotics (e.g., tetracycline) to block bacterial protein synthesis while not inhibiting the process in eukaryotic cells.

D. Populations of prokaryotes grow and adapt rapidly

Neither mitosis nor meiosis occur in the prokaryotes.

- Reproduction is asexual by *binary fission.*
- DNA synthesis is almost continuous.

Although meiosis and syngamy do not occur in prokaryotes, genetic recombination can take place through three mechanisms that transfer variable amounts of DNA:

- *Transformation* = The process by which external DNA is incorporated by bacterial cells
- *Conjugation* = The direct transfer of genes from one bacterium to another
- *Transduction* = The transfer of genes between bacteria by viruses

Growth in the numbers of cells is geometric in an environment with unlimited resources.

- Generation time is usually one to three hours, although it can be 20 minutes in optimal environments.
- At high concentrations of cells, growth slows due to accumulation of toxic wastes, lack of nourishment, among other things.
- Competition in natural environments is reduced by the release of antibiotic chemicals which inhibit the growth of other species.
- Optimal growth requirements vary depending upon the species.

Some bacteria survive adverse environmental conditions and toxins by producing endospores.

- *Endospore* = Resistant cell formed by some bacteria; contains one chromosome copy surrounded by a thick wall
  - When endospores form, the original cell replicates its chromosome and surrounds one copy with a durable wall. The original surrounding cell disintegrates, releasing the resistant endospore.
  - Since some endospores can survive boiling water for a short time, home canners and food canning industry must take special precautions to kill endospores of dangerous bacteria.
  - May remain dormant for many years until proper environmental conditions return.

Short generation times allow prokaryotic populations to adapt to rapidly changing environmental conditions.

- New mutations and genomes (from recombination) are screened by natural selection very quickly.
- This has resulted in the current diversity and success of prokaryotes as well as the variety of nutritional and metabolic mechanisms found in this group.

III. Nutritional and Metabolic Diversity

The prokaryotes exhibit some unique modes of nutrition as well as every type of nutrition found in eukaryotes. In addition, metabolic diversity is greater among prokaryotes than eukaryotes.

A. Prokaryotes can be grouped into four categories according to how they obtain energy and carbon

Prokaryotes exhibit a great diversity in how they obtain the necessary resources (energy and carbon) to synthesize organic compounds.

- Some obtain energy from light (phototrophs), while others use chemicals taken from the environment (chemotrophs).
- Many can utilize CO_2 as a carbon source (autotrophs) and others require at least one organic nutrient as a carbon source (heterotrophs).

Depending upon the energy source and the carbon source, prokaryotes have four possible nutritional modes (see Campbell, Table 27.1):

1. *Photoautotrophs* - Use light energy to synthesize organic compounds from CO_2. Include the cyanobacteria. (Actually all photosynthetic eukaryotes fit in this category.)
2. *Chemoautotrophs* - Require only CO_2 as a carbon source and obtain energy by oxidizing inorganic compounds such as H_2S, NH_3 and Fe^{2+}. This mode of nutrition is unique to certain prokaryotes (i.e. archaea of the genus *Sulfobolus*).
3. *Photoheterotrophs* - Use light to generate ATP from an organic carbon source. This mode of nutrition is unique to certain prokaryotes.
4. *Chemoheterotrophs* - Must obtain organic molecules for energy and as a source of carbon. Found in many bacteria as well as most eukaryotes.

1. Nutritional diversity among chemoheterotrophs

Most bacteria are chemoheterotrophs and can be divided into two subgroups: *saprobes* and *parasites.*

- Saprobes are decomposers that absorb nutrients from dead organic matter.
- Parasites are bacteria that absorb nutrients from body fluids of living hosts.

The chemoheterotrophs are a very diverse group, some have very strict requirements, while others are extremely versatile.

- *Lactobacillus* will grow well only when the medium contains all 20 amino acids, several vitamins, and other organic compounds.
- *E. coli* will grow on a medium which contains only a single organic ingredient (e.g., glucose or some other substitute).

Almost any organic molecule can serve as a carbon source for some species.

- Some bacteria are capable of degrading petroleum and are used to clean oil spills.
- Those compounds that cannot be used as a carbon source by bacteria are considered non-biodegradable (e.g., some plastics).

2. Nitrogen metabolism

While eukaryotes can only use some forms of nitrogen to produce proteins and nucleic acid, prokaryotes can metabolize most nitrogen compounds.

Prokaryotes are extremely important to the cycling of nitrogen through ecosystems.

- Some chemoautotrophic bacteria (*Nitrosomonas*) convert $NH_3 \rightarrow NO_2^-$.
- Other bacteria, such as *Pseudomonas*, denitrify NO_2^- or NO_3^- to atmospheric N_2.
- *Nitrogen fixation* ($N_2 \rightarrow NH_3$) is unique to certain prokaryotes (cyanobacteria) and is the only mechanism that makes atmospheric nitrogen available to organisms for incorporation into organic compounds.
- The nitrogen-fixing cyanobacteria are very self-sufficient, they need only light energy, CO_2, N_2, water, and a few minerals to grow.

3. Metabolic relationships to oxygen

Prokaryotes differ in their growth response to the presence of oxygen.

- *Obligate aerobes* = Prokaryotes that need O_2 for cellular respiration
- *Facultative anaerobes* = Prokaryotes that use O_2 when present, but in its absence can grow using fermentation
- *Obligate anaerobes* = Prokaryotes that are poisoned by oxygen
  - Some species live exclusively by fermentation.
  - Other species use inorganic molecules (other than O_2) as electron acceptors during anaerobic respiration.

B. The evolution of prokaryotic metabolism was both cause and effect of changing environments on Earth

Prokaryotes evolved all forms of nutrition and most metabolic pathways eons before eukaryotes arose.

- Evolution of these new metabolic capabilities was a response to the changing environment of the early atmosphere.
- As these new capabilities evolved, they changed the environment for subsequent prokaryotic communities.

Information from molecular systematics, comparisons of energy metabolism, and geological studies about Earth's early atmosphere have resulted in many hypotheses about the evolution of prokaryotes and their metabolic diversity.

1. The origins of metabolism

The first prokaryotes, which evolved 3.5 - 4.0 billion years ago, probably had few enzymes and were very simple. Moreover, living in an environment with virtually no oxygen, they would have been anaerobes.

The universal role of ATP implies that prokaryotes used that molecule for energy very early in their evolution.

- As ATP supplies were depleted, natural selection favored those prokaryotes that could regenerate ATP from ADP, leading to step-by-step evolution of glycolysis and other catabolic pathways.

Glycolysis is the only metabolic pathway common to all modern organisms and does not require O_2 (which was not abundant on early Earth).

- Some extant archaea and other obligate anaerobes that live by fermentation have forms of nutrition believed to be similar to those of the original prokaryotes.

Chemiosmotic ATP synthesis is also an ancient process since it is common to all three domains of life, but it more likely emerged later in prokaryotic evolution .

Many biologists believe that environmental conditions on early Earth would not have generated enough ATP or other organic molecules by abiotic synthesis to support chemoheterotrophs (see Campbell, Figure 27.8).

The most widely accepted view is that the first prokaryotes were chemoautotrophs that obtained their energy from inorganic chemicals and made their own energy currency molecules instead of absorbing ATP.

- Hydrogen sulfide and iron compounds were abundant and early cells could have obtained energy with their use.

2. The origin of photosynthesis

As the supply of free ATP and abiotically produced organic molecules was depleted, natural selection may have favored organisms that could make their own organic molecules from inorganic resources.

Light absorbing pigments in the earliest prokaryotes may have provided protection to the cells by absorbing excess light energy, especially ultraviolet, that could be harmful.

- These energized pigments may have then been coupled with electron transport systems to power ATP synthesis.
- *Bacteriorhodopsin*, the light-energy capturing pigment in the membrane of extreme halophiles (a group of archaea), uses light energy to pump H^+ out of the cell to produce a gradient of hydrogen ions. This gradient provides the power for production of ATP.
- This mechanism is being studied as a model system of solar energy conversion.

Components of electron transport chains that functioned in anaerobic respiration in other prokaryotes also may have been co-opted to provide reducing power. For example, H_2S could be used as a source of electrons and hydrogen for fixing CO_2.

- The nutritional modes of modern purple and green sulfur bacteria are believed the most similar to early prokaryotes.
- The colors of these bacteria are due to bacteriochlorophyll, their main photosynthetic pigment.

3. Cyanobacteria, the oxygen revolution, and the origins of cellular respiration

Eventually, some prokaryotes evolved that could use H_2O as the electron source. Thus evolved *cyanobacteria*, which released oxygen (see Campbell, Figure 27.9).

- Cyanobacteria evolved between 2.5 and 3.4 billion years ago.
- They lived with other bacteria in colonies that resulted in the formation of the stromatolites.

Oxygen released by photosynthesis may have first reacted with dissolved iron ions to precipitate as iron oxide (supported by geological evidence of deposits), preventing accumulation of free O_2.

- Precipitation of iron oxide would have eventually depleted the supply of dissolved iron and O_2 would have accumulated in the seas.
- As seas became saturated with O_2, the gas was released into the atmosphere.
- As O_2 accumulated, many species became extinct while others survived in anaerobic environments (including some archaea) and others evolved with antioxidant mechanisms.
- Aerobic respiration may have originated as a modification of electron transport chains used in photosynthesis. The purple nonsulfur bacteria are photoheterotrophs that still use a hybrid electron transport system between a photosynthetic and respiratory system.
- Other bacterial lineages reverted to chemoheterotrophic nutrition with electron transport chains adapted only to aerobic respiration.

All major forms of nutrition evolved among prokaryotes before the first eukaryotes arose.

IV. Phylogeny of Prokaryotes

A. Molecular systematics is leading to a phylogenetic classification of prokaryotes

The use of molecular systematics (especially ribosomal RNA comparisons) has shown that prokaryotes diverged into the archaea and bacteria lineages very early in prokaryotic evolution.

- Studies of ribosomal RNA indicate the presence of signature sequences.
  - *Signature sequences* = Domain-specific base sequences at comparable locations in ribosomal RNA or other nucleic acids
- Numerous other characteristics differentiate these two domains (see Campbell, Table 27.2).
- A somewhat surprising result of these types of studies has been the realization that archaea have at least as much in common with eukaryotes as they do with bacteria.

1. Domain Archaea

Some unique characteristics of archaea include:

- Cell walls lack peptidoglycan.
- Plasma membranes have a unique lipid composition.
- RNA polymerase and ribosomal protein are more like those of eukaryotes than of bacteria.

The archaea inhabit the most extreme environments of the Earth. Studies of these organisms have identified three main groups:

1. *Methanogens* are named for their unique form of energy metabolism.
   - They use H_2 to reduce CO_2 to CH_4 and are strict anaerobes.

- Some species are important decomposers in marshes and swamps (form marsh gas) and some are used in sewage treatment.
- Other species are important digestive system symbionts in termites and herbivores that subsist on cellulose diets.

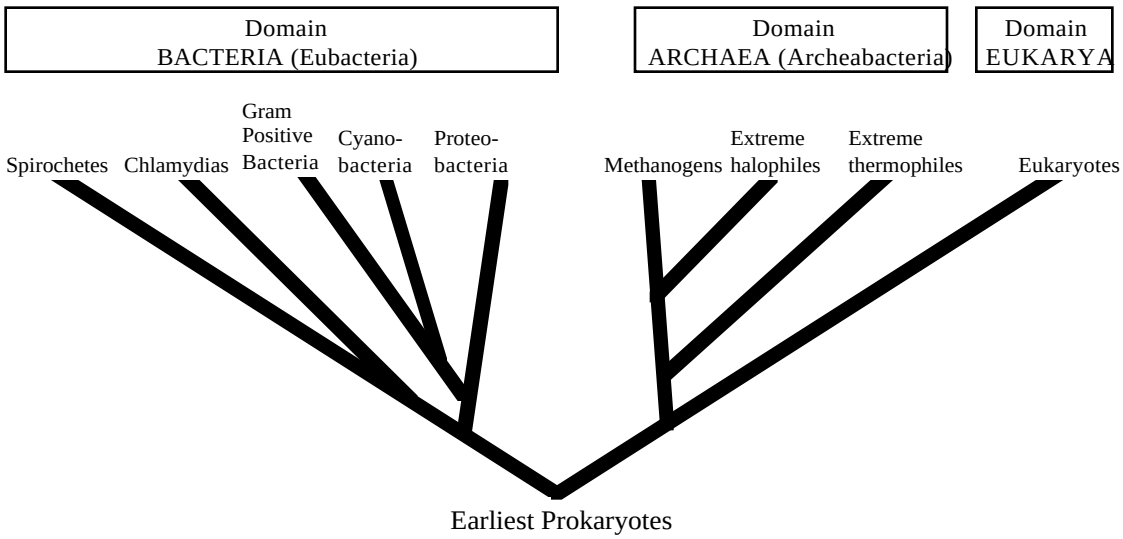
2. *Extreme halophiles* inhabit high salinity (15–20%) environments (e.g., Dead Sea).
   - Some species simply tolerate extreme salinities while others require such conditions (see Campbell, Figure 27.10).
   - They have the pigment bacteriorhodopsin in their plasma membrane which absorbs light to pump H^+ ions out of the cell.
   - This pigment is also responsible for the purple-red color of the colonies.
3. *Extreme thermophiles* inhabit hot environments.
   - They live in habitats of 60 – 80°C.
   - One sulfur-metabolizing thermophile inhabits water of 105°C near deep sea hydrothermal vents.

2. Domain Bacteria

Bacteria comprise a majority of the prokaryotes.

The major groups of bacteria include a very diverse assemblage of organisms. Among the thousands of known species are forms which exhibit every known mode of nutrition and energy metabolism.

Molecular systematics has provided an increased understanding of the once hazy relationships among members of this taxon. At present, most prokaryotic systematists recognize a dozen groups of bacteria (see Campbell, Figures 27.11 and Table 27.3).



V. Ecological Impact of Prokaryotes

A. Prokaryotes are indispensable links in the recycling of chemical elements in ecosystems

Prokaryotes are critical links in the recycling of chemical elements between the biological and physical components of ecosystems—a critical element in the continuation of life.

Decomposers = Prokaryotes that decompose dead organisms and waste of live organisms

- Return elements such as carbon and nitrogen to the environment in inorganic forms needed for reassimilation by other organisms, many of which are also prokaryotes.

Autotrophic bacteria = Bacteria that fix CO_2, thus supporting food chains through which organic nutrients pass

Cyanobacteria supplement plants in restoring oxygen to the atmosphere as well as fixing nitrogen into nitrogenous compounds used by other organisms.

Other prokaryotes also support cycling of nitrogen, sulfur, iron and hydrogen.

B. Many prokaryotes are symbiotic

Most prokaryotes form associations with other organisms; usually with other bacterial species possessing complementary metabolisms.

Symbiosis = Ecological relationships between organisms of different species that are in direct contact

- Usually the smaller organism, the *symbiont*, lives within or on the larger *host*.

Three categories of symbiosis:

1. *Mutualism* = Symbiosis in which both symbionts benefit

   Example: Nitrogen-fixing bacteria in root nodules of certain plants fix nitrogen to be used by the plant, which in turn furnishes sugar and other nutrients to the bacteria.

2. *Commensalism* = Symbiosis in which one symbiont benefits while neither helping nor harming the other symbiont
3. *Parasitism* = Symbiosis in which one symbiont (the *parasite*) benefits at the expense of the host

Symbiosis is believed to have played a major role not only in the evolution of prokaryotes, but also in the origin of early eukaryotes.

1. Prokaryotes and disease

About one-half of all of human disease is caused by bacteria. To cause a disease, the bacteria must invade, evade, or resist the host's internal defenses, long enough to grow and harm the host.

Some pathogens are opportunistic.

- *Opportunistic* = Normal inhabitants of the body that become pathogenic only when defenses are weakened by other factors such as poor nutrition or other infections

  Example: *Streptococcus pneumoniae* lives in the throat of most healthy humans, but can cause pneumonia if the host’s defenses are weakened.

Louis Pasteur, Joseph Lister, and others began linking disease to pathogenic microbes in the late 1800s, but Robert Koch was the first to determine a direct connection between specific bacteria and certain diseases.

- Koch identified the bacteria responsible for anthrax and tuberculosis, and his methods established the four criteria to use as guidelines in medical microbiology.
- *Koch's postulates* = Four criteria to substantiate a specific pathogen as the cause for a disease; they are:
  1. Find the same pathogen in each diseased individual.
  2. Isolate the pathogen from a diseased subject and grow it in a pure culture.

3. Use cultured pathogen to induce the disease in experimental animals.
4. Isolate the same pathogen in the diseased experimental animal.

Some pathogens cause disease by growth and invasion of tissues which disrupts the physiology of the host, while others cause disease by production of a toxin. Two major types of toxins have been found:

- *Exotoxins* = Proteins secreted by bacterial cells
  - Can cause disease without the organism itself being present; the toxin is enough
  - Among the most potent poisons known
  - Elicits specific symptoms

  Examples: Botulism toxin from *Clostridium botulinum* and cholera toxin from *Vibrio cholerae*

- *Endotoxins* = Toxic component of outer membranes of some gram-negative bacteria
  - All induce the general symptoms of fever and aches

  Examples: *Salmonella typhi* (typhoid fever) and other species of *Salmonella* that cause food poisoning

Improved sanitation measures and development of antibiotics have greatly reduced mortality due to bacterial diseases.

- Many of the antibiotics now in use are produced naturally by members of the genus *Streptomyces*. In its natural habitat (soil) such materials would reduce competition from other prokaryotes.
- Although beneficial, the excessive and improper use of antibiotics has resulted in the evolution of many antibiotic-resistant bacterial species, which now pose a major health problem.

C. Humans use prokaryotes in research and technology

Humans use the metabolic diversity of bacteria for a multitude of purposes. The range of these purposes has increased through the application of recombinant DNA technology.

- Pharmaceutical companies use cultured bacteria to make vitamins and antibiotics. More than half of the antibiotics used to treat bacterial diseases come from cultures of various species of *Streptomyces* maintained by pharmaceutical companies.
- As simple models of life to learn about metabolism and molecular biology. (*E. coli* is the best understood of all organisms.)
- Methanogens are used to digest organic wastes at sewage treatment plants.
- Some species of pseudomonads are used to decompose pesticides and other synthetic compounds (see Campbell, Figure 27.12).
- Industry uses bacterial cultures to produce products such acetone and butanol.
- The food industry uses bacteria to convert milk into yogurt and cheese.

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CHAPTER 28
THE ORIGINS OF EUKARYOTIC DIVERSITY

OUTLINE

I. Introduction to the Protists
   A. Protists are the most diverse of all eukaryotes
   B. Symbiosis was involved in the genesis of eukaryotes from prokaryotes

II. Protist Systematics and Phylogeny
   A. Monophyletic taxa are emerging from modern research in protist systematics
   B. Members of candidate kingdom Archaezoa lack mitochondria and may represent early eukaryotic lineages
   C. Candidate kingdom Euglenozoa includes both autotrophs and heterotrophic flagellates
   D. Surface cavities (alveoli) are diagnostic of candidate kingdom Alveolata
   E. A diverse assemblage of unicellular eukaryotes move by means of pseudopodia
   F. Slime molds have structural adaptations and life cycles that enhance their ecological role as decomposers
   G. Diatoms, golden algae, brown algae, and water molds are members of the candidate kingdom Stramenopila
   H. Structural and biochemical adaptations help seaweeds survive and reproduce at the ocean's margins
   I. Some algae have life cycles with alternating multicellular haploid and diploid generations
   J. Red algae (candidate kingdom Rhodophyta) lack flagella
   K. Green algae and plants probably had a common photoautotrophic ancestor
   L. Multicellularity originated independently many times

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. List the characteristics of protists.
2. Explain why some biologists prefer to use the term *undulipodia* for eukaryotic flagella and cilia.
3. Briefly summarize and compare the two major models of eukaryotic origins, the autogenous hypothesis and the endosymbiotic hypothesis.
4. Provide three major lines of evidence for the endosymbiotic hypothesis.
5. Explain why some critics are skeptical about the bacterial origins for chloroplasts and mitochondria.
6. Explain why modern biologists recommend expanding the original boundaries of the Kingdom Protista.

7. Explain what is meant by the statement that the Kingdom Protista is a polyphyletic group.
8. List five candidate kingdoms of protists and describe a major feature of each.
9. Describe amoeboid movement.
10. Outline the life cycle of *Plasmodium.*
11. Indicate the organism that causes African sleeping sickness and explain how it is spread and why it is difficult to control.
12. Describe the function of contractile vacuoles in freshwater ciliates.
13. Distinguish between macronuclei and micronuclei.
14. Using diagrams, describe conjugation in *Paramecium caudatum.*
15. Explain how accessory pigments can be used to classify algae and determine phylogenetic relationships among divisions.
16. Distinguish among the following algal groups based upon pigments, cell wall components, storage products, reproduction, number and position of flagella, and habitat:
    a. Dinoflagellata
    b. Bacillariophyta
    c. Chrysophyta
    d. Phaeophyta
    e. Rhodophyta
    f. Chlorophyta
17. Describe three possible evolutionary trends that led to multicellularity in the Chlorophyta.
18. Outline the life cycles of *Chlamydomonas, Ulva,* and *Laminaria* and indicate whether the stages are haploid or diploid.
19. Distinguish between isogamy and oogamy; sporophyte and gametophyte; and isomorphic and heteromorphic generations.
20. Compare the life cycles of plasmodial and cellular slime molds and describe the major differences between them.
21. Provide evidence that the oomycetes are not closely related to true fungi.
22. Give examples of oomycetes and describe their economic importance.
23. Explain the most widely accepted hypothesis for the evolution of multicellularity.

KEY TERMS

acritarchs
protozoa
algae
syngamy
plankton
serial endosymbiosis
flagellates
Euglenozoa
euglenoids
kinetoplastids
Alveolata
dinoflagellates
apicomplexans
sporozoites
ciliates
conjugation
pseudopodia
detritus
amoebas
heliozoans
radiolarians
forams
plasmodial slime molds
cellular slime molds
Stramenopila
diatoms
golden algae
water mold
white rust
brown algae
thallus
holdfast
stipe
blades
alternation of generations
sporophyte
gametophyte
heteromorphic
isomorphic
red algae
green algae
lichens
diatoms
laminarin
isogamy
anisogamy
oogamy

LECTURE NOTES

Using lenses he developed, Anton von Leeuwenhoek (17th century) was the first to describe the diversity of microscopic protists.

I. Introduction to the Protists

Protists are the earliest eukaryotic descendants of prokaryotes.

Protists arose a billion years before the emergence of other eukaryotes such as plants, fungi, and animals.

Precambrian rock dated to about 2.1 billion years of age contain *acritarchs*, the oldest commonly accepted fossils of protists.

- Remnants of the proper size and structure to be ruptured coats of cysts similar to those of extant protists.
- Adaptive radiation produced a diversity of protists over the next billion years.
- The variations present in these organisms were representative of the structure and function possible in eukaryotic cells.

A. Protists are the most diverse of all the eukaryotes

Because protists vary so much in structure and function, more so than any other group, few other general characteristics besides their being eukaryotes can be cited without exception.

There are about 60,000 extant species of protists.

- Most are unicellular, but colonial forms and even some simple multicellular forms exist.
- Their eukaryotic structure makes even the simplest protist more complex than prokaryotes.
- Primal eukaryotes not only gave rise to current protists, but also to plants, fungi, and animals.

Protists are considered the simplest eukaryotic organisms because most are unicellular.

- At the cellular level, protists are extremely complex.
- The unicellular protist is not analogous to a single plant or animal cell, but is a complete organism.
- The single cell of a protist must perform all the basic functions carried out by the specialized cells of plants and animals.

Protists are metabolically divers, and as a groups, they are the most nutritionally diverse of all eukaryotes.

- Almost all protists are aerobic, using mitochondria for cellular respiration.
- Anaerobic forms lack mitochondria and live in anaerobic environments or have mutualistic respiring bacteria.
- Some may be photoautotrophic, heterotrophic, or mixotrophic (combining photosynthesis and heterotrophy).
- The different modes of nutrition are used to separate protists into three categories: photosynthetic forms are typically referred to as algae, ingestive forms as protozoa, and absorptive protists. (Although the terms protozoa and algae are commonly used, they have no basis in phylogeny and no significance in taxonomy.)

Most protists have flagella or cilia (not homologous to prokaryotic flagella) at some time in life cycle.

- Eukaryotic cilia and flagella are extensions of the cytoplasm. (Prokaryotic flagella are attached to the cell surface.)
- These cilia and flagella have the same basic 9 + 2 microtubular ultrastructure, but cilia are shorter and more numerous.

The life cycles of protists are quite variable.

- Unique mitotic divisions occur in many groups.

- Some can reproduce asexually
- Some can also reproduce sexually or at least use *syngamy* (fusion of gametes) to trade genes between asexual reproductive episodes.
- Some form resistant cysts when stressed by harsh environments.

Protists are found in almost all moist environments: the seas, freshwater systems, and moist terrestrial habitats such as damp soil and leaf litter.

- They are important components of marine and freshwater plankton.
  - *Plankton* = Communities of organisms, mostly microscopic, that drift passively or swim weakly near the surface of oceans, ponds, and lakes
- Many are bottom dwellers in freshwater and marine habitats where they attach to rocks or live in the sand and silt.
- Photosynthetic species form mats at the still-water edges of lakes and ponds where they provide a food source for other protists.

Some protists are free-living, while others are symbiotic species found in the body fluids, tissues, or cells of host organisms.

- The nature of symbiosis ranges from mutualism to parasitism and many are important pathogens.

B. Symbiosis was involved in the genesis of eukaryotes from prokaryotes

There is a greater difference between prokaryotic and eukaryotic cells than between the cells of plants and animals.

During the genesis of eukaryotes, the following cellular structures and process unique to eukaryotes arose:

- A membrane-bound nucleus
- Mitochondria, chloroplasts, and the endomembrane system
- A cytoskeleton
- 9 + 2 flagella
- Multiple chromosomes consisting of linear DNA molecules compactly arranged with proteins
- Life cycles that involve mitosis, meiosis, and sex

The small size and simpler construction of the prokaryotic cell has many advantages but also imposes a number of limitations.

Examples:

- The number of metabolic activities that can occur at one time is smaller.
- The smaller size of the prokaryotic genome limits the number of genes which code for enzymes controlling these activities.

While prokaryotes are extremely successful, natural selection resulted in increasing complexity in some groups, trending toward:

- Multicellular forms, such as the cyanobacteria, which have different cells types with specialized functions
- Evolution of complex prokaryotic communities in which each species benefits from the metabolic activities of other species
- Compartmentalization of different functions within single cells

  The first eukaryotes resulted from this solution.

The evolution of the compartmentalized nature of eukaryotic cells may have resulted from two processes.

1. Specialization of plasma membrane invaginations

- Invaginations and subsequent specializations may have given rise to the nuclear envelope, endoplasmic reticulum, Golgi apparatus, and other components of the endomembrane system (see Campbell, Figure 28.2a).

2. Endosymbiotic associations of prokaryotes may have resulted in the appearance of some organelles.
   - Mitochondria, chloroplasts, and some other organelles evolved from prokaryotes living within other prokaryotic cells.

The hypothesis of *serial endosymbiosis* proposes that certain prokaryotic species, called *endosymbionts,* lived within larger prokaryotes. This theory was developed extensively by Lynn Margulis of University of Massachusetts (see Campbell, Figure 28.2b).

- Hypothesis focuses mainly on mitochondria and chloroplasts.
- Chloroplasts are descended from endosymbiotic photosynthesizing prokaryotes, such as cyanobacteria, living in larger cells.
- Mitochondria are postulated to be descendants of prokaryotic aerobic heterotrophs.

  May have been parasites or undigested prey of larger prokaryotes.

  The association progressed from parasitism or predation to mutualism.

  As the host and endosymbiont became more interdependent, they integrated into a single organism.
- Many extant organisms are involved in endosymbiotic relationships.

Evidence for the endosymbiotic origin of mitochondria and chloroplasts includes the similarities between these organelles and prokaryotes.

- Are of appropriate size to be descendants of bacteria
- Have inner membranes containing several enzymes and transport systems similar to those on prokaryotic plasma membranes
- Replicate by splitting processes similar to binary fission present in prokaryotes
- Have DNA that is circular and not associated with histones or other proteins, as in prokaryotes
- Contain their own tRNA, ribosomes, and other components for DNA transcription and translation into proteins
- Chloroplasts have ribosomes more similar to prokaryotic ribosomes (in size, biochemical characteristics, and antibiotic sensitivity) than to eukaryotic ribosomes.
- Mitochondrial ribosomes vary, but are also more similar to prokaryotic ribosomes.

Molecular systematics lends even more evidence to support the endosymbiotic theory.

- The rRNA of chloroplasts is more similar in base sequence to RNA from certain photosynthetic eubacteria than to rRNA in eukaryotic cytoplasm.

  Chloroplast rRNA is transcribed from genes in the chloroplast while eukaryotic rRNA is transcribed from nuclear DNA.
- Mitochondrial rRNA also has a base sequence which supports a prokaryotic origin.

A comprehensive theory for the origin of eukaryotic cells must also include the evolution of:

- 9 + 2 flagella and cilia, which are analogous, not homologous, to prokaryotic flagella.
- The origins of mitosis and meiosis which also utilize microtubules.

  Mitosis made it possible for large eukaryotic genomes to be reproduced.

Meiosis is essential to sexual reproduction.

Protists have the most varied sexual life histories of the eukaryotes.

II. Protist Systematics and Phylogeny

A. Monophyletic taxa are emerging from modern research in protist systematics

Classification schemes and the phylogeny they reflect are based on available information.

- These presentations are tentative and often change as additional information becomes available.

In 1969, Robert H. Whittaker popularized the five-kingdom taxonomic system and placed only unicellular eukaryotes in the kingdom Protista.

- During the 1970s and 1980s, the Kingdom Protista was expanded to include some multicellular organisms earlier classified as either plants or fungi.
- Studies of cell ultrastructure and life cycle details formed the basis for such taxonomic transfers.

  Seaweeds were found to exhibit characteristics which indicated a closer relationship with certain algae than to plants.

  Slime molds and water molds were found to be more closely related to certain protozoans than to fungi.
- The tendency was to place all eukaryotes that could not comfortably be fitted into the plants, fungi, or animals into this kingdom.

Molecular systematics, especially rRNA comparisons, have stimulated three main trends in eukaryotic systematics and taxonomy over the last decade.

1. Reassessment of the number and membership of protistan phyla
   - The phylum Sarcodina once housed all unicellular organisms that possessed pseudopodia, current classification splits these organisms into several phyla.
2. Arrangement of the phyla into a cladogram based largely on what molecular methods and cell structure comparisons reveal about evolutionary relationships of protists.
3. Reevaluation of the five-kingdom system and debate about the addition of new kingdoms.

At present, most systematists working on the origins of eukaryotes consider the Kingdom Protista and the five-kingdom system obsolete. This is based on the observation that the Kingdom Protista is polyphyletic.

The organization of protists into three groups as in the eight-kingdom system is still polyphyletic.

In light of current research, the organization of protists into five groups, candidate kingdoms (Archaezoa, Euglenozoa, Alveolata, Stramenopila, and Rhodophyta), is indicated. All but one of the five candidate kingdoms, Archaezoa, is monophyletic.

B. Members of candidate kingdom Archaezoa lack mitochondria and may represent early eukaryote lineages

An ancient lineage of eukaryotes branched away from the eukaryotic tree perhaps as early as two billion years ago (see Campbell, Figure 28.2). This group is referred to as the Archaezoa and contains only a few phyla.

- These organisms lack mitochondria and plastids and have relatively simple cytoskeletons.
- Their ribosomes have some characteristics more closely aligned with prokaryotes than with eukaryotes; rRNA sequencing indicates a closer relationship.

Giardia lamblia, a diplomonad, is a modern representative of the archaezoa (see Campbell, Figure 28.4).

- It is a flagellated, unicellular eukaryote that is parasitic in the human intestine.
- It is most commonly transmitted in the cyst form through water contaminated with human feces.

Giardia's importance to evolutionary biologists is related more to its characteristics than to its role as a human parasite.

- Diplomonads have two separate haploid nuclei which produce a "face-like" appearance.
  - Dual nuclei may be a vestige of early eukaryotic evolution.
- Prokaryotes have haploid genomes and some researchers postulate that early eukaryotes had a single haploid nucleus bounded by a nuclear envelope.
- In most modern eukaryotes, the diploid stages in the life cycle result from the fusion of haploid nuclei which form the diploid nucleus.
  - Diplomonads may represent an early mechanism in the evolution of diploidy in eukaryotes.

If the diplomonads diverged from the eukaryotic lineage before the process of nuclear fusion and meiosis evolved, their dual nuclei may be a clue to the past.

- This coupled with the absence of mitochondria in this group and other archaezoans is consistent with an origin occurring before the endosymbiotic relationships that gave rise to mitochondria in aerobic species.

C. Candidate kingdom Euglenozoa includes both autotrophic and heterotrophic flagellates

Protists with flagella are often informally referred to as *flagellates*.

Two groups of flagellates make up the monophyletic candidate kingdom Euglenozoa: euglenoids and kinetoplastids.

Euglenoids (e.g., *Euglena*) have the following characteristics:

- Anterior pocket or chamber from which one or two flagella project
- Production of paramylum, a glucose polymer
- Varying modes of nutrition depending on species
  - Autotrophic
  - Mixotrophic - chiefly autotrophic with some requirement for organic molecules (e.g., vitamins)
  - Heterotrophic

Kinetoplastids have the following characteristics:

- Possess a single large mitochondrion associated with a unique organelle, the kinetoplast, that contains extranuclear DNA
- Symbiotic; some are pathogenic to hosts
  - Species of *Trypanosoma* cause African sleeping sickness and are spread by the bite of the tsetse fly (see Campbell, Figure 28.5).

D. Subsurface cavities (alveoli) are diagnostic of candidate kingdom Alveolata

This candidate kingdom encompasses photosynthetic flagellates (dinoflagellates), a group of parasites (apicomplexans), and a distinctive group that move by means of cilia (ciliates).

All alveolates have small membrane-bound cavities, or alveoli, under their cell surfaces.

The function of alveoli is unknown; however, they may help to:

- Stabilize the cell surface

- Regulate water and ion transport

1. Dinoflagellates

Dinoflagellates are components of phytoplankton that provides the foundation of most marine food chains.

- May cause *red tides* by explosive growth (bloom)

  These dinoflagellates produce a toxin that is concentrated by invertebrates, including shellfish.

  The toxin is dangerous to humans consuming shellfish and causes the condition known as paralytic shellfish poisoning.
- Most are unicellular, some are colonial
- Cell surface is reinforced by cellulose plates with flagella in perpendicular grooves, creating its whirling movement and resulting in a characteristic shape (see Campbell, Figure 28.6)
- Some live as photosynthetic symbionts of the cnidarians that build coral reefs
- Some lack chloroplasts and live as parasites; a few carnivorous species are known
- Have brownish plastids containing chlorophyll *a*, chlorophyll *c* and a mix of carotenoids, including *peridinin* (found only in this phylum)
- Food is stored as starch
- Chromosomes lack histones and are always condensed
- Has no mitotic stages
- Kinetochores are attached to the nuclear envelope and chromosomes distributed to daughter cells by the splitting of the nucleus

2. Apicomplexans

All member of apicomplexans (formerly called sporozoans) are parasites of animals.

- The infectious cells produced in the life cycle are called *sporozoites*.
- The apex of sporozoites has organelles for penetrating host cells and tissues; the phylum is named for these apical organelles.
- Life cycles are intricate having both sexual and asexual reproduction, often requiring two or more different host species.

Several species of *Plasmodium* cause malaria (see Campbell, Figure 28.7).

- *Anopheles* mosquitoes serve as the intermediate host and humans as the final host.
- The incidence of malaria was greatly reduced by the use of insecticides against mosquitoes in the 1960s.
- More recently, incidence of malaria has increased due to insecticide-resistant strains of mosquitoes and drug-resistant strains of *Plasmodium.*

  This is a relatively common (300 million new cases per year) and potentially fatal tropical disease resulting in about two million deaths each year.

There has been little success in developing a vaccine against *Plasmodium.*

- The human immune system has little effect on the parasite.

  Plasmodium spends most of its life cycle in blood cells or liver cells.

  Plasmodium also has the ability to alter its surface proteins.
- The most promising treatment may lie in inhibiting the function of one or more processes in a *Plasmodium* plastid.

3. Ciliates (Ciliophorans)

Species within this group use cilia to move and feed.

- Most ciliates exist as solitary cells in fresh water.
- Cilia are relatively short and beat in synchrony.
- The cilia are associated with a submembranous system that coordinates the movement of thousands of cilia.
- Cilia may be dispersed over surface, or clustered in fewer rows or tufts.
- Some species move on leg-like *cirri* (many cilia bonded together).
- Other species have rows of tightly packed cilia that function together as locomotor membranelles (e.g., *Stentor*).

Ciliates are among the most complex of all cells (see Campbell, Figure 28.8).

They possess two types of nuclei: one large macronucleus and from one to several small micronuclei.

- Characteristics of the macronucleus:

  It is large and has over 50 copies of the genome.

  Genes are packaged in a large number of small units, each with hundreds of copies of just a few genes.

  It controls everyday functions of the cell by synthesizing RNA.

  It is also necessary for asexual reproduction during binary fission. The macronucleus elongates and splits instead of undergoing mitosis.

- Characteristics of the micronucleus:

  It is small and may number from 1 to 80 micronuclei, depending on the species.

  It does not function in growth, maintenance or asexual reproduction.

  It functions in *conjugation*, a sexual process which produces genetic variation (see Campbell, Figure 28.9).

- Note that in ciliates, meiosis and syngamy are separate from reproduction.

E. A diverse assemblage of unicellular eukaryotes move by means of pseudopodia

Three groups of unicellular eukaryotes move and feed by means of cellular extensions called *pseudopodia.*

The mode of nutrition among member groups varies:

- Most are heterotrophs.
  - Some actively seek and feed on bacteria, other protists, or *detritus* (dead organic matter)
  - Some are symbiotic species, including some parasites that cause human diseases

1. Rhizopods (amoebas)

This group (*Rhizopoda* = rootlike feet) includes the *amoebas* and their relatives.

- Simplest of protists; all are unicellular
- No flagellated stages in life cycle
- *Pseudopodia* form as cellular extensions and function in feeding and movement (see Campbell, Figure 28.10)

  The cytoskeleton of microtubules and microfilaments functions in amoeboid movement.

- All reproduction is by asexual mechanisms: no meiosis or sexual reproduction are known to occur.

- During mitosis, spindle fibers form, but typical stages of mitosis are not apparent in most species.

  The nuclear envelope persists during cell division in many genera.
- Rhizopods inhabit freshwater, marine, and soil habitats.
- Most are free-living, although some are parasitic.

2. Actinopods (heliozoans and radiozoans)

Actinopods (= ray feet) possess axopodia, a slender form of pseudopodia.

- *Axopodia* = Projections reinforced by bundles of microtubules thinly covered by cytoplasm
- Axopodia increase the surface area that comes into contact with the surrounding water.
- They help the organisms float and function in feeding.
- Small protists and other microorganisms stick to the axopodia and are phagocytized by the thin layer of cytoplasm. They are carried to the main portion of the cell by cytoplasmic streaming.

The two main groups of actinopods are the heliozoans and the radiolarians.

- Most are planktonic.
- *Heliozoans* live primarily in fresh water.
- *Radiolarians* are primarily marine and have delicate shells, usually made of silica.

3. Foraminiferans (forams)

Forams have porous, multi-chambered shells of organic material hardened by calcium carbonate.

- Forams are almost all marine with most living in the sand or attached to algae and rocks; some are planktonic.
- Cytoplasmic strands extend through the shell's pores and function in swimming, feeding, and shell formation.
- Many have symbiotic algae living beneath the shell that provide nourishment through photosynthesis.
- 90% of the described species are fossils.
- Foram shells are an important component of sediments and sedimentary rocks (see Campbell, Figure 28.12).

F. Slime molds have structural adaptations and life cycles that enhance their ecological role as decomposers

Two groups of protists called slime molds resemble fungi in appearance and lifestyle.

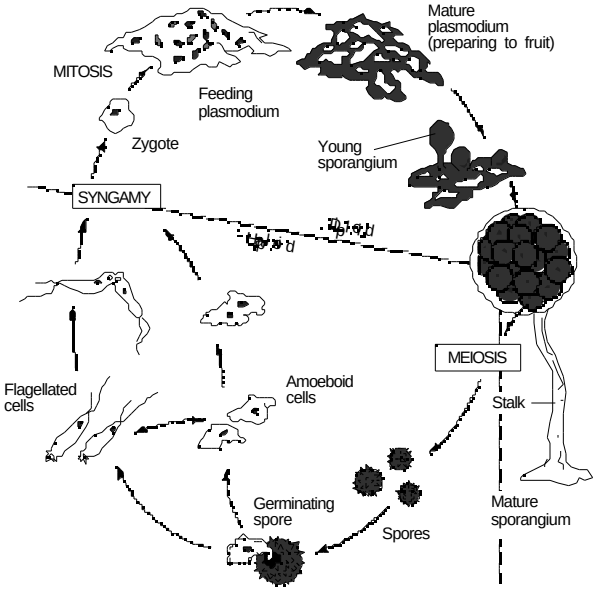
The resemblance of slime molds and water molds to true fungi is a result of convergent evolution of filamentous body structure.

- A filamentous body structure increases exposure to the environment and enhances their roles as decomposers.
- Slime molds differ from true fungi in their cellular organization, reproduction, and life cycles.

1. Plasmodial slime molds (Myxomycota)

The *plasmodial slime molds* are all heterotrophs and many are brightly pigmented.

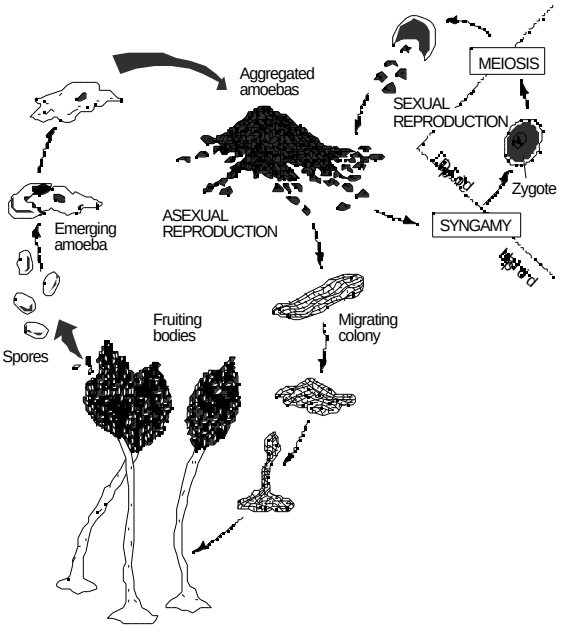
Plasmodium = Feeding stage of life cycle consisting of an amoeboid, coenocytic (multi-nucleated cytoplasm undivided by membranes) mass (see also Campbell, Figure 28.13).



- In most species, the nuclei of plasmodia are diploid and exhibit synchronous mitotic divisions.
- Cytoplasmic streaming within the plasmodium helps distribute nutrients and oxygen.
- Engulfs food by phagocytosis as it grows by extending pseudopodia.
- Live in moist soil, leaf mulch, and rotting logs.
- When stressed by drying or lack of food, the plasmodium ceases growth and forms sexually reproductive structures called fruiting bodies, or sporangia.

2. Cellular slime molds (Acrasiomycota)

This group possesses the following features:



- Feeding stage of life cycle consists of individual, solitary haploid cells.
- When the food supply is depleted, cells aggregate to form a mass similar to those of myxomycota but cells remain separate (not coenocytic) (see also Campbell, Figure 28.14).
- Fruiting bodies function in *asexual* reproduction (unlike plasmodial slime molds).
- Only a few have flagellated stages.

G. Diatoms, golden algae, brown algae, and water molds are members of the candidate kingdom Stramenopila

Stramenopila includes several groups of photosynthetic autotrophs (algae) and numerous heterotrophs.

The term "stramenopila" refers to numerous, fine, hairlike projections on the flagella, which is a characteristic feature of this group

1. Diatoms (Bacillariophyta)

- Diatoms are yellow or brown in color due to the presence of brown plastids.
- Many have a gliding movement produced by chemical secretions
- Usually reproduce asexually; sexual stages (egg and sperm production) are rare
- Some produce resistant cysts
- Mostly unicellular organisms with overlapping glasslike walls of hydrated silica in an organic matrix (see Campbell, Figure 28.15)
- Have the same photosynthetic pigments as in Chrysophyta
- Are components of freshwater and marine plankton
- Store food in laminarin (a glucose polymer) and in the form of oil
- Cellular regulation of ions counteracts weight of walls and maintains buoyancy

2. Golden algae (Chrysophyta)

- Algae named for their color, which results from accessory pigments (yellow and brown carotenoids and xanthophyll)
- Live among freshwater and marine plankton
- Most are unicellular, but some are colonial (see Campbell, Figure 28.16)
- Most are biflagellated, with both flagella attached near one end of the cell
- Survive environmental stress by forming resistant cysts
    - Microfossils resembling these cysts have been found in Precambrian rocks.

3. Water molds and their relatives (Oomycota)

This group includes water molds, white rusts, and downy mildews.

- All lack chloroplasts and are heterotrophic
- Have coenocytic hyphae (fine, branching filaments) that are analogous to fungal hyphae.
- Cell walls are made of cellulose rather than the chitin found in true fungi
- Diploid condition in the life cycle prevails in most species (see Campbell, Figure 28.17)
- Biflagellated cells are present in the life cycles, while fungi lack flagellated cells

In water molds:

- A large egg is fertilized by a smaller sperm cell to form a resistant zygote.
- These organisms are usually decomposers that grow on dead algae and animals in fresh water.
- Some are parasitic and grow on injured tissue, but they may also grow on the skin and gills of fish.

White rusts and downy mildews:

- Are usually parasitic on terrestrial plants

- Disperse by windblown spores, but also form flagellated zoospores at some point in their life cycle
- Some of the most important plant pathogens are members of this phylum.

4. Brown algae (Phaeophyta)

This is the largest and most complex of the algae. Color is due to accessory pigments.

- All are multicellular and most are marine inhabitants
- Have chlorophyll *a*, chlorophyll *c*, and the carotenoid pigment *fucoxanthin*
- Store carbohydrate food reserves in the form of laminarin
- Cell walls made of cellulose and *algin*

Many eukaryotes commonly called seaweeds are brown algae; however, red algae and green algae also are components of seaweeds.

H. Structural and biochemical adaptations help seaweeds survive and reproduce at the ocean's margins

Seaweeds are large, multicellular marine algae, which are found in the intertidal and subtidal zones of coastal waters.

- They are a diverse group and include members of the Phaeophyta (brown algae), Rhodophyta (red algae), and Chlorophyta (green algae).
- **The following emphasizes adaptations found in the red algae, however, many of these adaptations also apply to the brown algae and green algae seaweeds.**

The habitat of seaweeds, particularly the intertidal zone, poses several challenges to the survival of these organisms.

- Movement of the water due to wave action and winds produces a physically active habitat.
- Tidal rhythms result in the seaweeds being alternately covered by seawater and exposed to direct sunlight and the drying conditions of the air.

Seaweeds have evolved several unique structural and biochemical adaptations to survive the conditions of their habitats.

Structural adaptations found in seaweeds are a result of their complex multicellular anatomy. Some forms have differentiated tissues and organs analogous to those of plants.

- The body of a seaweed is called a *thallus*. It is plantlike in appearance but has no true roots, stems, or leaves.
- A thallus consists of a rootlike *holdfast* (maintains position), a stemlike *stipe* (supports the blades), and leaflike *blades* (large surfaces for photosynthesis) (see Campbell, Figure 28.18).
- Floats, which help suspend blades near the water surface, are present in some brown algae.
- Brown algae known as giant kelp occur beyond the intertidal zone where less harsh conditions exist and may have stipes which reach a length of up to 60 m (see Campbell, Figure 28.19).

Biochemical adaptations in some seaweeds reinforce the anatomical adaptations and enhance survival.

- Cellulose cell walls contain gel-forming polysaccharides (algin in brown algae; carageenan in red algae), which cushion the thalli against wave action and prevent desiccation during low tide.

- Some red algae retard grazing by marine invertebrates by incorporating large amounts of calcium carbonate into their cell walls, rendering them unpalatable.

Seaweeds are used by humans in a variety of ways:

- Brown and red alga are used as food in many parts of Asia.
- Algin, agar, and carageenan are extracted and used as thickeners for processed foods and lubricants in oil drilling.
- Agar is also used as a microbiological culture media.

I. Some algae have life cycles with alternating multicellular haploid and diploid generations

A variety of life cycles in the brown algae, the most complex involve alternation of generations. Also found in certain groups of red algae and green algae.

Alternation of generations = Alternation between multicellular haploid forms and multicellular diploid forms in a life history

The life cycle of *Lamanaria* is an example of a complex life cycle with an alteration of generations (see Campbell, Figure 28.20).

- The diploid individual is called a *sporophyte* because it produces reproductive cells called spores.
- The haploid individual is called the *gametophyte* because it produces gametes.
- The sporophyte and gametophyte generations of the life cycle take turns producing one another.
  - Spores released from the sporophyte develop into gametophytes.
  - Gametophytes produce gametes which fuse (fertilization) to form a diploid zygote that develops into a sporophyte.
- In *Laminaria,* the sporophyte and gametophyte generations are said to be *heteromorphic,* because they are morphologically different.
- In *Ulva*, a green algae exhibiting alteration of generations, the generations are referred to as *isomorphic* because they look alike.

J. Red algae (candidate kingdom Rhodophyta) lack flagella

The defining characteristic of red algae is that they do not have flagella in any of their life cycle stages, unlike other eukaryotic algae

- Current data suggests that red algae aren't ancient, but that flagella were lost during their evolution.
- Red algae probably arose about the same time as Stamenopiles.

Red algae are primarily warm, tropical, marine inhabitants, although some are found in fresh water and soil. Other features include:

- Chlorophyll *a*, carotenoids, phycobilins, and chlorophyll *d* in some
- Red color of plastids due to the accessory pigment, phycoerythrin

  Phycoerythrin is a phycobilin, a pigment found only in red algae and cyanobacteria.
- Color of the thallus may vary (even in a single species) with depth, as pigmentation changes to optimize photosynthesis.
  - Deep water forms are almost black, moderate depth forms are red, and shallow water forms are green.
  - One species has been discovered near the Bahamas at a depth of 260 meters.
  - Some tropical species lack pigmentation and survive as parasites on other red algae.

- Carbohydrate food reserves stored as floridean starch (similar to glycogen).
- Cell walls are cellulose with agar and carageenan.
- Most red algae are multicellular and the largest are designated as seaweeds.
- Most thalli are filamentous and are often branched forming an interwoven lacy network (see Campbell, Figure 28.21)

All red algae reproduce sexually.

- Have no flagellated stages, unlike other algal protists
- Alternation of generations is common

K. Green algae and plants probably had a common photoautotrophic ancestor

Green algae (Chlorophyta) are named for their grass-green chloroplasts, which are similar in ultrastructure and pigment composition to the organelles of organism s traditionally referred to as plants.

Molecular and structural features suggest that green algae and plants are closely related and were derived from a common ancestor different from that giving rise to stramenopiles and red algae.

- Some systematists argue for the inclusion of green algae in the plant kingdom.

At least 7000 species of green algae are known; most are freshwater, some are marine.

- Many unicellular types live as plankton, inhabit damp soil, coat snow surfaces, or are symbionts with protozoa or invertebrates.
- When living mutualistically with fungi they form the association known as *lichens*.
- Colonial forms are often filamentous ("pond scum").
- Multicellular forms may have large, complex structures resembling true plants and comprise a group of seaweeds.

Evolutionary trends probably produced colonial and multicellular forms from flagellated unicellular ancestors.

1. Formation of colonies of individual cells, as seen in *Volvox* (see Campbell, Figure 28.22a)
2. Repeated division of nuclei with no cytoplasmic division, as in *Caulerpa* (see Campbell, Figure 28.22b)
3. Formation of true multicellular forms, as in *Ulva* (see Campbell, Figure 28.22c)

Most green algae have complex life histories involving sexual and asexual reproductive stages.

- Nearly all reproduce sexually by way of biflagellated gametes.
- Some are conjugating algae (e.g., *Spirogyra*), which produce amoeboid gametes (see Campbell, Figure 28.23).

The life cycle of *Chlamydomonas* is a good example of the life history of a unicellular chlorophyte. Note: a mature *Chlamydomonas* is a single haploid cell (see Campbell, Figure 28.24) .

- During asexual reproduction, the flagella are resorbed and the cell divides twice by mitosis to form four cells (more in some species).

  The daughter cells develop and emerge as swimming zoospores. Zoospore development includes formation of flagella and cell walls.

  Zoospores grow into mature cells, thus completing asexual reproduction.

Sexual reproduction is stimulated by environmental stress from such things as a shortage of nutrients, drying of the pond, or some other factor.

During sexual reproduction, many gametes are produced by mitotic division within the wall of the parent cell. The gametes escape the parent cell wall.

Gametes of opposite mating strains (+ and −) pair off and cling together by the tips of their flagella.

The gametes are morphologically indistinguishable and their fusion is known as *isogamy*.

The slow fusion of the gametes forms a diploid zygote which secretes a resistant coat that protects it from harsh environmental conditions.

When dormancy of the zygote is broken, four haploid individuals (two of each mating type) are produced by meiosis.

These haploid cells emerge from the coat and develop into mature cells, thus completing the sexual life cycle.

Many features of *Chlamydomonas* sex are believed to have evolved early in the chlorophyte lineage. Using this basic life cycle, many refinements that evolved among the chlorophytes have been identified.

- Some green algae produce gametes that differ from vegetative cells and, in some species, the male gamete differs in size or morphology from the female gamete (*anisogamy*).
- Many species exhibit *oogamy*, a type of anisogamy in which a flagellated sperm fertilizes a nonmotile egg.
- Some multicellular species also exhibit alternation of generations.

  Ulva produces isomorphic thalli for its diploid sphorophyte and haploid gametophyte (see Campbell, Figure 28.25).

L. Multicellularity originated independently many times

Early eukaryotes were more complex than prokaryotes and this increase in complexity allowed for greater morphological variations to evolve.

- Extant protists are more complex in structure and show a greater diversity of morphology than the simpler prokaryotes.
- The ancestral stock which gave rise to new waves of adaptive radiations were the protists with multicellular bodies.

Multicellularity evolved several times among the early eukaryotes and gave rise to the multicellular algae, plants, fungi, and animals.

Most researchers believe that the earliest multicellular forms arose from unicellular ancestors as colonies or loose aggregates of interconnected cells (see Campbell, Figure 28.26).

- Multicellular algae, plants, fungi, and animals probably evolved from several lineages of protists that formed by amalgamations of individual cells.
- Evolution of multicellularity from colonial aggregates involved cellular specialization and division of labor.

  The earliest specialization may have been locomotor capabilities provided by flagella.

  As cells became more interdependent, some lost their flagella and performed other functions.
- Further division of labor may have separated sex cells from somatic cells.

  This type of specialization and cooperation is seen today in colonial species such as *Volvox* (a green alga).

  Gametes specialized for reproduction are dependent on somatic cells while developing.

The evolution of telomerase enzymes, which add nucleotides to the ends of DNA (telomeres; see chapter 19) and protects genes from degradation during DNA replication, may have been involved in gamete formation.

- Many additional steps were involved in the evolution of specialized somatic cells capable of performing all the nonreproductive function in a multicellular organism.

  Extensive division of labor exists among the different tissues that comprise the thalli of seaweeds.
- Multicellular forms more complex than filamentous algae appeared approximately 700 million years ago.
- A variety of animal fossils has been found in late Precambrian strata and many new forms evolved in the Cambrian period (about 570 million years ago).
- Seaweeds and other complex algae were also abundant during the Cambrian period.
- Primitive plants are believed to have evolved from certain green algae living in shallow waters about 400 million years ago.

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CHAPTER 29

PLANT DIVERSITY I: THE COLONIZATION OF LAND

OUTLINE

I. An Overview of Plant Evolution
   A. Structural, chemical, and reproductive adaptations enabled plants to colonize land
   B. The history of terrestrial adaptation is the key to modern plant diversity

II. The Origins of Plants
   A. Plants probably evolved from green algae called charophytes
   B. Alternation of generations in plants may have originated by delayed meiosis
   C. Adaptations to shallow water as preadapted plants for living on land

III. Bryophytes
   A. The embryophyte adaptation evolved in bryophytes
   B. The gametophyte is the dominant generation in the life cycles of byrophytes
   C. The three divisions of bryophytes are mosses, liverworts, and hornworts

IV. The Origin of Vascular Plants
   A. Additional terrestrial adaptations evolved as vascular plants descended from bryophyte-like ancestors
   B. The branched sporophytes of vascular plants amplified the production of spores and made complex bodies possible

V. Seedless Vascular Plants
   A. A sporophyte-dominant life cycle evolved in seedless vascular plants
   B. The three divisions of seedless vascular plants are lycophytes, horsetails, and ferns
   C. Seedless vascular plants formed vast “coal forests” during the carboniferous period

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. List characteristics that distinguish plants from organisms in the other kingdoms.
2. Diagram a generalized plant life cycle indicating which generation is the sporophyte/ gametophyte, which individuals are haploid/diploid, where meiosis occurs and where mitosis occurs.
3. Describe four major periods of plant evolution that opened new adaptive zones on land.
4. Distinguish between the categories division and phylum.
5. Using the classification scheme presented in the text, list the plant divisions; give the common name for each; and categorize them into nonvascular, vascular seedless and vascular seed plants.
6. Provide evidence to defend the position that plants evolved from green algae.
7. Describe two adaptations that made the bryophytes' move onto land possible.

8. Explain how bryophytes are still tied to water.
9. List and distinguish among three division of Bryophyta.
10. Diagram the life cycle of a moss including gamete production, fertilization, and spore production.
11. Compare environmental conditions faced by algae in an aquatic environment and plants in a terrestrial environment.
12. Provide evidence that suggests the division Bryophyta is a phylogenetic branch separate from vascular plants.
13. Describe adaptations of vascular plants, including modifications of the life cycle and modifications of the sporophyte, that have contributed to their success on land.
14. List and distinguish among the four extant divisions of seedless vascular plants.
15. Distinguish between homosporous and heterosporous.
16. Distinguish among spore, sporophyte, sporophyll and sporangium.
17. Diagram the life cycle of a fern including spore production, gamete production and fertilization.
18. Point out the major life cycle differences between mosses and ferns.
19. Describe how coal is formed and during which geological period the most extensive coal beds were produced.

KEY TERMS

stomata	sporophyte	sporangium	megaspores
cuticle	vascular tissue	mosses	microspores
secondary products	gymnosperm	liverworts	lycophytes
lignin	angiosperm	hornworts	epiphytes
sporopollenin	division	xylem	sporophylls
gametangia	charophyte	phloem	horsetails
embryophyte	antheridium	homosporous	ferns
gametophyte	archegonium	heterosporous	

LECTURE NOTES

Plants appeared on land about 475 million years ago, and the evolutionary history of the plant kingdom reflects increasing adaptation to the terrestrial environment. The colonization of land by plants transformed the biosphere. This transformation created new adaptive zones and paved the way for other organisms.

I. An Overview of Plant Evolution

A. Structural, chemical, and reproductive adaptations enabled plants to colonize land

1. Some characteristics of plants

Plants are multicellular eukaryotes that are photosynthetic autotrophs; however, not all organisms with these characteristics are plants. Plants share the following characteristics with their green algal ancestors:

- Chloroplasts with the photosynthetic pigments: chlorophyll *a*, chlorophyll *b*, and carotenoids
- Cell walls containing cellulose
- Food reserve that is starch stored in plastids

It is the set of structural, chemical, and reproductive adaptations associated with terrestrial life that distinguishes plants from algae. Plants have evolved complex bodies with cell specialization for different functions.

- Plants have developed structural specializations in order to extract the resources needed for photosynthesis (water, minerals, carbon dioxide, light) from the terrestrial environment (above and below ground).
  - In most plants, gas exchange occurs via *stomata*, special pores on the surfaces of leaves
- Chemical adaptation includes the secretion of a waxy *cuticle*, a coating on the surface of plants that helps prevent desiccation.
  - Cuticle waxes are *secondary products*, so named because they arise through metabolic pathways not common to all plants. (Cellulose is an example of a primary product).
  - Other secondary products include *lignin* (cell wall component of "woody" plants) and *sporopollenin* (a resilient polymer in the walls of spores and pollen grains).

2. Plants as embryophytes

With the move from an aquatic to terrestrial environment, a new mode of reproduction was necessary to solve two problems:

1. Gametes must be dispersed in a nonaquatic environment. Plants produce gametes within *gametangia*, organs with protective jackets of sterile (nonreproductive) cells that prevent gametes from drying out (see Campbell, Figure 29.1a). The egg is fertilized within the female organ.
2. Embryos must be protected against desiccation. The zygote develops into an embryo that is retained for awhile within the female gametangia's jacket of protective cells (see Campbell, Figure 29.1b). Emphasizing this terrestrial adaptation, plants are often referred to as *embryophytes*.

3. Alternation of generations: *a review*

Most plants reproduce sexually, and most are also capable of asexual propagation. All plants have life cycles with an *alternation of generations* (also occurs in some groups of algae).

- A haploid *gametophyte* generation produces and alternates with a diploid *sporophyte* generation. The sporophyte, in turn, produces gametophytes (see Campbell, Figure 29.2).
- The life cycles are heteromorphic; that is, sporophytes and gametophytes differ in morphology.
- The sporophyte is larger and more noticeable than the gametophyte in all plants but mosses and their relatives.

A comparison of life cycles among plant divisions is instructive because:

- It points to an important trend in plant evolution: reduction of the haploid gametophyte generation and dominance of the diploid sporophyte.
- Certain life cycle features are adaptations to a terrestrial environment; for example the replacement of flagellated sperm by pollen.

4. Some highlights of plant phylogeny

There are four major periods of plant evolution that opened new adaptive zones on land (see Campbell, Figure 29.3):

1. Origin of plants from aquatic ancestors (probably green algae) in the Ordovician about 475 million years ago (mya).
   - Cuticle and jacketed gametangia evolved which protected gametes and embryos.

- *Vascular tissue* evolved with conducting cells that transport water and nutrients throughout the plant.

2. Diversification of seedless vascular plants, such as ferns, during the early Devonian about 400 mya.
3. Origin of the seed near the end of the Devonian about 360 mya.
   - *Seed* = Plant embryo packaged with a store of food within a resistant coat
   - Early seed plants bore seeds as naked structures and evolved into *gymnosperms*, including conifers.
   - Conifers and ferns coexisted in the landscape for more than 200 million years.
4. Emergence of flowering plants during the early Cretaceous, about 130 mya.
   - Unlike gymnosperms, flowering plants bear seeds within the flower's protective ovaries.
   - Most contemporary plants are flowering plants or *angiosperms*.

5. Classification of plants

The major taxonomic category of plants is the *division*; it is comparable to phylum, the highest category in the animal kingdom.

- Divisions are subdivided into lower taxa (class, order, family, genus).
- Currently, eleven divisions of plants are recognized (see also Campbell, Table 29.1).

A CLASSIFICATION OF PLANTS

	Common Name	Approximate Number of Extant Species
Nonvascular Plants (byrophytes)		
Division Bryophyta	Mosses	12,000
Division Hepatophyta	Liverworts	6,500
Division Anthocerophyta	Hornworts	100
Vascular Plants		
Seedless Vascular Plants		
Division Lycophyta	Lycophytes	1,000
Division Sphenophyta	Horsetails	15
Division Pterophyta	Ferns	12,000
Seed Plants		
Gymnosperms		
Division Coniferophyta	Conifers	550
Division Cycadophyta	Cycads	100
Division Ginkgophyta	Ginkgo	1
Division Gnetophyta	Gnetae	70
Angiosperms		
Division Anthophyta	Flowering plants	250,000

II. The Origin of Plants

A. Plants probably evolved from green algae called charophytes

The green algae are likely the photosynthetic protists most closely related to plants. This conclusion is based on homologies in:

- Cell wall composition
- Structure and pigmentation of chloroplasts

Available evidence supports the hypothesis that plants and green algae called *charophytes* both evolved from a common ancestor (see Campbell, Figure 29.4). Researchers have found the following homologies between charophytes and plants:

1. Homologous chloroplasts
   - Green algae and plants both have the accessory pigments, chlorophyll *b* and beta-carotene.
   - Green algae and plants both have chloroplasts with thylakoid membranes stacked as grana.
   - Compared to chloroplast DNA of various green algae, plant chloroplast DNA most closely matches that of charophytes.
2. Biochemical similarity
   - Most green algae and plants contain cellulose in their cell walls. Charophytes are the most plantlike in wall composition with cellulose making up 20% - 26% to 26% of the wall material.
   - Charophyte peroxisomes are the only algal peroxisomes with the same enzyme composition as plant peroxisomes.
3. Similarity in mitosis and cytokinesis. During cell division in charophytes and plants:
   - The nuclear envelope completely disperses during late prophase.
   - The mitotic spindle persists until cytokinesis begins.
   - Cell plate formation during cytokinesis involves cooperation of microtubules, actin microfilaments, and vesicles.
4. Similarity in sperm ultrastructure. Charophyte sperm ultrastructure is more similar to certain plants than to other green algae.
5. Genetic relationship. DNA and rRNA similarities in charophytes and plants provides additional evidence for the hypothesis that charophytes are the closest relatives of plants.

B. Alternation of generations in plants may have originated by delayed meiosis

The alternation of haploid and diploid generations apparently evolved independently among various groups of algae.

- Since alternation of generations does not occur among modern charophytes, it is presumed that alternation of generations in plants has had a separate origin from alternation of generations in other algal groups.
- Its appearance in plants is thus *analogous*, not homologous, to the alternation of generations observed in various groups of algae.

How did alternation of generations evolve in plant ancestors?

Coleochaete, a modern charophyte, holds some clues:

- The Coleochaete thallus is haploid.

  In contrast to most algae, the parental thallus of *Coleochaete* retains the eggs, and after fertilization, the zygotes remain attached to the parent.

Nonreproductive cells of the thallus grow around each zygote, which enlarges, undergoes meiosis, and releases haploid swimming spores.

Haploid spores develop into new individuals.

The only diploid stage is the zygote; there is no alternation of multicellular diploid and haploid generations.

- If an ancestral charophyte delayed meiosis until after the zygote divided mitotically, there would be a multicellular diploid generation (sporophyte) still attached to the haploid parent (gametophyte). Such a life cycle would be an alternation of generations.
- If specialized gametophyte cells formed protective layers around a tiny sporophyte, this hypothetical ancestor would also be a primitive embryophyte (see Campbell, Figure 29.5).

What would be the adaptive advantage of delaying meiosis and forming a mass of diploid cells?

It may maximize the production of haploid spores.

- If the zygote undergoes meiosis directly, each fertilization event results in only a few haploid spores.
- Mitotic division of the zygote to form a multicellular sporophyte amplifies the sexual product. Many diploid cells can undergo meiosis producing a large number of haploid spores, enhancing the chances of survival in unfavorable environments.

C. Adaptations to shallow water preadapted plants for living on land

Some adaptations for life in shallow water could also have been adaptive for life on land.

- Many modern charophytes live in shallow water, and some ancient charophytes may have also lived in shallow-water habitats subject to occasional drying.
- About 440 million years ago, during the transition from Ordivician to Silurian, repeated glaciation and climatic changes caused fluctuations in the water levels of lakes and ponds.
- Natural selection may have favored shallow-water plants tolerant to periodic drying. Adaptations to shallow water may also have been preadaptive for terrestrial life.

Examples:
- Waxy cuticles
- Protection of gametes
- Protection of developing embryos

- Eventually, accumulated adaptations made it possible for ancestral plants to live permanently above the water line, opening a new adaptive zone with:

  Sunlight unfiltered by water and algae

  Soil rich in minerals

  Absence of terrestrial herbivores

III. Bryophytes

A. The embryophyte adaptation evolved in bryophytes

The *bryophytes* include plants found in three divisions:

- Bryophyta (mosses)
- Hepatophyta (liverworts)

- Anthocerophyta (hornworts)

Bryophytes display a pivotal adaptation that made the move onto land possible: the embryophyte condition.

- Gametangia protect developing gametes.
  a. *Antheridium*, or male gametangium, produces flagellated sperm cells.
  b. *Archegonium*, or female gametangium, produces a single egg; fertilization occurs within the archegonium, and the zygote develops into an embryo within the protective jacket of the female organ (embryophyte condition).

Bryophytes are not totally free from their ancestral aquatic habitat.

- They need water to reproduce. Their flagellated sperm cells must swim from the antheridium to the archegonium to fertilize the egg.
- Most have no vascular tissue to carry water from the soil to aerial plant parts; they imbibe water and distribute it throughout the plant by the relatively slow processes of diffusion, capillary action, and cytoplasmic streaming.

Bryophytes lack woody tissue and cannot support tall plants on land; they may sprawl horizontally as mats, but always have a low profile (see Campbell, Figure 29.6).

B. The gametophyte is the dominant generation in the life cycles of bryophytes

The life cycle of a bryophyte alternates between haploid and diploid generations (see Campbell, Figure 29.7)

- The sporophyte (2n) produces haploid spores by meiosis in a *sporangium*; the spores divide by mitosis to form new gametophytes.
- Contrary to the life cycles of vascular plants, the haploid gametophyte is the dominant generation in mosses and other bryophytes. Sporophytes are generally smaller and depend on the gametophyte for water and nutrients.

C. The three divisions of bryophytes are mosses, liverworts, and hornworts

1. Mosses (Division Bryophyta)

A tight pack of many moss plants forms a spongy mat that can absorb and retain water.

Each plant grips the substratum with *rhizoids*, elongate cells or cellular filaments.

Photosynthesis occurs mostly in the small stemlike and leaflike structures found in upper parts of the plant; these structures are not homologous with stems and leaves in vascular plants.

Mosses cover about 3% of the land surface, and they contain vast amounts of organic carbon (see Campbell, Figure 29.6).

2. Liverworts (Division Hepatophyta)

Liverworts are less conspicuous than mosses.

They sometimes have bodies divided into lobes.

They have a life cycle similar to mosses. Their sporangia have elaters, coil-shaped cells, that spring out of the capsule and disperse spores.

They can also reproduce asexually from gemmae (small bundles of cells that can bounce out of cups on the surface of the gametophyte when hit by rainwater) (see Campbell, Figure 29.8).

They display their greatest diversity in tropical forests.

3. Hornworts (Division Anthocerophyta)

Hornworts resemble liverworts, but sporophytes are horn-shaped, elongated capsules that grow from the matlike gametophyte (see Campbell, Figure 29.9).

Their photosynthetic cells have only one large chloroplast, unlike the many smaller ones of other plants.

Recent molecular evidence suggest that they are most closely related to vascular plants.

IV. The Origin of Vascular Plants

A. Additional terrestrial adaptations evolved as vascular plants descended from bryophyte-like ancestors

In addition to cuticles and jacketed sex organs, other adaptations for terrestrial life evolved in vascular plants as they colonized land:

1. *Regional specialization of the plant body*. Unlike aquatic environments, terrestrial environments spatially segregate the resources of water and light. This problem was solved as plants evolved subterranean roots that absorb water and minerals from the soil and an aerial shoot system of stems and leaves to make food.
2. *Structural support.* In aquatic environments, the denser medium of water buoys plants up toward the light, but in terrestrial environments plants must have structural support to stand upright in air. Such support was provided as the hard material lignin was embedded into the cellulose matrix of cell walls.
3. *Vascular system.* Regional specialization of the plant body presented the problem of transporting substances between the root and shoot systems. This problem was solved as a vascular system evolved with two types of conducting tissues:

   Xylem = Complex, plant vascular tissue that conducts water and minerals from the roots to the rest of the plant
   - Composed of dead, tube-shaped cells that form a microscopic water-pipe system
   - Cell walls are usually lignified, giving the plant structural support

   Phloem = Plant vascular tissue that conducts food throughout the plant
   - Composed of living cells arranged into tubules
   - Distributes sugars, amino acids, and other organic nutrients
4. *Pollen.* Pollination eliminated the need for water to transport gametes.
5. *Seeds*
6. *Increased dominance of the diploid sporophyte*

B. The branched sporophytes of vascular plants amplified the production of spores and made more complex bodies possible

Oldest fossilized vascular plant is *Cooksonia* (late Silurian):

- Discovered in both European and North American Silurian rocks; North America and Europe were probably connected during the late Silurian, about 400 million years ago
- Simple plant that displayed dichotomous branching and bulbous terminal sporangia on sporophyte (see Campbell, Figure 29.10)
- True roots and leaves were absent; the largest species was about 50 cm tall
- Grew in dense stands around marshes
- As vascular plants became more widespread, new species appeared

V. Seedless Vascular Plants

The earliest vascular plants were seedless and they dominated the Carboniferous forests. Modern flora includes three divisions of seedless vascular plants.

A. A sporophyte-dominant life cycle evolved in seedless vascular plants

The sporophyte (diploid) generation emerged as the larger and more complex plant from the time of *Cooksonia* and other early vascular plants. It is the dominant stage in the life cycle in all extant vascular plants.

The sporophyte-dominant life cycle is exemplified by ferns, one group of the seedless vascular plants (see Campbell, Figure 29.11).

- The familiar leafy plant is the sporophyte.
- Gametophytes are quite small and grow on or below the surface of the soil.

Vascular plants display two distinct reproductive strategies:

- The sporophyte of a *homosporous* plant produces a single type of spore (e.g., ferns); each spore develops into a bisexual gametophyte with both male (antheridia) and female (archegonia) sex organs.
- The sporophyte of a *heterosporous* plant produces two kinds of spores:

1. *Megaspores* develop into female gametophytes possessing archegonia.
2. *Microspores* develop into male gametophytes possessing antheridia.

B. The three divisions of seedless vascular plants are lycophytes, horsetails, and ferns

1. Lycophytes (division Lycophyta)

The division Lycophyta includes the club mosses and ground pines.

Lycophytes survived through the Devonian period and dominated land during the Carboniferous Period (340–280 million years ago).

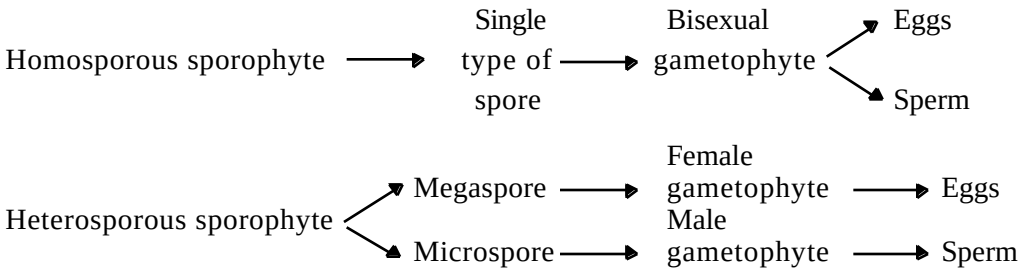
Some are temperate, low-growing plants with rhizomes and true leaves.

Some species of *Lycopodium* are *epiphytes*, plants that use another organism as a substratum, but are not parasites.

- The sporangia of *Lycopodium* are borne on *sporophylls*, leaves specialized for reproduction. In some, sporophylls are clustered at branch tips into club-shaped strobili—hence the name club moss.
- Spores develop into inconspicuous gametophytes. The non-photosynthetic gametophytes are nurtured by symbiotic fungi.

Most are homosporous.

Genus *Selaginella* is heterosporous.



2. Horsetails (division Sphenophyta)

The division Sphenophyta includes the horsetails; it survived through the Devonian and reached its zenith during the Carboniferous period.

The only existing genus is *Equisetum*, which (see Campbell, Figure 29.13):

- Lives in damp locations and has flagellated sperm
- Is homosporous
- Has a conspicuous sporophyte generation

- Has photosynthetic, free-living gametophytes (not dependent on the sporophyte for food)

3. Ferns (division Pterophyta)

Appearing in the Devonian, ferns radiated into diverse species that coexisted with tree lycopods and horsetails in the great Carboniferous forests.

- Ferns are the most well represented seedless plants in modern floras. There are more than 12,000 existing species of ferns; most diverse in the tropics.
- Fern leaves are generally much larger than those of lycopods and probably evolved in a different way.

  Lycopods have microphylls, small leaves that probably evolved as emergences from the stem that contained a single strand of vascular tissue.

  Ferns have megaphylls, leaves with a branched system of veins. Megaphylls probably evolved from webbing formed between separate branches growing close together.

Most ferns have fronds, compound leaves that are divided into several leaflets (see Campbell, Figure 29.11).

- The emerging frond is coiled into a fiddlehead that unfurls as it grows.
- Leaves may sprout directly from a prostrate stem (bracken and sword ferns) or from upright stems many meters tall (tropical tree ferns).

Ferns are *homosporous* and the conspicuous leafy fern plant is the sporophyte.

- Specialized sporophylls bear sporangia on their undersides; many ferns have sporangia arranged in clusters called sori and are equipped with springlike devices that catapult spores into the air, where they can be blown by the wind far from their origin.
- The spore is the dispersal stage.
- The free-living gametophyte is small and fragile, requiring a moist habitat.
- Water is necessary for fertilization, since flagellated sperm cells must swim from the antheridium to the archegonium, where fertilization takes place.
- The sporophyte embryo develops protected within the archegonium.

C. Seedless vascular plants formed vast "coal forests" during the Carboniferous period

During the Carboniferous period, the landscape was dominated by extensive swamp forests

- Organic rubble of the seedless plants mentioned above accumulated as peat.
- When later covered by the sea and sediments, heat and pressure transformed the peat into coal.

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CHAPTER 30

PLANT DIVERSITY II: THE EVOLUTION OF SEED PLANTS

OUTLINE

I. Overview of Reproductive Adaptations of Seed Plants
   A. The gametophytes of seed plants became even more reduced than the gametophytes of seedless vascular plants
   B. In seed plants, the seed replaced the spore as the main means of dispersing offspring
   C. Pollen became the vehicles for sperm cells in seed plants

II. Gymnosperms
   A. The Mesozoic era was the age of gymnosperms
   B. The four divisions of extant gymnosperms are the cycads, the ginkgo, the gnetophytes, and the conifers
   C. The life cycle of a pine demonstrates the key reproductive adaptations of seed plants

III. Angiosperms (Flowering Plants)
   A. Terrestrial adaptation continued with the refinement of vascular tissue in angiosperms
   B. The flower is the defining reproductive adaptation of angiosperms
   C. Fruits help disperse the seeds of angiosperms
   D. The life cycle of an angiosperm is a highly refined version of the alternation of generations common to all plants
   E. The radiation of angiosperms marks the transition from the Mesozoic to the Cenozoic era
   F. Angiosperms and animals have shaped one another's evolution
   G. Agriculture is based almost entirely on angiosperms

IV. The Global Impact of Plants
   A. Plants transformed the atmosphere and climate
   B. Plant diversity is a nonrenewable resource

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Describe the adaptations of seed plants that have contributed to their success on land.
2. List the four divisions of gymnosperms.
3. Describe the structures of ovulate and pollen cones of a pine and distinguish between the two.

4. Describe the life history of a pine and indicate which structures are part of the gametophyte generation and which are part of the sporophyte generation.
5. Point out the major life cycle differences in ferns and pines.
6. Distinguish between pollination and fertilization.
7. Describe a pine seed and indicate which structures are old sporophyte, gametophyte, and new sporophyte.
8. Describe how the needle-shaped leaves of pines and firs are adapted to dry conditions.
9. List and give examples of the two classes of Anthophyta.
10. Compare the life cycles of mosses, ferns, conifers, and flowering plants in terms of:
    a. Dominant life cycle stage (gametophyte/sporophyte)
    b. Whether they are homosporous or heterosporous
    c. Mechanism of gamete transfer
11. Describe some refinements in vascular tissue that occurred during angiosperm evolution.
12. Explain how evolution of the flower enhanced the reproductive efficiency of angiosperms.
13. Identify the following floral structures and describe a function for each:
    a. Sepals
    b. Petals
    c. Stamens
    d. Carpels
    e. Filament
    f. Anther
    g. Stigma
    h. Style
    i. Ovary
14. Describe four commonly recognized evolutionary trends in floral structure found in various angiosperm lineages.
15. Define fruit and explain how fruits are modified in ways that help disperse seeds.
16. Diagram the generalized life cycle of an angiosperm, identify which structures are haploid, and explain how it differs from the life cycle of a pine.
17. Explain the process of double fertilization and describe the fate of the polyploid nucleus.
18. Explain how an angiosperm seed differs from that of a pine.
19. Explain why paleobotanists have difficulty piecing together the origin of angiosperms and describe some current theories on how flowering plants may have evolved.
20. Explain how animals may have influenced the evolution of terrestrial plants, and vice versa.

KEY TERMS

nucellus	fiber	anther	cross-pollination
integuments	flower	stigma	double fertilization
ovule	sepal	style	cotyledons
seed	petal	ovary	endosperm
conifer	stamen	fruit	coevolution
tracheids	carpel	pollen grains	
vessel elements	filament	embryo sac	

LECTURE NOTES

The emergence of seed plants further transformed the Earth. Seeds and other adaptations of gymnosperms and angiosperms heightened the ability of plants to survive and reproduce in diverse terrestrial environments; these plants became the principal producers in the food webs of most ecosystems on land.

I. Overview of Reproductive Adaptations of Seed Plants

Three life cycle modifications contributed to terrestrial seed plant success:

1. Reduction of the gametophyte. They were retained in the moist reproductive tissue of the sporophyte generation (not independent).
2. Origin of the seed
   - Zygotes developed into embryos packaged with a food supply within a protective seed coat.
   - Seeds replaced spores as main means of dispersal.
3. Evolution of pollen. Plants were no longer tied to water for fertilization.

A. The gametophytes of seed plants became even more reduced than the gametophytes of seedless vascular plants

While the gametophytes of seedless vascular plants develop in the soil as an independent generation, those of seed plants are reduced in size and retained within the moist reproductive tissue of the sporophyte generation.

- This evolutionary trend reverses the gametophyte-sporophyte relationship observed in bryophytes (see Campbell, Figure 30.1).
- Dominance of the diploid generation may afford protection from solar radiation-induced mutations of the genome (damaging radiation is more extensive on land than in aquatic habitats).

B. In seed plants, the seed replaced the spore as the main means of dispersing offspring

The relatively harsh terrestrial environment led to the development of resistant structures for the dispersal of offspring.

- Bryophytes and seedless vascular plants produce and release hardy single-celled spores.
- Seeds are more hardy because of their multicellular nature.

A seed consists of a sporophyte embryo together with a food supply surrounded by a protective coat.

- The sporophytes do not release their spores, but retain them in their sporangia, as a result, the sporophyte also contains a gametophyte.

All seed plants are heterosporous in that they possess two different kinds of sporangia, each producing a different type of spore.

- Megasporangia produce megaspores that give rise to female egg-containing gametophytes.
- Microsporangia produce microspores that give rise to male sperm-containing gametophytes.

The development of the seed is associated with the megasporangia.

- The megasporangium of seed plants is not a chamber, but a fleshy structure called a *nucellus.*
- Additional tissues called *integuments* surround the megasporangium (contribute to the protective coat).

- The resulting structure—megaspore, megasporangium, and integuments—is called an *ovule* (see Campbell, Figure 30.2a).
- The female gametophyte develops within the wall of the megaspore and is nourished by the nucellus.
- If the egg cell of a female gametophyte is fertilized by a sperm cell (see Campbell, Figure 30.2b), the zygote develops into a sporophyte embryo.
- The resulting sporophyte-containing ovule develops into a seed (see Campbell, Figure 30.2c).

C. Pollen became the vehicles for sperm cells in seed plants

The microspores develop into pollen grains, which in turn, mature to form the male gametophores of seed plants.

- Pollen grains are coated with a resilient polymer, sporopollenin (see Chapter 29).
- Pollen grains can be carried away by wind or animals (e.g., bees) following release from microsporangia.

A pollen grain near an ovule will extend a tube through sperm cells into the female gametophyte within the ovule.

- In some gymnosperms, the sperm cells are flagellated (ancestral condition).
- Other gymnosperms (including conifers) and angiosperms do not have flagellated sperm cells.

II. Gymnosperms

A. The Mesozoic era was the age of gymnosperms

Gymnosperms appear in the fossil record much earlier than flowering plants. Gymnosperms most likely descended from Devonian progymnosperm and were seedless.

- Seeds evolved by the end of the Devonian.
- Adaptive radiation during the Carboniferous and Permian periods led to today's divisions.
- During the Permian, Earth became warmer and drier; therefore, lycopods, horsetails, and ferns (previously dominant) were largely replaced by conifers and their relatives, the cycads (two divisions of gymnosperms).
- This large change marks the end of the Paleozoic era and the beginning of the Mesozoic era.

Gymnosperms lack enclosed chambers (ovaries) in which seeds develop.

B. The four divisions of extant gymnosperms are the cycads, the ginkgo, the gnetophytes, and the conifers

Campbell, Figure 30.3, shows the four divisions of living gymnosperms.

The *conifers* are the largest division of gymnosperms.

- Most are evergreens: pines, firs, spruces, larches, yews, junipers, cedars, cypresses, and redwoods all belong to this division.
- Includes some of the tallest (redwoods and some eucalyptus); largest (giant sequoias); and oldest (bristle cone pine) living organisms.
- Most lumber and paper pulp is from conifer wood.

Needle-shaped conifer leaves are adapted to dry conditions.

- Thick cuticle covers the leaf
- Stomata are in pits, reducing water loss
- Despite the shape, needles are megaphylls, as are leaves of all seed plants.

C. The life cycle of a pine demonstrates the key reproductive adaptations of seed plants

The life cycle of pine, a representative conifer, is characterized by the following:

- The multicellular sporophyte is the most conspicuous stage; the pine tree is a sporophyte, with its sporangia located on cones.
- The multicellular gametophyte generation is reduced and develops from haploid spores that are retained within sporangia.

  The male gametophyte is the pollen grain; there is no antheridium.

  The female gametophyte consists of multicellular nutritive tissue and an archegonium that develops within an ovule.

Conifer life cycles are heterosporous; male and female gametophytes develop from different types of spores produced by separate cones.

- Trees of most pine species bear both pollen cones and ovulate cones, which develop on different branches.
- Pollen cones have microsporangia; cells in these sporangia undergo meiosis producing haploid microspores, small spores that develop into pollen grains—the male gametophytes.
- Ovulate cones have megasporangia; cells in these sporangia undergo meiosis producing large megaspores that develop into the female gametophyte (see Campbell, Figure 30.4). Each ovule initially includes a megasporangium (nucellus) enclosed in protective integuments with a single opening, the micropyle.

It takes nearly three years to complete the pine life cycle, which progresses through a complicated series of events to produce mature seeds.

- Windblown pollen falls onto the ovulate cone and is drawn into the ovule through the micropyle.
- The pollen grain germinates in the ovule, forming a pollen tube that begins to digest its way through the nucellus.
- A megaspore mother cell in the nucellus undergoes meiosis producing four haploid megaspores, one of which will survive; it grows and divides repeatedly by mitosis producing the immature female gametophyte.
- Two or three archegonia, each with an egg, then develop within the multicellular gametophyte.
- More than a year after pollination, the eggs are ready to be fertilized; two sperm cells have developed and the pollen tube has grown through the nucellus to the female gametophyte.
- Fertilization occurs when one of the sperm nuclei unites with the egg nucleus. All eggs in an ovule may be fertilized, but usually only one zygote develops into an embryo.
- The pine embryo, or new sporophyte, has a rudimentary root and several embryonic leaves. It is embedded in the female gametophyte, which nourishes the embryo until it is capable of photosynthesis. The ovule has developed into a pine seed, which consists of an embryo (2n), its food source (n), and a surrounding seed coat (2n) derived from the integuments of the parent tree.
- Scales of the ovulate cone separate, and the winged seeds are carried by the wind to new locations. Note, that with the seed plants, the seed has replaced the spore as the mode of dispersal.
- A seed that lands in a habitable place germinates, its embryo emerging as a pine seedling.

III. Angiosperms (Flowering Plants)

Flowering plants are the most widespread and diverse; 250,000 species are now known.

- There is only one division, Anthophyta, with two classes, Monocotyledones (monocots) and Dicotyledones (dicots).
- Most use insects and animals for transferring pollen, and therefore, are less dependent on wind and have less random pollination.

A. Terrestrial adaptation continued with refinement of vascular tissue in angiosperms

Vascular tissue became more refined during angiosperm evolution.

- Conifers have *tracheids* (see Campbell, Figure 30.5), water-conducting cells that are:
  - An early type of xylem cell
  - Elongated, tapered cells that function both in mechanical support and water movement up the plant
- Most angiosperms also have *vessel elements* that are:
  - Shorter, wider cells than the more primitive tracheids
  - Arranged end to end forming continuous tubes
  - Compared to tracheids, vessel elements are more specialized for conducting water, but less specialized for support
- Angiosperm xylem is reinforced by other cell types called *fibers*, which are:
  - Specialized for support with a thick lignified wall
  - Evolved in conifers. (Conifer xylem contains both fibers and tracheids, but not vessel elements.)

B. The flower is the defining reproductive adaptation of angiosperms

Flower = The reproductive structure of an angiosperm which is a compressed shoot with four whorls of modified leaves (see also Campbell, Figure 30.6)

Parts of the flower:

Sepals - Sterile, enclose the bud

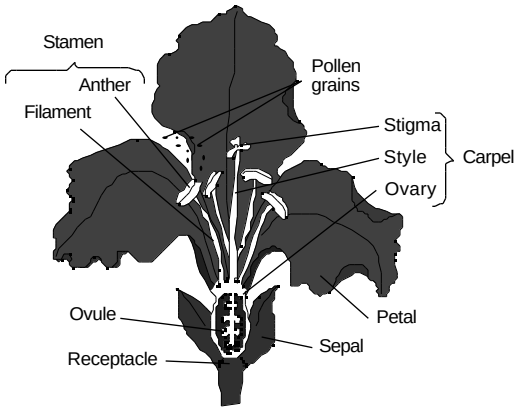
Petals – Sterile, aid in attracting pollinators

Stamen – Produces the pollen

Carpel – Evolved from a seed-bearing leaf that became rolled into a tube

Stigma – Part of the carpel that is a sticky structure that receives the pollen

Ovary – Part of the carpel that protects the ovules, which develop into seeds after fertilization



There are four evolutionary trends in various angiosperm lineages:

1. The number of floral parts have become reduced.
2. Floral parts have become fused.
3. Symmetry has changed from radial to bilateral.
4. The ovary has dropped below the petals and sepals, where the ovules are better protected.

C. Fruits help disperse the seeds of angiosperms

Fruit = A ripened ovary that protects dormant seeds and aids in their dispersal; some fruits (like apples) incorporate other floral parts along with the ovary (see Campbell, Figure 30.7)

Aggregate fruits = Several ovaries that are part of the same flower (e.g., raspberry)

Multiple fruit = One that develops from several separate flowers (e.g., pineapple)

Modifications of fruits that help disperse seeds include:

- Seeds within fruits that are shaped like kites or propellers to aid in wind dispersal
- Burr-like fruit that cling to animal fur
- Edible fruit with tough seeds which pass through the digestive tract of herbivores unharmed, dispersing seeds miles away

D. The life cycle of an angiosperm is a highly refined version of the alternation of generations common to all plants

Life cycles of angiosperms are heterosporous (in common with all seed plants) and the two types of sporangia are found in the flower (see Campbell, Figure 30.8):

- Microsporangia in anthers produce microspores that form male gametophytes.
- Megasporangia in ovules produce megaspores that develop into female gametophytes.

Immature male gametophytes:

- Are *pollen grains*, which develop within the anthers of stamens
- Each pollen grain has two haploid nuclei that will participate in *double fertilization* characteristic of angiosperms.

Female gametophytes:

- Do *not* produce an archegonium
- Are located within an ovule
- Consist of only a few cells: an *embryo sac* with eight haploid nuclei in seven cells (a large central cell has two haploid nuclei)
- One of the cells is the egg

An outline of the angiosperm life cycle follows:

- Pollen from the anther lands on the sticky stigma at the carpel's tip; most flowers do not self-pollinate, but have mechanisms to ensure *cross-pollination.*
- The pollen grain germinates on the stigma by growing a pollen tube down the style of the carpel.
- When it reaches the ovary, the pollen tube grows through its micropyle and discharges two sperm cells into the embryo sac.
- Double fertilization occurs as one sperm nucleus unites with the egg to form a diploid zygote; the other sperm nucleus fuses with two nuclei in the embryo sac's central cell to form triploid (3*n*) endosperm.
- After double fertilization, the ovule matures into a seed.

The seed is a mature ovule, consisting of:

1. Embryo. The zygote develops into an embryo with a rudimentary root and one (in monocots) or two (in dicots) *cotyledons* or seed leaves.
2. Endosperm. The triploid nucleus in the embryo sac divides repeatedly forming triploid endosperm, rich in starch and other food reserves.
3. Seed coat. This is derived from the integuments (outer layers of the ovules).

Monocots and dicots use endosperm differently.

- Monocot seeds store most food in the endosperm.
- Dicots generally restock most of the nutrients in the developing cotyledons.

In a suitable environment the seed coat ruptures and the embryo emerges as a seedling, using the food stored in the endosperm and cotyledons.

E. The radiation of angiosperms marks the transition from the Mesozoic to the Cenozoic era

Angiosperms showed a relatively sudden appearance in the fossil record with no clear transitional links to ancestors.

- Earliest fossils are early Cretaceous (approximately 130 million years ago)
- dominant, as they are today.

There are two theories about their sudden appearance:

1. Angiosperms originated where fossilization was unlikely (they are an artifact of an imperfect fossil record).
2. Angiosperms evolved and radiated relatively abruptly *(punctuated equilibrium*).

Perhaps angiosperms evolved from seed ferns, an extinct group of unspecialized gymnosperms.

F. Angiosperms and animals shaped one another's evolution

Terrestrial plants and animals have coevolved, a consequence of their interdependence.

Coevolution = Reciprocal evolutionary responses among two or more interacting species; adaptive change in one species is in response to evolutionary change in the other species.

Coevolution between angiosperms and their pollinators led to diversity of flowers.

- Some pollinators are specific for a particular flower. The pollinator has a monopoly on a food source and guarantees the flower's pollen will pollinate a flower of the same species (see Campbell, Figure 30.9).
- Often, the relationship between angiosperms and their pollinators is not species specific; a pollinator may not depend exclusively on one flower species, or a flower species may not depend exclusively on one species of pollinator. However, flower color, fragrance, and structure are usually adaptations for *types* of pollinators, such as various species of bees or hummingbirds.

Edible fruits of angiosperms have coevolved with animals that can disperse seeds. Animals become attracted to ripening fruits as they:

- Become softer, more fragrant, and higher in sugar
- Change to a color that attracts birds and mammals, animals which are large enough to disperse the seeds

G. Agriculture is based almost entirely on angiosperms

Angiosperms provide nearly all our food: fruit, vegetable crops, and grains, such as corn, rice, wheat.

Flowering plants are also used for other purposes, such as:

- Fiber
- Medication
- Perfume
- Decoration

Through agriculture, humans have influenced plant evolution by artificially selecting for plants that improved the quantity and quality of foods and other crops.

- Many of our agricultural plants are so genetically removed from their origins that they probably could not survive in the wild.

- As a consequence, cultivated crops that require human intervention to water, fertilize, provide protection from insects and disease, and even to plant their seeds, are vulnerable to natural and human-caused disasters.

V. The Global Impact of Plants

A. Plants transformed the atmosphere and the climate

In addition to being the primary producers of the terrestrial environment, plants also changed the physical environment of Earth.

- They decreased atmospheric carbon dioxide, resulting in global cooling.
- The cooler environment may have made terrestrial life more habitable for other organisms.

B. Plant diversity is a nonrenewable resource

Plant diversity is a nonrenewable resource, and the irrevocable extinction of plant species is occurring at an unprecedented rate.

- The exploding human population demands space and natural resources.
- The toll of habitat destruction is greatest in the tropics because this is where:

  Most species live

  More than half the human population lives and human population growth is fastest

  Most deforestation is caused by slash-and-burn clearing for agriculture

As the forest disappears, so do thousands of plant and animal species.

- Habitat destruction also endangers animal species that depend on plants in the tropical rainforest.
- Habitat destruction by humans has not been limited to the tropics. Europeans eliminated most of their forests centuries ago, and in North America, destruction of habitat is endangering many species.

There are many reasons to value plant diversity and to find ways to protect it.

- Ecosystems are living treasures that can regenerate only slowly.
- Humans depend on plants for products such as medicines, food and building materials.
- We still know so little of the 250,000 known plant species. (Food agriculture is based on only about two dozen species.)

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CHAPTER 31
FUNGI

OUTLINE

I. Introduction to Fungi
   A. Absorptive nutrition enables fungi to live as decomposers and symbionts
   B. Extensive surface area and rapid growth adapt fungi for absorptive nutrition
   C. Fungi reproduce by releasing spores that are produced either sexually or asexually

II. Diversity of Fungi
   A. Division Chytridiomycota: chytrids may provide clues about fungal origins
   B. Division Zygomycota: zygote fungi form resistant dikaryotic structures during sexual reproduction
   C. Division Ascomycota: sac fungi produce sexual spores in saclike asci
   D. Division Basidiomycota: club fungi have long-lived dikaryotic mycelia and a transient diploid stage
   E. Molds, yeasts, lichens, and mycorrhizae represent unique lifestyles that evolved independently in three fungal divisions

III. Ecological Impacts of Fungi
   A. Ecosystems depend on fungi as decomposers and symbionts
   B. Some fungi are pathogens
   C. Many animals, including humans, eat fungi

IV. Phylogenetic Relationships of Fungi
   A. Fungi and animals probably evolved from a common protistan ancestor

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. List characteristics that distinguish fungi from organisms in other kingdoms.
2. Explain how fungi acquire their nutrients.
3. Explain how non-motile fungi seek new food sources and how they disperse.
4. Describe the basic body plan of a fungus.
5. Distinguish between septate and aseptate (coenocytic) fungi.
6. Describe some advantages to the dikaryotic state.
7. Distinguish among fungi and list some common examples of each.
8. Describe asexual and sexual reproduction in Zygomycota, Ascomycota, and Basidiomycota, and the sexual structure that characterizes each group.
9. Explain the difference between conidia and ascospores.
10. Explain why ascomycetes can be useful to geneticists studying genetic recombination.
11. Explain why the Deuteromycota are called imperfect fungi.

12. Describe the anatomy of lichens and explain how they reproduce.
13. Provide evidence for both sides of the debate on whether symbiosis in lichens is parasitic or mutualistic.
14. Describe the ecological importance of lichens.
15. Explain why fungi are ecologically and commercially important.
16. Describe how the mutualistic relationship in mycorrhizae is beneficial to both the fungus and the plant, and explain its importance to natural ecosystems and agriculture.
17. Describe a scenario for fungal phylogeny and list two possible ancestors of Zygomycota.

KEY TERMS

absorption	plasmogamy	asci	imperfect fungi
hyphae	karyogamy	ascocarp	yeast
mycelium	dikaryon	conidia	lichen
septa	chytrids	basidium	soredia
chitin	zygote fungi	club fungus	
coenocytic	mycorrhizae	basidiocarps	
haustoria	sac fungi	mold	

LECTURE NOTES

I. Introduction to the Fungi

Fungi are eukaryotes, and nearly all are multicellular (although yeasts are unicellular). Their nutrition, structural organization, growth, and reproduction distinguish them from organisms in other kingdoms.

A. Absorptive nutrition enables fungi to live as decomposers and symbionts

Fungi are heterotrophs that acquire nutrients by *absorption*.

- They secrete hydrolytic enzymes and acids to decompose complex molecules into simpler ones that can be absorbed.
- Fungi are specialized into three main types:
  1. Saprobes, which absorb nutrients from dead organic material
  2. Parasitic fungi, which absorb nutrients from the cells of living hosts; some are pathogenic
  3. Mutualistic fungi, which absorb nutrients from a host, but reciprocate to benefit the host.

Fungi exist in diverse habitats and form symbioses with many organisms.

For example, fungi are found in:

- Terrestrial habitats
- Aquatic habitats, both freshwater and marine
- Symbiotic relationships with algae to form lichens

B. Extensive surface area and rapid growth adapt fungi for absorptive nutrition

The basic structural unit of a fungal vegetative body (*mycelium*) is the *hypha*. Except for yeasts, fungal bodies are diffuse, intertwining mats of hyphae that are organized around and within their food source (see Campbell, Figure 31.1).

These hyphae:

- Are composed of tubular walls containing *chitin*, a strong, flexible nitrogen-containing polysaccharide similar to that found in arthropod exoskeletons
- Provide enormous surface area for the absorptive mode of nutrition. Parasitic fungi have modified hyphae called *haustoria*, which are nutrient absorbing hyphae that penetrate host tissue, but remain outside the host cell membrane (see Campbell, Figure 31.2c).

Fungal hyphae may be aseptate or septate.

- Hyphae of aseptate fungi lack cross-walls and are *coenocytic*, formed from repeated nuclear division without cytokinesis (see Campbell, Figure 31.2b).
- Hyphae of septate fungi are divided into cells by crosswalls called *septa*. Pores in the septa allow organelles to move from cell to cell.

True fungi have no flagellated stages in their life cycle. This characteristic is partly why the Chytridiomycota and Oomycota have been moved to the Protista.

C. Fungi reproduce by releasing pores that are produced either sexually or asexually

Mycelial growth is adapted to the absorptive node of nutrition.

- Mycelia grow in length, not girth, which maximizes the surface area for absorption.
- Mycelia grow rapidly, as much as a kilometer of hyphae each day. Fast growth can occur because cytoplasmic streaming carries molecules synthesized by the mycelium to the growing hyphal tips.
- Since fungi are nonmotile, they cannot search for food or mates. Instead, they grow in hyphal length to reach new food sources and territory.

Fungal chromosomes and nuclei are relatively small, and the nuclei divide differently from most other eukaryotes.

- During mitosis, the nuclear envelope remains intact from prophase to anaphase; the spindle is inside the nuclear envelope.
- After anaphase, the nuclear envelope pinches in two, and the spindle disappears.

Fungi reproduce by releasing spores that are:

- Usually unicellular, haploid, and of various shapes and sizes.
- Produced either sexually (by meiosis) or asexually (by mitosis). In favorable conditions, fungi generally produce enormous numbers of spores asexually. For many fungi (not all), sexual reproduction occurs only as a contingency for stressful environmental conditions.
- The agent of dispersal responsible for the wide geographic distribution of fungi. Carried by wind or water, spores germinate if they land in a moist place with an appropriate substratum.

Except for transient diploid stages in sexual life cycles, fungal hyphae and spores are haploid. Some mycelia may, however, be genetically heterogeneous resulting from fusion of hyphae with different nuclei.

- The different nuclei may stay in separate parts of the same mycelium.
- Alternatively, the different nuclei may mingle and even exchange genes in a process similar to crossing over.

The sexual cycle in fungi differs from other eukaryotic organisms in that syngamy occurs in two stages that are separated in time.

Syngamy = The sexual union of haploid cells from two individuals. In fungi, syngamy occurs in two stages:

1. *Plasmogamy*, the fusion of cytoplasm
2. *Karyogamy*, the fusion of nuclei

After plasmogamy, haploid nuclei from each parent pair up, forming a *dikaryon*, but they do not fuse.

- Nuclear pairs in dikaryons may exist and divide synchronously for months or years.
- The dikaryotic condition has some advantages of diploidy; one haploid genome may compensate for harmful mutations in the other nucleus.
- Eventually, the haploid nuclei fuse forming a diploid cell that immediately undergoes meiosis.

II. Diversity of Fungi

There are four divisions of fungi (see Campbell, Figure 31.4). They differ in the:

- Structures involved in plasmogamy
- Length of time spent as a dikaryon
- Location of karyogamy; the fungal divisions are named for the sexual structures in which karyogamy occurs.

A. Division Chytridiomycota: chytrids may provide clues about fungal origins

The Chytridiomycota and Fungi may share a protistan ancestor.

- Chytrids were placed in the Kingdom Protista because they form flagellated zoospores and gametes—a protistan characteristic.
- However, chytrids and fungi share many characteristics, such as:

  An absorptive mode of nutrition

  Cell walls of chitin

  Most form hyphae

  Key enzymes and metabolic pathways that are not found in the other fungus-like protists (slime molds and water molds)

  Similar sequences of proteins and nucleic acids
- This evidence lends support for

  Combining the chytrids with fungi as a monophyletic group

  The hypothesis that chytrids are the most primitive fungi, diverging earliest in fungal phylogeny.

  The hypothesis that fungi evolved from protists with flagella, a feature retained by the chytrids.

B. Division Zygomycota: zygote fungi form resistant dikaryotic structures during sexual reproduction

Fungi in the division Zygomycota are characterized by the presence of dikaryotic *zygosporangia*, resistant structures formed during sexual reproduction.

- Zygomycetes are mostly terrestrial and live in soil or on decaying organic material.
- Some form *mycorrhizae*, mutualistic associations with plant roots (see Campbell, Figure 31.16).
- Zygomycete hyphae are coenocytic; septa are found only in reproductive cells.

See Campbell, Figure 31.6 for the life cycle of the zygomycete, *Rhizopus stolonifer*, a common bread mold.

- The mycelium consists of horizontal hyphae that spread out and penetrate the food source.
- Under favorable environmental conditions, *Rhizopus* reproduces asexually:

  Sporangia develop at the tips of upright hyphae.

  Mitosis produces hundreds of haploid spores that are dispersed through the air.

  If they land in a moist, favorable environment, spores germinate into new mycelia.
- In unfavorable conditions, *Rhizopus* begins its sexual cycle of reproduction:

  Mycelia of opposite mating types (+ and −) form gametangia that contain several haploid nuclei walled off by the septum.

  Plasmogamy of the + and − gametangia occurs, and the haploid nuclei pair up forming a dikaryotic zygosporangium that is metabolically inactive and resistant to desiccation and freezing.

  When conditions become favorable, karyogamy occurs between paired nuclei; the resulting diploid nuclei immediately undergo meiosis producing genetically diverse haploid spores.

  The zygosporangium germinates a sporangium that releases the genetically recombined haploid spores.

  If they land in a moist, favorable environment, spores germinate into new mycelia.

Even though air currents are not a very precise way to disperse spores, *Rhizopus* releases so many that enough land in hospitable places. Some zygomycetes, however, can actually aim their spores.

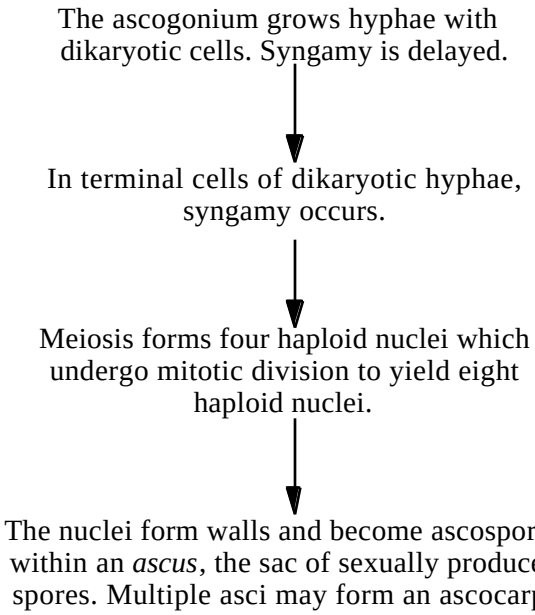
- For example, *Pilobolus*, a fungus that decomposes animal dung, bends sporangium-bearing hyphae toward light, where grass is likely to be growing.
- The sporangium is shot out of the hypha, dispersing spores away from the dung and onto surrounding grass. If an herbivore eats the grass and consumes the spores, the asexual life cycle is completed when the animal disperses the spores in its feces.

C. Division Ascomycota: sac fungi produce sexual spores in saclike asci

Ascomycetes include unicellular yeasts and complex multicellular cup fungi (see Campbell, Figure 31.7).

- Hyphae are septate.

- In asexual reproduction, the tips of specialized hyphae form *conidia*, which are chains of haploid, asexual spores that are usually wind dispersed.
- In sexual reproduction, haploid mycelia of opposite mating strains fuse. One acts as "female" and produces an ascogonium which receives haploid nuclei from the antheridium of the "male" (see Campbell, Figure 31.8).



Ascocarps = Fruiting structures of many asci packed together

- The ascospores of each ascus are lined up in a row in the order in which they formed from a single zygote, allowing geneticists to study genetic recombination.
- Unicellular yeasts appear dissimilar, but produce the equivalent of an ascus during sexual reproduction and bud during asexual reproduction in a manner similar to the formation of conidia. Thus, they are classified as ascomycetes.
- Includes important decomposers and both mutualistic and parasitic symbionts
- Many live symbiotically with algae as lichens.

D. Division Basidiomycota: club fungi have long-lived dikaryotic mycelia and a transient diploid stage

The division Basidiomycota, or *club fungi*, includes mushrooms, shelf fungi, puffballs, and stinkhorns (see Campbell, Figure 31.9).

Basidiomycetes:

- Are named for a transient diploid stage called the *basidium*, a club-shaped spore-producing structure.
- Are important decomposers of wood and other plant material. Saprobic basidiomycetes can decompose the complex polymer lignin, an abundant component of wood.
- Include mycorrhiza-forming mutualists and plant parasites. Many shelf fungi are tree parasites that function later as saprobes after the trees die.

- Include mushroom-forming fungi, only a few of which are strictly parasitic. About half are saprobic and the other half form mycorrhizae.
- Include the rusts and smuts, which are plant parasites.

Basidiomycete life cycles are characterized by a long-lived *dikaryotic* mycelium that reproduces sexually by producing fruiting bodies called *basidiocarps*. Refer to Campbell, Figure 31.10 for the life cycle of a mushroom-forming basidiomycete.

- Haploid basidiospores grow into short-lived haploid mycelia. Under certain environmental conditions, *plasmogamy* occurs between two haploid mycelia of opposite mating types (+ and −).
- The resulting dikaryotic mycelium grows; depending upon the species, it may form mycorrhizae with trees. Certain environmental cues stimulate the mycelium to produce mushrooms (basidiocarps). A "fairy ring" is an expanding ring of living mycelium that produces mushrooms above it; it slowly increases in diameter, about 30 cm per year.
- The mushroom cap supports and protects a large surface area of gills; karyogamy in the terminal, dikaryotic cells lining the gills produces diploid basidia.
- Each basidium immediately undergoes meiosis producing four haploid basidiospores. When mature, these sexual spores drop from the cap and are dispersed by wind.

Asexual reproduction occurs less often than in ascomycetes, but also results in conidia formation.

E. Molds, yeasts, lichens, and mycorrhizae represent unique lifestyles that evolved independently in three fungal divisions

1. Molds

Mold = A rapidly growing, asexually reproducing fungus

Molds may be saprobes or parasites on a great variety of substrates.

Molds only include asexual stages; they may be zygomycetes, ascomycetes, basidiomycetes or fungi with no known sexual stage.

Since molds are classified by their sexual stages (zygosporangium, ascogonium or basidium), molds with no known sexual stage cannot be classified as zygomycetes, ascomycetes, or basidiomycetes.

Molds with no known sexual stages are classified as Deuteromycota or *imperfect fungi*.

- Imperfect fungi reproduce asexually by producing spores.
- Deuteromycetes are sources of antibiotics. Penicillin is produced by some species of *Penicillium*, which are ascomycetes.
- Other commercial uses of imperfect fungi include flavoring for cheeses, such as blue cheese, Brie, Camembert and Roquefort; fermenting food products such as soybeans; and providing pharmaceuticals such as cyclosporine.
- Some deuteromycetes are predatory soil fungi that kill small animals such as soil nematodes (see Campbell, Figure 31.12).

2. Yeasts

Yeasts are unicellular fungi that inhabit liquid or moist habitats; some can alternate between mycelium or yeast, depending on the amount of liquid in the environment.

Yeasts reproduce:

- Asexually by simple cell division or by budding off from a parent cell; some are classified as Deuteromycota, if no sexual stages are known.
- Sexually by forming asci (Ascomycota) or basidia (Basidiomycota)

Though humans have used yeasts to raise bread and ferment alcoholic beverages for thousands of years, only recently have they been separated into pure culture for more controlled human use.

- *Saccharomyces cerevisiae* is the most important of all domesticated fungi (see Campbell, Figure 31.13). Highly active metabolically, this ascomycete is available as baker's and brewer's yeast.
- In an aerobic environment, baker's yeast respires, releasing small bubbles of carbon dioxide that leaven dough; cultured anaerobically, *Saccharomyces* ferments sugars to alcohol.
- Researchers use *Saccharomyces* to study eukaryotic molecular genetics because it is easy to culture and manipulate.

Some yeasts cause problems for humans.

- *Rhodotorula*, a pink yeast, grows on shower curtains and other moist surfaces.
- *Candida*, a normal inhabitant of moist human tissues, can become pathogenic when there is a change in pH or other environmental factor; or when an individual's immune system is compromised.

3. Lichens

Lichen = Highly integrated symbiotic association of algal cells (usually filamentous green algae or blue-green algae) with fungal hyphae (usually ascomycetes)

Though lichens vary in shape and physiology, some shared general features characterize the symbiotic relationship.

The alga, which is below the lichen's surface (see Campbell, Figure 31.15),

- Always provides the fungus with food
- May fix nitrogen (e.g.,. cyanobacteria)

The fungus provides a suitable environment for algal growth:

The hyphal mass:

- Absorbs needed minerals from airborne dust or rain
- Retains water and minerals
- Allows gas exchange
- Protects the algae

The fungus produces unique organic compounds with several functions.

- Fungal pigments shade the algae from intense sunlight.
- Toxic fungal compounds prevent lichens from being eaten by consumers.
- Fungal acid secretion aids the uptake of minerals.

Most of the lichen's mass is hyphal tissue which gives the lichen its shape and structure. Named for their fungal component, lichens are informally categorized as:

- Foliose (leafy)
- Fruticose (shrubby)

- Crustose (crusty)

Lichen reproduction occurs as a combined unit or as independent reproduction of the symbionts.

- Many lichen fungi reproduce sexually by forming ascocarps or rarely, basidiocarps.
- Lichen algae reproduce independently by asexual cell division.
- Symbiotic units commonly reproduce asexually by:

  Fragmentation of the parental lichen

  Formation of *soredia*, specialized reproductive structures that are small clusters of hyphae with embedded algae.

Though most evidence points to a mutualistic symbiosis, some debate that the relationship may actually be parasitic.

- The argument for mutualism is that fungi benefit the algae and that lichens can survive in habitats that are inhospitable to either organism alone.
- The argument for "controlled parasitism" is based on the fact that the fungus actually kills some algal cells, though not as fast as the algae replenishes itself.

Lichens are important pioneers, breaking down rock and allowing for colonization by other plants.

- Some can tolerate severe cold.
- Photosynthesis occurs when lichen water content is 65-75%.

Lichens are sensitive to air pollution due to their mode of mineral uptake.

4. Mycorrhizae

Mycorrhizae are specific, mutualistic associations of plant roots and fungi (see Campbell, Figure 31.16).

- The fungi increase the absorptive surface of roots and exchanges soil minerals.

Mycorrhizae are seen in 95% of all vascular plants.

They are necessary for optimal plant growth.

III. Ecological Impacts of Fungi

A. Ecosystems depend on fungi as decomposers and symbionts

Fungi and bacteria are the principal decomposers in ecosystems. Decomposition allows for the recycling of nutrients between biotic and abiotic components.

Fungi decompose food, wood, and even certain plastics.

Between 10% - 50% of the world's fruit harvest is lost each year to fungal attack.

B. Some fungi are pathogens

Many fungi are pathogenic (e.g., athletes foot, ringworm, and yeast infections).

Plants are particularly susceptible. For example, Dutch elm disease, caused by an ascomycete, drastically changed the landscape of northeastern United States.

Ergots = Purple structure on rye caused by an ascomycete

- Causes gangrene, hallucinations, and burning sensations (St. Anthony's fire).
- Produces lysergic acid, from which LSD is made.

Toxins from fungi may be used in weak doses for medical purposes such as treating high blood pressure.

C. Many animals, including humans, eat fungi

Fungi are consumed as food by a variety of animals, including humans.

- In the U.S., mushroom (basidiomycete) consumption is usually restricted to one species of *Agaricus*, which is cultivated commercially on compost in the dark.
- In many other countries, however, people eat a variety of cultivated and wild mushrooms.
- Truffles prized by gourmets are underground ascocarps of mycelia that are mycorrhizal on tree roots (see Campbell, Figure 31.17). The fruiting bodies (ascocarps) release strong odors that attract mammals and insects—consumers that excavate the truffles and disperse their spores.
- Since it is difficult for novices to distinguish between poisonous and edible mushrooms, only qualified experts at identification should collect wild mushrooms for eating.

IV. Phylogenetic Relationships of Fungi

A. Fungi and animals probably evolved from a common protistan ancestor

The presence of flagella in the most primitive group of fungi, the chytrids, suggests that the ancestors of fungi were flagellated and that the lack of flagella in the other fungi divisions is a secondary condition.

There is compelling evidence that animals and fungi diverged from a common protistan ancestor.

- Animals also probably evolved from flagellated protists.
- Proteins and rRNA comparisons indicate that fungi and animals are more closely related to each other than either is to plants. Molecular systematists believe the most likely protistan ancestor common to fungi and animals was a choanoflagellate.

Perhaps the fungi are a consequence of adaptive radiation when life began to colonize land.

- The oldest undisputed fossils are 450-500 million years old.
- All major groups of fungi evolved by the end of the Carboniferous period (approximately 300 million years ago).
- Plants and fungi moved from water to land together. Fossils of the first vascular plants have mycorrhizae.

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CHAPTER 32
INTRODUCTION TO ANIMAL EVOLUTION

OUTLINE

I. What Is an Animal?
II. An Overview of Animal Phylogeny and Diversity
  A. Parazoans lack true tissues
  B. Evolution of body cavities led to more complex animals
  C. Coelomates branched into protostomes and deuterostomes
III. The Origins of Animal Diversity
  A. Most animal phyla originated in a relatively brief span of geological time
  B. Developmental genetics may clarify our understanding of the Cambrian diversification

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. List characteristics that distinguish animals from organisms in the other four kingdoms.
2. Distinguish between radial and bilateral symmetry.
3. Outline the major phylogenetic branches of the animal kingdom, which are based upon grade of organization; symmetry and embryonic germ layers; absence or presence of a body cavity; and protostome-deuterostome dichotomy.
4. Distinguish among acoelomate, pseudocoelomate and coelomate.
5. Distinguish between spiral and radial cleavage; determinant and indeterminate cleavage; schizocoelous and enterocoelous.
6. Compare developmental differences between protostomes and deuterostomes including:
   a. Plane of cleavage
   b. Determination
   c. Fate of the blastopore
   d. Coelom formation
7. Compare and contrast two hypotheses about animal origins from unicellular ancestors: syncytial hypothesis and colonial hypothesis.
8. Explain why it is difficult to resolve what the first animals looked like.
9. Describe two views about discontinuities between Ediacaran and Cambrian fauna.

KEY TERMS

ingestion	bilateral symmetry	archenteron	deuterostomes
cleavage	dorsal	mesoderm	spiral cleavage
blastula	ventral	diploblastic	determinate cleavage
gastrulation	anterior	triploblastic	radial cleavage
larva	posterior	acoelomates	indeterminate cleavage
metamorphosis	bilateria	pseudocoelom	blastopore

parazoa	cephalization	pseudocoelomates	schizocoelous
eumetazoa	germ layers	coelomates	enterocoelous
radial symmetry	ectoderm	coelom	Ediacaran period
radiata	endoderm	protostomes	Cambrian explosion

LECTURE NOTES

Over one million species of animals are living today; 95% of these are invertebrates.

- Grouped into about 35 phyla depending on the taxonomic view followed.
- Most are aquatic.
- The most familiar belong to the subphylum Vertebrata of the phylum Chordata. This is only about 5% of the total.

I. What Is an Animal?

Although there is great animal diversity, most animals share the following characteristics:

- Multicellular, eukaryotic organisms
- Heterotrophy is by ingestion.
  - *Ingestion* = Eating other organisms or decomposing organic matter (detritus). This mode of nutrition distinguishes animals from the plants and fungi.
  - Carbohydrate reserves generally are stored as glycogen.
- No cell walls are present, but animals do have intercellular junctions: desmosomes, gap junctions, and tight junctions.
- Highly differentiated body cells which are organized into tissues, organs and organ systems for such specialized functions as digestion, internal transport, gas exchange, movement, coordination, excretion, and reproduction.
- Nervous tissue (impulse conduction) and muscle tissue (movement) are unique to animals.
- Reproduction is typically sexual with flagellated sperm fertilizing nonmotile eggs to form diploid zygotes. A diploid stage dominates the life cycle.
  - The zygote undergoes a series of mitotic divisions known as *cleavage* which produces a *blastula* in most animals.
  - *Gastrulation* occurs after the blastula has formed; during this process, the embryonic forms of adult body tissues are produced.
  - Development in some animals is direct to maturation while the life cycles of others include *larvae* which undergo *metamorphosis* into a sexually mature adults.
  - *Larva* = Free-living, sexually immature forms

The seas contain the greatest diversity of animal phyla, although many groups live in fresh water and terrestrial habitats.

II. An Overview of Animal Phylogeny and Diversity

Animals diversified so rapidly during the late Precambrian and early Cambrian periods that it is difficult to determine the exact sequence of branching from the fossil record.

- To reconstruct the evolutionary history of the animal phyla, zoologists use information from comparative anatomy, embryology of living animals, and molecular systematics.

- Most zoologists agree that the animal kingdom is monophyletic and that the ancestral organism was probably a colonial flagellated protist related to choanoflagellates (see Campbell, Figure 32.2).

A. Parazoans lack true tissues

Sponges (Phylum Porifera) represent an early branch of the animal kingdom (see Campbell, Figure 32.3:

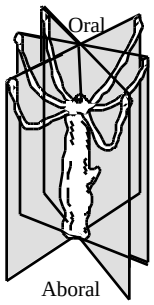
- Have unique development and simple anatomy that separates them from other animals
- Lack true tissues, therefore, they are called parazoa ("beside the animals")

The presence of true tissues is characteristic of nearly all the other groups of animals, collectively known as *eumetazoa*. True tissues permitted the evolution of a more complex anatomy.

B. Radiata and bilateria are the major branches of eumetazoans

The division of eumetazoans into two branches is based partly on body symmetry (see Campbell, Figure 32.3).

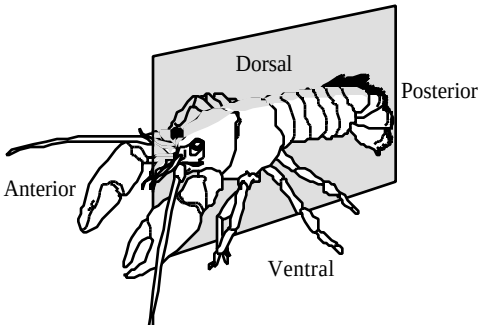
- *Radiata* exhibit *radial symmetry* (see also Campbell, Figure 32.4).
  - These animals have an oral (top) and aboral (bottom) side, but no front, back, left, or right sides.



- *Bilateria* exhibit *bilateral symmetry* (see also Campbell, Figure 32.5).

  Bilaterally symmetrical animals have *dorsal* (top), *ventral* (bottom), *anterior* (head), *posterior* (tail), left and right body surfaces.

  These animals exhibit *cephalization* (an evolutionary trend toward concentration of sensory structures at the anterior end).



Care must be taken when assigning an animal to an evolutionary line as symmetry may change between the larval and adult forms. The phylum Echinodermata shows a secondary radial symmetry in adults, which evolved as an adaptation to their sedentary lifestyle. They are actually in the bilateria.

Examination of development and body plan can define the radiata-bilateria split better than symmetry.

- The early embryo of all eumetazoans undergoes *gastrulation*. Concentric germ layers develop which form the various tissues and organs as development continues.

  The radiata (e.g., Phylum Cnidaria, Phylum Ctenophores) develop only two germ layers (*ectoderm* and *endoderm*) and are termed *diploblastic*.

  The bilateria (e.g., all eumetazoan phyla except Phylum Cnidaria and Phylum Ctenophores) develop three germ layers (ectoderm, endoderm, and *mesoderm*) and are termed *triploblastic*.

The germ layers of an early embryo include:

1. Ectoderm
   - Covers the surface of the embryo

- Forms the animal's outer covering and the central nervous system in some phyla

2. Endoderm
   - Innermost germ layer which lines the *archenteron* (primitive gut)
   - Forms the lining of the digestive tract, and outpocketings give rise to the liver and lungs of vertebrates
3. Mesoderm
   - Located between the ectoderm and endoderm in triploblastic animals
   - Forms the muscles and most organs located between the digestive tract and outer covering of the animal

C. Evolution of body cavities led to more complex animals

Triploblastic animals can also be grouped on the basis of whether a body cavity develops and how that cavity develops.

Animals in which no body cavity develops are termed acoelomate.

- *Acoelomate* = An animal body plan characterized by no body cavity present between the digestive tract and the outer body wall (see Campbell, Figure 32.5a)
- The area between the digestive tract and outer wall is filled with cells, producing a solid body (e.g., Phylum Platyhelminthes).

Animals in which a body cavity develops may be termed pseudocoelomate or coelomate, depending on how the cavity develops.

- *Pseudocoelomate* = Animal body plan characterized by a fluid-filled body cavity that separates the digestive tract and the outer body wall. (See Campbell, Figure 32.5b.)
- This cavity (the *pseudocoelom*) is not completely lined with tissue derived from mesoderm (e.g., Phylum Nematoda).
- *Coelomate* = Animal body plan characterized by a fluid-filled body cavity completely lined with tissue derived from mesoderm (the *coelom*) that separates the digestive tract from the outer body wall (see Campbell, Figure 32.5c)
- Mesenteries connect the inner and outer mesoderm layers and suspend the internal organs in the coelom (e.g., Annelida).
- The fluid-filled body cavities:
  - Cushion the organs, thus preventing injury
  - Allow internal organs can grow and move independently of the outer body wall.
  - Serve as a hydrostatic skeleton in soft bodied coelomates such as earthworms.

In addition to the presence of a body cavity, acoelomates differ from pseudocoelomates and eucoelomates by not having a blood vascular system.

D. Coelomates branched into protostomes and deuterostomes

Distinguished by differences in development, the coelomate phyla can be divided into two distinct evolutionary lines (see Campbell, Figure 32.3):

1. *Protostomes* (e.g., mollusks, annelids, arthropods)
2. *Deuterostomes* (e.g., echinoderms, chordates)

Developmental differences between protostomes and deuterostomes include: cleavage patterns, coelom formation, and fate of the blastopore.

1. Cleavage

Most protostomes undergo spiral cleavage and determinate cleavage during their development.

- *Spiral cleavage* = Cleavage in which the planes of cell division are diagonal to the vertical axis of the embryo (see Campbell, Figure 32.6)
- *Determinate cleavage* = Cleavage in which the developmental fate of each embryonic cell is established very early; a cell isolated from the four-cell stage of an embryo will not develop fully.

Deuterostomes undergo radial cleavage and indeterminate cleavage during their development.

- *Radial cleavage* = Cleavage during which the cleavage planes are either parallel or perpendicular to the vertical axis of the embryo
- *Indeterminate cleavage* = Cleavage in which each early embryonic cell retains the capacity to develop into a complete embryo if isolated from other cells; this type of cleavage in the human zygote results in identical twins.

2. Coelom formation

Schizocoelous = Descriptive term for coelom development during which, as the archenteron forms, the coelom begins as splits within the solid mesodermal mass; coelom formation found in protostomes (see Campbell, Figure 32.6b)

Enterocoelous = Coelom development during which the mesoderm arises as lateral outpocketings of the archenteron with hollows that become the coelomic cavities; coelom formation found in deuterostomes (see Campbell, Figure 32.6c)

3. Blastopore fate

Blastopore = The first opening of the archenteron which forms during gastrulation

- In protostomes, the blastopore forms the mouth.
- In deuterostomes, the blastopore forms the anus.

SUMMARY OF PROTOSTOME - DEUTEROSTOME SPLIT	
PROTOSTOMES	**DEUTEROSTOMES**
Spiral cleavage	Radial cleavage
Determinate cleavage	Indeterminate cleavage
Blastopore forms the mouth	Blastopore forms the anus
Schizocoelous coelom formation	Enterocoelous coelom formation

III. The Origins of Animal Diversity

A. Most animal phyla originated in a relatively brief span of geological time

The animal kingdom probably originated from colonial protists related to choanoflagellates.The diversification that produced many phyla occurred in a relatively short time on the geological scale. This evolutionary episode is called the *Cambrian explosion.*

The Cambrian explosion encompassed a 20-million-year time span at the beginning of the Cambrian period (ca. 545 to 525 million years ago).

- Nearly all of the major animal body plans seen today evolved during this time.
- New taxa appeared later but were variations on the basic plans already evolved. For example, mammals evolved about 220 million years ago, but are only a variation of the chordate body plan which evolved during the Cambrian explosion.

A much less diverse fauna preceded the Cambrian explosion.

- This Precambrian fauna dated back to the *Ediacaran period* (700 million years ago).

  This period is named for the Ediacara Hills of Australia where Precambrian animal fossils were first discovered.

  Fossils similar in age to these have since been discovered on other continents.
- Most Ediacaran fossils appear to be cnidarians although bilaterial animals are also indicated by fossilized burrows probably left by worms.

The diversity of Cambrian animals is represented in three fossil beds:

- The Burgess Shale in British Columbia is the best known (see Campbell, Figure 32.7).
- A fossil bed in Greenland and one in the Yunnan region of China predate the Burgess Shale by 10 million years.

Two contrasting interpretations of Burgess Shale fossils have been proposed:

1. The Cambrian explosion resulted in a large number of phyla which included the current phyla, many of which are now extinct.

   During the mass extinction at the end of the Cambrian, only the base stock of 35 or so extant phyla survived.
2. The diversity of the Cambrian fossils represents ancient variations within the taxonomic boundaries of extant phyla.

   As these fossils undergo continued study, many are classified into extant phyla. Thus, the number of exclusively Cambrian fossils is decreasing.

B. Developmental genetics may clarify our understanding of the Cambrian diversification

Several hypotheses about external factors have been proposed as explanations for the Cambrian explosion and the lack of subsequent major diversification.

1. The Cambrian explosion was an adaptive radiation resulting from the origin of the first animals.

   These early animals diversified as they adapted to the various, previously unoccupied, ecological niches.
2. Predator-prey relationships emerged and triggered diverse evolutionary adaptations.

   Various kinds of shells and different forms of locomotion evolved as defense mechanisms against predation.

   Predators also evolved new mechanisms to capture prey.
3. Major environmental change provided an opportunity for diversification during the Cambrian explosion.

   The accumulation of atmospheric oxygen may have finally reached a concentration to support the more active metabolism needed for feeding and other activities by mobile animals.

Other hypotheses for the Cambrian explosion have emphasized internal changes in the organisms.

1. The origin of mesoderm may have stimulated diversification of the body plan.

   This third tissue layer permits development of more complex anatomical structure.

2. Variation in genes that control pattern formation during animal development may have played a role in diversification.

   Some of the genes that determine features such as segmentation and placement of appendages and other structures are common to diverse animal phyla.

   Variation in expression of these genes during development results in morphological differences that distinguish the phyla.

   This same kind of variation in expression may have resulted in the relatively rapid origin of diverse animal types during the Cambrian explosion.

   The phyla, once developed, may have become locked into developmental patterns that permitted subtle variation to allow speciation and the origin of lower taxa, but prevented large scale morphological evolution resulting in new phyla.

The hypotheses presented for external and internal factors are not mutually exclusive. A combination of factors may have combined to produce the Cambrian explosion.

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