CHAPTER 1

INTRODUCTION: THEMES IN THE STUDY OF LIFE

OUTLINE

I. Life's Hierarchical Order
   A. The living world is a hierarchy, with each level of biological structure building on the level below it
   B. Each level of biological structure has emergent properties
   C. Cells are an organism's basic units of structure and function
   D. The continuity of life is based on heritable information in the form of DNA
   E. Structure and function are correlated at all levels of biological organization
   F. Organisms are open systems that interact continuously with their environments
   G. Regulatory mechanisms ensure a dynamic balance in living systems

II. Evolution, Unity, and Diversity
   A. Diversity and unity are the dual faces of life on Earth
   B. Evolution is the core theme of biology

III. Science as a Process
   A. Testable hypotheses are the hallmarks of the scientific process
   B. Science and technology are functions of society
   C. Biology is a multidisciplinary adventure

OBJECTIVES

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

1. Briefly describe unifying themes that pervade the science of biology.
2. Diagram the hierarchy of structural levels in biology.
3. Explain how the properties of life emerge from complex organization.
4. Describe seven emergent properties associated with life.
5. Distinguish between holism and reductionism.
6. Explain how technological breakthroughs contributed to the formulation of the cell theory and our current knowledge of the cell.
7. Distinguish between prokaryotic and eukaryotic cells.
8. Explain, in their own words, what is meant by "form fits function."
9. List the five kingdoms of life and distinguish among them.
10. Briefly describe how Charles Darwin's ideas contributed to the conceptual framework of biology.
11. Outline the scientific method.
12. Distinguish between inductive and deductive reasoning.
13. Explain how science and technology are interdependent.

KEY TERMS

emergent property	holism	evolution	control group
population	reductionism	natural selection	variable
community	prokaryotic	scientific method	experimental group
ecosystem	eukaryotic	hypothesis	deductive reasoning
biome	taxonomy	inductive reasoning	scientific theory
biogenesis			

LECTURE NOTES

Biology, the study of life, is a human endeavor resulting from an innate attraction to life in its diverse forms (E.O. Wilson's *biophilia*).

The science of biology is enormous in scope.

- It reaches across size scales from submicroscopic molecules to the global distribution of biological communities.
- It encompasses life over huge spans of time from contemporary organisms to ancestral life forms stretching back nearly four billion years.

As a science, biology is an ongoing process.

- As a result of new research methods developed over the past few decades, there has been an information explosion.
- Technological advances yield new information that may change the conceptual framework accepted by the majority of biologists.

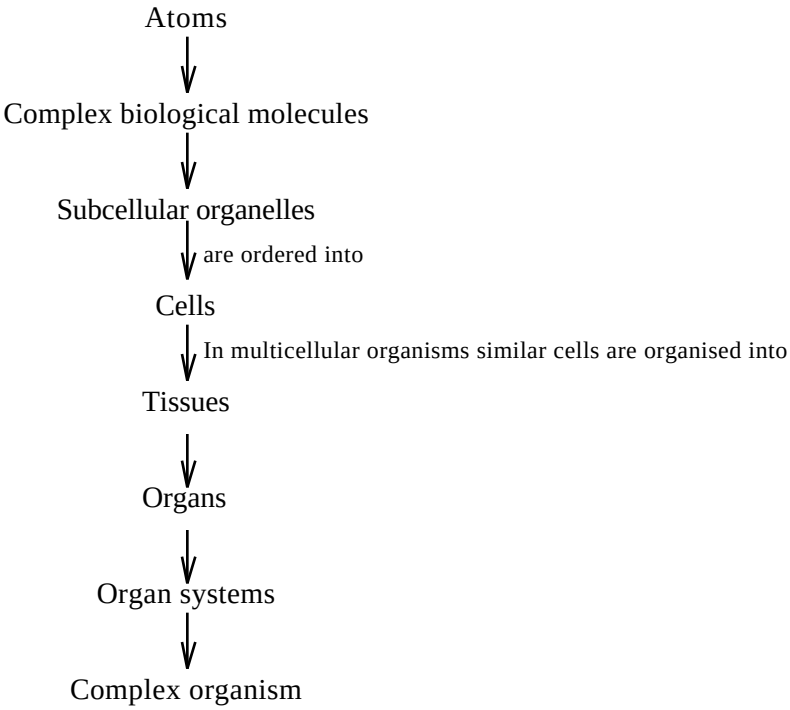
With rapid information flow and new discoveries, biology is in a continuous state of flux. There are, however, enduring unifying themes that pervade the science of biology:

- A hierarchy of organization
- The cellular basis of life
- Heritable information
- The correlation between structure and function
- The interaction of organisms with their environment
- Unity in diversity
- Evolution: the core theme
- Scientific process: the hypothetico-deductive method

I. Life's Hierarchical Order

A. The living world is a hierarchy, with each level of biological structure building on the level below it

A characteristic of life is a high degree of order. Biological organization is based on a hierarchy of structural levels, with each level building on the levels below it.



There are levels of organization beyond the individual organism:

Population	=	Localized group of organisms belonging to the same species
Community	=	Populations of species living in the same area
Ecosystem	=	An energy-processing system of community interactions that include abiotic environmental factors such as soil and water
Biomes	=	Large scale communities classified by predominant vegetation type and distinctive combinations of plants and animals
Biosphere	=	The sum of all the planet's ecosystems

B. Each level of biological organization has emergent properties

Emergent property = Property that emerges as a result of interactions between components.

- With each step upward in the biological hierarchy, new properties emerge that were not present at the simpler organizational levels.
- Life is difficult to define because it is associated with numerous emergent properties that reflect a hierarchy of structural organization.

Some of the emergent properties and processes associated with life are the following:

1. *Order.* Organisms are highly ordered, and other characteristics of life emerge from this complex organization.

2. *Reproduction.* Organisms reproduce; life comes only from life (*biogenesis*).
3. *Growth and Development.* Heritable programs stored in DNA direct the species-specific pattern of growth and development.
4. *Energy Utilization.* Organisms take in and transform energy to do work, including the maintenance of their ordered state.
5. *Response to Environment.* Organisms respond to stimuli from their environment.
6. *Homeostasis.* Organisms regulate their internal environment to maintain a steady-state, even in the face of a fluctuating external environment.
7. *Evolutionary Adaptation.* Life evolves in response to interactions between organisms and their environment.

Because properties of life emerge from complex organization, it is impossible to fully explain a higher level of order by breaking it into its parts.

Holism = The principle that a higher level of order cannot be meaningfully explained by examining component parts in isolation.

- An organism is a living whole greater than the sum of its parts.
- For example, a cell dismantled to its chemical ingredients is no longer a cell.

It is also difficult to analyze a complex process without taking it apart.

Reductionism = The principle that a complex system can be understood by studying its component parts.

- Has been a powerful strategy in biology
- Example: Watson and Crick deduced the role of DNA in inheritance by studying its molecular structure.

The study of biology balances the reductionist strategy with the goal of understanding how the parts of cells, organisms, and populations are functionally integrated.

C. Cells are an organism's basic units of structure and function

The cell is an organism's basic unit of structure and function.

- Lowest level of structure capable of performing all activities of life.
- All organisms are composed of cells.
- May exist singly as unicellular organisms or as subunits of multicellular organisms.

The invention of the microscope led to the discovery of the cell and the formulation of the cell theory.

- Robert Hooke (1665) reported a description of his microscopic examination of cork. Hooke described tiny boxes which he called "cells" (really cell walls). The significance of this discovery was not recognized until 150 years later.
- Antonie van Leeuwenhok (1600's) used the microscope to observe living organisms such as microorganisms in pond water, blood cells, and animal sperm cells.
- Matthias Schleiden and Theodor Schwann (1839) reasoned from their own microscopic studies and those of others, that all living things are made of cells. This formed the basis for the *cell theory*.
- The cell theory has since been modified to include the idea that all cells come from preexisting cells.

Over the past 40 years, use of the electron microscope has revealed the complex ultrastructure of cells.

- Cells are bounded by *plasma membranes* that regulate passage of materials between the cell and its surroundings.
- All cells, at some stage, contain DNA.

Based on structural organization, there are two major kinds of cells: *prokaryotic* and *eukaryotic*.

Prokaryotic cell = Cell lacking membrane-bound organelles and a membrane-enclosed nucleus.

- Found only in the archaebacteria and bacteria
- Generally much smaller than eukaryotic cells
- Contains DNA that is *not* separated from the rest of the cell, as there is no membrane-bound nucleus
- Lacks membrane-bound organelles
- Almost all have tough external walls

Eukaryotic cell = Cell with a membrane-enclosed nucleus and membrane-enclosed organelles.

- Found in protists, plants, fungi, and animals
- Subdivided by internal membranes into different functional compartments called *organelles*
- Contains DNA that is segregated from the rest of the cell. DNA is organized with proteins into *chromosomes* that are located within the *nucleus*, the largest organelle of most cells.
- *Cytoplasm* surrounds the nucleus and contains various organelles of different functions
- Some cells have a tough *cell wall* outside the plasma membrane (e.g., plant cells). Animal cells lack cell walls.

Though structurally different, eukaryotic and prokaryotic cells have many similarities, especially in their chemical processes.

D. The continuity of life is based on heritable information in the form of DNA

Biological instructions for an organism's complex structure and function are encoded in DNA.

- Each DNA molecule is made of four types of chemical building blocks called *nucleotides*.
- The linear sequence of these four nucleotides encode the precise information in a gene, the unit of inheritance from parent to offspring.
- An organism's complex structural organization is specified by an enormous amount of coded information.

Inheritance is based on:

- A complex mechanism for copying DNA.
- Passing the information encoded in DNA from parent to offspring.

All forms of life use essentially the same genetic code.

- A particular nucleotide sequence provides the same information to one organism as it does to another.
- Differences among organisms reflect differences in nucleotide sequence.

E. Structure and function are correlated at all levels of biological organization

There is a relationship between an organism's structure and how it works. Form fits function.

- Biological structure gives clues about what it does and how it works.
- Knowing a structure's function gives insights about its construction.

- **This correlation is apparent at many levels of biological organization.**

F. Organisms are open systems that interact continuously with their environments

Organisms interact with their environment, which includes other organisms as well as abiotic factors.

- Both organism and environment are affected by the interaction between them.
- Ecosystem dynamics include two major processes:
  1. Nutrient cycling
  2. Energy flow (see Campbell, Figure 1.7)

G. Regulatory mechanisms ensure a dynamic balance in living systems

Regulation of biological processes is critical for maintaining the ordered state of life.

Many biological processes are self-regulating; that is, the product of a process regulates that process (= feedback regulation; see Campbell, Figure 1.8).

- Positive feedback speeds a process up
- Negative feedback slows a process down

Organisms and cells also use chemical mediators to help regulate processes.

- The hormone insulin, for example, signals cells in vertebrate organisms to take up glucose. As a result, blood glucose levels go down.
- In certain forms of diabetes mellitus, insulin is deficient and cells do not take up glucose as they should, and as a result, blood glucose levels remain high.

II. Evolution, Unity, and Diversity

A. Diversity and unity are the dual faces of life on Earth

Biological diversity is enormous.

- Estimates of total diversity range from five million to over 30 million species.
- About 1.5 million species have been identified and named, including approximately 260,000 plants, 50,000 vertebrates, and 750,000 insects.

To make this diversity more comprehensible, biologists classify species into categories.

Taxonomy = Branch of biology concerned with naming and classifying organisms.

- Taxonomic groups are ranked into a hierarchy from the most to least inclusive category: *domain*, *kingdom*, *phylum*, *class*, *order*, *family*, *genus*, *species*.
- A six-kingdom system recognizes two prokaryotic groups and divides the Monera into the Archaebacteria and Eubacteria.
- The kingdoms of life recognized in the traditional five-kingdom system are Monera, Protista, Plantae, Fungi, and Animalia (see Campbell, Figure 1.10).

There is unity in the diversity of life forms at the lower levels of organization. Unity of life forms is evident in:

- A universal genetic code.
- Similar metabolic pathways (e.g., glycolysis).
- Similarities of cell structure (e.g., flagella of protozoans and mammalian sperm cells).

B. Evolution is the core theme of biology

Evolution is the one unifying biological theme.

- Life evolves. Species change over time and their history can be described as a branching tree of life.
- 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.

- All life is connected and can be traced back to primeval prokaryotes that existed more than three billion years ago.

In 1859, Charles Darwin published *On the Origin of Species* in which he made two major points:

1. Species change, and contemporary species arose from a succession of ancestors through a process of "descent with modification."
2. A mechanism of evolutionary change is *natural selection.*

Darwin synthesized the concept of natural selection based upon the following observations:

- Individuals in a population of any species vary in many inheritable traits.
- Populations have the potential to produce more offspring than will survive or than the environment can support.
- Individuals with traits best suited to the environment leave a larger number of offspring, which increases the proportion of inheritable variations in the next generation. This differential reproductive success is what Darwin called *natural selection*.

Organisms' adaptations to their environments are the products of natural selection.

- Natural selection *does not create* adaptations; it merely increases the frequency of inherited variants that arise by chance.
- Adaptations are the result of the editing process of natural selection. When exposed to specific environmental pressures, certain inheritable variations favor the reproductive success of some individuals over others.

Darwin proposed that cumulative changes in a population over long time spans could produce a new species from an ancestral one.

Descent with modification accounts for both the unity and diversity of life.

- Similarities between two species may be a reflection of their descent from a common ancestor.
- Differences between species may be the result of natural selection modifying the ancestral equipment in different environmental contexts.

III. Science as a Process

A. Testable hypotheses are the hallmarks of the scientific process

As the science of life, biology has the characteristics associated with science in general.

Science is a way of knowing. It is a human endeavor that emerges from our curiosity about ourselves, the world, and the universe. Good scientists are people who:

- Ask questions about nature and believe those questions are answerable.
- Are curious, observant, and passionate in their quest for discovery.
- Are creative, imaginative, and intuitive.
- Are generally skeptics.

Scientific method = Process which outlines a series of steps used to answer questions.

- Is not a rigid procedure.
- Based on the conviction that natural phenomena have natural causes.
- Requires evidence to logically solve problems.

The key ingredient of the scientific process is the *hypothetico-deductive method,* which is an approach to problem-solving that involves:

1. Asking a question and formulating a tentative answer or hypothesis by inductive reasoning.
2. Using deductive reasoning to make predictions from the hypothesis and then testing the validity of those predictions.

Hypothesis = Educated guess proposed as a tentative answer to a specific question or problem.

Inductive reasoning = Making an inference from a set of specific observations to reach a general conclusion.

Deductive reasoning = Making an inference from general premises to specific consequences, which logically follow if the premises are true.

- Usually takes the form of *If...then* logic.
- In science, deductive reasoning usually involves predicting experimental results that are expected *if* the hypothesis is true.

Some students cannot make the distinction between inductive and deductive reasoning. An effective teaching strategy is to let them actually experience both processes. To illustrate inductive reasoning, provide an every day scenario with enough pieces of information for student to hypothesize a *plausible* explanation for some event. Demonstrate deductive reasoning by asking students to solve a simple problem, based upon given assumptions.

Useful hypotheses have the following characteristics:

- *Hypotheses are possible causes.* Generalizations formed by induction are not necessarily hypotheses. Hypotheses should also be tentative explanations for observations or solutions to problems.
- *Hypotheses reflect past experience with similar questions.* Hypotheses are not just blind propositions, but are *educated* guesses based upon available evidence.
- *Multiple hypotheses should be proposed whenever possible.* The disadvantage of operating under only one hypothesis is that it might restrict the search for evidence in support of this hypothesis; scientists might bias their search, as well as neglect to consider other possible solutions.
- *Hypotheses must be testable via the hypothetico-deductive method.* Predictions made from hypotheses must be testable by making observations or performing experiments. This limits the scope of questions that science can answer.
- *Hypotheses can be eliminated, but not confirmed with absolute certainty.* If repeated experiments consistently disprove the predictions, then we can assume that the hypothesis is false. However, if repeated experimentation supports the deductions, we can only assume that the hypothesis *may* be true; accurate predictions can be made from false hypotheses. The more deductions that are tested and supported, the more confident we can be that the hypothesis is true.

Another feature of the scientific process is the controlled experiment which includes control and experimental groups.

Control group = In a controlled experiment, the group in which all variables are held constant.

- Controls are a necessary basis for comparison with the experimental group, which has been exposed to a single treatment variable.
- Allows conclusions to be made about the effect of experimental manipulation.
- Setting up the best controls is a key element of good experimental design.

Variable = Condition of an experiment that is subject to change and that may influence an experiment's outcome.

Experimental group = In a controlled experiment, the group in which one factor or treatment is varied.

Science is an ongoing process that is a self-correcting way of knowing. Scientists:

- Build on prior scientific knowledge.
- Try to replicate the observations and experiments of others to check on their conclusions.

- Share information through publications, seminars, meetings, and personal communication.

What really advances science is not just an accumulation of facts, but a new concept that collectively explains observations that previously seemed to be unrelated.

- Newton, Darwin, and Einstein stand out in the history of science because they synthesized ideas with great explanatory power.
- *Scientific theories* are comprehensive conceptual frameworks which are well supported by evidence and are widely accepted by the scientific community.

B. Science and technology are functions of society

Science and technology are interdependent.

- Technology extends our ability to observe and measure, which enables scientists to work on new questions that were previously unapproachable.
- Science, in turn, generates new information that makes technological inventions possible.
- Example: Watson and Crick's scientific discovery of DNA structure led to further investigation that enhanced our understanding of DNA, the genetic code, and how to transplant foreign genes into microorganisms. The biotechnology industry has capitalized on this knowledge to produce valuable pharmaceutical products such as human insulin.

We have a love-hate relationship with technology.

- Technology has improved our standard of living.
- The consequence of using technology also includes the creation of new problems such as increased population growth, acid rain, deforestation, global warming, nuclear accidents, ozone holes, toxic wastes, and endangered species.
- Solutions to these problems have as much to do with politics, economics, culture and values as with science and technology.

A better understanding of nature must remain the goal of science. Scientists should:

- Try to influence how technology is used.
- Help educate the public about the benefits and hazards of specific technologies.

C. Biology is a multidisciplinary adventure

Biology is a multidisciplinary science that integrates concepts from chemistry, physics and mathematics. Biology also embraces aspects of humanities and the social sciences.

REFERENCES

Campbell, N. *Biology*. 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Moore, J.A. "Science as a Way of Knowing–Evolutionary Biology." *American Zoologist*, 24(2): 470-475, 1980.

CHAPTER 2
THE CHEMICAL CONTEXT OF LIFE

OUTLINE

I. Chemical Elements and Compounds
   A. Matter consists of chemical elements in pure form and in combinations called compounds
   B. Life requires about 25 chemical elements

II. Atoms and Molecules
   A. Atomic structure determines the behavior of an element
   B. Atoms combine by chemical bonding to form molecules
   C. Weak chemical bonds play important roles in the chemistry of life
   D. A molecule's biological function is related to its shape
   E. Chemical reactions make and break chemical bonds

OBJECTIVES

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

1. Define element and compound.
2. State four elements essential to life that make up 96% of living matter.
3. Describe the structure of an atom.
4. Define and distinguish among atomic number, mass number, atomic weight, and valence.
5. Given the atomic number and mass number of an atom, determine the number of neutrons.
6. Explain why radioisotopes are important to biologists.
7. Explain how electron configuration influences the chemical behavior of an atom.
8. Explain the octet rule and predict how many bonds an atom might form.
9. Explain why the noble gases are so unreactive.
10. Define electronegativity and explain how it influences the formation of chemical bonds.
11. Distinguish among nonpolar covalent, polar covalent and ionic bonds.
12. Describe the formation of a hydrogen bond and explain how it differs from a covalent or ionic bond.
13. Explain why weak bonds are important to living organisms.
14. Describe how the relative concentrations of reactants and products affect a chemical reaction.

KEY TERMS

matter	atomic weight	valence electron	polar covalent bond
element	isotope	valence shell	ion
trace element	radioactive isotope	chemical bond	cation
atom	energy	covalent bond	anion
neutron	potential energy	molecule	ionic bond
proton	energy level	structural formula	hydrogen bond
electron	energy	molecular formula	chemical reactions
atomic nucleus	potential energy	double covalent bond	reactants
dalton	energy level	valence	products
atomic number	electron shell	electronegativity	chemical equilibrium
mass number	orbital	nonpolar covalent bond	

LECTURE NOTES

I. Chemical Elements and Compounds

A. Matter consists of chemical elements in pure form and in combinations called compounds

Chemistry is fundamental to an understanding of life, because living organisms are made of matter.

Matter = Anything that takes up space and has mass.

Mass = A measure of the amount of matter an object contains.

> You might want to distinguish between mass and weight for your students. *Mass* is the measure of the amount of matter an object contains, and it stays the same regardless of changes in the object's position. *Weight* is the measure of how strongly an object is pulled by earth's gravity, and it varies with distance from the earth's center. The key point is that the mass of a body does not vary with its position, whereas weight does. So, for all practical purposes—as long as we are earthbound—weight can be used as a measure of mass.

B. Life requires about 25 chemical elements

Element = A substance that cannot be broken down into other substances by chemical reactions.

- All matter is made of elements.
- There are 92 naturally occurring elements.
- They are designated by a symbol of one or two letters.

About 25 of the 92 naturally occurring elements are essential to life. Biologically important elements include:

C = carbon
O = oxygen
H = hydrogen
N = nitrogen

] make up 96% of living matter

Ca = calcium
P = phosphorus
K = potassium
S = sulfur
Na = sodium
Cl = chlorine
Mg = magnesium
Trace elements

make up remaining 4% of an organism's weight

Trace element = Element required by an organism in extremely minute quantities.

- Though required by organisms in small quantity, they are indispensable for life
- Examples: B, Cr, Co, Cu, F, I, Fe, Mn, Mo, Se, Si, Sn, V and Zn

Elements can exist in combinations called compounds.

- *Compound* = A pure substance composed of two or more elements combined in a fixed ratio.
- Example: NaCl (sodium chloride)
- Has unique emergent properties beyond those of its combined elements (Na and Cl have very different properties from NaCl). See Campbell, Figure 2.2.

Since a compound is the next structural level above element or atom, this is an excellent place to emphasize the concept of emergent properties, an integral theme found throughout the text and course.

II. Atoms and Molecules

A. Atomic structure determines the behavior of an element

Atom = Smallest possible unit of matter that retains the physical and chemical properties of its element.

- Atoms of the same element share similar chemical properties.
- Atoms are made up of *subatomic particles*.

1. Subatomic particles

The three *most stable* subatomic particles are:

1. *Neutrons* [no charge (neutral)].
2. *Protons* [+1 electrostatic charge].
3. *Electrons* [-1 electrostatic charge].

NEUTRON	PROTON	ELECTRON
No charge	+1 charge	-1 charge
Found together in a dense core called the *nucleus* (positively charged because of protons)		Orbits around nucleus (held by electrostatic attraction to positively charged nucleus)
1.009 dalton	1.007 dalton	1/2000 dalton
Masses of both are about the same (about 1 dalton)		Mass is so small, usually not used to calculate atomic mass

NOTE: The *dalton* is a unit used to express mass at the atomic level. One dalton (d) is equal to 1.67×10^{-24} g.

If an atom is electrically neutral, the number of protons equals the number of electrons, which yields an electrostatically balanced charge.

2. Atomic number and atomic weight

Atomic number = Number of protons in an atom of a particular element.

- All atoms of an element have the same atomic number.
- Written as a subscript to the left of the element's symbol (e.g., $_{11}$Na)
- In a neutral atom, # protons = # electrons.

Mass number = Number of protons and neutrons in an atom.

- Written as a superscript to left of an element's symbol (e.g., ^{23}Na)
- Is approximate mass of the whole atom, since the mass of a proton and the mass of a neutron are both about 1 dalton
- Can deduce the number of neutrons by subtracting *atomic number* from *mass number*
- Number of neutrons can vary in an element, but number of protons is constant
- Is not the same as an element's *atomic weight*, which is the weighted mean of the masses of an element's constituent isotopes

In a large classroom with up to 300 students, it can be difficult to interact. Try putting examples on an overhead transparency and soliciting student input to complete the information. It is a quick way to check for understanding and to actively involve students.

Examples:

(Mass #) 23
(Atomic #) 11 Na # of electrons _____

# of protons _____

# of neutrons _____

$^{12}_{6}$C # of electrons _____

# of protons _____

# of neutrons _____

3. Isotopes

Isotopes = Atoms of an element that have the same atomic number but different mass number.

- They have the same number of protons, but a different number of neutrons.
- Under natural conditions, elements occur as mixtures of isotopes.
- Different isotopes of the same element react chemically in same way.
- Some isotopes are radioactive.

Radioactive isotope = Unstable isotope in which the nucleus spontaneously decays, emitting subatomic particles and/or energy as radioactivity.

- Loss of nuclear particles may transform one element to another (e.g., $^{14}_{6}C \rightarrow {}^{14}_{7}N$).
- Has a fixed half life.
  - *Half life* = Time for 50% of radioactive atoms in a sample to decay.

Biological applications of radioactive isotopes include:

a. Dating geological strata and fossils

- Radioactive decay is at a fixed rate.
- By comparing the ratio of radioactive and stable isotopes in a fossil with the ratio of isotopes in living organisms, one can estimate the age of a fossil.
- The ratio of ^{14}C to ^{12}C is frequently used to date fossils less than 50,000 years old.

b. Radioactive tracers
- Chemicals labelled with radioactive isotopes are used to trace the steps of a biochemical reaction or to determine the location of a particular substance within an organism (see Campbell, p. XX, Methods: The Use of Radioactive Tracers in Biology).
- Radioactive isotopes are useful as biochemical tracers because they chemically react like the stable isotopes and are easily detected at low concentrations.
- Isotopes of P, N, and H were used to determine DNA structure.
- Used to diagnose disease (e.g., PET scanner)
- Because radioactivity can damage cell molecules, radioactive isotopes can also be hazardous

c. Treatment of cancer
- e.g., radioactive cobalt

4. The energy levels of electrons

Electrons = Light negatively charged particles that orbit around nucleus.

- Equal in mass and charge
- Are the only stable subatomic particles directly involved in chemical reactions
- Have *potential energy* because of their position relative to the positively charged nucleus

Energy = Ability to do work

Potential energy = Energy that matter stores because of its position or location.

- There is a natural tendency for matter to move to the lowest state of potential energy.
- Potential energy of electrons is not infinitely divisible, but exists only in discrete amounts called *quanta*.
- Different fixed potential energy states for electrons are called *energy levels* or *electron shells* (see Campbell, Figure 2.7).
- Electrons with lowest potential energy are in energy levels closest to the nucleus.
- Electrons with greater energy are in energy levels further from nucleus.

Electrons may move from one energy level to another. In the process, they gain or lose energy equal to the difference in potential energy between the old and new energy level.

5. Electron orbitals

Orbital = Three-dimensional space where an electron will most likely be found 90% of the time (see Campbell, Figure 2.8).

- Viewed as a three-dimensional probability cloud (a statistical concept)
- No more than two electrons can occupy same orbital.

First energy level:

- Has one spherical *s* orbital (1*s* orbital)
- Holds a maximum of two electrons

Second energy level

- Holds a maximum of eight electrons
- One spherical *s* orbital (2*s* orbital)
- Three dumbbell-shaped *p* orbitals each oriented at right angles to the other two ($2p_x$, $2p_y$, $2p_z$ orbitals)

Higher energy levels:

- Contain *s* and *p* orbitals
- Contain additional orbitals with more complex shapes

6. Electron configuration and chemical properties

An atom's electron configuration determines its chemical behavior.

- *Electron configuration* = Distribution of electrons in an atom's electron shells

The first 18 elements of a periodic chart are arranged sequentially by atomic number into three rows (periods). In reference to these *representative* elements, note the following:

- Outermost shell of these atoms never have more than four orbitals (one *s* and three *p*) or eight electrons.
- Electrons must first occupy lower electron shells before the higher shells can be occupied. (This is a reflection of the natural tendency for matter to move to the lowest possible state of potential energy—the most stable state.)
- Electrons are added to each of the *p* orbitals singly, before they can be paired.
- If an atom does not have enough electrons to fill all shells, the outer shell will be the only one partially filled. Example: O_2 with a total of eight electrons:

OXYGEN

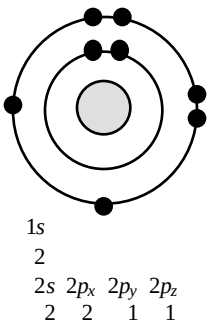
$_8O$

Two electrons have the 1*s* orbital of the first electron shell.

First two electrons in the second shell are both in the 2*s* orbital.

Next three electrons each have a *p* orbital ($2p_x$, $2p_y$, $2p_z$).

Eighth electron is paired in the $2p_x$ orbital.



Chemical properties of an atom depend upon the number of valence electrons.

- *Valence electrons* = Electrons in the outermost energy shell (valence shell).

Octet rule = Rule that a valence shell is complete when it contains eight electrons (except H and He).

- An atom with a complete valence shell is unreactive or *inert*.
- Noble elements (e.g., helium, argon, and neon) have filled outer shells in their elemental state and are thus inert.
- An atom with an incomplete valence shell is chemically reactive (tends to form chemical bonds until it has eight electrons to fill the valence shell).
- Atoms with the same number of valence electrons show similar chemical behavior.

NOTE: The consequence of this unifying chemical principle is that the valence electrons are responsible for the atom's bonding capacity. This rule applies to most of the representative elements, but *not all*.

B. Atoms combine by chemical bonding to form molecules

Atoms with incomplete valence shells tend to fill those shells by interacting with other atoms. These interactions of electrons among atoms may allow atoms to form chemical bonds.

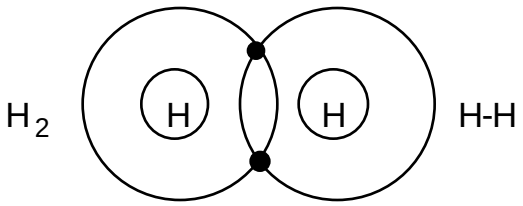
- *Chemical bonds* = Attractions that hold molecules together

Molecules = Two or more atoms held together by chemical bonds.

1. Covalent bonds

Covalent bond = Chemical bond between atoms formed by *sharing* a pair of valence electrons.

- Strong chemical bond
- Example: molecular hydrogen (H_2); when two hydrogen atoms come close



enough for their 1*s* orbitals to overlap, they *share* electrons, thus completing the valence shell of each atom.

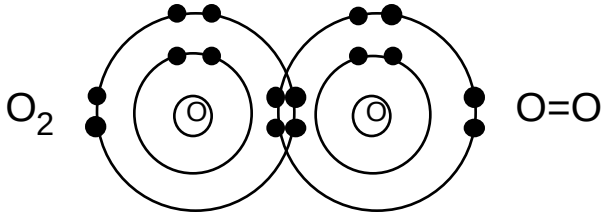
Structural formula = Formula which represents the atoms and bonding within a molecule (e.g., H-H). The line represents a shared pair of electrons.

Molecular formula = Formula which indicates the number and type of atoms (e.g., H_2).

Single covalent bond = Bond between atoms formed by sharing a single pair of valence electrons.

- Atoms may freely rotate around the axis of the bond.

Double covalent bond = Bond formed when atoms share *two* pairs of valence electrons (e.g., O_2).



Molecules = Two or more atoms held together by chemical bonds.

Triple covalent bond = Bond formed when atoms share three pairs of valence electrons (e.g., N_2 or N°N).

NOTE: Double and triple covalent bonds are rigid and do not allow rotation.

Valence = Bonding capacity of an atom which is the number of covalent bonds that must be formed to complete the outer electron shell.

- Valences of some common elements: hydrogen = 1, oxygen = 2, nitrogen = 3, carbon = 4, phosphorus = 3 (sometimes 5 as in biologically important compounds, e.g., ATP), sulfur = 2.

Compound = A pure substance composed of two or more elements combined in a fixed ratio.

- Example: water (H_2O), methane (CH_4)
- Note that two hydrogens are necessary to complete the valence shell of oxygen in water, and four hydrogens are necessary for carbon to complete the valence shell in methane.

2. Nonpolar and polar covalent bonds

Electronegativity = Atom's ability to attract and hold electrons.

- The more electronegative an atom, the more strongly it attracts shared electrons.
- Scale determined by Linus Pauling:

  O = 3.5

  N = 3.0

  S and C = 2.5

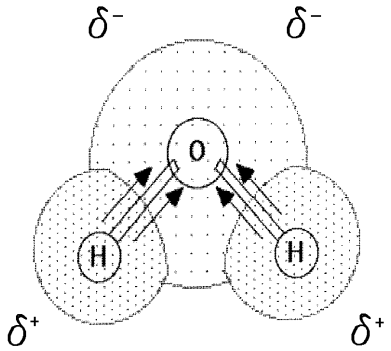
  P and H = 2.1

Nonpolar covalent bond = Covalent bond formed by an equal sharing of electrons between atoms.

- Occurs when electronegativity of both atoms is about the same (e.g., CH_4)
- Molecules made of one element usually have nonpolar covalent bonds (e.g., H_2, O_2, Cl_2, N_2).

Polar covalent bond = Covalent bond formed by an unequal sharing of electrons between atoms.

- Occurs when the atoms involved have different electronegativities.
- Shared electrons spend more time around the more electronegative atom.
- In H_2O, for example, the oxygen is strongly electronegative, so negatively charged electrons spend more time around the oxygen than the hydrogens. This causes the oxygen atom to have a slight negative charge and the hydrogens to have a slight positive charge (see also Campbell, Figure 2.11).



3. Ionic bonds

Ion = Charged atom or molecule.

Anion = An atom that has gained one or more electrons from another atom and has become negatively charged; a negatively charged ion.

Cation = An atom that has lost one or more electrons and has become positively charged; a positively charged ion.

Ionic bond = Bond formed by the electrostatic attraction after the complete transfer of an electron from a donor atom to an acceptor.

- The acceptor atom attracts the electrons because it is much more electronegative than the donor atom.
- Are strong bonds in crystals, but are fragile bonds in water; salt crystals will readily dissolve in water and dissociate into ions.
- Ionic compounds are called salts (e.g., NaCl or table salt) (see Campbell, Figure 2.13).

NOTE: The *difference* in electronegativity between interacting atoms determines if electrons are shared equally (nonpolar covalent), shared unequally (polar covalent), gained or lost (ionic bond). Nonpolar covalent bonds and ionic bonds are two extremes of a continuum from interacting atoms with similar electronegativities to interacting atoms with very different electronegativities.

C. Weak chemical bonds play important roles in the chemistry of life

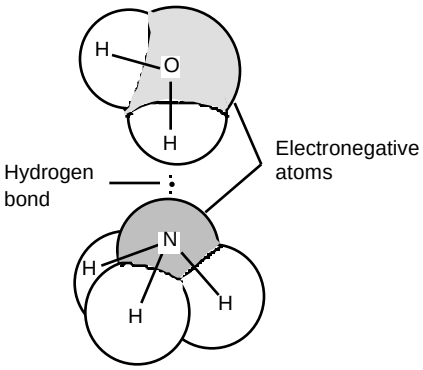
Biologically important weak bonds include the following:

- Hydrogen bonds, ionic bonds in aqueous solutions, and other weak forces such as Van der Waals and hydrophobic interactions
- Make chemical signaling possible in living organisms because they are only temporary associations. Signal molecules can briefly and reversibly bind to receptor molecules on a cell, causing a short-lived response.
- Can form between molecules or between different parts of a single large molecule.
- Help stabilize the three-dimensional shape of large molecules (e.g., DNA and proteins).

1. Hydrogen bonds

Hydrogen bond = Bond formed by the charge attraction when a hydrogen atom covalently bonded to one electronegative atom is attracted to another electronegative atom.

- Weak attractive force that is about 20 times easier to break than a covalent bond
- Is a charge attraction between oppositely charged portions of polar molecules
- Can occur between a hydrogen that has a slight positive charge when covalently bonded to an atom with high electronegativity (usually O and N)
- Example: NH_3 in H_2O (see Campbell, Figure 2.14)



2. Van der Waals interactions

Weak interactions that occur between atoms and molecules that are very close together and result from charge asymetry in electron clouds.

D. A molecule's biological function is related to its shape

A molecule has a charasteric size and shape.

The function of many molecules depends upon their shape

> Insulin causes glucose uptake into liver and muscle cells of veterbrates because the shape of the insulin molecule is recognized by specific receptors on the target cell.

- Molecules with only two atoms are linear.
- Molecules with more than two atoms have more complex shapes.

When an atom forms covalent bonds, orbitals in the valence shell rearrange into the most stable configuration. To illustrate, consider atoms with valence electrons in the *s* and three *p* orbitals:

- The *s* and three *p* orbitals *hybridize* into four new orbitals.
- The new orbitals are teardrop shaped, extend from the nucleus and spread out as far apart as possible.
- Example: If outer tips of orbitals in methane (CH_4) are connected by imaginary lines, the new molecule has a tetrahedral shape with C at the center (see Campbell, Figure 2.15).

E. Chemical reactions make and break chemical bonds

Chemical reactions = process of making and breaking chemical bonds leading to changes in the composition of matter.

- Process where *reactants* undergo changes into *products*.
- Matter is conserved, so all reactant atoms are only rearranged to form products.
- Some reactions go to completion (all reactants converted to products), but most reactions are *reversible*. For example:

$$3H_2 + N_2 \rightleftharpoons 2NH_3$$

- The relative concentration of reactants and products affects reaction rate (the higher the concentration, the greater probability of reaction).

Chemical equilibrium = Equilibrium established when the rate of forward reaction equals the rate of the reverse reaction.

- Is a *dynamic* equilibrium with reactions continuing in both directions
- Relative concentrations of reactants and products stop changing.

Point out to students that chemical equilibrium does NOT mean that the concentrations of reactants and products are equal.

REFERENCES

Atkins, P.W. *Atoms, Electrons and Change*. W.H. Freeman and Company, 1991.

Campbell, N., et al. *Biology*. 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Weinberg, S. *The Discovery of Subatomic Particles*. New York, San Francisco: W.H. Freeman and Company, 1983.

Brown, T.L., H. E. Le May, Jr., and B. Bursten. *Chemistry: The Central Science*. 7th ed. Upper Saddle River, New Jersey: Prentice Hall, 1997.

CHAPTER 3

WATER AND THE FITNESS OF THE ENVIRONMENT

OUTLINE

I. Water's Polarity and Its Effects
   A. The polarity of water molecules results in hydrogen bonding
   B. Organisms depend on the cohesion of water molecules
   C. Water moderates temperatures on Earth
   D. Oceans and lakes don't freeze solid because ice floats
   E. Water is the solvent of life

II. The Dissociation of Water
   A. Organisms are sensitive to changes in pH

III. Acid Precipitation Threatens the Fitness of the Environment

OBJECTIVES

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

1. Describe how water contributes to the fitness of the environment to support life.
2. Describe the structure and geometry of a water molecule, and explain what properties emerge as a result of this structure.
3. Explain the relationship between the polar nature of water and its ability to form hydrogen bonds.
4. List five characteristics of water that are emergent properties resulting from hydrogen bonding.
5. Describe the biological significance of the cohesiveness of water.
6. Distinguish between heat and temperature.
7. Explain how water's high specific heat, high heat of vaporization and expansion upon freezing affect both aquatic and terrestrial ecosystems.
8. Explain how the polarity of the water molecule makes it a versatile solvent.
9. Define molarity and list some advantages of measuring substances in moles.
10. Write the equation for the dissociation of water, and explain what is actually transferred from one molecule to another.
11. Explain the basis for the pH scale.
12. Explain how acids and bases directly or indirectly affect the hydrogen ion concentration of a solution.
13. Using the bicarbonate buffer system as an example, explain how buffers work.

14. Describe the causes of acid precipitation, and explain how it adversely affects the fitness of the environment.

KEY TERMS

polar molecule	Celsius scale	solute	hydrogen ion
cohesion	calorie	solvent	molarity
adhesion	kilocalorie	aqueous solution	hydroxide ion
surface tension	joule	hydrophilic	acid
kinetic energy	specific heat	hydrophobic	base
heat	evaporative cooling	mole	pH scale
temperature	solution	molecular weight	buffer
acid precipitation			

LECTURE NOTES

Water contributes to the fitness of the environment to support life.

- Life on earth probably evolved in water.
- Living cells are 70%-95% H_2O.
- Water covers about 3/4 of the earth.
- In nature, water naturally exists in all three physical states of matter—solid, liquid and gas.

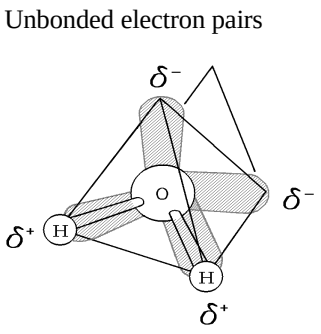
Water's extraordinary properties are emergent properties resulting from water's structure and molecular interactions.

I. Water's Polarity and Its Effects

A. The polarity of water molecules results in hydrogen bonding

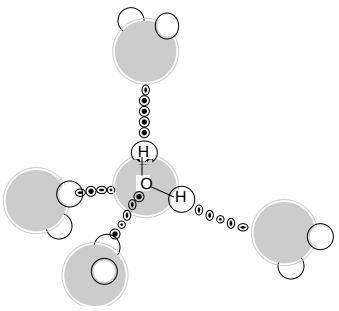
Water is a *polar* molecule. Its polar bonds and asymmetrical shape give water molecules opposite charges on opposite sides.

- Four valence orbitals of O point to corners of a tetrahedron.
- Two corners are orbitals with unshared pairs of electrons and weak negative charge.
- Two corners are occupied by H atoms which are in polar covalent bonds with O. Oxygen is so electronegative, that shared electrons spend more time around the O causing a weak positive charge near H's.



Hydrogen bonding orders water into a higher level of structural organization.

- The polar molecules of water are held together by hydrogen bonds.
- Positively charged H of one molecule is attracted to the negatively charged O of another water molecule.
- Each water molecule can form a maximum of four hydrogen bonds with neighboring water molecules.



Water has extraordinary properties that emerge as a consequence of its polarity and hydrogen-bonding. Some of these properties are that water:

- has cohesive behavior
- resists changes in temperature
- has a high heat of vaporization and cools surfaces as it evaporates
- expands when it freezes
- is a versatile solvent

B. Organisms depend on the cohesion of water molecules.

Cohesion = Phenomenon of a substance being held together by hydrogen bonds.

- Though hydrogen bonds are transient, enough water molecules are hydrogen bonded at any given time to give water more structure than other liquids.
- Contributes to upward water transport in plants by holding the water column together. *Adhesion* of water to vessel walls counteracts the downward pull of gravity.

Surface tension = Measure of how difficult it is to stretch or break the surface of a liquid.

- Water has a greater surface tension than most liquids; function of the fact that at the air/H_2O interface, surface water molecules are hydrogen bonded to each other and to the water molecules below.
- Causes H_2O to bead (shape with smallest area to volume ratio and allows maximum hydrogen bonding).

C. Water moderates temperatures on Earth

1. Heat and temperature

Kinetic energy = The energy of motion.

Heat = Total kinetic energy due to molecular motion in a body of matter.

Temperature = Measure of heat intensity due to the *average* kinetic energy of molecules in a body of matter.

Calorie (cal) = Amount of heat it takes to raise the temperature of one gram of water by one degree Celsius. Conversely, one calorie is the amount of heat that one gram of water releases when it cools down by one degree Celsius. NOTE: The "calories" on food packages are actually kilocalories (kcal).

Kilocalorie (kcal or Cal) = Amount of heat required to raise the temperature of one kilogram of water by one degree Celsius (1000 cal).

Celsius Scale at Sea Level		Scale Conversion		
100°C (212°F)	= water boils	°C	=	$\frac{5(°F - 32)}{9}$
37°C (98.6°F)	= human body temperature	°F	=	$\frac{9° C}{5} + 32$
23°C (72°F)	= room temperature	°K	=	°C + 273
0°C (32°F)	= water freezes			

2. Water's high specific heat

Water has a high *specific heat*, which means that it resists temperature changes when it absorbs or releases heat.

Specific heat = Amount of heat that must be absorbed or lost for one gram of a substance to change its temperature by one degree Celsius.

Specific heat of water = One calorie per gram per degree Celsius (1 cal/g/°C).

- As a result of hydrogen bonding among water molecules, it takes a relatively large heat loss or gain for each 1°C change in temperature.
- Hydrogen bonds must absorb heat to break, and they release heat when they form.
- Much absorbed heat energy is used to disrupt hydrogen bonds before water molecules can move faster (increase temperature).

A large body of water can act as a heat sink, absorbing heat from sunlight during the day and summer (while warming only a few degrees) and releasing heat during the night and winter as the water gradually cools. As a result:

- Water, which covers three-fourths of the planet, keeps temperature fluctuations within a range suitable for life.
- Coastal areas have milder climates than inland.
- The marine environment has a relatively stable temperature.

3. Evaporative cooling

Vaporization (evaporation) = transformation from liquid to a gas.

- Molecules with enough kinetic energy to overcome the mutual attraction of molecules in a liquid, can escape into the air.

Heat of vaporization = Quantity of heat a liquid must absorb for 1 g to be converted to the gaseous state.

- For water molecules to evaporate, hydrogen bonds must be broken which requires heat energy.
- Water has a relatively high heat of vaporization at the boiling point

(540 cal/g or 2260 J/g; Joule = 0.239 cal).

Evaporative cooling = Cooling of a liquid's surface when a liquid evaporates

(see Campbell, Figure 3.4).

- The surface molecules with the highest kinetic energy are most likely to escape into gaseous form; the average kinetic energy of the remaining surface molecules is thus lower.

Water's high heat of vaporization:

- Moderates the Earth's climate.
  - Solar heat absorbed by tropical seas dissipates when surface water evaporates (evaporative cooling).
  - As moist tropical air moves poleward, water vapor releases heat as it condenses into rain.
- Stabilizes temperature in aquatic ecosystems (evaporative cooling).
- Helps organisms from overheating by *evaporative cooling.*

D. Oceans and lakes don’t freeze solid because ice floats

Because of hydrogen bonding, water is less dense as a solid than it is as a liquid. Consequently, ice floats.

- Water is densest at 4°C.
- Water contracts as it cools to 4°C.
- As water cools from 4°C to freezing (0°C), it expands and becomes *less dense* than liquid water (ice floats).
- When water begins to freeze, the molecules do not have enough kinetic energy to break hydrogen bonds.
- As the crystalline lattice forms, each water molecule forms a maximum of four hydrogen bonds, which keeps water molecules further apart than they would be in the liquid state; see Campbell, Figure 3.5.

Expansion of water contributes to the fitness of the environment for life:

- Prevents deep bodies of water from freezing solid from the bottom up.
- Since ice is less dense, it forms on the surface first. As water freezes it releases heat to the water below and insulates it.
- Makes the transitions between seasons less abrupt. As water freezes, hydrogen bonds form releasing heat. As ice melts, hydrogen bonds break absorbing heat.

E. Water is the solvent of life

Solution = A liquid that is a completely homogenous mixture of two or more substances.

Solvent = Dissolving agent of a solution.

Solute = Substance dissolved in a solution.

Aqueous solution = Solution in which water is the solvent.

Water is a versatile solvent owing to the *polarity* of the water molecule.

Hydrophilic

{

Ionic compounds dissolve in water (see Campbell, Figure 3.8).

- Charged regions of polar water molecules have an electrical attraction to charged ions.
- Water surrounds individual ions, separating and shielding them from one another.

Polar compounds in general, are water-soluble.

- Charged regions of polar water molecules have an affinity for oppositely charged regions of other polar molecules.

Hydrophobic { Nonpolar compounds (which have symmetric distribution in charge) are NOT water-soluble.

1. Hydrophilic and hydrophobic substances

Ionic and polar substances are *hydrophilic*, but nonpolar compounds are hydrophobic.

Hydrophilic = (Hydro = water; philo = loving); property of having an affinity for water.

- Some large hydrophilic molecules can absorb water without dissolving.

Hydrophobic = (Hydro = water; phobos = fearing); property of not having an affinity for water, and thus, not being water-soluble.

2. Solute concentration in aqueous solutions

Most biochemical reactions involve solutes dissolved in water. There are two important quantitative properties of aqueous solutions: solute concentration and pH.

Molecular weight = Sum of the weight of all atoms in a molecule (expressed in daltons).

Mole = Amount of a substance that has a mass in grams numerically equivalent to its molecular weight in daltons.

For example, to determine a mole of sucrose ($C_{12}H_{22}O_{11}$):

- Calculate molecular weight:

C = 12 dal	12 dal × 12 =	144 dal
H = 1 dal	1 dal × 22 =	22 dal
O = 16 dal	16 dal × 11 =	176 dal
		342 dal

- Express it in grams (342 g).

Molarity = Number of moles of solute per liter of solution

- To make a 1M sucrose solution, weigh out 342 g of sucrose and add water up to 1L.

Advantage of measuring in moles:

- Rescales weighing of single molecules in daltons to grams, which is more practical for laboratory use.
- A mole of one substance has the *same* number of molecules as a mole of any other substance (6.02×10^{23}; Avogadro's number).
- Allows one to combine substances in fixed ratios of molecules.

II. The Dissociation of Water

Occasionally, the hydrogen atom that is shared in a hydrogen bond between two water molecules, shifts from the oxygen atom to which it is covalently bonded to the unshared orbitals of the oxygen atom to which it is hydrogen bonded.

- Only a *hydrogen ion* (proton with a +1 charge) is actually transferred.
- Transferred proton binds to an unshared orbital of the second water molecule creating a *hydronium ion* (H_3O^+).
- Water molecule that lost a proton has a net negative charge and is called a *hydroxide ion* (OH^-).

$$H_2O + H_2O \rightleftharpoons H_3O^+ + OH^-$$

- By convention, ionization of H_2O is expressed as the *dissociation* into H^+ and OH^-.

$$H_2O \rightleftharpoons H^+ + OH^-$$

- Reaction is reversible.

- At equilibrium, most of the H_2O is *not* ionized.

A. Organisms are sensitive to changes in pH

1. Acids and bases

At equilibrium in pure water at 25°C:

- Number of H+ ions = number of OH- ions.
- $[H^+] = [OH^-] = \frac{1}{10{,}000{,}000}$ $M = 10^{-7}$ M
- Note that brackets indicate molar concentration.

This is a good place to point out how *few* water molecules are actually dissociated (only 1 out of 554,000,000 molecules).

ACID	BASE
Substance that *increases* the relative $[H^+]$ of a solution. Also removes OH^- because it tends to combine with H^+ to form H_2O. For example: (in water) $HCl \rightarrow H^+ + Cl^-$	Substance that *reduces* the relative $[H^+]$ of a solution. May alternately increase $[OH^-]$. For example: A base may reduce $[H^+]$ directly: $NH_3 + H^+ \rightleftharpoons NH_4^+$ A base may reduce $[H^+]$ indirectly: $NaOH \rightarrow Na^+ + OH^-$ $OH^- + H^+ \rightarrow H_2O$

A solution in which:

- $[H^+] = [OH^-]$ is a neutral solution.
- $[H^+] > [OH^-]$ is an acidic solution.
- $[H^+] < [OH^-]$ is a basic solution.

Strong acids and bases dissociate completely in water.

- Example: HCl and NaOH
- Single arrows indicate complete dissociation.

  $NaOH \rightarrow Na^+ + OH^-$

Weak acids and bases dissociate only partially and reversibly.

- Examples: NH_3 (ammonia) and H_2CO_3 (carbonic acid)
- Double arrows indicate a reversible reaction; at equilibrium there will be a fixed ratio of reactants and products.

$$\underset{\text{Carbonic acid}}{H_2CO_3} \rightleftharpoons \underset{\text{Bicarbonate ion}}{HCO_3^-} + \underset{\text{Hydrogen ion}}{H^+}$$

2. The pH scale

In any aqueous solution:

$[H^+][OH^-] = 1.0 \times 10^{-14}$

For example:

- In a neutral solution, $[H^+] = 10^{-7}$ M and $[OH^-] = 10^{-7}$ M.
- In an acidic solution where the $[H^+] = 10^{-5}$ M, the $[OH^-] = 10^{-9}$ M.
- In a basic solution where the $[H^+] = 10^{-9}$ M, the $[OH^-] = 10^{-5}$ M.

pH scale = Scale used to measure degree of acidity. It ranges from 0 to 14.

pH = Negative $\log_{10}$ of the $[H^+]$ expressed in moles per liter.

- pH of 7 is a neutral solution.
- pH < 7 is an acidic solution.
- pH > 7 is a basic solution.

- Most biological fluids are within the pH range of 6 to 8. There are some exceptions such as stomach acid with pH = 1.5. (See Campbell, Figure 3.9)
- Each pH unit represents a *tenfold* difference (scale is logarithmic), so a slight change in pH represents a large change in actual $[H^+]$.

To illustrate this point, project the following questions on a transparency and cover the answer. The students will frequently give the wrong response (3×), and they are surprised when you unveil the solution.

How much greater is the $[H^+]$ in a solution with pH 2 than in a solution with pH 6?

ANS: pH 2 = $[H^+]$ of $1.0 \times 10^{-2} = \frac{1}{100}$ M

pH 6 = $[H^+]$ of $1.0 \times 10^{-6} = \frac{1}{1{,}000{,}000}$ M

10,000 times greater.

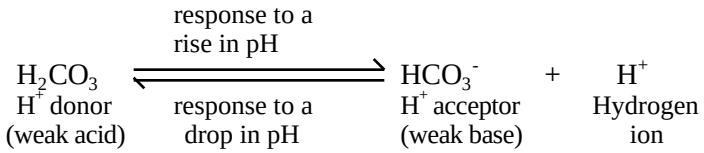
3. Buffers

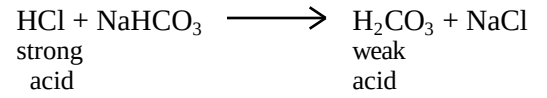
By minimizing wide fluctuations in pH, buffers help organisms maintain the pH of body fluids within the narrow range necessary for life (usually pH 6-8).

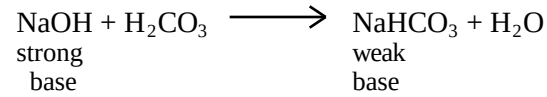
Buffer = Substance that minimizes large sudden changes in pH.

- Are combinations of H^+-donor and H^+-acceptor forms in a solution of weak acids or bases
- Work by accepting H^+ ions from solution when they are in excess and by donating H^+ ions to the solution when they have been depleted

  Example: Bicarbonate buffer







III. Acid Precipitation Threatens the Fitness of the Environment

Acid precipitation = Rain, snow, or fog more strongly acidic than pH 5.6.

- Has been recorded as low as pH 1.5 in West Virginia
- Occurs when sulfur oxides and nitrogen oxides in the atmosphere react with water in the air to form acids which fall to Earth in precipitation
- Major oxide source is the combustion of fossil fuels by industry and cars
- Acid rain affects the fitness of the environment to support life.
  - Lowers soil pH which affects mineral solubility. May leach out necessary mineral nutrients and increase the concentration of minerals that are potentially toxic to vegetation in higher concentration (e.g., aluminum). This is contributing to the decline of some European and North American forests.

- Lowers the pH of lakes and ponds, and runoff carries leached out soil minerals into aquatic ecosystems. This adversely affects aquatic life. Example: In the Western Adirondack Mountains, there are lakes with a pH < 5 that have no fish.

What can be done to reduce the problem?

- Add industrial pollution controls.
- Develop and use antipollution devices.
- Increase involvement of voters, consumers, politicians, and business leaders.

The political issues surrounding acid rain can be used to enhance student awareness and make this entire topic more relevant and interesting to the students.

REFERENCES

Campbell, N., et al. *Biology*. 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Gould, R. *Going Sour: Science and Politics of Acid Rain*. Boston: Birkhauser, 1985.

Henderson, L. J. *The Fitness of the Environment*. Boston: Beacon Press, 1958.

Mohnen, V.A. "The Challenge of Acid Rain." *Scientific American*, August 1988.

CHAPTER 4
CARBON AND MOLECULAR DIVERSITY

OUTLINE

I. The Importance of Carbon
   A. Organic chemistry is the study of carbon compounds
   B. Carbon atoms are the most versatile building blocks of molecules
   C. Variation in carbon skeletons contributes to the diversity of organic molecules

II. Functional Groups
   A. Functional groups also contribute to the molecular diversity of life

OBJECTIVES

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

1. Summarize the philosophies of *vitalism* and *mechanism*, and explain how they influenced the development of organic chemistry, as well as mainstream biological thought.
2. Explain how carbon's electron configuration determines the kinds and number of bonds carbon will form.
3. Describe how carbon skeletons may vary, and explain how this variation contributes to the diversity and complexity of organic molecules.
4. Distinguish among the three types of isomers: structural, geometric and enantiomers.
5. Recognize the major functional groups, and describe the chemical properties of organic molecules in which they occur.

KEY TERMS

organic chemistry	enantiomer	aldehyde	amine
hydrocarbon	functional group	ketone	sulfhydryl group
isomer	hydroxyl group	carboxyl group	thiol
structural isomer	alcohol	carboxylic acid	phosphate group
geometric isomer	carbonyl group	amino group	

LECTURE NOTES

Aside from water, most biologically important molecules are carbon-based (*organic*).

The structural and functional diversity of organic molecules emerges from the ability of carbon to form large, complex and diverse molecules by bonding to itself and to other elements such as H, O, N, S, and P.

I. The Importance of Carbon

A. Organic chemistry is the study of carbon compounds

Organic chemistry = The branch of chemistry that specializes in the study of carbon compounds.

Organic molecules = Molecules that contain carbon

Vitalism = Belief in a life force outside the jurisdiction of chemical/physical laws.

- Early 19th century organic chemistry was built on a foundation of vitalism because organic chemists could not artificially synthesize organic compounds. It was believed that only living organisms could produce organic compounds.

Mechanism = Belief that all natural phenomena are governed by physical and chemical laws.

- Pioneers of organic chemistry began to synthesize organic compounds from inorganic molecules. This helped shift mainstream biological thought from vitalism to mechanism.
- For example, Friedrich Wohler synthesized urea in 1828; Hermann Kolbe synthesized acetic acid.
- Stanley Miller (1953) demonstrated the possibility that organic compounds could have been produced under the chemical conditions of primordial Earth.

B. Carbon atoms are the most versatile building blocks of molecules

The carbon atom:

- Usually has an atomic number of 6; therefore, it has 4 valence electrons.
- Usually completes its outer energy shell by sharing valence electrons in four covalent bonds. (Not likely to form ionic bonds.)

Emergent properties, such as the kinds and number of bonds carbon will form, are determined by their *tetravalent* electron configuration.

- It makes large, complex molecules possible. The carbon atom is a central point from which the molecule branches off into four directions.
- It gives carbon covalent compatibility with many different elements. The four major atomic components of organic molecules are as follows:
- It determines an organic molecule's three-dimensional shape, which may affect molecular function. For example, when carbon forms four single covalent bonds, the four valence orbitals hybridize into teardrop-shaped orbitals that angle from the carbon atoms toward the corners of an imaginary tetrahedron.

Students have problems visualizing shapes of organic molecules in three dimensions. Specific examples can be enhanced by an overhead transparency of ball-and-stick or space-filling models. A large three-dimensional molecular model that can be held up in front of class works best (see Campbell, Figure 4.2)

C. Variation in carbon skeletons contributes to the diversity of organic molecules

Covalent bonds link carbon atoms together in long chains that form the skeletal framework for organic molecules. These carbon skeletons may vary in:

- Length
- Shape (straight chain, branched, ring)
- Number and location of double bonds
- Other elements covalently bonded to available sites

This variation in carbon skeletons contributes to the complexity and diversity of organic molecules (see Campbell, Figure 4.4).

Hydrocarbons = Molecules containing only carbon and hydrogen

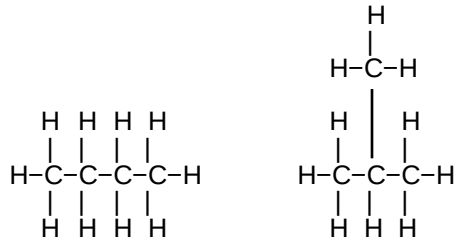
- Are major components of fossil fuels produced from the organic remains of organisms living millions of years ago, though they are not prevalent in living organisms.
- Have a diversity of carbon skeletons which produce molecules of various lengths and shapes.
- As in hydrocarbons, a carbon skeleton is the framework for the large diverse organic molecules found in living organisms. Also, some biologically important molecules may have regions consisting of hydrocarbon chains (e.g. fats).
- Hydrocarbon chains are hydrophobic because the C−C and C−H bonds are nonpolar.

1. Isomers

Isomers = Compounds with the same molecular formula but with different structures and hence different properties. Isomers are a source of variation among organic molecules.

There are three types of isomers (see Campbell, Figure 4.6):

Structural isomers = Isomers that differ in the covalent arrangement of their atoms.



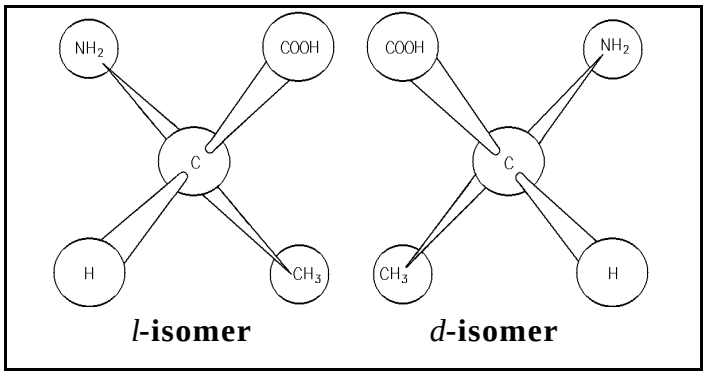
- Number of possible isomers increases as the carbon skeleton size increases.
- May also differ in the location of double bonds.

Geometric isomers = Isomers which share the same covalent partnerships, but differ in their spatial arrangements.

- Result from the fact that double bonds will not allow the atoms they join to rotate freely about the axis of the bonds.
- Subtle differences between isomers affects their biological activity.

Enantiomers = Isomers that are mirror images of each other.

- Can occur when four different atoms or groups of atoms are bonded to the same carbon (*asymmetric carbon*).
- There are two different spatial arrangements of the four groups around the asymmetric carbon. These arrangements are mirror images.
- Usually one form is biologically active and its mirror image is not.



It is often helpful to point at the pharmacological significance of enantiomers, e.g., Campbell, Figure 4.7.

II. Functional Groups

A. Functional groups also contribute to the molecular diversity of life

Small characteristic groups of atoms (functional groups) are frequently bonded to the carbon skeleton of organic molecules. These functional groups:

- Have specific chemical and physical properties.
- Are the regions of organic molecules which are commonly chemically reactive.
- Behave consistently from one organic molecule to another.
- Depending upon their number and arrangement, determine unique chemical properties of organic molecules in which they occur.

As with hydrocarbons, diverse organic molecules found in living organisms have carbon skeletons. In fact, these molecules can be viewed as hydrocarbon derivatives with functional groups in place of H, bonded to carbon at various sites along the molecule.

1. The hydroxyl group

Hydroxyl group = A functional group that consists of a hydrogen atom bonded to an oxygen atom, which in turn is bonded to carbon (−OH).

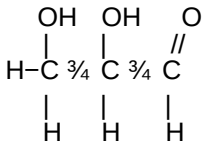
- Is a *polar* group; the bond between the oxygen and hydrogen is a polar covalent bond.
- Makes the molecule to which it is attached *water soluble*. Polar water molecules are attracted to the polar hydroxyl group which can form hydrogen bonds.
- Organic compounds with hydroxyl groups are called *alcohols*.

2. The carbonyl group

Carbonyl group = Functional group that consists of a carbon atom double-bonded to oxygen (−CO).

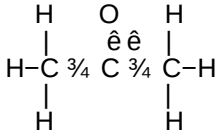
- Is a *polar* group. The oxygen can be involved in hydrogen bonding, and molecules with his functional group are *water soluble*.
- Is a functional group found in sugars.

- If the carbonyl is at the end off the carbon skeleton, the compound is an *aldehyde*.



Glyceraldehyde

- If the carbonyl is at the end of the carbon skeleton, the compound is a *ketone*.

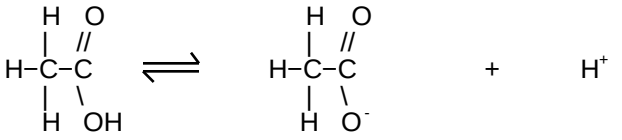


Acetone

3. The carboxyl group

Carboxyl group = Functional group that consists of a carbon atom which is both double-bonded to an oxygen and single-bonded to the oxygen of a hydroxyl group (−COOH).

- Is a polar group and water soluble. The covalent bond between oxygen and hydrogen is so polar, that the hydrogen reversibly dissociates as H^+. This polarity results from the combined effect of the two electronegative oxygen atoms bonded to the same carbon.



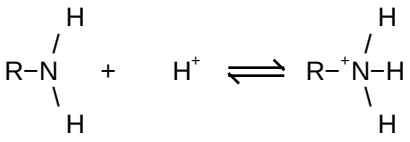
Acetic acid | Acetate ion | Hydrogen ion

- Since it donates protons, this group has acidic properties. Compounds with this functional group are called *carboxylic acids*.

4. The amino group

Amino group = Functional group that consists of a nitrogen atom bonded to two hydrogens and to the carbon skeleton ($-NH_2$).

- Is a polar group and soluble in water.
- Acts as a weak base. The unshared pair of electrons on the nitrogen can accept a proton, giving the amino group a +1 charge.



Amine | Ammonium ion

- Organic compounds with this function group are called *amines*.

5. The Sulfhydryl group

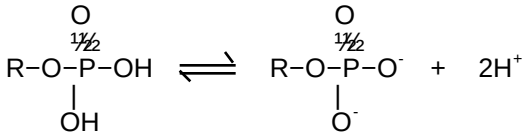
Sulfhydryl group = Functional group which consists of an atom of sulfur bonded to an atom of hydrogen (−SH).

- Help stabilize the structure of proteins. (Disulfide bridges will be discussed with tertiary structure of proteins in Chapter 5, Structure and Function of Macromolecules.)
- Organic compounds with this functional group are called *thiols*.

6. The phosphate group

Phosphate group = Functional group which is the dissociated form of phosphoric acid (H_3PO_4).

- Loss of two protons by dissociation leaves the phosphate group with a negative charge.



- Has acid properties since it loses protons.
- Polar group and soluble in water.
- Organic phosphates are important in cellular energy storage and transfer. (ATP is discussed with energy for cellular work in Chapter 6: Introduction to Metabolism.)

In lecture, you may also choose to include the methyl group ($-CH_3$) as an example of a nonpolar hydrophobic functional group. This is helpful later in the course in explaining how nonpolar amino acids contribute to the tertiary structure of proteins including integral membrane proteins.

To impress upon students how important functional groups are in determining chemical behavior of organic molecules, use the following demonstration: show a comparison of estradiol and testosterone and ask students to find the differences in functional groups. Ask one male and female student to stand up or show pictures of sexual dimorphism in other vertebrates. Point out that differences between males and females are due to slight variation in functional groups attached to sex hormones.

REFERENCES

Campbell, N. et al. *Biology*. 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Lehninger, A.L., D.L. Nelson and M.M. Cox. *Principles of Biochemistry*. 2nd ed. New York: Worth, 1993.

Whitten, K.W. and K.D. Gailey. *General Chemistry*. 4th ed. New York: Saunders, 1992.

CHAPTER 5

THE STRUCTURE AND FUNCTION OF MACROMOLECULES

OUTLINE

I. Polymer Principles
   A. Most macromolecules are polymers
   B. A limitless variety of polymers can be built from a small set of monomers
II. Carbohydrates: Fuel and Building Material
   A. Sugars, the smallest carbohydrates, serve as fuel and carbon sources
   B. Polysaccharides, the polymers of sugars, have storage and structural roles
III. Lipids: Diverse Hydrophobic Molecules
   A. Fats store large amounts of energy
   B. Phospholipids are major components of cell membranes
   C. Steroids include cholesterol and certain hormones
IV. Proteins: The Molecular Tools of the Cell
   A. A polypeptide is a polymer of amino acids connected in a specific sequence
   B. A protein's function depends on its specific conformation
V. Nucleic Acids: Informational Polymers
   A. Nucleic acids store and transmit hereditary information
   B. A nucleic acid strand is a polymer of nucleotides
   C. Inheritance is based on replication of the DNA double helix
   D. We can use DNA and proteins as tape measures of evolution

OBJECTIVES

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

1. List the four major classes of biomolecules.
2. Explain how organic polymers contribute to biological diversity.
3. Describe how covalent linkages are formed and broken in organic polymers.
4. Describe the distinguishing characteristics of carbohydrates, and explain how they are classified.
5. List four characteristics of a sugar.
6. Identify a glycosidic linkage and describe how it is formed.
7. Describe the important biological functions of polysaccharides.
8. Distinguish between the glycosidic linkages found in starch and cellulose, and explain why the difference is biologically important.
9. Explain what distinguishes lipids from other major classes of macromolecules.
10. Describe the unique properties, building block molecules and biological importance of the three important groups of lipids: fats, phospholipids and steroids.
11. Identify an ester linkage and describe how it is formed.

12. Distinguish between a saturated and unsaturated fat, and list some unique emergent properties that are a consequence of these structural differences.
13. Describe the characteristics that distinguish proteins from the other major classes of macromolecules, and explain the biologically important functions of this group.
14. List and recognize four major components of an amino acid, and explain how amino acids may be grouped according to the physical and chemical properties of the side chains.
15. Identify a peptide bond and explain how it is formed.
16. Explain what determines protein conformation and why it is important.
17. Define primary structure and describe how it may be deduced in the laboratory.
18. Describe the two types of secondary protein structure, and explain the role of hydrogen bonds in maintaining the structure.
19. Explain how weak interactions and disulfide bridges contribute to tertiary protein structure.
20. Using collagen and hemoglobin as examples, describe quaternary protein structure.
21. Define denaturation and explain how proteins may be denatured.
22. Describe the characteristics that distinguish nucleic acids from the other major groups of macromolecules.
23. Summarize the functions of nucleic acids.
24. List the major components of a nucleotide, and describe how these monomers are linked together to form a nucleic acid.
25. Distinguish between a pyrimidine and a purine.
26. List the functions of nucleotides.
27. Briefly describe the three-dimensional structure of DNA.

KEY TERMS

polymer	cellulose	polypeptide	quaternary structure
monomer	chitin	amino acid	denaturation
condensation reaction	lipid	protein	chaperone proteins
dehydration reaction	fat	conformation	gene
hydrolysis	fatty acid	peptide bond	nucleic acid
carbohydrate	triacylglycerol	primary structure	deoxyribonucleic acid
monosaccharide	saturated fatty acid	secondary structure	ribonucleic acid
disaccharide	unsaturated fatty acid	alpha (α) helix	nucleotide
glycosidic linkage	steroid	pleated sheet	pyrimidine
polysaccharide	cholesterol	tertiary structure	purine
starch	protein	hydrophobic interaction	ribose
glycogen	conformation	disulfide bridges	polynucleotide
double helix			

LECTURE NOTES

The topic of macromolecules lends itself well to illustrate three integral themes that permeate the text and course:

1. There is a natural hierarchy of structural level in biological organization.
2. As one moves up the hierarchy, new properties emerge because of interactions among subunits at the lower levels.
3. Form fits function.

I. Polymer Principles

A. Most macromolecules are polymers

Polymer = (Poly = many; mer = part); large molecule consisting of many identical or similar subunits connected together.

Monomer = Subunit or building block molecule of a polymer

Macromolecule = (Macro = large); large organic polymer

- Formation of macromolecules from smaller building block molecules represents another level in the hierarchy of biological organization.
- There are four classes of macromolecules in living organisms:
  1. Carbohydrates
  2. Lipids
  3. Proteins
  4. Nucleic acids

Most polymerization reactions in living organisms are condensation reactions.

- *Polymerization reactions* = Chemical reactions that link two or more small molecules to form larger molecules with repeating structural units.
- *Condensation reactions* = Polymerization reactions during which monomers are covalently linked, producing net removal of a water molecule for each covalent linkage.
  - One monomer loses a hydroxyl (–OH), and the other monomer loses a hydrogen (–H).
  - Removal of water is actually indirect, involving the formation of "activated" monomers (discussed in Chapter 6, Introduction to Metabolism).
  - Process requires energy.
  - Process requires biological catalysts or enzymes.

Hydrolysis = (Hydro = water; lysis = break); a reaction process that breaks covalent bonds between monomers by the addition of water molecules.

- A hydrogen from the water bonds to one monomer, and the hydroxyl bonds to the adjacent monomer.
- Example: Digestive enzymes catalyze hydrolytic reactions which break apart large food molecules into monomers that can be absorbed into the bloodstream.

B. An immense variety of polymers can be built from a small set of monomers

Structural variation of macromolecules is the basis for the enormous diversity of life.

- There is *unity* in life as there are only about 40 to 50 common monomers used to construct macromolecules.
- There is *diversity* in life as new properties emerge when these universal monomers are arranged in different ways.

II. Carbohydrates: Fuel and Building Material

A. Sugars, the smallest carbohydrates, serve as fuel and carbon sources

Carbohydrates = Organic molecules made of sugars and their polymers

- Monomers or building block molecules are simple sugars called *monosaccharides*.
- Polymers are formed by condensation reactions.
- Carbohydrates are classified by the number of simple sugars.

1. Monosaccharides

Monosaccharides = (Mono = single; sacchar = sugar); simple sugar in which C, H, and O occur in the ratio of (CH_2O).

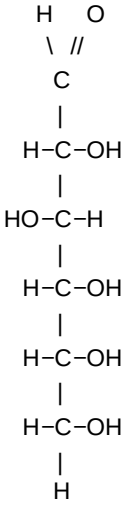
- Are major nutrients for cells; glucose is the most common
- Can be produced (glucose) by photosynthetic organisms from CO_2, H_2O, and sunlight
- Store energy in their chemical bonds which is harvested by cellular respiration
- Their carbon skeletons are raw material for other organic molecules.
- Can be incorporated as monomers into disaccharides and polysaccharides

Characteristics of a sugar:

a. An –OH group is attached to each carbon except one, which is double bonded to an oxygen (*carbonyl*).

Aldehyde

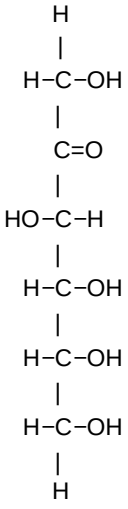
Terminal carbon forms a double bond with oxygen.



Glucose (aldose)

Ketone

Carbonyl group is within the carbon skeleton.

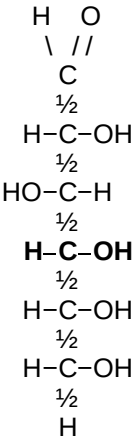


Fructose (ketose)

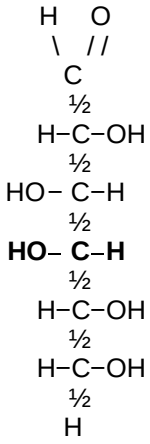
b. Size of the carbon skeleton varies from three to seven carbons. The *most common* monosaccharides are:

Classification	Number of Carbons	Example
Triose	3	Glyceraldehyde
Pentose	5	Ribose
Hexose	6	Glucose

c. Spatial arrangement around asymmetric carbons may vary. For example, glucose and galactose are enantiomers.



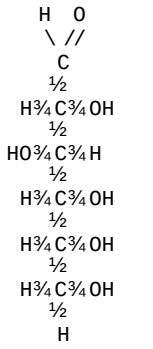
Glucose



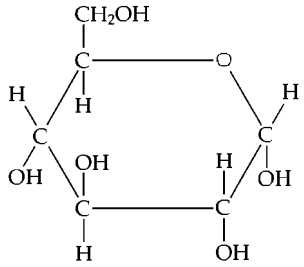
Galactose

The small difference between isomers affects molecular shape which gives these molecules distinctive biochemical properties.

d. In aqueous solutions, many monosaccharides form rings. Chemical equilibrium favors the ring structure.



Linear Form of Glucose

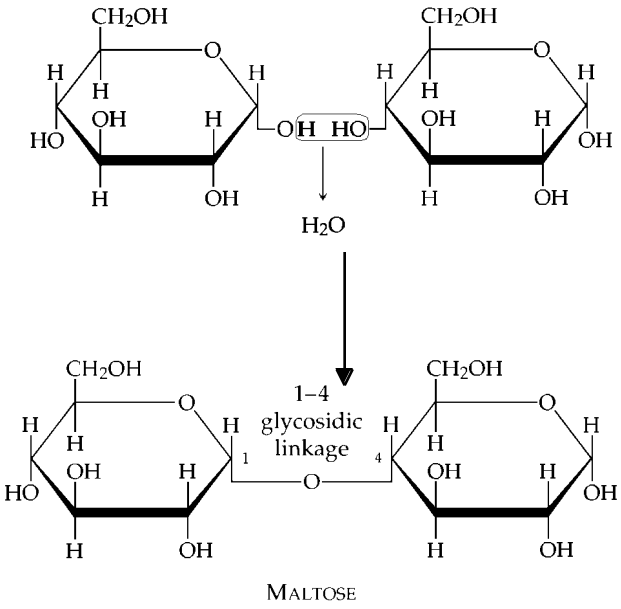


Ring Form of Glucose

2. Disaccharides

Disaccharide = (Di = two; sacchar = sugar); a double sugar that consists of two monosaccharides joined by a *glycosidic linkage.*

Glycosidic linkage = Covalent bond formed by a condensation reaction between two sugar monomers; for example, maltose:



MALTOSE

Examples of disaccharides include:

Disaccharide	Monomers	General Comments
Maltose	Glucose + Glucose	Important in brewing beer
Lactose	Glucose + Galactose	Present in milk
Sucrose	Glucose + Fructose	Table sugar; most prevalent disaccharide; transport form in plants

B. Polysaccharides, the polymers of sugars, have storage and structural roles

Polysaccharides = Macromolecules that are polymers of a few hundred or thousand monosaccharides.

- Are formed by linking monomers in enzyme-mediated condensation reactions
- Have two important biological functions:
  1. Energy storage (starch and glycogen)
  2. Structural support (cellulose and chitin)

1. Storage polysaccharides

Cells hydrolyze storage polysaccharides into sugars as needed. Two most common storage polysaccharides are *starch* and *glycogen.*

Starch = Glucose polymer that is a storage polysaccharide in plants.

- Helical glucose polymer with α 1-4 linkages (see Campbell, Figure 5.6)
- Stored as granules within plant organelles called *plastids*
- *Amylose*, the simplest form, is an unbranched polymer.
- *Amylopectin* is branched polymer.
- Most animals have digestive enzymes to hydrolyze starch.
- Major sources in the human diet are potato tubers and grains (e.g., wheat, corn, rice, and fruits of other grasses).

Glycogen = Glucose polymer that is a storage polysaccharide in animals.

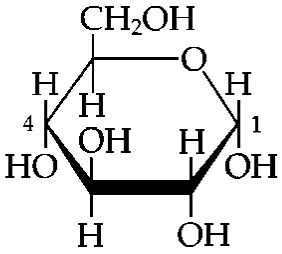
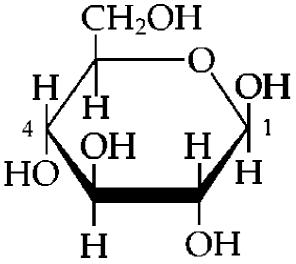
- Large glucose polymer that is more highly branched (α 1-4 and 4-6 linkages) than amylopectin
- Stored in the muscle and liver of humans and other vertebrates

2. Structural polysaccharides

Structural polysaccharides include *cellulose* and *chitin*.

Cellulose = Linear unbranched polymer of *D*-glucose in (α 1-4, β 4-6) linkages.

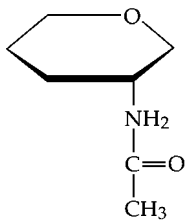
- A major structural component of plant cell walls
- Differs from starch (also a glucose polymer) in its glycosidic linkages (see Campbell, Figure 5.7)

STARCH	CELLULOSE
Glucose monomers are in α *configuration* (–OH group on carbon one is *below* the ring's plane).	Glucose monomers are in β *configuration* (–OH group on carbon one is *above* the ring's plane).
	
Monomers are connected with α 1-4 linkage.	Monomers are connected with β 1-4 linkage.

- Cellulose and starch have different three-dimensional shapes and properties as a result of differences in glycosidic linkages.
- Cellulose reinforces plant cell walls. Hydrogen bonds hold together parallel cellulose molecules in bundles of *microfibrils* (see Campbell, Figure 5.8)
- Cellulose cannot be digested by most organisms, including humans, because they lack an enzyme that can hydrolyze the β 1-4 linkage. (Exceptions are some symbiotic bacteria, other microorganisms and some fungi.)

Chitin = A structural polysaccharide that is a polymer of an amino sugar (see Campbell, Figure 5.9).

- Forms exoskeletons of arthropods
- Found as a building material in the cell walls of some fungi
- Monomer is an *amino sugar*, which is similar to *beta*-glucose with a nitrogen-containing group replacing the hydroxyl on carbon 2.



III. Lipids: Diverse Hydrophobic Molecules

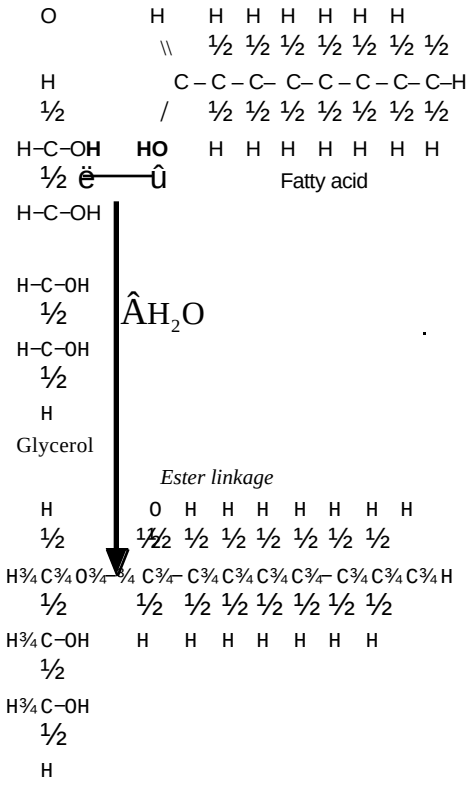
Lipids = Diverse group of organic compounds that are insoluble in water, but will dissolve in nonpolar solvents (e.g., ether, chloroform, benzene). Important groups are *fats, phospholipids,* and *steroids*.

A. Fats store large amounts of energy

Fats = Macromolecules are constructed from (see Campbell, Figure 5.10):

1. Glycerol, a three-carbon alcohol
2. Fatty acid (carboxylic acid)
   - Composed of a carboxyl group at one end and an attached *hydrocarbon chain* ("tail")
   - Carboxyl functional group ("head") has properties of an acid.
   - Hydrocarbon chain has a long carbon skeleton usually with an even number of carbon atoms (most have 16 – 18 carbons).
   - Nonpolar C–H bonds make the chain hydrophobic and not water soluble.

During the formation of a fat, enzyme-catalyzed condensation reactions link glycerol to fatty acids by an ester linkage.



Ester linkage = Bond formed between a hydroxyl group and a carboxyl group.

Each of glycerol's three hydroxyl groups can bond to a fatty acid by an ester linkage producing a fat.

Triacylglycerol = A fat composed of three fatty acids bonded to one glycerol by ester linkages (triglyceride).

Some characteristics of fat include:

- Fats are insoluble in water. The long fatty acid chains are hydrophobic because of the many nonpolar C–H bonds.
- The source of variation among fat molecules is the fatty acid composition.
- Fatty acids in a fat may all be the same, or some (or all) may differ.
- Fatty acids may vary in length.
- Fatty acids may vary in the number and location of carbon-to-carbon double bonds.

SATURATED FAT	UNSATURATED FAT
No double bonds between carbons in fatty acid tail	One or more double bonds between carbons in fatty acid tail
Carbon skeleton of fatty acid is bonded to maximum number of hydrogens (*saturated* with hydrogens)	Tail kinks at each C=C, so molecules do not pack closely enough to solidify at room temperature
Usually a solid at room temperature	Usually a liquid at room temperature
Most animal fats	Most plant fats
e.g., bacon grease, lard and butter (see Campbell, Figure 5.11)	e.g., corn, peanut and olive oil

- In many commercially prepared food products, unsaturated fats are artificially hydrogenated to prevent them from separating out as oil (e.g., peanut butter and margarine).

Fat serves many useful functions:

- Energy storage. One gram of fat stores twice as much energy as a gram of polysaccharide. (Fat has a higher proportion of energy rich C–H bonds.)
- More compact fuel reservoir than carbohydrate. Animals store more energy with less weight than plants which use starch, a bulky form of energy storage.
- Cushions vital organs in mammals (e.g., kidney).
- Insulates against heat loss (e.g., in mammals such as whales and seals).

B. Phospholipids

Phospholipids = Compounds with molecular building blocks of glycerol, two fatty acids, a phosphate group, and usually, an additional small chemical group attached to the phosphate (see Campbell , Figure 5.12)

- Differ from fat in that the third carbon of glycerol is joined to a *negatively charged* phosphate group
- Can have small variable molecules (usually charged or polar) attached to phosphate
- Are diverse depending upon differences in fatty acids and in phosphate attachments
- Show ambivalent behavior toward water. Hydrocarbon tails are hydrophobic and the polar head (phosphate group with attachments) is hydrophilic.
- Cluster in water as their hydrophobic portions turn away from water. One such cluster, a *micelle*, assembles so the hydrophobic tails turn toward the water-free interior and the hydrophilic phosphate heads arrange facing outward in contact with water (see Campbell, Figure 5.13).
- Are major constituents of cell membranes. At the cell surface, phospholipids form a bilayer held together by hydrophobic interactions among the hydrocarbon tails. Phospholipids in water will spontaneously form such a bilayer.

C. Steroids

Steroids = Lipids which have four fused carbon rings with various functional groups attached.

Cholesterol is an important steroid.

- Is the precursor to many other steroids including vertebrate sex hormones and bile acids.
- Is a common component of animal cell membranes.
- Can contribute to atherosclerosis.

IV. Proteins: The Molecular Tools of the Cell

Polypeptide chains = Polymers of amino acids that are arranged in a specific linear sequence and are linked by peptide bonds.

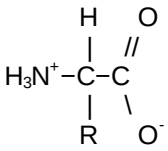
Protein = A macromolecule that consists of one or more *polypeptide chains* folded and coiled into specific conformations.

- Are abundant, making up 50% or more of cellular dry weight
- Have important and varied functions in the cell:
  1. Structural support
  2. Storage (of amino acids)
  3. Transport (e.g., hemoglobin)
  4. Signaling (chemical messengers)
  5. Cellular response to chemical stimuli (receptor proteins)
  6. Movement (contractile proteins)
  7. Defense against foreign substances and disease-causing organisms (antibodies)
  8. Catalysis of biochemical reactions (enzymes)
- Vary extensively in structure; each type has a unique three-dimensional shape (conformation)
- Though they vary in structure and function, they are commonly made of only 20 amino acid monomers.

A. A polypeptide is a polymer of amino acids connected in a specific sequence

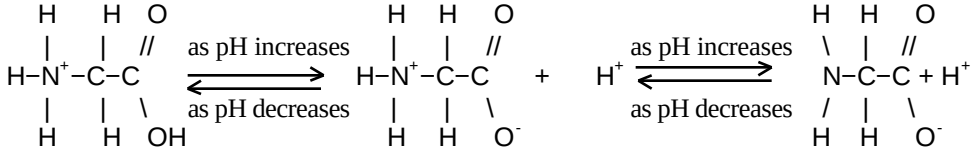
Amino acid = Building block molecule of a protein; most consist of an asymmetric carbon, termed the *alpha carbon*, which is covalently bonded to a(n):

1. Hydrogen atom.
2. Carboxyl group.
3. Amino group.
4. Variable R group (side chain) specific to each amino acid. Physical and chemical properties of the side chain determine the uniqueness of each amino acid.



(At pH's normally found in the cell, both the carboxyl and amino groups are ionized.)

Amino acids contain both carboxyl and amino functional groups. Since one group acts as a weak acid and the other group acts as a weak base, an amino acid can exist in three ionic states. The pH of the solution determines which ionic state predominates.



Cation — Zwitterion (dipolar ion) — Anion

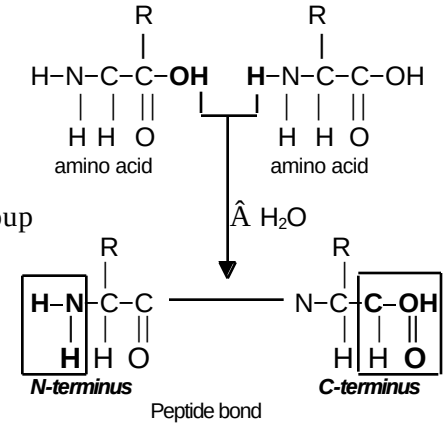
The twenty common amino acids can be grouped by properties of side chains (see Campbell, Figure 5.15):

1. Nonpolar side groups (hydrophobic). Amino acids with nonpolar groups are less soluble in water.
2. Polar side groups (hydrophilic). Amino acids with polar side groups are soluble in water. Polar amino acids can be grouped further into:
   a. Uncharged polar
   b. Charged polar
      - Acidic side groups. Dissociated carboxyl group gives these side groups a negative charge.
      - Basic side groups. An amino group with an extra proton gives these side groups a net positive charge.

Polypeptide chains are polymers that are formed when amino acid monomers are linked by peptide bonds (see Campbell, Figure 5.16).

Peptide bond = Covalent bond formed by a condensation reaction that links the carboxyl group of one amino acid to the amino group of another.

- Has polarity with an amino group on one end (*N-terminus*) and a carboxyl group on the other (*C-terminus*).
- Has a backbone of the repeating sequence −N−C−C−N−C−C−.



Polypeptide chains:

- Range in length from a few monomers to more than a thousand.
- Have unique linear sequences of amino acids.

B. A protein's function depends on its specific conformation

A protein's function depends upon its unique *conformation.*

Protein conformation = Three-dimensional shape of a protein.

Native conformation = Functional conformation of a protein found under normal biological conditions.

- Enables a protein to recognize and bind specifically to another molecule (e.g., hormone/receptor, enzyme/substrate, and antibody/antigen)
- Is a consequence of the specific linear sequence of amino acids in the polypeptide
- Is produced when a newly formed polypeptide chain coils and folds spontaneously, mostly in response to hydrophobic interactions
- Is stabilized by chemical bonds and weak interactions between neighboring regions of the folded protein

1. Four levels of protein structure

The correlation between form and function in proteins is an emergent property resulting from superimposed levels of protein structure (see Campbell, Figure 5.24):

- Primary structure
- Secondary structure

- Tertiary structure
- When a protein has two or more polypeptide chains, it also has quaternary structure.

a. Primary structure

Primary structure = Unique sequence of amino acids in a protein.

- Determined by genes
- Slight change can affect a protein's conformation and function (e.g., sickle-cell hemoglobin; see Campbell, Figure 5.19).
- Can be sequenced in the laboratory. A pioneer in this work was Frederick Sanger who determined the amino acid sequence in insulin (late 1940s and early 1950s). This laborious process involved:
  1) Determination of amino acid composition by complete acid hydrolysis of peptide bonds and separation of resulting amino acids by chromatography. Using these techniques, Sanger identified the amino acids and determined the relative proportions of each.
  2) Determination of amino acid sequence by partial hydrolysis with enzymes and other catalysts to break only specific peptide bonds. Sanger deductively reconstructed the primary structure from fragments with overlapping segments.
- Most of the sequencing process is now automated.

b. Secondary structure

Secondary structure = Regular, repeated coiling and folding of a protein's polypeptide backbone (see Campbell, Figure 5.20).

- Contributes to a protein's overall conformation.
- Stabilized by hydrogen bonds between peptide linkages in the protein's backbone (carbonyl and amino groups).
- The major types of secondary structure are alpha (α) helix and beta (β) pleated sheet.

1) Alpha (α) helix

Alpha (α) helix = Secondary structure of a polypeptide that is a helical coil stabilized by hydrogen bonding between every fourth peptide bond (3.6 amino acids per turn).

- Described by Linus Pauling and Robert Corey in 1951.
- Found in fibrous proteins (e.g., α-keratin and collagen) for most of their length and in some portions of globular proteins.

2) Beta (β) pleated sheet

Beta (β) pleated sheet = Secondary protein structure which is a sheet of antiparallel chains folded into accordion pleats.

- Parallel regions are held together by either intrachain or interchain hydrogen bonds (between adjacent polypeptides).
- Make up the dense core of many globular proteins (e.g., lysozyme) and the major portion of some fibrous proteins (e.g., fibroin, the structural protein of silk).

c. Tertiary structure

Tertiary structure = The three-dimensional shape of a protein. The irregular contortions of a protein are due to bonding between and among side chains (R groups) and to interaction between R groups and the aqueous environment (see Campbell, Figure 5.22).

Types of bonds contributing to tertiary structure are weak interactions and covalent linkage (both may occur in the same protein).

1) Weak interactions

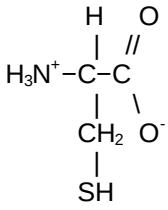
Protein shape is stabilized by the cumulative effect of weak interactions. These weak interactions include:

- Hydrogen bonding between polar side chains.
- Ionic bonds between charged side chains.
- *Hydrophobic interactions* between nonpolar side chains in protein's interior.

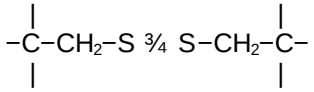
Hydrophobic interactions = (Hydro = water; phobos = fear); the clustering of hydrophobic molecules as a result of their mutual exclusion from water.

2) Covalent linkage

Disulfide bridges form between two cysteine monomers brought together by folding of the protein. This is a strong bond that reinforces conformation.



Cysteine



Disulfide Bridge
(S of one cysteine sulfhydryl, bonds to the S of a second cysteine.)

d. Quaternary structure

Quaternary structure = Structure that results from the interactions between and among several polypeptides chains (subunits) (see Campbell, Figure 5.23).

- Example: Collagen, a fibrous protein with three helical polypeptides supercoiled into a triple helix; found in animal connective tissue, collagen's supercoiled quaternary structure gives it strength.
- Some globular proteins have subunits that fit tightly together. Example: Hemoglobin, a globular protein that has four subunits (two α chains and two β chains)

2. What determines protein conformation?

A protein's three-dimensional shape is a consequence of the interactions responsible for secondary and tertiary structure.

- This conformation is influenced by physical and chemical environmental conditions.
- If a protein's environment is altered, it may become *denatured* and lose its native conformation.

Denaturation = A process that alters a protein's native conformation and biological activity. Proteins can be denatured by:

- Transfer to an organic solvent. Hydrophobic side chains, normally inside the protein's core, move towards the outside. Hydrophilic side chains turn away from the solvent towards the molecule's interior.
- Chemical agents that disrupt hydrogen bonds, ionic bonds and disulfide bridges.
- Excessive heat. Increased thermal agitation disrupts weak interactions (see Campbell, Figure 5.25).

The fact that some denatured proteins return to their native conformation when environmental conditions return to normal is evidence that a protein's amino acid sequence (primary structure) determines conformation. It influences where and which interactions will occur as the molecule arranges into secondary and tertiary structure.

3. The protein-folding problem

Even though primary structure ultimately determines a protein's conformation, three-dimensional shape is difficult to predict solely on the basis of amino acid sequence. It is difficult to find the rules of protein folding because:

- Most proteins pass through several intermediate stages in the folding process; knowledge of the final conformation does not reveal the folding process required to create it.
- A protein's native conformation may be dynamic, alternating between several shapes.

Using recently developed techniques, researchers hope to gain new insights into protein folding:

- Biochemists can now track a protein as it passes through its intermediate stages during the folding process.
- *Chaperone proteins* have just been discovered that temporarily brace a folding protein.

Rules of protein folding are important to molecular biologists and the biotechnology industry. This knowledge should allow the design of proteins for specific purposes.

V. Nucleic Acids: Informational Polymers

A. Nucleic acids store and transmit hereditary information

Protein conformation is determined by primary structure. Primary structure, in turn, is determined by *genes*; hereditary units that consist of DNA, a type of *nucleic acid.*

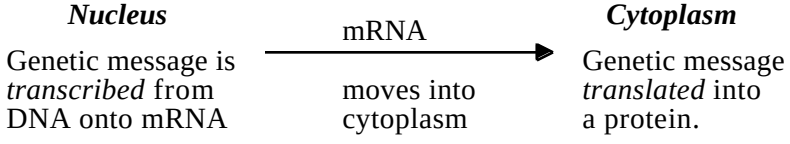
There are two types of nucleic acids.

1. Deoxyribonucleic acid (DNA)

- Contains coded information that programs all cell activity.
- Contains directions for its own replication.
- Is copied and passed from one generation of cells to another.
- In eukaryotic cells, is found primarily in the nucleus.
- Makes up *genes* that contain instructions for protein synthesis. Genes do not directly make proteins, but direct the synthesis of mRNA.

2. Ribonucleic acid (RNA)

- Functions in the actual synthesis of proteins coded for by DNA.
- Sites of protein synthesis are on *ribosomes* in the cytoplasm.
- Messenger RNA (mRNA) carries encoded genetic message from the nucleus to the cytoplasm.
- The flow of genetic information goes from DNA → RNA → protein (see Campbell, Figure 5.26).



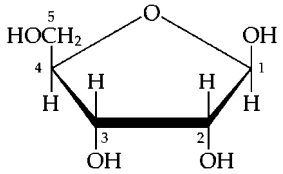
B. A nucleic acid strand is a polymer of nucleotides

Nucleic acid = Polymer of *nucleotides* linked together by condensation reactions.

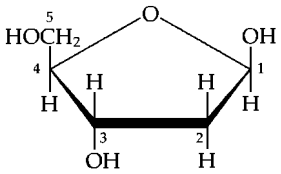
Nucleotide = Building block molecule of a nucleic acid; made of (1) a five-carbon sugar covalently bonded to (2) a phosphate group and (3) a nitrogenous base.

1. Pentose (5-carbon sugar)

There are two pentoses found in nucleic acids: ribose and deoxyribose.



Ribose is the pentose in RNA.



Deoxyribose is the pentose in DNA. (It lacks the −OH group at the number two carbon.)

2. Phosphate

The phosphate group is attached to the number 5 carbon of the sugar.

3. Nitrogenous base

There are two families of *nitrogenous bases*:

Pyrimidine = Nitrogenous base characterized by a six-membered ring made up of carbon and nitrogen atoms. For example:

- Cytosine (C)
- Thymine (T); found only in DNA
- Uracil (U); found only in RNA

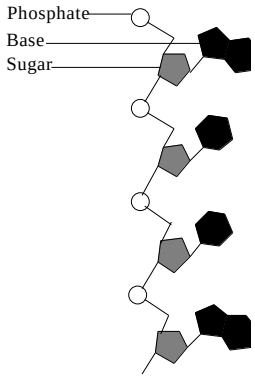
Purine = Nitrogenous base characterized by a five-membered ring fused to a six-membered ring. For example:

- Adenine (A)
- Guanine (G)

Nucleotides have various functions:

- Are monomers for nucleic acids.
- Transfer chemical energy from one molecule to another (e.g., ATP).
- Are electron acceptors in enzyme-controlled redox reactions of the cell (e.g., NAD).

A nucleic-acid polymer or polynucleotise, results from joining nucleotides together by covalent bonds called *phosphodiester linkages*. The bond is formed between the phosphate of one nucleotide and the sugar of the next.



- Results in a backbone with a repeating pattern of sugar-phosphate-sugar-phosphate.
- Variable nitrogenous bases are attached to the sugar-phosphate backbone.
- Each gene contains a unique linear sequence of nitrogenous bases which codes for a unique linear sequence of amino acids in a protein.

C. Inheritance is based on precise replication of the DNA double helix

In 1953, James Watson and Francis Crick proposed the *double helix* as the three dimensional structure of DNA.

- Consists of two nucleotide chains wound in a double helix.
- Sugar-phosphate backbones are on the outside of the helix.
- The two polynucleotide strands of DNA are held together by hydrogen bonds between the paired nitrogenous bases and by van der Waals attraction between the stacked bases (see Campbell, Figure 5.28).
- Base-pairing rules are that adenine (A) always pairs with thymine (T); guanine (G) always pairs with cytosine (C).
- Two strands of DNA are complimentary and thus can serve as templates to make new complementary strands. It is this mechanism of precise copying that makes inheritance possible.
- Most DNA molecules are long, containing thousands or millions of base pairs.

D. We can use DNA and proteins as tape measures of evolution

Closely related species have more similar sequences of DNA and amino acids, than more distantly related species. Using this type of molecular evidence, biologists can deduce evolutionary relationships among species.

Chapters 16 and 17 are devoted to DNA and protein synthesis. Since any discussion of DNA function must include the details of DNA structure, it may be more practical and less time-consuming to cover nucleic acids later in the course.

REFERENCES

Alberts, B., et al. *Essential Cell Biology: An Introduction to the Molecular Biology of the Cell.* New York: Garland Publishing, Inc., 1998.

Campbell, N., et al. *Biology*. 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Lehninger, A.L., D.L. Nelson and M.M. Cox. *Principles of Biochemistry*. 2nd ed. New York: Worth, 1993.

Brown, T.L., H. E. Le May, Jr., and B. Bursten. *Chemistry: The Central Science*. 7th Ed. Upper Saddle River, New Jersey: Prentice Hall, 1997.

CHAPTER 6
AN INTRODUCTION TO METABOLISM

OUTLINE

I. Metabolism, Energy and Life
   A. The chemistry of life is organized into metabolic pathways
   B. Organisms transform energy
   C. The energy transformations of life are subject to two laws of thermodynamics
   D. Organisms live at the expense of free energy
   E. ATP powers cellular work by coupling exergonic to endergonic reactions

II. Enzymes
   A. Enzymes speed up metabolic reactions by lowering energy barriers
   B. Enzymes are substrate-specific
   C. The active site is an enzyme's catalytic center
   D. A cell's physical and chemical environment affects enzyme activity

III. The Control of Metabolism
   A. Metabolic control often demends on allosteric regulation
   B. The location of enzymes within a cell helps order metabolism

OBJECTIVES

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

1. Explain the role of catabolic and anabolic pathways in the energy exchanges of cellular metabolism.
2. Distinguish between kinetic and potential energy.
3. Distinguish between open and closed systems.
4. Explain, in their own words, the First and Second Laws of Thermodynamics.
5. Explain why highly ordered living organisms do not violate the Second Law of Thermodynamics.
6. Distinguish between entropy and enthalpy.
7. Write the Gibbs equation for free energy change.
8. Explain how changes in enthalpy, entropy and temperature influence the maximum amount of usable energy that can be harvested from a reaction.
9. Explain the usefulness of free energy.
10. List two major factors capable of driving spontaneous processes.
11. Distinguish between exergonic and endergonic reactions.
12. Describe the relationship between equilibrium and free energy change for a reaction.

13. Describe the function of ATP in the cell.
14. List the three components of ATP and identify the major class of macromolecules to which it belongs.
15. Explain how ATP performs cellular work.
16. Explain why chemical disequilibrium is essential for life.
17. Describe the energy profile of a chemical reaction including activation energy (E_A), free energy change (ΔG) and transition state.
18. Describe the function of enzymes in biological systems.
19. Explain the relationship between enzyme structure and enzyme specificity.
20. Explain the *induced fit* model of enzyme function and describe the catalytic cycle of an enzyme.
21. Describe several mechanisms by which enzymes lower activation energy.
22. Explain how substrate concentration affects the rate of an enzyme-controlled reaction.
23. Explain how enzyme activity can be regulated or controlled by environmental conditions, cofactors, enzyme inhibitors and allosteric regulators.
24. Distinguish between allosteric activation and cooperativity.
25. Explain how metabolic pathways are regulated.

KEY TERMS

metabolism	first law of thermodynamics	catalyst	noncompetitive inhibitors
catabolic pathways	second law of thermodynamics	activation energy	allosteric site
anabolic pathways	free energy	substrate	feedback inhibition
bioenergetics	exergonic reaction	active site	cooperativity
energy	endergonic reaction	induced fit	entropy
kinetic energy	energy coupling	cofactors	spontaneous reaction
potential energy	ATP	coenzymes	
thermodynamics	phosphorylated intermediate	competitive inhibitors	

LECTURE NOTES

I. Metabolism, Energy and Life

A. The chemistry of life is organized into metabolic pathways

Metabolism = Totality of an organism's chemical processes (see Campbell, Figure 6.1).

- Property emerging from specific molecular interactions within the cell.
- Concerned with managing cellular resources: material and energy.

Metabolic reactions are organized into pathways that are orderly series of enzymatically controlled reactions. *Metabolic pathways* are generally of two types:

Catabolic pathways = Metabolic pathways that *release energy* by breaking down complex molecules to simpler compounds (e.g., cellular respiration which degrades glucose to carbon dioxide and water; provides energy for cellular work).

Anabolic pathways = Metabolic pathways that *consume energy* to build complicated molecules from simpler ones (e.g., photosynthesis which synthesizes glucose from CO_2 and H_2O; any synthesis of a macromolecule from its monomers).

Metabolic reactions may be coupled, so that energy released from a catabolic reaction can be used to drive an anabolic one.

It may be useful at this point to illustrate energy exchanges in metabolic reactions.. When respiration is introduced in Chapters 9 and 10, you can use this concept again as a transition.

B. Organisms transform energy

Energy = Capacity to do work

Kinetic energy = Energy in the process of doing work (energy of motion). For example:

- Heat (thermal energy) is kinetic energy expressed in random movement of molecules.
- Light energy from the sun is kinetic energy which powers photosynthesis.

Potential energy = Energy that matter possesses because of its location or arrangement (energy of position). For example:

- In the earth's gravitational field, an object on a hill or water behind a dam have potential energy.
- Chemical energy is potential energy stored in molecules because of the arrangement of nuclei and electrons in its atoms.

Energy can be transformed from one form to another. For example:

- Kinetic energy of sunlight can be transformed into the potential energy of chemical bonds during photosynthesis.
- Potential energy in the chemical bonds of gasoline can be transformed into kinetic mechanical energy which pushes the pistons of an engine.

C. The energy transformations of life are subject to two laws of thermodynamics

Thermodynamics = Study of energy transformations

First Law of Thermodynamics = Energy can be transferred and transformed, but it cannot be created or destroyed (energy of the universe is constant).

Second Law of Thermodynamics = Every energy transfer or transformation makes the universe more disordered (every process increases the *entropy* of the universe).

Entropy = Quantitative measure of disorder that is proportional to randomness (designated by the letter S).

Closed system = Collection of matter under study which is isolated from its surroundings.

Open system = System in which energy can be transferred between the system and its surroundings.

It is important to distinguish between open and closed systems and to spend lecture time on the second law of thermodynamics. Students often ask: "How is the evolution of complex life forms possible if it violates the second law of thermodynamics?" Thoughtful preparation of an answer beforehand will be well worth the effort.

The entropy of a system may decrease, but the entropy of the system *plus its surroundings* must always increase. Highly ordered living organisms do not violate the second law because they are open systems. For example, animals:

- Maintain highly ordered structure at the expense of increased entropy of their surroundings.
- Take in complex high energy molecules as food and extract chemical energy to create and maintain order.
- Return to the surroundings simpler low energy molecules (CO_2 and water) and heat.

Energy can be transformed, but part of it is dissipated as heat which is largely unavailable to do work. Heat energy *can* perform work if there is a heat gradient resulting in heat flow from warmer to cooler.

Combining the first and second laws; the *quantity* of energy in the universe is constant, but its *quality* is not.

D. Organisms live at the expense of free energy

1. Free energy: a criterion for spontaneous change

Not all of a system's energy is available to do work. The amount of energy that is available to do work is described by the concept of *free energy*. Free energy (G) is related to the system's total energy (H) and its entropy (S) in the following way:

$G = H - TS$

where:

G = Gibbs free energy (energy available to do work)

H = enthalpy or total energy

T = temperature in °K

S = entropy

Free energy (G) = Portion of a system's energy available to do work; is the difference between the total energy (*enthalpy*) and the energy *not* available for doing work (TS).

The maximum amount of usable energy that can be harvested from a particular reaction is the system's free energy change from the initial to the final state. This change in free energy (ΔG) is given by the Gibbs-Helmholtz equation at constant temperature and pressure:

$\Delta G = \Delta H - T\Delta S$

where:

ΔG = change in free energy

ΔH = change in total energy (enthalpy)

ΔS = change in entropy

T = absolute temperature in °K (which is °C + 273)

To put these thermodynamic concepts in the context of chemical reactions, you also may briefly discuss the other component of the Gibbs-Helmholtz equation – ΔH or change in enthalpy measured as the *heat of reaction.* Students should understand that during a chemical reaction, reactant molecules must absorb energy for their bonds to break, and that energy is released when bonds form between the rearranged atoms of the products. Consequently, the net energy consumed or released when reactants are converted to products is the *net difference* between the energy consumed to break chemical bonds of reactants and the energy released from the formation of the products.

Significance of free energy:

a. Indicates the maximum amount of a system's energy which is available to do work.

b. Indicates whether a reaction will occur spontaneously or not.

- A *spontaneous reaction* is one that will occur without additional energy.
- In a spontaneous process, ΔG or free energy of a system *decreases* ($\Delta G<0$).

- A decrease in enthalpy ($-\Delta H$) and an increase in entropy ($+\Delta S$) reduce the free energy of a system and contribute to the spontaneity of a process.
- A higher temperature enhances the effect of an entropy change. Greater kinetic energy of molecules tends to disrupt order as the chances for random collisions increase.
- When enthalpy and entropy changes in a system have an opposite effect on free energy, temperature may determine whether the reaction will be spontaneous or not (e.g., protein denaturation by increased temperature).
- High energy systems, including high energy chemical systems, are unstable and tend to change to a more stable state with a lower free energy.

2. Free energy and equilibrium

There is a relationship between chemical equilibrium and the free energy change (ΔG) of a reaction:

- As a reaction approaches equilibrium, the free energy of the system decreases (spontaneous and exergonic reaction).
- When a reaction is pushed away from equilibrium, the free energy of system increases (non-spontaneous and endergonic reaction).
- When a reaction reaches equilibrium, $\Delta G = 0$, because there is no net change in the system.

3. Free energy and metabolism

a. Reactions can be classified based upon their free energy changes:

Exergonic reaction = A reaction that proceeds with a net loss of free energy.

Endergonic reaction = An energy-requiring reaction that proceeds with a net gain of free energy; a reaction that absorbs free energy from its surroundings.

Exergonic Reaction	Endergonic Reaction
Chemical products have *less* free energy than the reactant molecules.	Products store *more* free energy than reactants.
Reaction is energetically downhill.	Reaction is energetically uphill.
Spontaneous reaction.	Non-spontaneous reaction (requires energy input).
ΔG is negative.	ΔG is positive.
$-\Delta G$ is the maximum amount of work the reaction can perform.	$+\Delta G$ is the minimum amount of work required to drive the reaction.

If a chemical process is exergonic, the reverse process must be endergonic. For example:

- For each mole of glucose oxidized in the exergonic process of cellular respiration, 2870 kJ are released ($\Delta G = -2870$ kJ/mol or -686 kcal/mol).
- To produce a mole of glucose, the endergonic process of photosynthesis requires an energy input of 2870 kJ ($\Delta G = +2870$ kJ/mol or +686 kcal/mol).

From this point on, the text uses joules and kilojoules as energy units and puts the caloric equivalent in parentheses. The *joule* (J) is the metric unit of energy; some handy conversions follow:

joule (*J*)	=	0.239 cal
Kilojoule (*kJ*)	=	1000 J or 0.239 kcal
calorie (cal)	=	4.184 J

In cellular metabolism, endergonic reactions are driven by coupling them to reactions with a greater negative free energy (exergonic). ATP plays a critical role in this energy coupling.

b. Metabolic disequilibrium

Since many metabolic reactions are reversible, they have the potential to reach equilibrium.

- At equilibrium, $\Delta G = 0$, so the system can do no work.
- Metabolic disequilibrium is a necessity of life; a cell at equilibrium is dead.
- In the cell, these potentially reversible reactions are pulled forward away from equilibrium, because the products of some reactions become reactants for the next reaction in the metabolic pathway.
- For example, during cellular respiration a steady supply of high energy reactants such as glucose and removal of low energy products such as CO_2 and H_2O, maintain the disequilibrium necessary for respiration to proceed.

E. ATP powers cellular work by coupling exergonic to endergonic reactions

ATP is the immediate source of energy that drives most cellular work, which includes:

- *Mechanical work* such as beating of cilia, muscle contraction, cytoplasmic flow, and chromosome movement during mitosis and meiosis.
- *Transport work* such as pumping substances across membranes.
- *Chemical work* such as the endergonic process of polymerization.

1. The structure and hydrolysis of ATP

ATP (adenosine triphosphate) = Nucleotide with unstable phosphate bonds that the cell hydrolyzes for energy to drive endergonic reactions. ATP consists of:

- Adenine, a nitrogenous base.
- Ribose, a five-carbon sugar.
- Chain of three phosphate groups.

Unstable bonds between the phosphate groups can be hydrolyzed in an exergonic reaction that releases energy.

- When the terminal phosphate bond is hydrolyzed, a phosphate group is removed producing ADP (adenosine diphosphate).

$$ATP + H_2O \longrightarrow ADP + \textcircled{P}$$

- Under standard conditions in the laboratory, this reaction releases −31 kJ/mol (−7.3 kcal/mol).
- In a living cell, this reaction releases −55 kJ/mol (–13 kcal/mol)—about 77% more than under standard conditions.

The terminal phosphate bonds of ATP are unstable, so:

- The products of the hydrolysis reaction are more stable than the reactant.
- Hydrolysis of the phosphate bonds is thus exergonic as the system shifts to a more stable state.

2 How ATP performs work

Exergonic hydrolysis of ATP is coupled with endergonic processes by transferring a phosphate group to another molecule.

- Phosphate transfer is enzymatically controlled.
- The molecule acquiring the phosphate (*phosphorylated* or *activated intermediate*) becomes more reactive.

For example, conversion of glutamic acid to glutamine (see Campbell, Figure 6.7):

$$\underset{\text{glutamic acid}}{Glu} + \underset{\text{ammonia}}{NH_3} \longrightarrow \underset{\text{glutamine}}{Gln} \quad \Delta G = +14.2 \text{ kJ/mol } (+3.4 \text{ kcal/mol}) \text{ (endergonic)}$$

Two step process of energy coupling with ATP hydrolysis:

1. Hydrolysis of ATP and phosphorylation of glutamic acid.

$$Glu + ATP \longrightarrow \underset{\substack{\text{unstable}\\ \text{phosphorylated}\\ \text{intermediate}}}{Glu\text{–}\textcircled{P}} + ADP$$

2. Replacement of the phosphate with the reactant ammonia.

$$Glu\text{–}\textcircled{P} + NH_3 \longrightarrow Gln + \textcircled{P}$$

Overall ΔG:

$$\begin{aligned} Glu + NH_3 &\longrightarrow Gln & \Delta G &= +14.2 \text{ kJ/mol}\\ ATP &\longrightarrow ADP + \textcircled{P} & \Delta G &= -31.0 \text{ kJ/mol}\\ & & \text{Net } \Delta G &= -16.8 \text{ kJ/mol} \end{aligned}$$

(Overall process is exergonic)

3. The regeneration of ATP

ATP is continually regenerated by the cell.

- Process is rapid (10^7 molecules used and regenerated/sec/cell).
- Reaction is endergonic.

$$ADP + \textcircled{P} \longrightarrow ATP \quad \Delta G = +31 \text{ kJ/mol } (+7.3 \text{ kcal/mol})$$

· Energy to drive the endergonic regeneration of ATP comes from the exergonic process of cellular respiration.

II. Enzymes

A. Enzymes speed up metabolic reactions by lowering energy barriers

Free energy change indicates whether a reaction will occur spontaneously, but does not give information about the speed of reaction.

- A chemical reaction will occur spontaneously if it releases free energy ($-\Delta G$), but it may occur too slowly to be effective in living cells.
- Biochemical reactions require *enzymes* to speed up and control reaction rates.

Catalyst = Chemical agent that accelerates a reaction without being permanently changed in the process, so it can be used over and over.

Enzymes = Biological catalysts made of protein.

Before a reaction can occur, the reactants must absorb energy to break chemical bonds. This initial energy investment is the *activation energy*.

Free energy of activation (*activation energy*) = Amount of energy that reactant molecules must absorb to start a reaction (E_A).

Transition state = Unstable condition of reactant molecules that have absorbed sufficient free energy to react.

Energy profile of an exergonic reaction:

1. Reactants must absorb enough energy (E_A) to reach the transition state (uphill portion of the curve). Usually the absorption of thermal energy from the surroundings is enough to break chemical bonds.
2. Reaction occurs and energy is released as new bonds form (downhill portion of the curve).
3. ΔG for the overall reaction is the difference in free energy between products and reactants. In an exergonic reaction the free energy of the products is less than reactants.

Even though a reaction is energetically favorable, there must be an initial investment of activation energy (E_A).

The breakdown of biological macromolecules is exergonic. However, these molecules react *very slowly* at cellular temperatures because they cannot absorb enough thermal energy to reach transition state.

In order to make these molecules reactive when necessary, cells use biological catalysts called *enzymes*, which:

- Are proteins.
- Lower E_A, so the transition state can be reached at cellular temperatures.
- Do *not change* the nature of a reaction (ΔG), but only speed up a reaction that would have occurred anyway.
- Are very selective for which reaction they will catalyze.

B. Enzymes are substrate-specific

Enzymes are specific for a particular *substrate*, and that specificity depends upon the enzyme's three-dimensional shape.

Substrate = The substance an enzyme acts on and makes more reactive.

- An enzyme binds to its substrate and catalyzes its conversion to product. The enzyme is released in original form.

  Substrate + enzyme ⟶ enzyme-substrate complex ⟶ product + enzyme

- The substrate binds to the enzyme's *active site*.

Active site = Restricted region of an enzyme molecule which binds to the substrate.

- Is usually a pocket or groove on the protein's surface.
- Formed with only a few of the enzyme's amino acids.
- Determines enzyme specificity which is based upon a compatible fit between the shape of an enzyme's active site and the shape of the substrate.
- Changes its shape in response to the substrate.
  - As substrate binds to the active site, it *induces* the enzyme to change its shape.
  - This brings its chemical groups into positions that enhance their ability to interact with the substrate and catalyze the reaction.

Induced fit = Change in the shape of an enzyme's active site, which is induced by the substrate (see Campbell, Figure 6.11).

C. The active site is an enzyme's catalytic center

The entire enzymatic cycle is quite rapid (see Campbell, Figure 6.12).

Steps in the catalytic cycle of enzymes:

1. Substrate binds to the active site forming an *enzyme-substrate complex*. Substrate is held in the active site by weak interactions (e.g., hydrogen bonds and ionic bonds).
2. *Induced fit* of the active site around the substrate. Side chains of a few amino acids in the active site catalyze the conversion of substrate to product.
3. Product departs active site and the enzyme emerges in its original form. Since enzymes are used over and over, they can be effective in very small amounts.

Enzymes lower activation energy and speed up reactions by several mechanisms:

- Active site can hold two or more reactants in the proper position so they may react.
- Induced fit of the enzyme's active site may distort the substrate's chemical bonds, so less thermal energy (lower ΔG) is needed to break them during the reaction.
- Active site might provide a micro-environment conducive to a particular type of reaction (e.g., localized regions of low pH caused by acidic side chains on amino acids at the active site).
- Side chains of amino acids in the active site may participate directly in the reaction.

The initial substrate concentration partly determines the rate of an enzyme controlled reaction.

- The higher the substrate concentration, the faster the reaction - up to a limit.
- If substrate concentration is high enough, the enzyme becomes *saturated* with substrate. (The active sites of all enzymes molecules are engaged.)
- When an enzyme is saturated, the reaction rate depends upon how fast the active sites can convert substrate to product.
- When enzyme is saturated, reaction rate may be increased by adding more enzyme.

D. A cell's physical and chemical environment affects enzyme activity

Each enzyme has optimal environmental conditions that favor the most active enzyme conformation.

1. Effects of temperature and pH

Optimal temperature allows the greatest number of molecular collisions without denaturing the enzyme.

- Enzyme reaction rate increases with increasing temperature. Kinetic energy of reactant molecules increases with rising temperature, which increases substrate collisions with active sites.
- Beyond the optimal temperature, reaction rate slows. The enzyme denatures when increased thermal agitation of molecules disrupts weak bonds that stabilize the active conformation.
- Optimal temperature range of most human enzymes is 35°– 40°C.

Optimal pH range for most enzymes is pH 6 – 8.

- Some enzymes operate best at more extremes of pH.
- For example, the digestive enzyme, pepsin, found in the acid environment of the stomach has an optimal pH of 2.

2. Cofactors

Cofactors = Small nonprotein molecules that are required for proper enzyme catalysis.

- May bind tightly to active site.
- May bind loosely to both active site and substrate.
- Some are inorganic (e.g., metal atoms of zinc, iron or copper).
- Some are organic and are called *coenzymes* (e.g., most vitamins).

3. Enzyme inhibitors

Certain chemicals can selectively inhibit enzyme activity (see Campbell, Figure 6.14).

- Inhibition may be *irreversible* if the inhibitor attaches by covalent bonds.
- Inhibition may be *reversible* if the inhibitor attaches by weak bonds.

Competitive inhibitors = Chemicals that resemble an enzyme's normal substrate and compete with it for the active site.

- Block active site from the substrate.
- If reversible, the effect of these inhibitors can be overcome by increased substrate concentration.

Noncompetitive inhibitors = Enzyme inhibitors that do not enter the enzyme's active site, but bind to another part of the enzyme molecule.

- Causes enzyme to change its shape so the active site cannot bind substrate.
- May act as metabolic poisons (e.g., DDT, many antibiotics).
- Selective enzyme inhibition is an essential mechanism in the cell for regulating metabolic reactions.

III. The Control of Metabolism

A. Metabolic pathways are regulated by controlling enzyme activity.

Metabolic control often depends on allosteric regulation

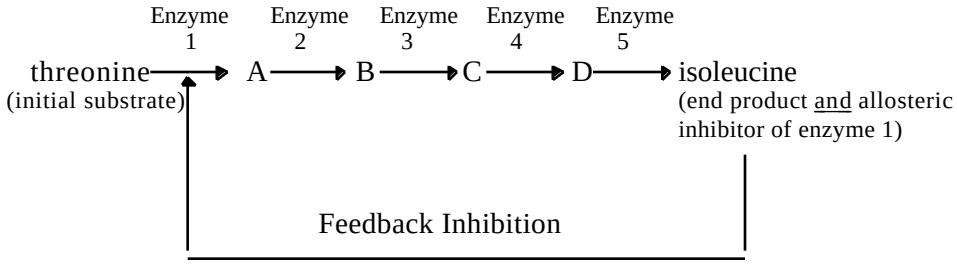
1. Allosteric regulation

Allosteric site = Specific receptor site on some part of the enzyme molecule other than the active site.

- Most enzymes with allosteric sites have two or more polypeptide chains, each with its own active site. Allosteric sites are often located where the subunits join.
- Allosteric enzymes have two conformations, one catalytically active and the other inactive (see Campbell, Figure 6.15) .
- Binding of an *activator* to an allosteric site stabilizes the active conformation.
- Binding of an *inhibitor* (noncompetitive inhibitor) to an allosteric site stabilizes the inactive conformation.
- Enzyme activity changes continually in response to changes in the relative proportions of activators and inhibitors (e.g., ATP/ADP).
- Subunits may interact so that a single activator or inhibitor at one allosteric site will affect the active sites of the other subunits.

2. Feedback inhibition

Feedback inhibition = Regulation of a metabolic pathway by its end product, which inhibits an enzyme within the pathway. For example:



Prevents the cell from wasting chemical resources by synthesizing more product than is necessary (see also Campbell, Figure 6.16).

3. Cooperativity

Substrate molecules themselves may enhance enzyme activity.

Cooperativity = The phenomenon where substrate binding to the active site of one subunit induces a conformational change that enhances substrate binding at the active sites of the other subunits (see Campbell, Figure 6.17).

B. The localization of enzymes within the cell helps order metabolism

Cellular structure orders and compartmentalizes metabolic pathways (see Campbell, Figure 6.18).

- Some enzymes and enzyme complexes have fixed locations in the cell because they are incorporated into a membrane.
- Other enzymes and their substrates may be localized within membrane-enclosed eukaryotic organelles (e.g., chloroplasts and mitochondria).

REFERENCES

Atkins, P.W. *The Second Law*. New York, Oxford: W.H. Freeman and Company, 1984. A beautifully written, understandable description of the Second Law of Thermodynamics; addresses the role of the Second Law in life processes.

Campbell, N., et al. *Biology*. 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Lehninger, A.L., D.L. Nelson and M.M. Cox. *Principles of Biochemistry*. 2nd ed. New York: Worth, 1993.

CHAPTER 7
A TOUR OF THE CELL

OUTLINE

I. How We Study Cells
   A. Microscopes provide windows to the world of the cell
   B. Cell biologists can isolate organelles to study their functions
II. A Panoramic View of the Cell
   A. Prokaryotic and eukaryotic cells differ in size and complexity
   B. Internal membranes compartmentalize the functions of a eukaryotic cell
III. The Nucleus and Ribosomes
   A. The nucleus contains a eukaryotic cell's genetic library
   B. Ribosomes build a cell's proteins
IV. The Endomembrane System
   A. The endoplasmic reticulum manufactures membranes and performs many other biosynthetic functions
   B. The Golgi apparatus finishes, sorts, and ships cell products
   C. Lysosomes are digestive compartments
   D. Vacuoles have diverse functions in cell maintenance
V. Other Membranous Organelles
   A. Peroxisomes consume oxygen in various metabolic functions
   B. Mitochondria and chloroplasts are the main energy transformers of cells
VI. The Cytoskeleton
   A. Provides structural support to cells for cell motility and regulation
VII. Cell Surfaces and Junctions
   A. Plant cells are encased by cell walls
   B. The extracellular matrix (ECM) of animal cells functions in support, adhesion, movement and development
   C. Intercellular junctions help integrate cells into higher levels of structure and function

OBJECTIVES

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

1. Describe techniques used to study cell structure and function.
2. Distinguish between magnification and resolving power.
3. Describe the principles, advantages and limitations of the light microscope, transmission electron microscope and the scanning electron microscope.
4. Describe the major steps of cell fractionation and explain why it is a useful technique.

5. Distinguish between prokaryotic and eukaryotic cells.
6. Explain why there are both upper and lower limits to cell size.
7. Explain why compartmentalization is important in eukaryotic cells.
8. Describe the structure and function of the nucleus, and briefly explain how the nucleus controls protein synthesis in the cytoplasm.
9. Describe the structure and function of a eukaryotic ribosome.
10. List the components of the *endomembrane system*, describe their structures and functions and summarize the relationships among them.
11. Explain how impaired lysosomal function causes the symptoms of storage diseases.
12. Describe the types of vacuoles and explain how their functions differ.
13. Explain the role of *peroxisomes* in eukaryotic cells.
14. Describe the structure of a *mitochondrion* and explain the importance of compartmentalization in mitochondrial function.
15. Distinguish among *amyloplast, chromoplast* and *chloroplast.*
16. Identify the three functional compartments of a chloroplast, and explain the importance of compartmentalization in chloroplast function.
17. Describe probable functions of the cytoskeleton.
18. Describe the structure, monomers and functions of microtubules, microfilaments and intermediate filaments.
19. Explain how the ultrastructure of cilia and flagella relates to their function.
20. Describe the development of plant cell walls.
21. Describe the structure and list some functions of the extracellular matrix in animal cells.
22. Describe the structure of intercellular junctions found in plant and animal cells, and relate their structure to function.

KEY TERMS

light microscope
resolving power
organelle
electron microscope
TEM
SEM
cell fractionation
ultracentrifuges
cytoplasm
prokaryotic cell
nucleoid
eukaryotic cell
cytoplasm
cytosol
plasma membrane
nucleus
nuclear lamina
chromatin
chromosome
nucleolus
ribosome
endomembrane system
endoplasmic reticulum (ER)
smooth ER
rough ER
glycoprotein
transport vesicles
Golgi apparatus
phagocytosis
food vacuole
contractile vacuole
central vacuole
peroxisome
mitochondria
chloroplast
cristae
mitochondrial matrix
plastid
thylakoid
granlakoids
stroma
cytoskeleton
microtubules
microfilaments
integrin
centrosome
centriole
flagella
cilia
basal body
dynein
actin
myosin
pseudopodia
cytoplasmic streaming
cell wall
primary cell wall
middle lamella
secondary cell wall
extracellular matrix
collagen
proteoglycan
fibronectin
intermediate filaments
plasmodesmata
tight junctions
desmosomes
gap junctions

LECTURE NOTES

All organisms are made of cells, the organism's basic unit of structure and function.

The cell as a microcosm can be used to illustrate four themes integral to the text and course:

1. Theme of emergent properties. Life at the cellular level arises from interactions among cellular components.
2. Correlation of structure and function. Ordered cellular processes (e.g., protein synthesis, respiration, photosynthesis, cell-cell recognition, cellular movement, membrane production and secretion) are based upon ordered structures.
3. Interaction of organisms within their environment. Cells are excitable responding to environmental stimuli. In addition, cells are open systems that exchange materials and energy with their environment.
4. Unifying theme of evolution. Evolutionary adaptations are the basis for the correlation between structure and function.

> Students often find this material boring. A good set of micrographs and line drawings in the form of slides or transparencies will help. If the class size is small enough, a tour of an electron microscopy facility will help stimulate interest.

I. How We Study Cells

A. Microscopes provide windows to the world of the cell

The microscope's invention and improvement in the seventeenth century led to the discovery and study of cells.

In 1665, Robert Hooke described cells using a *light microscope*. Modern light microscopy is based upon the same principles as microscopy first used by Renaissance scientists.

- Visible light is focused on a specimen with a *condenser lens*.
- Light passing through the specimen is refracted with an *objective lens* and an *ocular lens*. The specimen's image is thus magnified and inverted for the observer.

Two important concepts in microscopy are *magnification* and *resolving power*.

- *Magnification* = How much larger an object is made to appear compared to its real size.
- *Resolving power* = Minimum distance between two points that can still be distinguished as two separate points.
- Resolution of a light microscope is limited by the wavelength of visible light. Maximum possible resolution of a light microscope is 0.2 μm.
- Highest magnification in a light microscope with maximum resolution is about 1000 times.
- By the early 1900s, optics in light microscopes were good enough to achieve the best resolution, so improvements since then have focused on improving contrast.

In the 1950s, researchers began to use the *electron microscope* which far surpassed the resolving power of the light microscope.

- Resolving power is inversely related to wavelength. Instead of light, electron microscopes use electron beams which have much shorter wavelengths than visible light.
- Modern electron microscopes have a practical resolving power of about 2 nm.
- Enhanced resolution and magnification allowed researchers to clearly identify subcellular *organelles* and to study cell *ultrastructure*.

- Two types of electron microscopes are the *transmission electron microscope* (TEM) and the *scanning electron microscope*.

The *transmission electron microscope* (TEM) aims an electron beam at a thin section of specimen which may be stained with metals to absorb electrons and enhance contrast.

- Electrons *transmitted* through the specimen are focused and the image magnified by using electromagnetic lenses (rather than glass lenses) to bend the trajectories of the charged electrons.
- Image is focused onto a viewing screen or film.
- Used to study internal cellular ultrastructure.

The *scanning electron microscope* (SEM) is useful for studying the surface of a specimen.

- Electron beam *scans* the surface of the specimen usually coated with a thin film of gold.
- Scanning beam excites secondary electrons on the sample's surface.
- Secondary electrons are collected and focused onto a viewing screen.
- SEM has a great depth of field and produces a three-dimensional image.

Disadvantages of an electron microscope:

- Can usually only view dead cells because of the elaborate preparation required.
- May introduce structural artifacts.

In laboratory, it would be useful to give students electron micrographs of organelles to identify and label. Many are disappointed when they view wet mounts of cells or prepared slides with their light microscopes and cannot find the detail seen in the micrographs. Clearly, some students have no conception of the resolution and magnifying power of an electron microscope. It would be helpful to indicate a size scale on micrographs you might use in lecture.

B. Cell biologists can isolate organelles to study their function

Modern cell biology integrates the study of cell structure (*cytology*) with the study of cell function. Cell fractionation is a technique that enables researchers to isolate organelles without destroying their function (see Campbell, Figure 7.3).

Cell fractionation = Technique which involves centrifuging disrupted cells at various speeds and durations to isolate components of different sizes, densities, and shapes.

- Development of the *ultracentrifuge* made this technique possible.
- Ultracentrifuges can spin as fast as 80,000 rpm, applying a force of 500,000 g.

The process of cell fractionation involves the following:

- Homogenization of tissue and its cells using pistons, blenders, or ultrasound devices.
- Centrifugation of the resulting homogenate at a slow speed. Nuclei and other larger particles settle at the bottom of the tube, forming a *pellet*.
- The unpelleted fluid or *supernatant* is decanted into another tube and centrifuged at a faster speed, separating out smaller organelles.
- The previous step is repeated, increasing the centrifugation speed each time to collect smaller and smaller cellular components from successive pellets.
- Once the cellular components are separated and identified, their particular metabolic functions can be determined.

II. A Panoramic View of the Cell

A. Prokaryotic and eukaryotic cells differ in size and complexity

Living organisms are made of either prokaryotic or eukaryotic cells—two major kinds of cells, which can be distinguished by structural organization.

Prokaryotic (pro = before; karyon = kernel)	Eukaryotic (Eu = true; karyon = kernel)
Found only in bacteria and archaebacteria	Found in the Kingdoms Protista, Fungi, Plantae, and Animalia
No true nucleus; lacks nuclear envelope	True nucleus; bounded by nuclear envelope
Genetic material in *nucleoid* region	Genetic material within nucleus
No membrane-bound organelles (see Campbell, Figure 7.4)	Contains cytoplasm with *cytosol* and membrane-bound *organelles*

Cytoplasm = Entire region between the nucleus and cell membrane

Cytosol = Semi-fluid medium found in the cytoplasm

1. Cell size

Size ranges of cells:

Cell Type	Diameter
Mycoplasmas	0.1 - 1.0 μm
Most bacteria	1.0 - 10.0 μm
Most eukaryotic cells	10.0 - 100.0 μm

Range of cell size is limited by metabolic requirements. The lower limits are probably determined by the smallest size with enough:

- DNA to program metabolism.
- ribosomes, enzymes and cellular components to sustain life and reproduce.

The upper limits of size are imposed by the surface area to volume ratio. As a cell increases in size, its volume grows proportionately more than its surface area (see Campbell, Figure 7.5).

- The surface area of the plasma membrane must be large enough for the cell volume, in order to provide an adequate exchange surface for oxygen, nutrients and wastes.

B. Internal membranes compartmentalize the functions of a eukaryotic cell

The average eukaryotic cell has a thousand times the volume of the average prokaryotic cell, but only a hundred times the surface area. Eukaryotic cells compensate for the small surface area to volume ratio by having internal membranes which:

- Partition the cell into compartments.
- Have unique lipid and protein compositions depending upon their specific functions.
- May participate in metabolic reactions since many enzymes are incorporated directly into the membrane.
- Provide localized environmental conditions necessary for specific metabolic processes.

- Sequester reactions, so they may occur without interference from incompatible metabolic processes elsewhere in the cell (see Campbell, Figure 7.6).

III. The Nucleus and Ribosomes

A. The nucleus contains a eukaryotic cell's genetic library

Nucleus = A generally conspicuous membrane-bound cellular organelle in a eukaryotic cell; contains most of the genes that control the entire cell (see Campbell, Figure 7.9).

- Averages about 5 μm diameter.
- Enclosed by a *nuclear envelope.*

Nuclear envelope = A double membrane which encloses the nucleus in a eukaryotic cell.

- Is two lipid bilayer membranes separated by a space of about 20 to 40 nm. Each lipid bilayer has its own specific proteins.
- Attached to proteins on the envelope's nuclear side is a network of protein filaments, the *nuclear lamina*, which stabilizes nuclear shape.
- Is perforated by pores (100 nm diameter), which are ordered by an octagonal array of protein granules.
    - The envelope's inner and outer membranes are fused at the lip of each pore.
    - Pore complex regulates molecular traffic into and out of the nucleus.
- There is new evidence of an intranuclear framework of fibers, the *nuclear matrix*.

The nucleus contains most of the cell's DNA which is organized with proteins into a complex called *chromatin.*

Chromatin = Complex of DNA and histone proteins, which makes up *chromosomes* in eukaryotic cells; appears as a mass of stained material in nondividing cells.

Chromosomes = Long threadlike association of genes, composed of *chromatin* and found in the nucleus of eukaryotic cells.

- Each species has a characteristic chromosome number.
- Human cells have 46 chromosomes, except egg and sperm cells, which have half or 23.

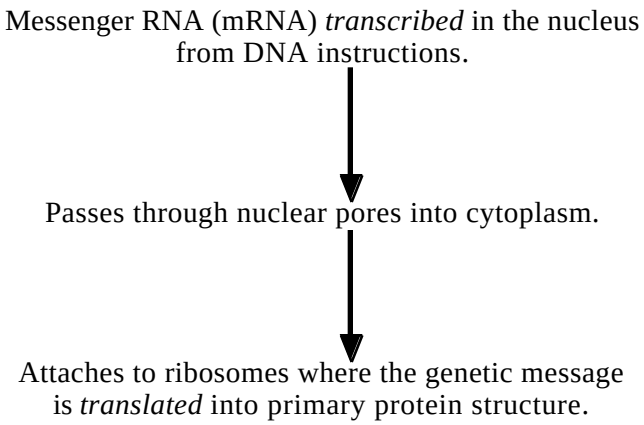
The most visible structure within the nondividing nucleus is the *nucleolus.*

Nucleolus = Roughly spherical region in the nucleus of nondividing cells, which consists of *nucleolar organizers* and ribosomes in various stages of production.

- May be two or more per cell.
- Packages ribosomal subunits from:
    - rRNA transcribed in the nucleolus.
    - RNA produced elsewhere in the nucleus.
    - Ribosomal proteins produced and imported from the cytoplasm.
- Ribosomal subunits pass through nuclear pores to the cytoplasm, where their assembly is completed.

Nucleolar organizers = Specialized regions of some chromosomes, with multiple copies of genes for rRNA (ribosomal RNA) synthesis.

The nucleus controls protein synthesis in the cytoplasm:



B. Ribosomes build a cell's proteins

Ribosome = A cytoplasmic organelle that is the site for protein synthesis (see Campbell, Figure 7.10).

- Are complexes of RNA and protein
- Constructed in the nucleolus in eukaryotic cells
- Cells with high rates of protein synthesis have prominent nucleoli and many ribosomes (e.g., human liver cell has a few million).

> Since most organelles are membrane-bound, students frequently ask if the ribosome has a membrane. They can deductively answer the question themselves if they are reminded that prokaryotes have ribosomes as well.

Ribosomes function either free in the cytosol or bound to endoplasmic reticulum. Bound and free ribosomes are structurally identical and interchangeable.

Free ribosomes = Ribosomes suspended in the cytosol.

- Most proteins made by free ribosomes will function in the cytosol.

Bound ribosomes = Ribosomes attached to the outside of the endoplasmic reticulum.

- Generally make proteins that are destined for membrane inclusion or export.
- Cells specializing in protein secretion often have many bound ribosomes (e.g., pancreatic cells).

IV. The Endomembrane System

Biologists consider many membranes of the eukaryotic cell to be part of an *endomembrane system.*

- Membranes may be interrelated *directly* through physical contact.
- Membranes may be related *indirectly* through *vesicles.*

Vesicles = Membrane-enclosed sacs that are pinched off portions of membranes moving from the site of one membrane to another.

Membranes of the endomembrane system vary in structure and function, and the membranes themselves are dynamic structures changing in composition, thickness and behavior.

The endomembrane system includes:

- Nuclear envelope
- Endoplasmic reticulum
- Golgi apparatus
- Lysosomes
- Vacuoles

- Plasma membrane (not actually an *endomembrane*, but related to endomembrane system)

A. The endoplasmic reticulum manufactures membranes and performs many other biosynthetic functions

Endoplasmic reticulum (ER) = (Endoplasmic = within the cytoplasm; reticulum = network); extensive membranous network of tubules and sacs (*cisternae*) which sequesters its internal lumen (*cisternal space*) from the cytosol.

- Most extensive portion of endomembrane system.
- Continuous with the outer membrane of the nuclear envelope; therefore, the space between the membranes of the nuclear envelope is continuous with cisternal space.

There are two distinct regions of ER that differ in structure and function: smooth ER and rough ER (see Campbell, Figure 7.11).

1. Functions of smooth ER

Appears smooth in the electron microscope because its cytoplasmic surface lacks ribosomes. Smooth ER functions in diverse metabolic processes:

a. Participates in the synthesis of lipids, phospholipids and steroids

- For example, vertebrate, particularly mammalian sex hormones and steroids secreted by the adrenal gland.
- Cells that produce and secrete these products are rich in smooth ER (e.g., testes, ovaries, skin oil glands).

b. Participates in carbohydrate metabolism

- Smooth ER in liver contains an embedded enzyme that catalyzes the final step in the conversion of glycogen to glucose (removes the phosphate from glucose-phosphate).

c. Detoxifies drugs and poisons

- Smooth ER, especially in the liver, contains enzymes which detoxify drugs and poisons.
- Enzymes catalyze the addition of hydroxyl groups to drugs and poisons. This makes them soluble in the cytosol, so they may be excreted from the body.
- Smooth ER in liver cells proliferates in response to barbiturates, alcohol and other drugs. This, in turn, may increase drug tolerance.

d. Stores calcium ions necessary for muscle contraction

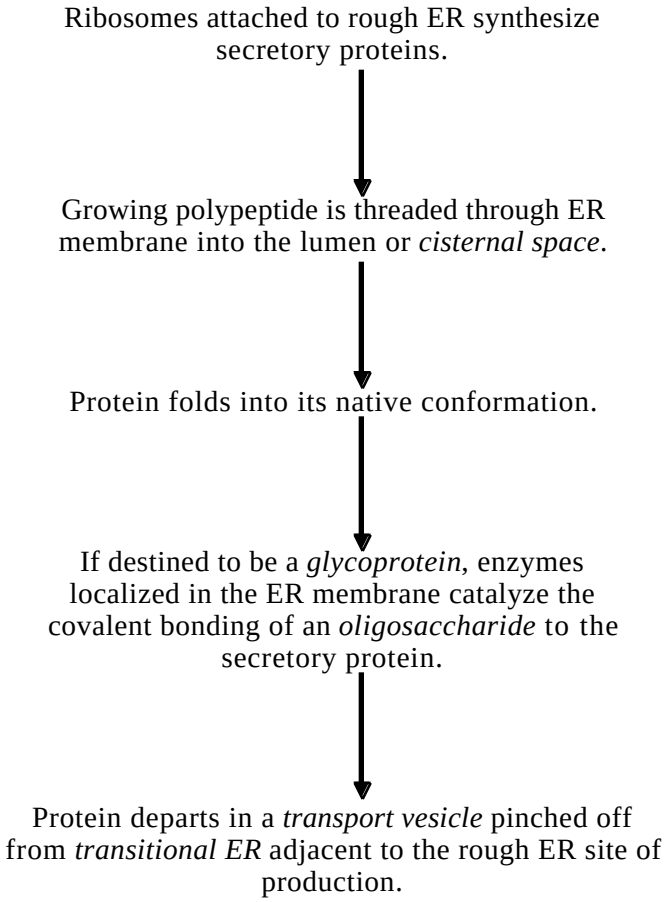
- In a muscle cell, the ER membrane pumps Ca^{++} from the cytosol into the cisternal space.
- In response to a nerve impulse, Ca^{++} leaks from the ER back into the cytosol, which triggers muscle cell contraction.

2. Rough ER and protein synthesis

Rough ER:

- Appears rough under an electron microscope because the cytoplasmic side is studded with ribosomes.
- Is continuous with outer membrane of the nuclear envelope (which may also be studded with ribosomes on the cytoplasmic side).
- Manufactures secretory proteins and membrane.

Proteins destined for secretion are synthesized by ribosomes attached to rough ER:



Glycoprotein = Protein covalently bonded to carbohydrate.

Oligosaccharide = Small polymer of sugar units.

Transport vesicle = Membrane vesicle in transit from one part of the cell to another.

It may be useful to point out the protein that will be packaged into vesicles (e.g., hydrolytic enzymes within lysosomes) to be inserted into membranes (e.g., membrane-bound enzymes, receptors) is also synthesized by ribosomes attached to the ER.

3. Rough ER and membrane production

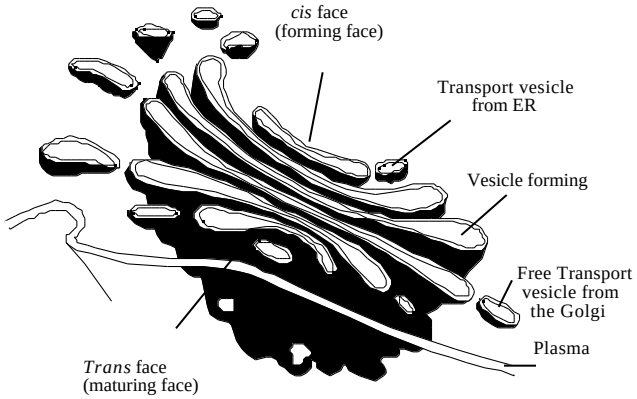
Membranes of rough ER grow *in place* as newly formed proteins and phospholipids are assembled:

- Membrane proteins are produced by ribosomes. As a polypeptide grows, it is inserted directly into the rough ER membrane where it is anchored by hydrophobic regions of the proteins.
- Enzymes within the ER membrane synthesize phospholipids from raw materials in the cytosol.
- Newly expanded ER membrane can be transported as a vesicle to other parts of the cell.

B. The Golgi apparatus finishes, sorts, and ships cell products

Many transport vesicles leave the ER and travel to the *Golgi apparatus.*

Golgi apparatus = Organelle made of stacked, flattened membranous sacs (*cisternae*), that modifies, stores and routes products of the endoplasmic reticulum (see Campbell, Figure 7.12).



- Membranes of the cisternae sequester cisternal space from the cytosol.
- Vesicles may transport macromolecules between the Golgi and other cellular structures.
- Has a distinct polarity. Membranes of cisternae at opposite ends differ in thickness and composition.
- Two poles are called the *cis face* (forming face) and the *trans face* (maturing face).
- *Cis face*, which is closely associated with transitional ER, receives products by accepting transport vesicles from the ER. A vesicle fuses its membrane to the cis face of the Golgi and empties its soluble contents into the Golgi's cisternal space.
- *Trans face* pinches off vesicles from the Golgi and transports molecules to other sites.

Enzymes in the Golgi modify products of the ER in stages as they move through the Golgi stack from the *cis* to the *trans* face:

- Each cisternae between the *cis* and *trans* face contains unique combinations of enzymes.
- Golgi products in transit from one cisternae to the next, are carried in transport vesicles.

During this process, the Golgi:

- Alters some membrane phospholipids.
- Modifies the oligosaccharide portion of glycoproteins.
- Manufactures certain macromolecules itself (e.g., hyaluronic acid).
- Targets products for various parts of the cell.
  - Phosphate groups or oligosaccharides may be added to Golgi products as molecular identification tags.
  - Membranous vesicles budded from the Golgi may have external molecules that recognize docking sites on the surface of certain other organelles.
- Sorts products for secretion. Products destined for secretion leave the *trans* face in vesicles which eventually fuse with the plasma membrane.

C. Lysosomes are digestive compartments

Lysosome = An organelle which is a membrane-enclosed bag of hydrolytic enzymes that digest all major classes of macromolecules (see Campbell, Figure 7.13).

- Enzymes include lipases, carbohydrases, proteases, and nucleases.
- Optimal pH for lysosomal enzymes is about pH 5.
- Lysosomal membrane performs two important functions:
  - Sequesters potentially destructive hydrolytic enzymes from the cytosol.
  - Maintains the optimal acidic environment for enzyme activity by pumping H^+s inward from the cytosol to the lumen.
- Hydrolytic enzymes and lysosomal membrane are synthesized in the rough ER and processed further in the Golgi apparatus.
- Lysosomes probably pinch off from the *trans* face of the Golgi apparatus (see Campbell, Figure 7.14).

1. Functions of lysosomes

a. Intracellular digestion

Phagocytosis = (Phago = to eat; cyte = cell); cellular process of ingestion, in which the plasma membrane engulfs particulate substances and pinches off to form a particle-containing *vacuole*.

- Lysosomes may fuse with food-filled vacuoles, and their hydrolytic enzymes digest the food.
- Examples are *Amoeba* and other protists which eat smaller organisms or food particles.
- Human cells called *macrophages* phagocytize bacteria and other invaders.

b. Recycle cell's own organic material

- Lysosomes may engulf other cellular organelles or part of the cytosol and digest them with hydrolytic enzymes (autophagy).
- Resulting monomers are released into the cytosol where they can be recycled into new macromolecules.

c. Programmed cell destruction

Destruction of cells by their own lysosomes is important during metamorphosis and development.

2. Lysosomes and human disease

Symptoms of inherited *storage diseases* result from impaired lysosomal function. Lack of a specific lysosomal enzyme causes substrate accumulation which interferes with lysosomal metabolism and other cellular functions.

- In Pompe's disease, the missing enzyme is a carbohydrase that breaks down glycogen. The resulting glycogen accumulation damages the liver.
- Lysosomal lipase is missing or inactive in Tay-Sachs disease, which causes lipid accumulation in the brain.

D. Vacuoles have diverse functions in cell maintenance

Vacuole = Organelle which is a membrane-enclosed sac that is larger than a vesicle (transport vesicle, lysosome, or microbody).

Vacuole types and functions:

Food vacuole = Vacuole formed by phagocytosis which is the site of intracellular digestion in some protists and macrophages (see Campbell, Figure 7.14).

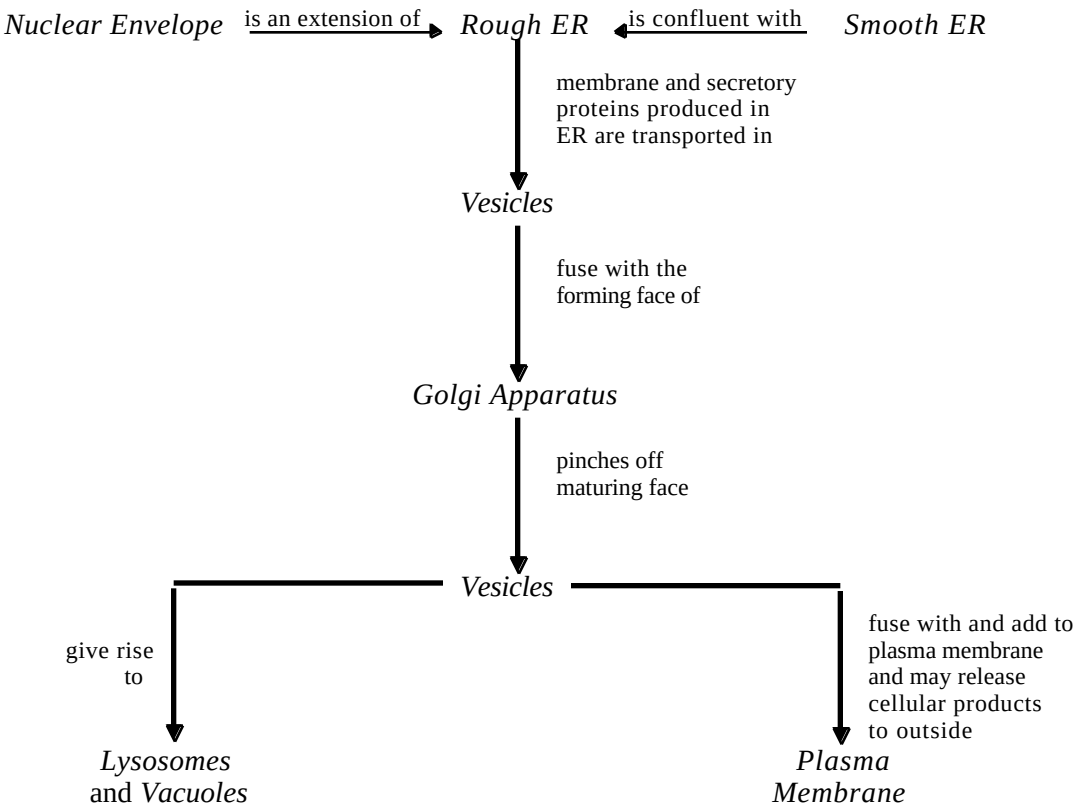
Contractile vacuole = Vacuole that pumps excess water from the cell; found in some freshwater protozoa.

Central vacuole = Large vacuole found in most mature plant cells (see Campbell, Figure 7.15)

- Is enclosed by a membrane called the *tonoplast* which is part of the endomembrane system
- Develops by the coalescence of smaller vacuoles derived from the ER and Golgi apparatus
- Is a versatile compartment with many functions:
  - Stores organic compounds (e.g., protein storage in seeds)
  - Stores inorganic ions (e.g., K^+ and Cl^-)
  - Sequesters dangerous metabolic by-products from the cytoplasm
  - Contains soluble pigments in some cells (e.g., red and blue pigments in flowers)
  - May protect the plant from predators by containing poisonous or unpalatable compounds
  - Plays a role in plant growth by absorbing water and elongating the cell
  - Contributes to the large ratio of membrane surface area to cytoplasmic volume. (There is only a thin layer of cytoplasm between the tonoplast and plasma membrane.)

E. A summary of relationships among endomembranes

Components of the endomembrane system are related through direct contact or through vesicles (see Campbell, Figure 7.16).



V. Other Membranous Organelles

A. Peroxisomes consume oxygen in various metabolic functions

Peroxisomes = Membrane-bound organelles that contain specialized teams of enzymes for specific metabolic pathways; all contain peroxide-producing oxidases.

- Bound by a single membrane
- Found in nearly all eukaryotic cells
- Often have a granular or crystalline core which is a dense collection of enzymes (see Campbell, Figure 7.17)
- Contain peroxide-producing oxidases that transfer hydrogen from various substrates to oxygen, producing hydrogen peroxide

$$RH_2 + O_2 \xrightarrow{\text{oxidase}} R + H_2O_2$$

- Contain catalase, an enzyme that converts toxic hydrogen peroxide to water

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

- Peroxisomal reactions have many functions, some of which are:
  - Breakdown of fatty acids into smaller molecules (acetyl CoA). The products are carried to the mitochondria as fuel for cellular respiration.
  - Detoxification of alcohol and other harmful compounds. In the liver, peroxisomes enzymatically transfer H from poisons to O_2.
- Specialized peroxisomes (*glyoxysomes*) are found in heterotrophic fat-storing tissue of germinating seeds.
  - Contain enzymes that convert lipid to carbohydrate.
  - These biochemical pathways make energy stored in seed oils available for the germinating seedling.
- Current thought is that peroxisome biogenesis occurs by pinching off from preexisting peroxisomes. Necessary lipids and enzymes are imported from the cytosol.

B. Mitochondria and chloroplasts are the main energy transformers of cells

Mitochondria and chloroplasts are organelles that transduce energy acquired from the surroundings into forms useable for cellular work.

- Enclosed by *double* membranes (see Campbell, Figure 7.18).
- Membranes are not part of endomembrane system. Rather than being made in the ER, their membrane proteins are synthesized by free ribosomes in the cytosol and by ribosomes located within these organelles themselves.
- Contain ribosomes and some DNA that programs a small portion of their own protein synthesis, though most of their proteins are synthesized in the cytosol programmed by nuclear DNA.
- Are semiautonomous organelles that grow and reproduce within the cell.

> You may want to just *briefly* mention mitochondria and chloroplasts at this point in the course. Because structure is so closely tied to function, the organelle structure must be covered again in detail with cellular respiration and photosynthesis. In deference to time, it may be more practical to discuss it just once with the metabolism lectures.

1. Mitochondria

Mitochondria = Organelles which are the sites of cellular respiration, a catabolic oxygen-requiring process that uses energy extracted from organic macromolecules to produce ATP.

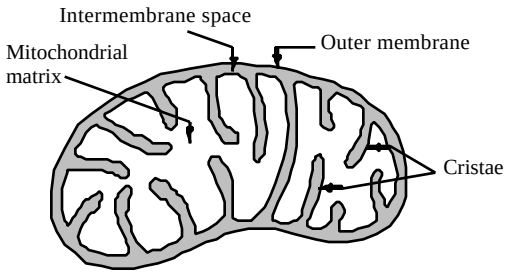
- Found in nearly all eukaryotic cells
- Number of mitochondria per cell varies and directly correlates with the cell's metabolic activity
- Are about 1 mm in diameter and 1-10 mm in length
- Are dynamic structures that move, change their shape and divide

Structure of the mitochondrion:

- Enclosed by two membranes that have their own unique combination of proteins embedded in phospholipid bilayers (see Campbell, Figure 7.18)
- Smooth *outer membrane* is highly permeable to small solutes, but it blocks passage of proteins and other macromolecules
- Convoluted *inner membrane* contains embedded enzymes that are involved in cellular respiration. The membrane's many infoldings or *cristae* increase the surface area available for these reactions to occur.
- The inner and outer membranes divide the mitochondrion into two internal compartments:

a. Intermembrane space

- Narrow region between the inner and outer mitochondrial membranes.
- Reflects the solute composition of the cytosol, because the outer membrane is permeable to small solute molecules.



b. Mitochondrial matrix

- Compartment enclosed by the inner mitochondrial membrane
- Contains enzymes that catalyze many metabolic steps of cellular respiration
- Some enzymes of respiration and ATP production are actually embedded in the inner membrane.

2. Chloroplasts

Plastids = A group of plant and algal membrane-bound organelles that include *amyloplasts, chromoplasts* and *chloroplasts*.

Amyloplasts = (Amylo = starch); colorless plastids that store starch; found in roots and tubers.

Chromoplasts = (Chromo = color); plastids containing pigments other than chlorophyll; responsible for the color of fruits, flowers and autumn leaves.

Chloroplasts = (Chloro = green); chlorophyll-containing plastids which are the sites of photosynthesis.

- Found in eukaryotic algae, leaves and other green plant organs.
- Are lens-shaped and measure about 2 mm by 5 mm.
- Are dynamic structures that change shape, move and divide.

Structure of the chloroplast:

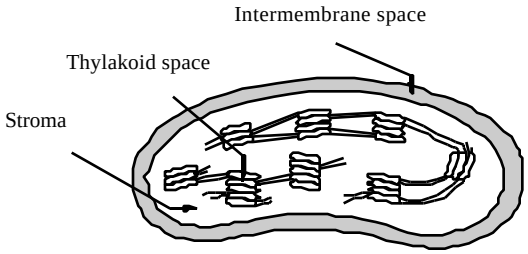
Chloroplasts are divided into three functional compartments by a system of membranes (see also Campbell, Figure 7.19):

a. Intermembrane space

The chloroplast is bound by a double membrane which partitions its contents from the cytosol. A narrow *intermembrane space* separates the two membranes.

b. Thylakoid space

Thylakoids form another membranous system within the chloroplast. The thylakoid membrane segregates the interior of the chloroplast into two compartments: *thylakoid space* and *stroma*.



- *Thylakoid space* = Space inside the thylakoid
- *Thylakoids* = Flattened membranous sacs inside the chloroplast
- Chlorophyll is found in the thylakoid membranes.
- Thylakoids function in the steps of photosynthesis that initially convert light energy to chemical energy.
- Some thylakoids are stacked into *grana*.

Grana = (Singular, *granum*); stacks of thylakoids in a chloroplast.

c. Stroma

Photosynthetic reactions that use chemical energy to convert carbon dioxide to sugar occur in the stroma.

Stroma = Viscous fluid outside the thylakoids

VI. The Cytoskeleton

A. Provides structural support to the cells for cell motility and regulation

It was originally thought that organelles were suspended in a formless cytosol. Technological advances in both light and electron microscopy (e.g., high voltage E.M.) revealed a three-dimensional view of the cell, which showed a network of fibers throughout the cytoplasm—the *cytoskeleton.* The cytoskeleton plays a major role in organizing the structures and activities of the cell.

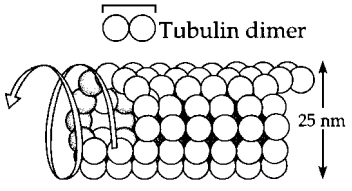
Cytoskeleton = A network of fibers throughout the cytoplasm that forms a dynamic framework for support and movement and regulation (see Campbell, Figure 7.20).

- Gives mechanical support to the cell and helps maintain its shape
- Enables a cell to change shape in an adaptive manner
- Associated with motility by interacting with specialized proteins called *motor molecules* (e.g., organelle movement, muscle contraction, and locomotor organelles)
- Play a regulatory role by mechanically transmitting signals from cell's surface to its interior
- Constructed from at least three types of fibers: *microtubules* (thickest), *microfilaments* (thinnest), and *intermediate filaments* (intermediate in diameter) (see Campbell, Table 7.2)

1. Microtubules

Found in cytoplasm of all eukaryotic cells, *microtubules*:

- Are straight *hollow* fibers about 25 nm in diameter and 200 nm – 25 μm in length
- Are constructed from globular proteins called *tubulin* that consists of one α-tubulin and one β-tubulin molecule



- Begin as two-dimensional sheets of tubulin units, which roll into tubes
- Elongate by adding tubulin units to its ends
- May be disassembled and the tubulin units recycled to build microtubules elsewhere in the cell

Functions of microtubules include:

- Cellular support; these microtubule function as compression-resistant girders to reinforce cell shape
- Tracks for organelle movement (see Campbell, Figure 7.21). Protein *motor molecules* (e.g., kinesin) interact with microtubules to translocate organelles (e.g., vesicles from the Golgi to the plasma membrane).
- Separation of chromosomes during cell division

a. Centrosomes and centrioles

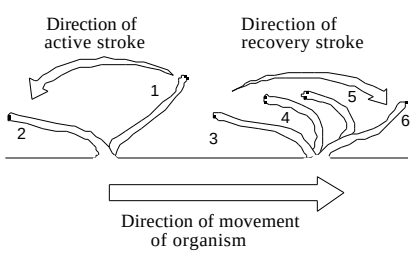
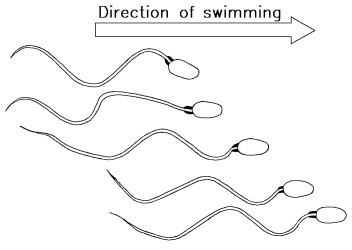
Centriole = Pair of cylindrical structures located in the centrosome of in animal cells, composed of nine sets of triplet microtubules arranged in a ring (see Campbell, Figure 7.22).

- Are about 150 nm in diameter and are arranged at right angles to each other.
- Pair of centrioles located within the centrosome, replicate during cell division.
- May organize microtubule assembly during cell division, but must not be mandatory for this function since plants lack centrioles.

b. Cilia and flagella

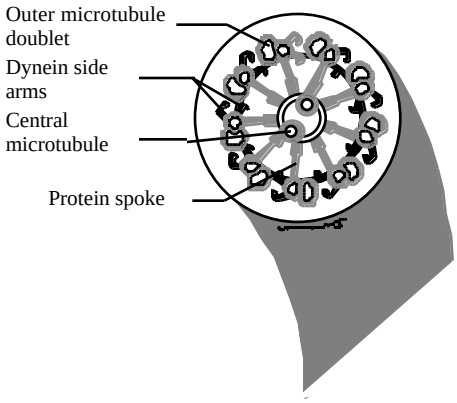
Cilia and *flagella* = Locomotor organelles found in eukaryotes that are formed from a specialized arrangement of microtubules.

- Many unicellular eukaryotic organisms are propelled through the water by cilia or flagella and motile sperm cells (animals, algae, some plants) are flagellated.
- May function to draw fluid across the surface of stationary cells (e.g., ciliated cells lining trachea).

Cilia **(singular, cilium)**	**Flagella** **(singular, flagellum)**
Occur in large numbers on cell surface.	One or a few per cell.
Shorter; 2-20 mm in length.	Longer; 10-200 mm in length.
Work like oars, alternating power with recovery strokes. Creates force in a direction perpendicular to the axis of the cilium.	Undulating motion that creates force in the same direction as the axis of the flagellum.
	

Ultrastructure of cilia and flagella:

- Are extensions of plasma membrane with a core of microtubules (see Campbell, Figure 7.24)
- Microtubular core is made of nine doublets of microtubules arranged in a ring with two single microtubules in the center (*9 + 2 pattern*).
- Each doublet is a pair of attached microtubules. One of the pair shares a portion of the other's wall.
- Each doublet is connected to the center of the ring by *radial spokes* that end near the central microtubules.



9+2 Pattern in Cross Section

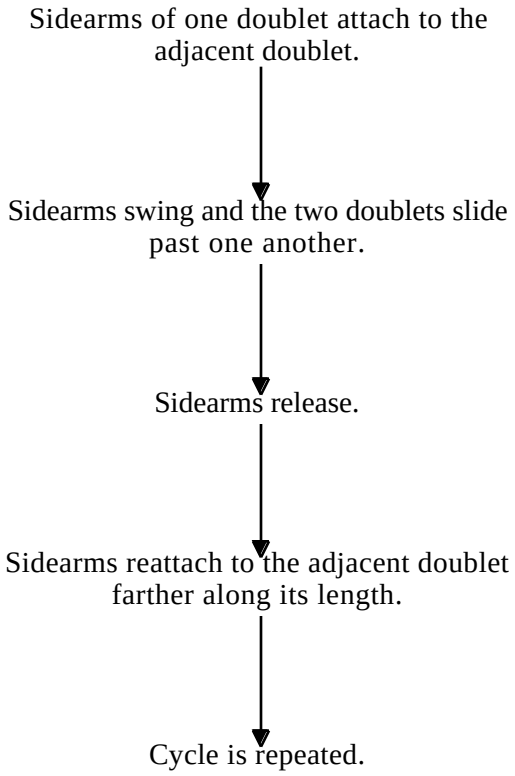
- Each doublet is attached to the neighboring doublet by a pair of *side arms*. Many pairs of side arms are evenly spaced along the doublet's length.
- Structurally identical to centrioles, *basal bodies* anchor the microtubular assemblies.

Basal body = A cellular structure, identical to a centriole, that anchors the microtubular assembly of cilia and flagella.

- Can convert into a centriole and vice versa
- May be a template for ordering tubulin into the microtubules of *newly* forming cilia or flagella. As cilia and flagella continue to grow, new tubulin subunits are added to the tips, rather than to the bases.

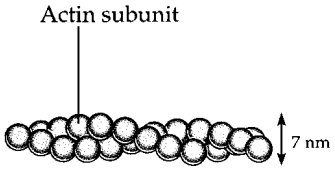
The unique ultrastructure of cilia and flagella is necessary for them to function:

- Sidearms are made of *dynein*, a large protein motor molecule that changes its conformation in the presence of ATP as an energy source.
- A complex cycle of movements caused by dynein's conformational changes, makes the cilium or flagellum bend (see Campbell, Figure 7.25):
- In cilia and flagella, linear displacement of dynein sidearms is translated into a *bending* by the resistance of the *radial spokes*. Working against this resistance, the "dynein-walking" distorts the microtubules, causing them to bend.



2. Microfilaments (actin filaments)

Structure of microfilaments (see Campbell, Figure 7.26):



- Solid rods about 7 nm in diameter
- Built from globular protein monomers, *G-actin*, which are linked into long chains
- Two actin chains are wound into a helix

Function of microfilaments:

a. Provide cellular support

- Bear tension (pulling forces)
- In combination with other proteins, they form a three-dimensional network just inside plasma membrane that helps support cell shape.
- In animal cells specialized for transport, bundles of microfilaments make up the core of microvilli (e.g., intestinal epithelial wall).

b. Participate in muscle contraction

- Along the length of a muscle cell, parallel actin microfilaments are interdigitated with thicker filaments made of the protein *myosin*, a motor molecule (see Campbell, Figure 7.27a).
- With ATP as the energy source, a muscle cell shortens as the thin actin filaments slide across the myosin filaments. Sliding results from the swinging of myosin cross-bridges intermittently attached to actin.

c. Responsible for localized contraction of cells

Small actin-myosin aggregates exist in some parts of the cell and cause localized contractions. Examples include:

- Contracting ring of microfilaments pinches an animal cell in two during cell division

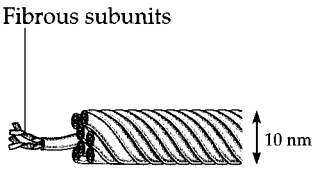
- Elongation and contraction of *pseudopodia* during *amoeboid movement*
- Involved in cytoplasmic streaming (cyclosis) found in plant cells

Cytoplasmic streaming (cyclosis) = Flowing of the entire cytoplasm around the space between the vacuole and plasma membrane in a plant cell (see Campbell, Figure 7.27c).

3. Intermediate filaments

Structure of intermediate filaments:

- Filaments that are intermediate in diameter (8-12 nm) between microtubules and microfilaments (see Campbell, Figure 7.26)
- Diverse class of cytoskeletal elements that differ in diameter and composition depending upon cell type
- Constructed from *keratin* subunits
- More permanent than microfilaments and microtubules



Function of intermediate filaments:

1. Specialized for bearing tension; may function as the framework for the cytoskeleton
2. Reinforce cell shape (e.g., nerve axons)
3. Probably fix organelle position (e.g., nucleus)
4. Compose the nuclear lamina, lining the nuclear envelope's interior

VII. Cell Surfaces and Junctions

A. Plant cells are encased by cell walls

Most cells produce coats that are external to the plasma membrane.

1. Cell walls

Plant cells can be distinguished from animal cells by the presence of a *cell wall*:

- Thicker than the plasma membrane (0.1–2 μm)
- Chemical composition varies from cell to cell and species to species.
- Basic design includes strong *cellulose* fibers embedded in a matrix of other polysaccharides and proteins.
- Functions to protect plant cells, maintain their shape, and prevent excess water uptake
- Has membrane-lined channels, *plasmodesmata*, that connect the cytoplasm of neighboring cells

Plant cells develop as follows:

- Young plant cell secretes a thin, flexible *primary cell wall*. Between primary cell walls of adjacent cells is a *middle lamella* made of *pectins*, a sticky polysaccharide that cements cells together.
- Cell stops growing and strengthens its wall. Some cells:
  1. secrete hardening substances into primary wall.
  2. add a *secondary cell wall* between plasma membrane and primary wall.

Secondary cell wall is often deposited in layers with a durable matrix that supports and protects the cell (see Campbell, Figure 7.28).

B. The extracellular matrix (ECM) of animal cells functions in support, adhesion, movement, and development

Animal cells lack walls, but they do have an elaborate *extracellular matrix (ECM)*.

Extracellular matrix (ECM) = Meshwork of macromolecules outside the plasma membrane of animal cells. This ECM is:

- locally secreted by cells.
- composed mostly of glycoproteins, the most abundant of which is *collagen* that:
  - accounts for about half of the total protein in the vertebrate body.
  - forms strong extracellular fibers embedded in a meshwork of carbohydrate-rich glycoproteins called *proteoglycans*.

Some cells are attached:

- directly to the collagen and proteoglycan of their extracellular matrix.
- or to the ECM by another class of glycoproteins–*fibronectins*.

Fibronectins bind to transmembrane receptor proteins called *integrins* that:

- bond on their cytoplasmic side to microfilaments of the cytoskeleton.
- integrate cytoskeletal responses to ECM changes and vice versa.

The extracellular matrix:

- provides support and anchorage for cells.
- functions in a cell's dynamic behavior. For example, some embryonic cells migrate along specific pathways by orienting their intracellular microfilaments to the pattern of extracellular fibers in the ECM (see Campbell, Figure 7.29).
- helps control gene activity in the cell's nucleus. Perhaps the transcription of specific genes is a response to chemical signals triggered by communication of mechanical stimuli across the plasma membrane from the ECM through integrins to the cytoskeleton.

C. Intercellular junctions help integrate cells into higher levels of structure and function

Neighboring cells often adhere and interact through special patches of direct physical contact.

Intercellular junctions in plants:

Plasmodesmata (singular, plasmodesma) = Channels that perforate plant cell walls, through which cytoplasmic strands communicate between adjacent cells.

- Lined by plasma membrane. Plasma membranes of adjacent cells are continuous through a plasmodesma.
- Allows free passage of water and small solutes. This transport is enhanced by *cytoplasmic streaming*.

Intercellular junctions in animals (see Campbell, Figure 7.30):

Tight junctions = Intercellular junctions that hold cells together tightly enough to block transport of substances through the intercellular space.

- Specialized membrane proteins in adjacent cells bond directly to each other allowing no space between membranes.
- Usually occur as belts all the way around each cell, that block intercellular transport.
- Frequently found in epithelial layers that separate two kinds of solutions.

Desmosomes = Intercellular junctions that rivet cells together into strong sheets, but still permit substances to pass freely through intracellular spaces. The desmosome is made of:

- Intercellular glycoprotein filaments that penetrate and attach the plasma membrane of both cells.
- A dense disk inside the plasma membrane that is reinforced by *intermediate filaments* made of *keratin* (a strong structural protein).

Gap junctions = Intercellular junctions specialized for material transport between the cytoplasm of adjacent cells.

- Formed by two connecting protein rings (*connexon*), each embedded in the plasma membrane of adjacent cells. The proteins protrude from the membranes enough to leave an intercellular gap of 2–4 nm.
- Have pores with diameters (1.5 nm) large enough to allow cells to share smaller molecules (e.g., inorganic ions, sugars, amino acids, vitamins), but not macromolecules such as proteins.
- Common in animal embryos and cardiac muscle where chemical communication between cells is essential.

REFERENCES

Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts and J.D. Watson. *Molecular Biology of the Cell.* 2nd ed. New York: Garland, 1994.

Becker, W.M. and D.W. Deamer. *The World of the Cell.* 3rd ed. Redwood City, California: Benjamin/Cummings, 1996.

Campbell, N., et al. *Biology*. 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

deDuve, C. *A Guided Tour of the Living Cell*. Volumes I and II. New York: Scientific American Books, 1984. Literally, a guided tour of the cell with the reader as "cytonaut." This is an excellent resource for lecture material and enjoyable reading.

CHAPTER 8
MEMBRANE STRUCTURE AND FUNCTION

OUTLINE

I. Membrane Structure
   A. Membrane models have evolved to fit new data: *science as a process*
   B. A membrane is a fluid mosaic of lipids, proteins, and carbohydrates

II. Traffic Across Membranes
   A. A membrane's molecular organization results in selective permeability
   B. Passive transport is diffusion across a membrane
   C. Osmosis is the passive transport of water
   D. Cell survival depends on balancing water uptake and loss
   E. Specific proteins facilitate the passive transport of selected solutes
   F. Active transport is the pumping of solutes against their gradients
   G. Some ion pumps generate voltage across membranes
   H. In cotransport, a membrane protein couples the transport of one solute to another
   I. Exocytosis and endocytosis transport large molecules

OBJECTIVES

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

1. Describe the function of the plasma membrane.
2. Explain how scientists used early experimental evidence to make deductions about membrane structure and function.
3. Describe the Davson-Danielli membrane model and explain how it contributed to our current understanding of membrane structure.
4. Describe the contribution J.D. Robertson, S.J. Singer, and G.L. Nicolson made to clarify membrane structure.
5. Describe the fluid properties of the cell membrane and explain how membrane fluidity is influenced by membrane composition.
6. Explain how hydrophobic interactions determine membrane structure and function.
7. Describe how proteins are spatially arranged in the cell membrane and how they contribute to membrane function.
8. Describe factors that affect selective permeability of membranes.
9. Define diffusion; explain what causes it and why it is a spontaneous process.
10. Explain what regulates the rate of passive transport.
11. Explain why a concentration gradient across a membrane represents potential energy.
12. Define osmosis and predict the direction of water movement based upon differences in solute concentration.
13. Explain how bound water affects the osmotic behavior of dilute biological fluids.
14. Describe how living cells with and without walls regulate water balance.

15. Explain how transport proteins are similar to enzymes.
16. Describe one model for facilitated diffusion.
17. Explain how active transport differs from diffusion.
18. Explain what mechanisms can generate a membrane potential or electrochemical gradient.
19. Explain how potential energy generated by transmembrane solute gradients can be harvested by the cell and used to transport substances across the membrane.
20. Explain how large molecules are transported across the cell membrane.
21. Give an example of receptor-mediated endocytosis.
22. Explain how membrane proteins interface with and respond to changes in the extracellular environment.

KEY TERMS

selective permeability	hypotonic	membrane potential
amphipathic	isotonic	electrochemical gradient
fluid mosaic model	osmosis	electrogenic pump
integral proteins	osmoregulation	proton pump
peripheral proteins	turgid	cotransport
transport proteins	plasmolysis	exocytosis
diffusion	facilitated diffusion	phagocytosis
concentration gradient	gated channels	pinocytosis
passive transport	active transport	receptor-mediated endocytosis
hypertonic	sodium-potassium pump	ligands

LECTURE NOTES

I. Membrane Structure

The *plasma membrane* is the boundary that separates the living cell from its nonliving surroundings. It makes life possible by its ability to discriminate in its chemical exchanges with the environment. This membrane:

- Is about 8 nm thick
- Surrounds the cell and controls chemical traffic into and out of the cell
- Is *selectively permeable*; it allows some substances to cross more easily than others
- Has a unique structure which determines its function and solubility characteristics

This is an opportune place to illustrate how form fits function. It is remarkable how much early models contributed to the understanding of membrane structure, since biologists proposed these models without the benefit of "seeing" a membrane with an electron microscope.

A. Membrane models have evolved to fit new data: *science as a process*

Membrane function is determined by its structure. Early models of the plasma membrane were deduced from *indirect* evidence:

1. Evidence: Lipid and lipid soluble materials enter cells more rapidly than substances that are insoluble in lipids (C. Overton, 1895).

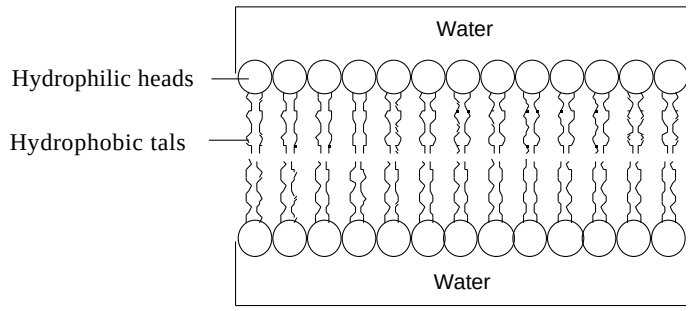
   Deduction: Membranes are made of lipids.

Deduction: Fat-soluble substance move through the membrane by dissolving in it ("like dissolves like").

2. Evidence: Amphipathic phospholipids will form an artificial membrane on the surface of water with only the hydrophilic heads immersed in water (Langmuir, 1917).

   Amphipathic = Condition where a molecule has both a hydrophilic region and a hydrophobic region.

   Deduction: Because of their molecular structure, phospholipids can form membranes (see also Campbell, Figure 8.1a).



3. Evidence: Phospholipid content of membranes isolated from red blood cells is just enough to cover the cells with two layers (Gorter and Grendel, 1925).

   Deduction: Cell membranes are actually phospholipid bilayers, two molecules thick (see Campbell, Figure 8.1b).

4. Evidence: Membranes isolated from red blood cells contain proteins as well as lipids.

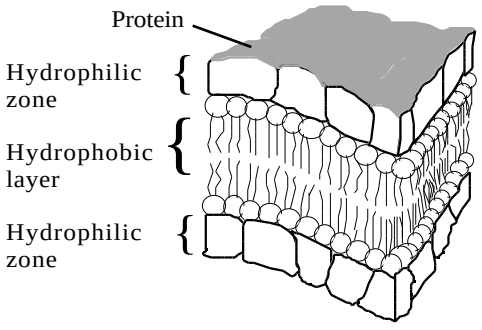
   Deduction: There is protein in biological membranes.

5. Evidence: Wettability of the surface of an actual biological membrane is greater than the surface of an artificial membrane consisting only of a phospholipid bilayer.

   Deduction: Membranes are coated on both sides with proteins, which generally absorb water.

Incorporating results from these and other solubility studies, J.F. Danielli and H. Davson (1935) proposed a model of cell membrane structure (see Campbell, Figure 8.2a):

- Cell membrane is made of a phospholipid bilayer sandwiched between two layers of globular protein.
- The polar (hydrophilic) heads of phospholipids are oriented towards the protein layers forming a hydrophilic zone.
- The nonpolar (hydrophobic) tails of phospholipids are oriented in between polar heads forming a hydrophobic zone.
- The membrane is approximately 8 nm thick.



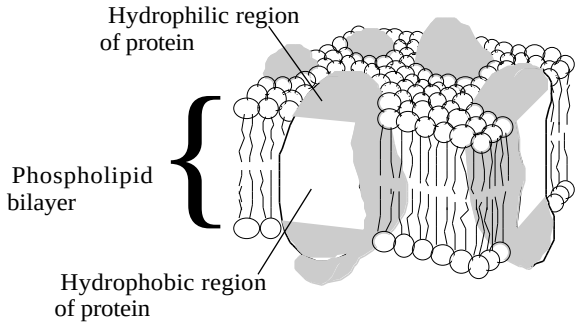
In the 1950s, electron microscopy allowed biologists to visualize the plasma membrane for the first time and provided support for the Davson-Danielli model. Evidence from electron micrographs:

1. Confirmed the plasma membrane was 7 to 8 nm thick (close to the predicted size if the Davson-Danielli model was modified by replacing globular proteins with protein layers in pleated-sheets).
2. Showed the plasma membrane was trilaminar, made of two electron-dense bands separated by an unstained layer. It was assumed that the heavy metal atoms of the stain adhered to the hydrophilic proteins and heads of phospholipids and not to the hydrophobic core.
3. Showed internal cellular membranes that looked similar to the plasma membrane. This led biologists (J.D. Robertson) to propose that all cellular membranes were symmetrical and virtually identical.

Though the phospholipid bilayer is probably accurate, there are problems with the Davson-Danielli model:

1. Not all membranes are identical or symmetrical.
   - Membranes with different functions also differ in chemical composition and structure.
   - Membranes are bifacial with distinct inside and outside faces.
2. A membrane with an outside layer of proteins would be an unstable structure.
   - Membrane proteins are not soluble in water, and, like phospholipid, they are *amphipathic*.
   - Protein layer not likely because its hydrophobic regions would be in an aqueous environment, and it would also separate the hydrophilic phospholipid heads from water.

In 1972, S.J. Singer and G.L. Nicolson proposed the *fluid mosaic model* which accounted for the amphipathic character of proteins (see Campbell, Figure 8.2b). They proposed:



- Proteins are individually embedded in the phospholipid bilayer, rather than forming a solid coat spread upon the surface.
- Hydrophilic portions of both proteins and phospholipids are maximally exposed to water resulting in a stable membrane structure.
- Hydrophobic portions of proteins and phospholipids are in the nonaqueous environment inside the bilayer.
- Membrane is a mosaic of proteins bobbing in a fluid bilayer of phospholipids.
- Evidence from freeze fracture techniques have confirmed that proteins are embedded in the membrane. Using these techniques, biologists can delaminate membranes along the middle of the bilayer. When viewed with an electron microscope, proteins appear to penetrate into the hydrophobic interior of the membrane (see Campbell, Methods Box).

B. A membrane is a fluid mosaic of lipids, proteins and carbohydrates

1. The fluid quality of membranes

Membranes are held together by hydrophobic interactions, which are weak attractions (see Campbell, Figure 8.3).

- Most membrane lipids and some proteins can drift laterally within the membrane.
- Molecules rarely flip transversely across the membrane because hydrophilic parts would have to cross the membrane's hydrophobic core.
- Phospholipids move quickly along the membrane's plane averaging 2 μm per second.
- Membrane proteins drift more slowly than lipids (see Campbell, Figure 8.4). The fact that proteins drift laterally was established experimentally by fusing a human and mouse cell (Frye and Edidin, 1970):

Membrane proteins of a human and mouse cell were labeled with different green and red fluorescent dyes.

↓

Cells were fused to form a hybrid cell with a continuous membrane.

↓

Hybrid cell membrane had initially distinct regions of green and red dye.

↓

In less than an hour, the two colors were intermixed.

- Some membrane proteins are tethered to the cytoskeleton and cannot move far.

Membranes must be fluid to work properly. Solidification may result in permeability changes and enzyme deactivation.

- Unsaturated hydrocarbon tails enhance membrane fluidity, because kinks at the carbon-to-carbon double bonds hinder close packing of phospholipids.
- Membranes solidify if the temperature decreases to a critical point. Critical temperature is lower in membranes with a greater concentration of unsaturated phospholipids.
- Cholesterol, found in plasma membranes of eukaryotes, modulates membrane fluidity by making the membrane:
  - Less fluid at warmer temperatures (e.g., 37°C body temperature) by restraining phospholipid movement.
  - More fluid at lower temperatures by preventing close packing of phospholipids.
- Cells may alter membrane lipid concentration in response to changes in temperature. Many cold tolerant plants (e.g., winter wheat) increase the unsaturated phospholipid concentration in autumn, which prevents the plasma membranes from solidifying in winter.

2. Membranes as mosaics of structure and function

A membrane is a *mosaic* of different proteins embedded and dispersed in the phospholipid bilayer (see Campbell, Figure 8.5). These proteins vary in both structure and function, and they occur in two spatial arrangements:

a. *Integral proteins* are generally transmembrane protein with hydrophobic regions that completely span the hydrophobic interior of the membrane (see Campbell, Figure 8.6).

b. *Peripheral proteins*, which are not embedded but attached to the membrane's surface.

- May be attached to integral proteins or held by fibers of the ECM
- On cytoplasmic side, may be held by filaments of cytoskeleton

Membranes are bifacial. The membrane's synthesis and modification by the ER and Golgi determines this asymmetric distribution of lipids, proteins and carbohydrates:

- Two lipid layers may differ in lipid composition.
- Membrane proteins have distinct directional orientation.
- When present, carbohydrates are restricted to the membrane's exterior.
- Side of the membrane facing the lumen of the ER, Golgi and vesicles is topologically the same as the plasma membrane's outside face (see Campbell, Figure 8.7).
- Side of the membrane facing the cytoplasm has always faced the cytoplasm, from the time of its formation by the endomembrane system to its addition to the plasma membrane by the fusion of a vesicle.
- Campbell, Figure 8.8, provides an overview of the six major kinds of function exhibited by proteins of the plasma membrane.

3. Membrane carbohydrates and cell-cell recognition

Cell-cell recognition = The ability of a cell to determine if other cells it encounters are alike or different from itself.

Cell-cell recognition is crucial in the functioning of an organism. It is the basis for:

- Sorting of an animal embryo's cells into tissues and organs
- Rejection of foreign cells by the immune system

The way cells recognize other cells is probably by keying on cell markers found on the external surface of the plasma membrane. Because of their diversity and location, likely candidates for such cell markers are membrane carbohydrates:

- Usually branched *oligosaccharides* (<15 monomers)
- Some covalently bonded to lipids (*glycolipids*)
- Most covalently bonded to proteins (*glycoproteins*)
- Vary from species to species, between individuals of the same species and among cells in the same individual

II. Traffic Across Membranes

A. A membrane's molecular organization results in selective permeability

The *selectively permeable* plasma membrane regulates the type and rate of molecular traffic into and out of the cell.

Selective permeability = Property of biological membranes which allows some substances to cross more easily than others. The selective permeability of a membrane depends upon:

- Membrane solubility characteristics of the phospholipid bilayer
- Presence of specific integral transport proteins

1. Permeability of the lipid bilayer

The ability of substances to cross the hydrophobic core of the plasma membrane can be measured as the rate of transport through an artificial phospholipid bilayer:

a. Nonpolar (hydrophobic) molecules

- Dissolve in the membrane and cross it with ease (e.g., hydrocarbons, O, CO_2)
- If two molecules are equally lipid soluble, the smaller of the two will cross the membrane faster.

b. Polar (hydrophilic) molecules

- Small, polar uncharged molecules (e.g., H_2O, ethanol) that are small enough to pass between membrane lipids, will easily pass through synthetic membranes.
- Larger, polar uncharged molecules (e.g., glucose) will *not* easily pass through synthetic membranes.
- All ions, even small ones (e.g., Na^+, H^+) have difficulty penetrating the hydrophobic layer.

2. Transport proteins

Small polar molecules and nonpolar molecules rapidly pass through the plasma membrane as they do an artificial membrane.

Unlike artificial membranes, however, biological membranes *are* permeable to specific ions and certain polar molecules of moderate size. These hydrophilic substances avoid the hydrophobic core of the bilayer by passing through *transport proteins*.

Transport proteins = Integral membrane proteins that transport specific molecules or ions across biological membranes (see Campbell, Figure 8.8a)

- May provide a hydrophilic tunnel through the membrane.
- May bind to a substance and physically move it across the membrane.
- Are specific for the substance they translocate.

B. Passive transport is diffusion across a membrane

Students have particular trouble with the concepts of *gradient* and *net* movement, yet their understanding of diffusion depends upon having a working knowledge of these terms.

Concentration gradient = Regular, graded concentration change over a distance in a particular direction.

Net directional movement = Overall movement away from the center of concentration, which results from random molecular movement in *all* directions.

Diffusion = The *net* movement of a substance down a *concentration gradient* (see Campbell, Figure 8.9).

- Results from the intrinsic kinetic energy of molecules (also called thermal motion, or heat)
- Results from random molecular motion, even though the *net* movement may be directional
- Diffusion continues until a dynamic equilibrium is reached—the molecules continue to move, but there is no net directional movement.

In the absence of other forces (e.g., pressure) a substance will diffuse from where it is more concentrated to where it is less concentrated.

- A substance diffuses down its concentration gradient.
- Because it decreases free energy, diffusion is a spontaneous process ($-\Delta G$). It increases entropy of a system by producing a more random mixture of molecules.
- A substance diffuses down its own concentration gradient and is not affected by the gradients of other substances.

Much of the traffic across cell membranes occurs by diffusion and is thus a form of *passive transport.*

Passive transport = Diffusion of a substance across a biological membrane.

- Spontaneous process which is a function of a concentration gradient when a substance is more concentrated on one side of the membrane.
- Passive process which does not require the cell to expend energy. It is the potential energy stored in a concentration gradient that drives diffusion.
- Rate of diffusion is regulated by the permeability of the membrane, so some molecules diffuse more freely than others.
- Water diffuses freely across most cell membranes.

C. Osmosis is the passive transport of water

Hypertonic solution = A solution with a greater solute concentration than that inside a cell.

Hypotonic solution = A solution with a lower solute concentration compared to that inside a cell.

Isotonic solution = A solution with an equal solute concentration compared to that inside a cell.

These terms are a source of confusion for students. It helps to point out that these are only relative terms used to compare the osmotic concentration of a solution to the osmotic concentration of a cell.

Osmosis = Diffusion of water across a selectively permeable membrane (see Campbell, Figure 8.10).

- Water diffuses down its concentration gradient.
- Example: If two solutions of different concentrations are separated by a selectively permeable membrane that is permeable to water but not to solute, water will diffuse from the hypoosmotic solution (solution with the lower osmotic concentration) to the hyperosmotic solution (solution with the higher osmotic concentration).
- Some solute molecules can reduce the proportion of water molecules that can freely diffuse. Water molecules form a hydration shell around hydrophilic solute molecules and this bound water cannot freely diffuse across a membrane.
- In dilute solutions including most biological fluids, it is the different in the proportion of the unbound water that causes osmosis, rather than the actual difference in water concentration.
- Direction of osmosis is determined by the difference in total solute concentration, regardless of the type or diversity of solutes in the solutions.
- If two isotonic solutions are separated by a selectively permeable membrane, water molecules diffuse across the membrane in both directions at an equal rate. There is no net movement of water.

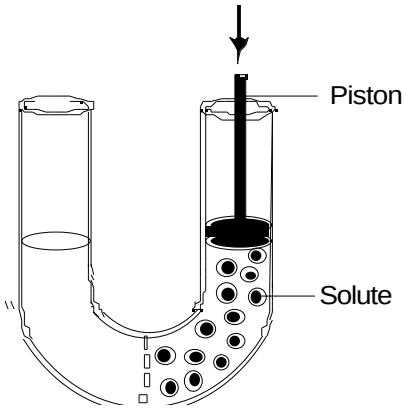
Clarification of this point is often necessary. Students may need to be reminded that even though there is no *net* movement of water across the membrane (or osmosis), the water molecules do not stop moving. At equilibrium, the water molecules move in both directions at the same rate.

Osmotic concentration = Total solute concentration of a solution

Osmotic pressure = Measure of the tendency for a solution to take up water when separated from pure water by a selectively permeable membrane.

- Osmotic pressure of pure water is zero.
- Osmotic pressure of a solution is proportional to its osmotic concentration. (The greater the solute concentration, the greater the osmotic pressure.)

Osmotic pressure can be measured by an *osmometer*:



- In one type of osmometer, pure water is separated from a solution by a selectively permeable membrane that is permeable to water but not solute.
- The tendency for water to move into the solution by osmosis is counteracted by applying enough pressure with a piston so the solution's volume will stay the same.
- The amount of pressure required to prevent net movement of water into the solution is the *osmotic pressure*.

D. Cell survival depends on balancing water uptake and loss

1. Water balance of cells without walls

Since animal cells lack cell walls, they are not tolerant of excessive osmotic uptake or loss of water (see Campbell, Figure 8.11).

- In an isotonic environment, the volume of an animal cell will remain stable with no net movement of water across the plasma membrane.
- In a hypertonic environment, an animal cell will lose water by osmosis and *crenate* (shrivel).
- In a hypotonic environment, an animal cell will gain water by osmosis, swell and perhaps *lyse* (cell destruction).

Organisms without cell walls prevent excessive loss or uptake of water by:

- Living in an isotonic environment (e.g., many marine invertebrates are isosmotic with sea water).
- Osmoregulating in a hypo- or hypertonic environment. Organisms can regulate water balance (osmoregulation) by removing water in a hypotonic environment (e.g., *Paramecium* with contractile vacuoles in fresh water) or conserving water and pumping out salts in a hypertonic environment (e.g., bony fish in seawater) (see Campbell, Figure 8.12).

2. Water balance of cells with walls

Cells of prokaryotes, some protists, fungi and plants have cell walls outside the plasma membrane.

- In a hypotonic environment, water moves by osmosis into the plant cell, causing it to swell until internal pressure against the cell wall equals the osmotic pressure of the cytoplasm. A dynamic equilibrium is established (water enters and leaves the cell at the same rate and the cell becomes turgid).
- *Turgid* = Firmness or tension such as found in walled cells that are in a hypoosmotic environment where water enters the cell by osmosis.
  - Ideal state for most plant cells.
  - Turgid cells provide mechanical support for plants.
  - Requires cells to be hyperosmotic to their environment.
- In an isotonic environment, there is no net movement of water into or out of the cell.
  - Plant cells become *flaccid* or limp.
  - Loss of structural support from turgor pressure causes plants to wilt.
- In a hypertonic environment, walled cells will lose water by osmosis and will *plasmolyze*, which is usually lethal.

Plasmolysis = Phenomenon where a walled cell shrivels and the plasma membrane pulls away from the cell wall as the cell loses water to a hypertonic environment.

E. Specific proteins facilitate the passive transport of selected solutes

Facilitated diffusion = Diffusion of solutes across a membrane, with the help of transport proteins.

- Is passive transport because solute is transported down its concentration gradient.
- Helps the diffusion of many polar molecules and ions that are impeded by the membrane's phospholipid bilayer.

Transport proteins share some properties of enzymes:

- Transport proteins are *specific* for the solutes they transport. There is probably a specific binding site analogous to an enzyme's active site.
- Transport proteins can be *saturated* with solute, so the maximum transport rate occurs when all binding sites are occupied with solute.
- Transport proteins can be inhibited by molecules that resemble the solute normally carried by the protein (similar to competitive inhibition in enzymes).

Transport proteins differ from enzymes in they do not usually catalyze chemical reactions.

One model for facilitated diffusion (see Campbell, Figure 8.13):

- Transport protein most likely remains in place in the membrane and translocates solute by alternating between two conformations.
- In one conformation, transport protein binds solute; as it changes to another conformation, transport protein deposits solute on the other side of the membrane.
- The solute’s binding and release may trigger the transport protein’s conformational change.

Other transport proteins are selective channels across the membrane.

- The membrane is thus permeable to specific solutes that can pass through these channels.
- Some selective channels (gated channels) only open in response to electrical or chemical stimuli. For example, binding of neurotransmitter to nerve cells opens gated channels so that sodium ions can diffuse into the cell.

In some inherited disorders, transport proteins are missing or are defective (e.g., cystinuria, a kidney disease caused by missing carriers for cystine and other amino acids which are normally reabsorbed from the urine).

F. Active transport is the pumping of solutes against their gradients

Active transport = Energy-requiring process during which a transport protein pumps a molecule across a membrane, *against* its concentration gradient.

- Is energetically uphill (+ΔG) and requires the cell to expend energy.
- Helps cells maintain steep ionic gradients across the cell membrane (e.g., Na^+, K^+, Mg^{++}, Ca^{++} and Cl^-).
- Transport proteins involved in active transport harness energy from ATP to pump molecules against their concentration gradients.

An example of an active transport system that translocates ions against steep concentration gradients is the *sodium-potassium pump*. Major features of the pump are:

1. The transport protein oscillates between two conformations:
   a. High affinity for Na^+ with binding sites oriented towards the cytoplasm.
   b. High affinity for K^+ with binding sites oriented towards the cell's exterior.
2. ATP phosphorylates the transport protein and powers the conformational change from Na^+ receptive to K^+ receptive.
3. As the transport protein changes conformation, it translocates bound solutes across the membrane.
4. Na^+K^+-pump translocates three Na^+ ions out of the cell for every two K^+ ions pumped into the cell. (Refer to Campbell, Figure 8.14 for the specific sequence of events.)

G. Some ion pumps generate voltage across membranes

Because anions and cations are unequally distributed across the plasma membrane, all cells have voltages across their plasma membranes.

Membrane potential = Voltage across membranes

- Ranges from -50 to -200 mv. As indicated by the negative sign, the cell's inside is negatively charged with respect to the outside.
- Affects traffic of charged substances across the membrane
- Favors diffusion of cations into cell and anions out of the cell (because of electrostatic attractions)

Two forces drive passive transport of ions across membranes:

1. Concentration gradient of the ion
2. Effect of membrane potential on the ion

Campbell, Figure 8.15, reviews the distinction between active and passive transport.

Electrochemical gradient = Diffusion gradient resulting from the combined effects of membrane potential and concentration gradient.

- Ions may not always diffuse down their concentration gradients, but they *always* diffuse down their electrochemical gradients.
- At equilibrium, the distribution of ions on either side of the membrane may be different from the expected distribution when charge is not a factor.
- Uncharged solutes diffuse down concentration gradients because they are unaffected by membrane potential.

Factors which contribute to a cell's membrane potential (net negative charge on the inside):

1. Negatively charged proteins in the cell's interior.

2. Plasma membrane's selective permeability to various ions. For example, there is a net loss of positive charges as K^+ leaks out of the cell faster than Na^+ diffuses in.
3. The sodium-potassium pump. This electrogenic pump translocates 3 Na^+ out for every 2 K^+ in - a net loss of one positive charge per cycle.

Electrogenic pump = A transport protein that generates voltage across a membrane (see Campbell, Figure 8.16).

- Na^+/K^+ ATPase is the major electrogenic pump in animal cells.
- A *proton pump* is the major electrogenic pump in plants, bacteria, and fungi. Also, mitochondria and chloroplasts use a proton pump to drive ATP synthesis.
- Voltages created by electrogenic pumps are sources of potential energy available to do cellular work.

This is a good place to emphasize that electrochemical gradients represent potential energy. Spending lecture time on cotransport and the proton pump will help prepare your students for the upcoming topic of chemiosmosis.

H. In cotransport, a membrane protein couples the transport of one solute to another

Cotransport = Process where a single ATP-powered pump actively transports one solute and indirectly drives the transport of other solutes against their concentration gradients.

One mechanism of cotransport involves two transport proteins:

1. ATP-powered pump actively transports one solute and creates potential energy in the gradient it creates.
2. Another transport protein couples the solute's downhill diffusion as it leaks back across the membrane with a second solute's uphill transport against its concentration gradient.

For example, plants use a proton pump coupled with sucrose-H^+ symport to load sucrose into specialized cells of vascular tissue. Both solutes, H^+ and sucrose, must bind to the transport protein for cotransport to take place (see Campbell, Figure 8.17).

I. Exocytosis and endocytosis transport large molecules

Water and small molecules cross membranes by:

1. Passing through the phospholipid bilayer.
2. Being translocated by a transport protein.

Large molecules (e.g., proteins and polysaccharides) cross membranes by the processes of *exocytosis* and *endocytosis*.

Exocytosis	Endocytosis
Process of exporting macromolecules from a cell by fusion of vesicles with the plasma membrane.	Process of importing macromolecules into a cell by forming vesicles derived from the plasma membrane.
Vesicle usually budded from the ER or Golgi and migrates to plasma membrane.	Vesicle forms from a localized region of plasma membrane that sinks inward; pinches off into the cytoplasm.
Used by secretory cells to export products (e.g., insulin in pancreas, or neuro-transmitter from neuron).	Used by cells to incorporate extracellular substances.

There are three types of endocytosis: (1) *phagocytosis,* (2) *pinocytosis* and (3) *receptor-mediated endocytosis* (see Campbell, Figure 8.18).

Phagocytosis = (cell eating); endocytosis of solid particles

- Cell engulfs particle with *pseudopodia* and pinches off a food vacuole.
- Vacuole fuses with a lysosome containing hydrolytic enzymes that will digest the particle.

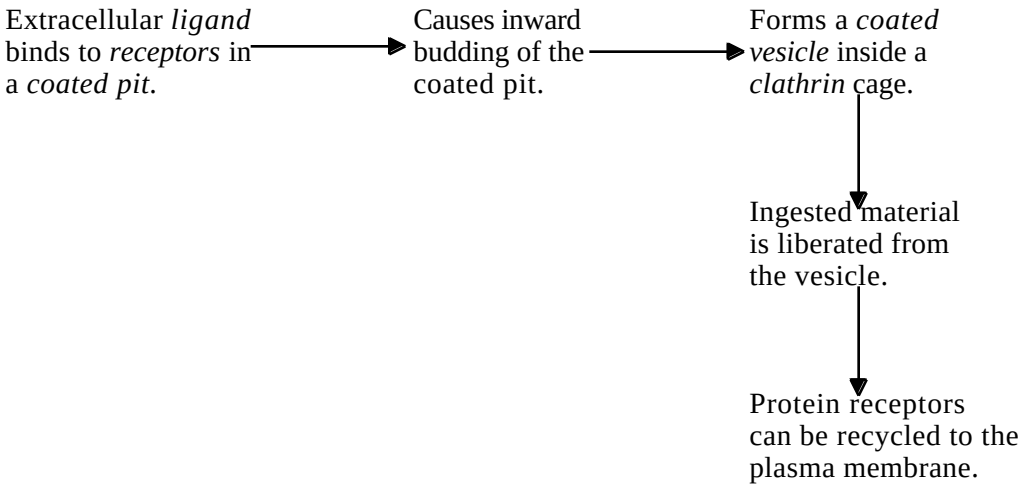
Pinocytosis = (cell drinking); endocytosis of fluid droplets

- Droplets of extracellular fluid are taken into small vesicles.
- The process is not discriminating. The cell takes in all solutes dissolved in the droplet.

Receptor-mediated endocytosis = The process of importing specific macromolecules into the cell by the inward budding of vesicles formed from *coated pits*; occurs in response to the binding of specific *ligands* to receptors on the cell's surface.

- More discriminating process than pinocytosis.
- A molecule that binds to a specific receptor site of another molecule is called a *ligand.*
- Membrane-embedded proteins with specific receptor sites exposed to the cell's exterior, cluster in regions called *coated pits.*
- A layer of *clathrin,* a fibrous protein, lines and reinforces the *coated pit* on the cytoplasmic side and probably helps deepen the pit to form a vesicle.

Progressive stages of receptor-mediated endocytosis:



Receptor-mediated endocytosis enables cells to acquire bulk quantities of specific substances, even if they are in low concentration in extracellular fluid. For example, cholesterol enters cells by receptor-mediated endocytosis.

- In the blood, cholesterol is bound to lipid and protein complexes called *low-density lipoproteins* (LDLs).
- These LDLs bind to LDL receptors on cell membranes, initiating endocytosis.
- An inherited disease call familial hypercholesterolemia is characterized by high cholesterol levels in the blood. The LDL receptors are defective, so cholesterol cannot enter the cells by endocytosis and thus accumulates in the blood, contributing to the development of atherosclerosis.

In a nongrowing cell, the amount of plasma membrane remains relatively constant.

- Vesicle fusion with the plasma membrane offsets membrane loss through endocytosis.
- Vesicles provide a mechanism to rejuvenate or remodel the plasma membrane.

REFERENCES

Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts and J.D. Watson. *Molecular Biology of the Cell.* 3rd ed. New York: Garland, 1994.

Becker, W.M. and D.W. Deamer. *The World of the Cell.* 3rd ed. Redwood City, California: Benjamin/Cummings, 1996.

Campbell, N. et al. *Biology.* 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

deDuve, C. *A Guided Tour of the Living Cell.* Volumes I and II. New York: Scientific American Books, 1984. Literally a guided tour of the cell with the reader as "cytonaut." This is an excellent resource for lecture material and enjoyable reading.

Kleinsmith, L.J. and V.M. Kish. *Principles of Cell Biology.* New York: Harper and Row, Publ., 1988.

CHAPTER 9

CELLULAR RESPIRATION: HARVESTING CHEMICAL ENERGY

OUTLINE

I. Principles of Energy Conservation
   A. Cellular respiration and fermentation are catabolic (energy-yielding) pathways
   B. Cells must recycle the ATP they use for work
   C. Redox reactions release energy when electrons move closer to electronegative atoms
   D. Electrons "fall" from organic molecules to oxygen during cellular respiration
   E. The "fall" of electrons during respiration is stepwise, via NAD^+ and an electron transport chain

II. The Process of Cellular Respiration
   A. Respiration involves glycolysis, the Krebs cycle, and electron transport: *an overview*
   B. Glycolysis harvests chemical energy by oxidizing glucose to pyruvate: *a closer look*
   C. The Krebs cycle completes the energy-yielding oxidation of organic molecules: *a closer look*
   D. The inner mitochondrial membrane couples electron transport to ATP synthesis: *a closer look*
   E. Cellular respiration generates many ATP molecules for each sugar molecule it oxidizes: *a review*

III. Related Metabolic Processes
   A. Fermentation enables some cells to produce ATP without the help of oxygen
   B. Glycolysis and the Krebs cycle connect to many other metabolic pathways
   C. Feedback mechanisms control cellular respiration

OBJECTIVES

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

1. Diagram energy flow through the biosphere.
2. Describe the overall summary equation for cellular respiration.
3. Distinguish between substrate-level phosphorylation and oxidative phosphorylation.
4. Explain how exergonic oxidation of glucose is coupled to endergonic synthesis of ATP.
5. Define oxidation and reduction.
6. Explain how redox reactions are involved in energy exchanges.
7. Define coenzyme and list those involved in respiration.
8. Describe the structure of coenzymes and explain how they function in redox reactions.

9. Describe the role of ATP in coupled reactions.
10. Explain why ATP is required for the preparatory steps of glycolysis.
11. Describe how the carbon skeleton of glucose changes as it proceeds through glycolysis.
12. Identify where in glycolysis that sugar oxidation, substrate-level phosphorylation and reduction of coenzymes occur.
13. Write a summary equation for glycolysis and describe where it occurs in the cell.
14. Describe where pyruvate is oxidized to acetyl CoA, what molecules are produced and how it links glycolysis to the Krebs cycle.
15. Describe the location, molecules in and molecules out for the Krebs cycle.
16. Explain at what point during cellular respiration glucose is completely oxidized.
17. Explain how the exergonic "slide" of electrons down the electron transport chain is coupled to the endergonic production of ATP by chemiosmosis.
18. Describe the process of chemiosmosis.
19. Explain how membrane structure is related to membrane function in chemiosmosis.
20. Summarize the net ATP yield from the oxidation of a glucose molecule by constructing an ATP ledger which includes coenzyme production during the different stages of glycolysis and cellular respiration.
21. Describe the fate of pyruvate in the absence of oxygen.
22. Explain why fermentation is necessary.
23. Distinguish between aerobic and anaerobic metabolism.
24. Describe how food molecules other than glucose can be oxidized to make ATP.
25. Describe evidence that the first prokaryotes produced ATP by glycolysis.
26. Explain how ATP production is controlled by the cell and what role the allosteric enzyme, phosphofructokinase, plays in this process.

KEY TERMS

fermentation	Krebs cycle	anaerobic
cellular respiration	oxidative phosphorylation	alcohol fermentation
redox reactions	substrate-level phosphorylation	lactic acid fermentation
oxidation	acetyl CoA	facultative anaerobe
reduction	cytochrome (cyt)	beta oxidation
reducing agent	ATP synthase	
oxidizing agent	chemiosmosis	
NAD+	proton-motive force	
glycolysis	aerobic	

LECTURE NOTES

I. Principles of Energy Conservation

As open systems, cells require outside energy sources to perform cellular work (e.g., chemical, transport, and mechanical).

Energy flows into most ecosystems as sunlight.

↓

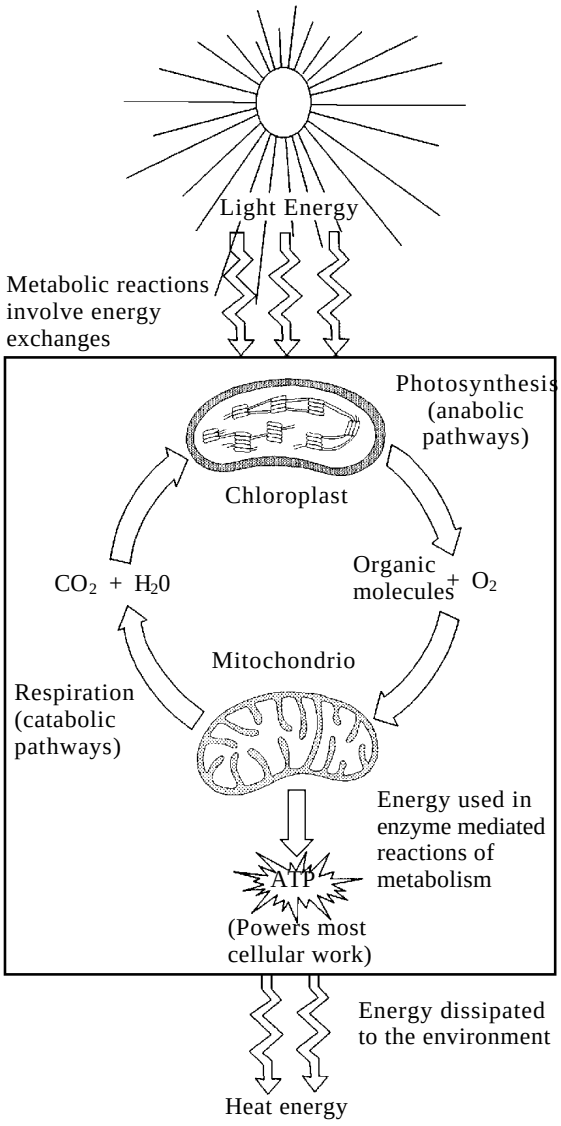
Photosynthetic organisms trap a portion of the light energy and transform it into chemical bond energy of organic molecules. O_2 is released as a byproduct.

↓

Cells use some of the chemical bond energy in organic molecules to make ATP–the energy source for cellular work.

↓

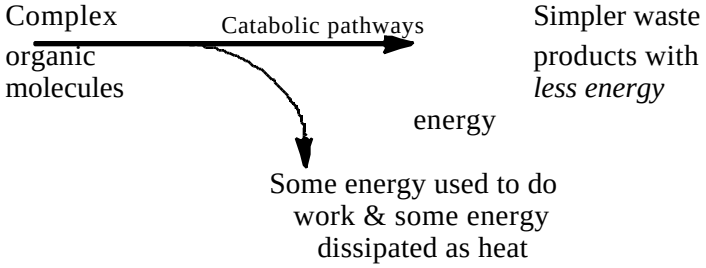
Energy leaves living organisms as it dissipates as heat.



The products of respiration (CO_2 and H_2O) are the raw materials for photosynthesis. Photosynthesis produces glucose and oxygen, the raw materials for respiration.

Chemical elements essential for life are recycled, but energy is not.

How do cells harvest chemical energy?



A. Cellular respiration and fermentation are catabolic (energy-yielding) pathways

Fermentation = An ATP-producing catabolic pathway in which both electron donors and acceptors are organic compounds.

- Can be an anaerobic process
- Results in a partial degradation of sugars

Cellular respiration = An ATP-producing catabolic process in which the ultimate electron acceptor is an inorganic molecule, such as oxygen.

- Most prevalent and efficient catabolic pathway
- Is an exergonic process ($\Delta G = -2870$ kJ/mol or -686 kcal/mol)
- Can be summarized as:

  Organic compounds + Oxygen (food) $\longrightarrow$ Carbon dioxide + Water + Energy
- Carbohydrates, proteins, and fats can all be metabolized as fuel, but cellular respiration is most often described as the oxidation of glucose:

  $C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O$ + Energy (ATP + Heat)

B. Cells recycle the ATP they use for work

The catabolic process of cellular respiration transfers the energy stored in food molecules to *ATP*.

ATP (adenosine triphosphate) = Nucleotide with high energy phosphate bonds that the cell hydrolyzes for energy to drive endergonic reactions.

- The cell taps energy stored in ATP by enzymatically transferring terminal phosphate groups from ATP to other compounds. (Recall that direct hydrolysis of ATP would release energy as heat, a form unavailable for cellular work. See Chapter 6.)
- The compound receiving the phosphate group from ATP is said to be *phosphorylated* and becomes more reactive in the process.
- The phosphorylated compound loses its phosphate group as cellular work is performed; inorganic phosphate and ADP are formed in the process (see Campbell, Figure 9.2).
- Cells must replenish the ATP supply to continue cellular work. Cellular respiration provides the energy to regenerate ATP from ADP and inorganic phosphate.

C. Redox reactions release energy when electrons move closer to electronegative atoms

1. An introduction to redox reactions

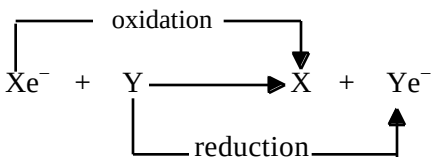
Oxidation-reduction reactions = Chemical reactions which involve a partial or complete transfer of electrons from one reactant to another; called *redox reactions* for short.

Oxidation = Partial or complete loss of electrons

Reduction = Partial or complete gain of electrons

Generalized redox reaction:

Electron transfer requires both a donor and acceptor, so when one reactant is oxidized the other is reduced.



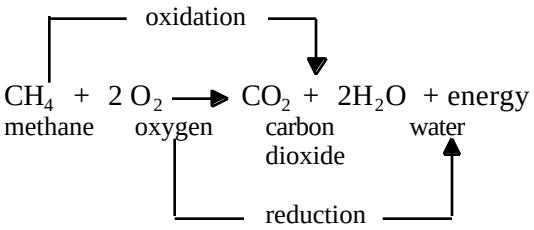
Where:

X = Substance being oxidized; acts as a *reducing agent* because it reduces Y.

Y = Substance being reduced; as an *oxidizing agent* because it oxidizes X.

Not all redox reactions involve a complete transfer of electrons, but, instead, may just change the degree of sharing in covalent bonds (see Campbell, Figure 9.3).

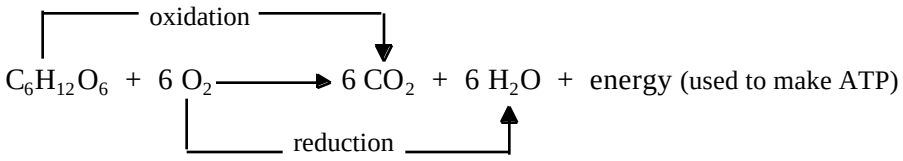
- Example: Covalent electrons of methane are equally shared, because carbon and hydrogen have similar electronegativities.
- As methane reacts with oxygen to form carbon dioxide, electrons shift away from carbon and hydrogen to the more electronegative oxygen.



- Since electrons lose potential energy when they shift toward more electronegative atoms, redox reactions that move electrons closer to oxygen release energy.
- Oxygen is a powerful oxidizing agent because it is so electronegative.

D. Electrons "fall" from organic molecules to oxygen during cellular respiration

Cellular respiration is a redox process that transfers hydrogen, including electrons with high potential energy, from sugar to oxygen.



- Valence electrons of carbon and hydrogen lose potential energy as they shift toward electronegative oxygen.
- Released energy is used by cells to produce ATP.
- Carbohydrates and fats are excellent energy stores because they are rich in C to H bonds.

Without the activation barrier, glucose would combine spontaneously with oxygen.

- Igniting glucose provides the activation energy for the reaction to proceed; a mole of glucose yields 686 kcal (2870 kJ) of heat when burned in air.
- Cellular respiration does not oxidize glucose in one explosive step, as the energy could not be efficiently harnessed in a form available to perform cellular work.
- Enzymes lower the activation energy in cells, so glucose can be slowly oxidized in a stepwise fashion during glycolysis and Krebs cycle.

E. The "fall" of electrons during respiration is stepwise, via NAD^+ and an electron transport chain

Hydrogens stripped from glucose are not transferred directly to oxygen, but are first passed to a special electron acceptor—*NAD^+*.

Nicotinamide adenine dinucleotide (NAD^+) = A *dinucleotide* that functions as a *coenzyme* in the redox reactions of metabolism (see Campbell, Figure 9.4).

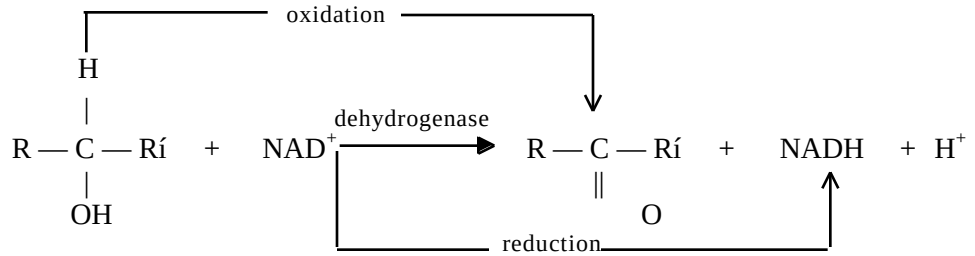
- Found in all cells
- Assists enzymes in electron transfer during redox reactions of metabolism

Coenzyme = Small nonprotein organic molecule that is required for certain enzymes to function.

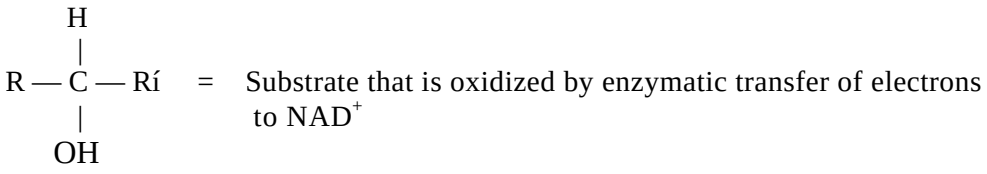
Dinucleotide = A molecule consisting of two nucleotides.

During the oxidation of glucose, NAD^+ functions as an oxidizing agent by trapping energy-rich electrons from glucose or food. These reactions are catalyzed by enzymes called *dehydrogenases*, which:

- Remove a pair of hydrogen atoms (two electrons and two protons) from substrate
- Deliver the two electrons and *one* proton to NAD^+
- Release the remaining proton into the surrounding solution



Where:



NAD^+ = Oxidized coenzyme (net positive charge)

NADH = Reduced coenzyme (electrically neutral)

The high energy electrons transferred from substrate to NAD^+ are then passed down the *electron transport chain* to oxygen, powering ATP synthesis (*oxidative phosphorylation*).

Some instructors find it difficult to drive this point home. Surprisingly, some students can recall the intermediate steps of glycolysis or the Krebs cycle, but cannot explain in general terms how energy from food is transferred to ATP. Campbell, Figure 9.16 can be used to give students an overview when respiration is introduced; it is useful to refer to it here so students can place the process you are describing in context. It can be used again later as a summary to bring closure to the topic.

Electron transport chains convert some of the chemical energy extracted from food to a form that can be used to make ATP (see Campbell, Figure 9.5). These transport chains:

- Are composed of electron-carrier molecules built into the inner mitochondrial membrane. Structure of this membrane correlates with its functional role (form fits function).
- Accept energy-rich electrons from reduced coenzymes (NADH and $FADH_2$); and during a series of redox reactions, pass these electrons down the chain to oxygen, the final electron acceptor. The electronegative oxygen accepts these electrons, along with hydrogen nuclei, to form water.
- Release energy from energy-rich electrons in a controlled stepwise fashion; a form that can be harnessed by the cell to power ATP production. If the reaction between hydrogen and oxygen during respiration occurred in a single explosive step, much of the energy released would be lost as heat, a form unavailable to do cellular work.

Electron transfer from NADH to oxygen is exergonic, having a free energy change of –222 kJ/mole (–53 kcal/mol).

- Since electrons lose potential energy when they shift toward a more electronegative atom, this series of redox reactions releases energy.
- Each successive carrier in the chain has a higher electronegativity than the carrier before it, so the electrons are pulled downhill towards oxygen, the final electron acceptor and the molecule with the highest electronegativity.

II. The Process of Cellular Respiration

A. Respiration involves glycolysis, the Krebs cycle, and electron transport: *an overview*

There are three metabolic stages of cellular respiration (see Campbell, Figure 9.6):

1. Glycolysis
2. Krebs cycle
3. Electron transport chain (ETC) and oxidative phosphorylation

Glycolysis is a catabolic pathway that:

- Occurs in the cytosol
- Partially oxidizes glucose (6C) into two *pyruvate* (3C) molecules

The *Krebs cycle* is a catabolic pathway that:

- Occurs in the mitochondrial matrix
- Completes glucose oxidation by breaking down a *pyruvate* derivative (acetyl CoA) into carbon dioxide

Glycolysis and the Krebs cycle produce:

- A small amount of ATP by substrate-level phosphorylation
- NADH by transferring electrons from substrate to NAD^+ (Krebs cycle also produces $FADH_2$ by transferring electrons to FAD)

The *electron transport chain*:

- Is located at the inner membrane of the mitochondrion
- Accepts energized electrons from reduced coenzymes (NADH and $FADH_2$) that are harvested during glycolysis and Krebs cycle. Oxygen pulls these electrons down the electron transport chain to a lower energy state.
- Couples this exergonic slide of electrons to ATP synthesis or oxidative phosphorylation. This process produces *most* (90%) of the ATP.

Oxidative phosphorylation = ATP production that is coupled to the exergonic transfer of electrons from food to oxygen.

A small amount of ATP is produced directly by the reactions of glycolysis and Krebs cycle. This mechanism of producing ATP is called substrate-level phosphorylation.

Substrate-level phosphorylation = ATP production by direct enzymatic transfer of phosphate from an intermediate substrate in catabolism to ADP.

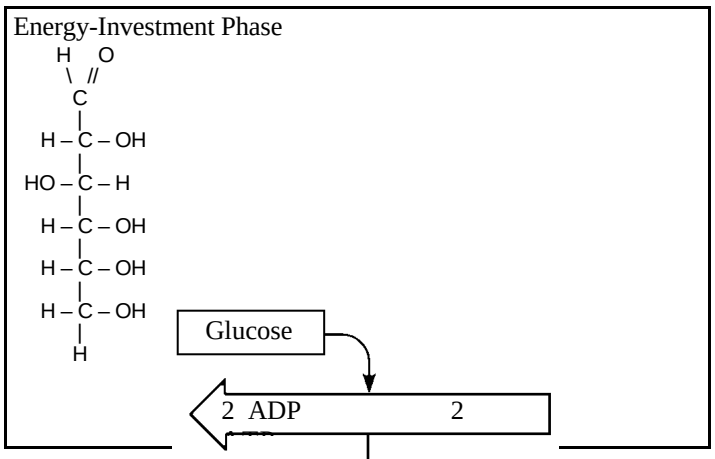
B. Glycolysis harvests chemical energy by oxidizing glucose to pyruvate: *a closer look*

Glycolysis = (Glyco = sweet, sugar; lysis = to split); catabolic pathway during which six-carbon glucose is split into two three-carbon sugars, which are then oxidized and rearranged by a step-wise process that produces two pyruvate molecules.

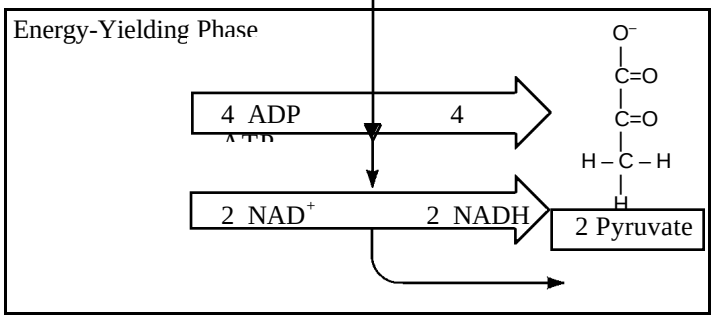
- Each reaction is catalyzed by specific enzymes dissolved in the cytosol.
- No CO_2 is released as glucose is oxidized to pyruvate; all carbon in glucose can be accounted for in the two molecules of pyruvate.
- Occurs whether or not oxygen is present.

The reactions of glycolysis occur in two phases:

Energy-investment phase. The cell uses ATP to phosphorylate the intermediates of glycolysis.



Energy-yielding phase. Two three-carbon intermediates are oxidized. For each glucose molecule entering glycolysis:



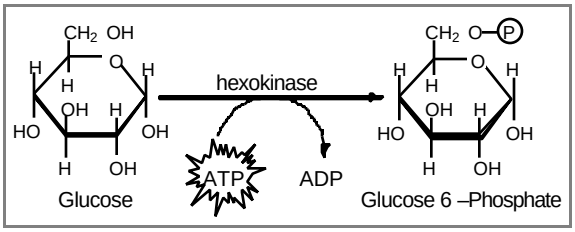
1. A net gain of two ATPs is produced by substrate-level phosphorylation.
2. Two molecules of NAD^+ are reduced to NADH. Energy conserved in the high-energy electrons of NADH can be used to make ATP by oxidative phosphorylation.

You may not want students to memorize the structures or steps of glycolysis, but you should expect them to understand the process, where it occurs, and the major molecules required and produced. It may be helpful to summarize a lecture with an overhead transparency.

Energy-investment phase:

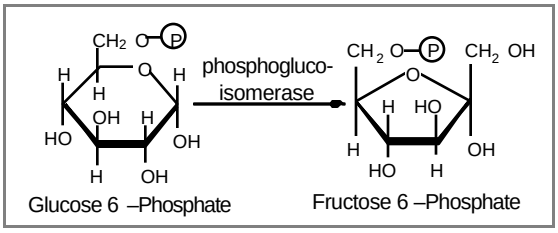
The *energy investment phase* includes five preparatory steps that split glucose in two. This process actually *consumes* ATP.

Step 1: Glucose enters the cell, and carbon six is phosphorylated. This ATP-coupled reaction:

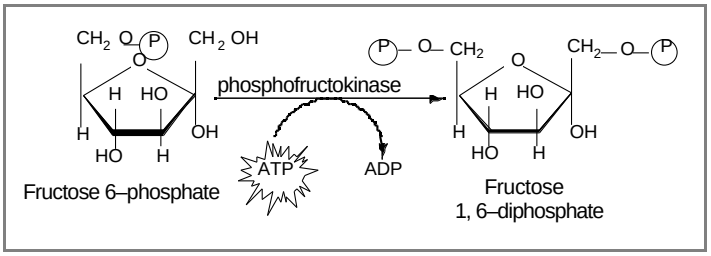


- Is catalyzed by *hexokinase* (*kinase* is an enzyme involved in phosphate transfer)
- Requires an initial investment of ATP
- Makes glucose more chemically reactive
- Produces glucose-6-phosphate; since the plasma membrane is relatively impermeable to ions, addition of an electrically charged phosphate group traps the sugar in the cell.

Step 2: An *isomerase* catalyzes the rearrangement of glucose-6-phosphate to its isomer, fructose-6-phosphate.

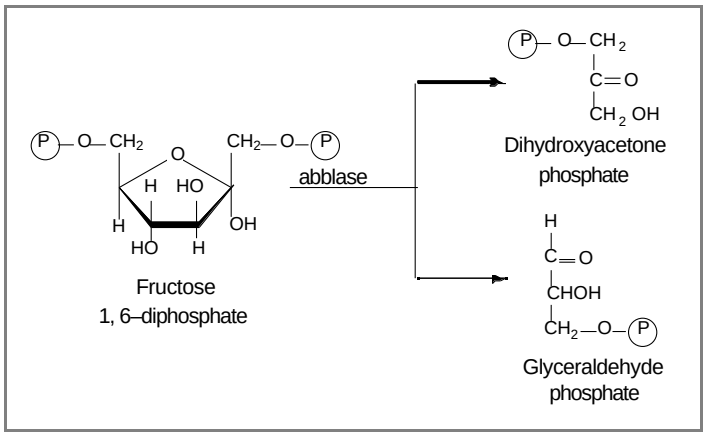


Step 3: Carbon one of fructose-6-phosphate is phosphorylated. This reaction:



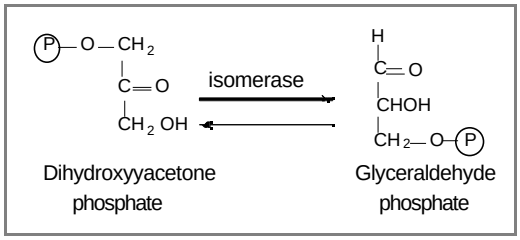
- Requires an investment of another ATP.
- Is catalyzed by *phosphofructokinase*, an allosteric enzyme that controls the rate of glycolysis. This step commits the carbon skeleton to glycolysis, a catabolic process, as opposed to being used to synthesize glycogen, an anabolic process.

Step 4: *Aldolase* cleaves the six-carbon sugar into two isomeric three-carbon sugars.



- This is the reaction for which glycolysis is named.
- For each glucose molecule that begins glycolysis, there are *two* product molecules for this and each succeeding step.

Step 5: An isomerase catalyzes the reversible conversion between the two three-carbon sugars. This reaction:

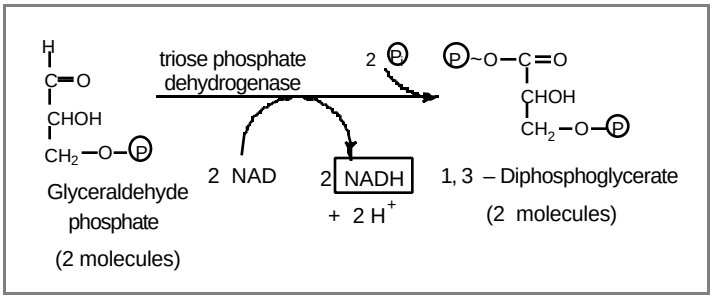


- Never reaches equilibrium because only one isomer, *glyceraldehyde phosphate*, is used in the next step of glycolysis.
- Is thus pulled towards the direction of glyceraldehyde phosphate, which is removed as fast as it forms.
- Results in the net effect that, for each glucose molecule, *two* molecules of glyceraldehyde phosphate progress through glycolysis.

Energy-yielding phase:

The *energy-yielding phase* occurs after glucose is split into two three-carbon sugars. During these reactions, sugar is oxidized, and ATP and NADH are produced.

Step 6: An enzyme catalyzes two sequential reactions:

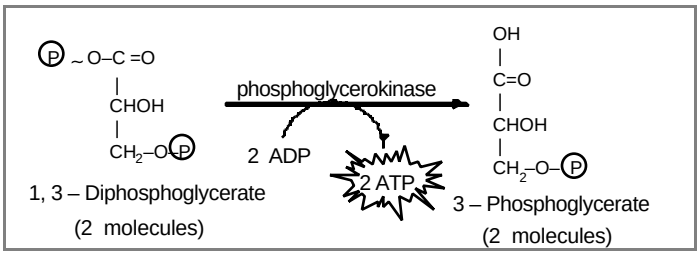


1. Glyceraldehyde phosphate is oxidized and NAD^+ is reduced to $NADH + H^+$.
   - This reaction is very exergonic and is coupled to the endergonic phosphorylation phase ($\Delta G = -10.3$ kcal/ mol).
   - For every glucose molecule, 2 NADH are produced.

2. Glyceraldehyde phosphate is phosphorylated on carbon one.
   - The phosphate source is inorganic phosphate, which is always present in the cytosol.
   - The new phosphate bond is a high energy bond with even more potential to transfer a phosphate group than ATP.

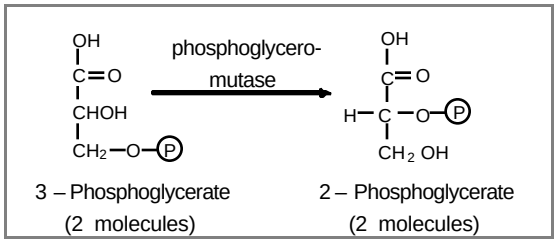
Step 7: ATP is produced by substrate-level phosphorylation.

- In a very exergonic reaction, the phosphate group with the high energy bond is transferred from 1,3-diphosphoglycerate to ADP.



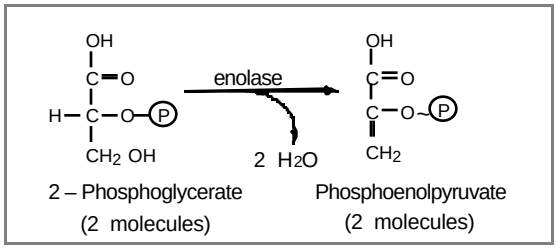
- For each glucose molecule, two ATP molecules are produced. The ATP ledger now stands at zero as the initial debt of two ATP from steps one and three is repaid.

Step 8: In preparation for the next reaction, a phosphate group on carbon three is enzymatically transferred to carbon two.



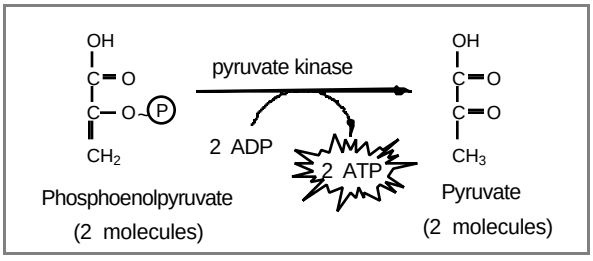
Step 9: Enzymatic removal of a water molecule:

- Creates a double bond between carbons one and two of the substrate.
- Rearranges the substrate's electrons, which transforms the remaining phosphate bond into an unstable bond.



Step 10: ATP is produced by substrate-level phosphorylation.

- In a highly exergonic reaction, a phosphate group is transferred from PEP to ADP.
- For each glucose molecule, this step produces two ATP.



Summary equation for glycolysis:

$$\begin{aligned} & C_6H_{12}O_6 \\ + \; & 2\ NAD^+ \\ + \; & 2\ ADP + 2\,\textcircled{P} \end{aligned} \longrightarrow \begin{aligned} & 2\ C_3H_4O_3\ (Pyruvate) \\ + \; & 2\ NADH + 2\ H^+ \\ + \; & 2\ ATP \\ + \; & 2\ H_2O \end{aligned}$$

- Glucose has been oxidized into two pyruvate molecules.

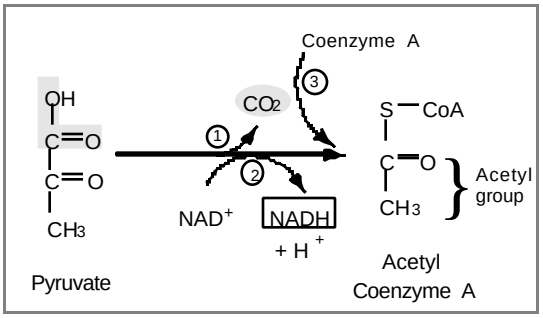
- The process is exergonic ($\Delta G = -140$ kcal/mol or –586 kJ/mol); most of the energy harnessed is conserved in the high-energy electrons of NADH and in the phosphate bonds of ATP.

C. The Krebs cycle completes the energy-yielding oxidation of organic molecules: *a closer look*

Most of the chemical energy originally stored in glucose still resides in the two pyruvate molecules produced by glycolysis. The fate of pyruvate depends upon the presence or absence of oxygen. If oxygen is present, pyruvate *enters the mitochondrion* where it is *completely oxidized* by a series of enzyme-controlled reactions.

1. Formation of acetyl CoA

The junction between glycolysis and the Krebs cycle is the oxidation of pyruvate to acetyl CoA (see Campbell, Figure 9.10):



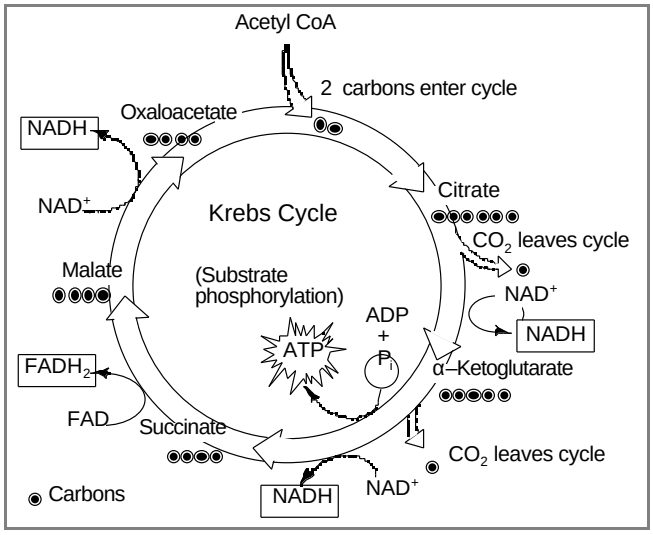
- Pyruvate molecules are translocated from the cytosol into the mitochondrion by a carrier protein in the mitochondrial membrane.
- This step is catalyzed by a *multienzyme complex* which:
  1. Removes CO_2 from the carboxyl group of pyruvate, changing it from a three-carbon to a two-carbon compound. This is the first step where CO_2 is released.
  2. Oxidizes the two-carbon fragment to acetate, while reducing NAD^+ to NADH. Since glycolysis produces two pyruvate molecules per glucose, there are *two* NADH molecules produced.
  3. Attaches coenzyme A to the acetyl group, forming acetyl CoA. This bond is unstable, making the acetyl group very reactive.

2. Krebs cycle

The Krebs cycle reactions oxidize the remaining acetyl fragments of acetyl CoA to CO_2. Energy released from this exergonic process is used to reduce coenzyme (NAD^+ and FAD) and to phosphorylate ATP (substrate-level phosphorylation).

NOTE: The FAD dinucleotide upon reduction accepts two electrons and two protons)

- A German-British scientist, Hans Krebs, elucidated this catabolic pathway in the 1930s.
- The Krebs cycle, which is also known as the *citric acid cycle* or *TCA cycle*, has eight enzyme-controlled steps that occur in the *mitochondrial matrix* (see Campbell, Figure 9.11).



For every turn of Krebs cycle:

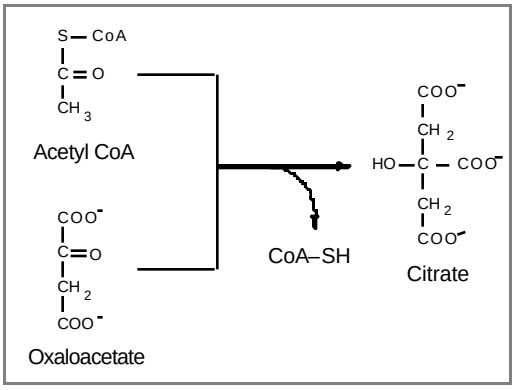
- Two carbons enter in the acetyl fragment of acetyl CoA.
- Two different carbons are oxidized and leave as CO_2.
- Coenzymes are reduced; three NADH and one $FADH_2$ are produced.
- One ATP molecule is produced by substrate-level phosphorylation.
- Oxaloacetate is regenerated.

For every glucose molecule split during glycolysis:

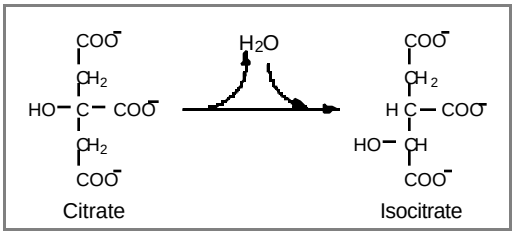
- Two acetyl fragments are produced.
- It takes two turns of Krebs cycle to complete the oxidation of glucose.

Steps of the Krebs cycle (see Campbell, Figure 9.12):

Step 1: The unstable bond of acetyl CoA breaks, and the *two-carbon* acetyl group bonds to the *four-carbon* oxaloacetate to form *six-carbon* citrate.

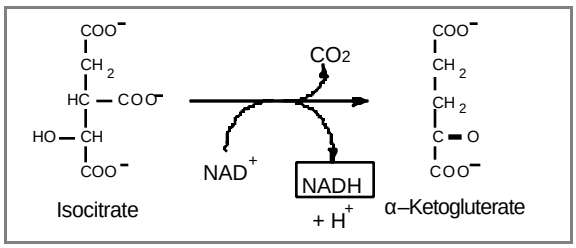


Step 2: Citrate is isomerized to isocitrate.



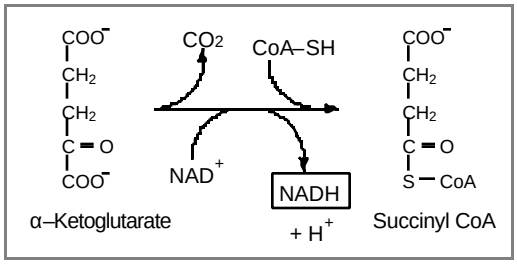
Step 3: Two major events occur during this step:

- Isocitrate loses CO_2 leaving a *five-carbon* molecule.
- The five-carbon compound is oxidized and NAD^+ is reduced.



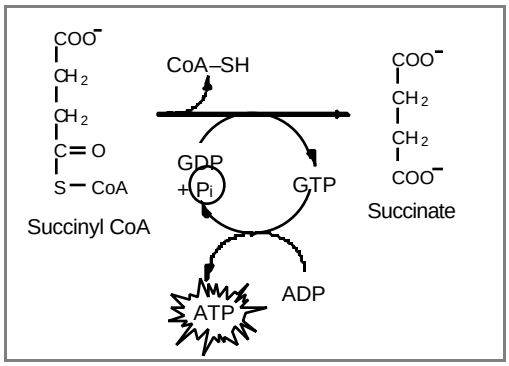
Step 4: A multienzyme complex catalyzes:

- Removal of CO_2
- Oxidation of the remaining *four-carbon* compound and reduction of NAD^+
- Attachment of CoA with a high energy bond to form succinyl CoA



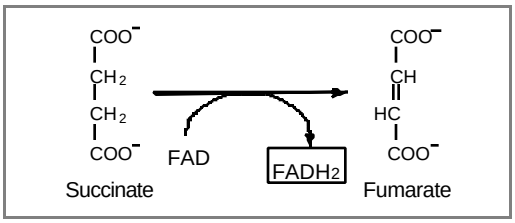
Step 5: Substrate-level phosphorylation occurs in a series of enzyme catalyzed reactions:

- The high energy bond of succinyl CoA breaks, and some energy is conserved as CoA is displaced by a phosphate group.
- The phosphate group is transferred to GDP to form GTP and succinate.
- GTP donates a phosphate group to ADP to form ATP.

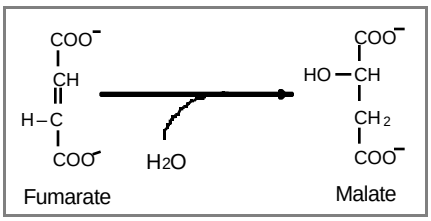


Step 6: Succinate is oxidized to fumarate and FAD is reduced.

- Two hydrogens are transferred to FAD to form $FADH_2$.
- The dehydrogenase that catalyzes this reaction is bound to the inner mitochondrial membrane.

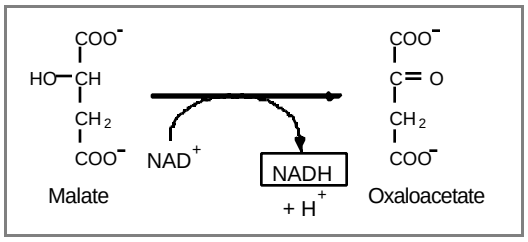


Step 7: Water is added to fumarate which rearranges its chemical bonds to form malate.



Step 8: Malate is oxidized and NAD^+ is reduced.

- A molecule of NADH is produced.
- Oxaloacetate is regenerated to begin the cycle again.



Two turns of the Krebs cycle produce two ATPs by substrate-level phosphorylation. However, *most* ATP output of respiration results from *oxidative phosphorylation.*

- Reduced coenzymes produced by the Krebs cycle (six NADH and two $FADH_2$ per glucose) carry high energy electrons to the electron transport chain.
- The ETC couples electron flow down the chain to ATP synthesis.

D. The inner mitochondrial membrane couples electron transport to ATP synthesis: *a closer look*

Only a few molecules of ATP are produced by substrate-level phosphorylation:

- Two net ATPs per glucose from glycolysis
- Two ATPs per glucose from the Krebs cycle

Most molecules of ATP are produced by oxidative phosphorylation.

- At the end of the Krebs cycle, most of the energy extracted from glucose is in molecules of NADH and $FADH_2$.
- These reduced coenzymes link glycolysis and the Krebs cycle to oxidative phosphorylation by passing their electrons down the electron transport chain to oxygen. (Though the Krebs cycle occurs only under aerobic conditions, it does not use oxygen directly. The ETC and oxidative phosphorylation require oxygen as the final electron acceptor.)
- This exergonic transfer of electrons down the ETC to oxygen is coupled to ATP synthesis.

1. The pathway of electron transport

The *electron transport chain* is made of electron carrier molecules embedded in the inner mitochondrial membrane.

- Each successive carrier in the chain has a higher electronegativity than the carrier before it, so the electrons are pulled downhill towards oxygen, the final electron acceptor and the molecule with the highest electronegativity.
- Except for ubiquinone (Q), most of the carrier molecules are proteins and are tightly bound to *prosthetic groups* (nonprotein cofactors).
- Prosthetic groups alternate between reduced and oxidized states as they accept and donate electrons.

Protein Electron Carriers	Prosthetic Group
flavoproteins	flavin mononucleotide (FMN)
iron-sulfur proteins	iron and sulfur
cytochromes	heme group

Heme group = Prosthetic group composed of four organic rings surrounding a single iron atom

Cytochrome = Type of protein molecule that contains a heme prosthetic group and that functions as an electron carrier in the electron transport chains of mitochondria and chloroplasts

- There are several cytochromes, each a slightly different protein with a heme group.
- It is the iron of cytochromes that transfers electrons.

Sequence of electron transfers along the electron transport chain (see also, Campbell, Figure 9.13):

NADH is oxidized and *flavoprotein* is reduced as high energy electrons from NADH are transferred to FMN.

↓

Flavoprotein is oxidized as it passes electrons to an *iron-sulfur protein*, Fe·S.

↓

Iron-sulfur protein is oxidized as it passes electrons to *ubiquinone* (Q).

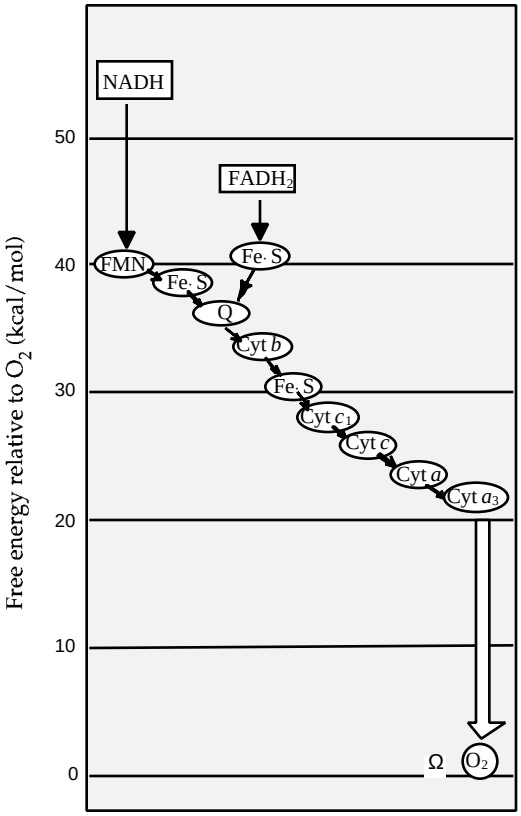
↓

Ubiquinone passes electrons on to a succession of electron carriers, most of which are cytochromes.

↓

Cyt a_3, the last cytochrome passes electrons to molecular oxygen, O_2.

↓



As molecular oxygen is reduced it also picks up two protons from the medium to form water. For every two NADHs, one O_2 is reduced to two H_2O molecules.

- $FADH_2$ also donates electrons to the electron transport chain, but those electrons are added at a lower energy level than NADH.
- The electron transport chain does not make ATP directly. It *generates a proton gradient across the inner mitochondrial membrane*, which stores potential energy that can be used to phosphorylate ADP.

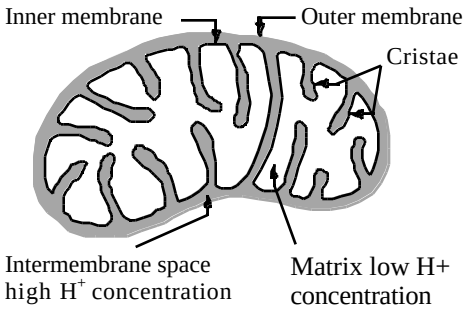
2. Chemiosmosis: the energy-coupling mechanism

The mechanism for coupling exergonic electron flow from the oxidation of food to the endergonic process of oxidative phosphorylation is *chemiosmosis.*

Chemiosmosis = The coupling of exergonic electron flow down an electron transport chain to endergonic ATP production by the creation of a proton gradient across a membrane. The proton gradient drives ATP synthesis as protons diffuse back across the membrane.

- Proposed by British biochemist, Peter Mitchell (1961)

- The term *chemiosmosis* emphasizes a coupling between (1) chemical reactions (phosphorylation) and (2) transport processes (proton transport).
- Process involved in oxidative phosphorylation and photophosphorylation.



The site of oxidative phosphorylation is the inner mitochondrial membrane, which has many copies of a protein complex, *ATP synthase*. This complex:

- Is an enzyme that makes ATP
- Uses an existing *proton gradient* across the inner mitochondrial membrane to power ATP synthesis

Cristae, or infoldings of the inner mitochondrial membrane, increase the surface area available for chemiosmosis to occur.

Membrane structure correlates with the prominent functional role membranes play in chemiosmosis:

- Using energy from exergonic electron flow, the *electron transport chain* creates the proton gradient by pumping H^+s from the *mitochondrial matrix*, across the *inner membrane* to the *intermembrane space*.
- This proton gradient is maintained, because the membrane's phospholipid bilayer is impermeable to H^+s and prevents them from leaking back across the membrane by diffusion.
- *ATP synthases* use the potential energy stored in a proton gradient to make ATP by allowing H^+ to diffuse down the gradient, back across the membrane. Protons diffuse through the ATP synthase complex, which causes the phosphorylation of ADP (see Figure 9.15).

How does the electron transport chain pump hydrogen ions from the matrix to the intermembrane space? The process is based on spatial organization of the electron transport chain in the membrane. Note that:

- Some electron carriers of the transport chain transport only electrons.
- Some electron carriers accept and release protons along with electrons. These carriers are spatially arranged so that protons are picked up from the matrix and are released into the intermembrane space.

Most of the electron carriers are organized into three complexes: 1) NADH dehydrogenase complex; 2) cytochrome *b*-c_1 complex; and 3) cytochrome oxidase complex (see Campbell, Figure 9.14).

- Each complex is an asymmetric particle that has a specific orientation in the membrane.
- As complexes transport electrons, they also harness energy from this exergonic process to pump protons across the inner mitochondrial membrane.

Mobile carriers transfer electrons between complexes. These mobile carriers are:

1. Ubiquinone (Q). Near the matrix, Q accepts electrons from the NADH dehydrogenase complex, diffuses across the lipid bilayer, and passes electrons to the cytochrome *b*-c_1 complex.
2. Cytochrome *c* (Cyt *c*). Cyt *c* accepts electrons from the cytochrome *b*-c_1 complex and conveys them to the cytochrome oxidase complex.

When the transport chain is operating:

- The pH in the intermembrane space is one or two pH units lower than in the matrix.
- The pH in the intermembrane space is the same as the pH of the cytosol because the outer mitochondrial membrane is permeable to protons.

The H^+ gradient that results is called a *proton-motive force* to emphasize that the gradient represents potential energy.

Proton motive force = Potential energy stored in the proton gradient created across biological membranes that are involved in chemiosmosis

- This force is an *electrochemical gradient* with two components:
  1. Concentration gradient of protons (chemical gradient)
  2. Voltage across the membrane because of a higher concentration of positively charged protons on one side (electrical gradient)
- It tends to drive protons across the membrane back into the matrix.

Chemiosmosis couples exergonic chemical reactions to endergonic H^+ transport, which creates the proton-motive force used to drive cellular work, such as:

- ATP synthesis in mitochondria (*oxidative phosphorylation*). The energy to create the proton gradient comes from the oxidation of glucose and the ETC.
- ATP synthesis in chloroplasts (*photophosphorylation*). The energy to create the proton gradient comes from light trapped during the energy-capturing reactions of photosynthesis.
- ATP synthesis, transport processes, and rotation of flagella in bacteria. The proton gradient is created across the plasma membrane. Peter Mitchell first postulated chemiosmosis as an energy-coupling mechanism based on experiments with bacteria.

3. Biological themes and oxidative phosphorylation

The working model of how mitochondria harvest the energy of food illustrates many of the text's integrative themes in the study of life:

- Energy conversion and utilization
- Emergent properties - Oxidative phosphorylation is an emergent property of the intact mitochondrion that uses a precise interaction of molecules.
- Correlation of structure and function - The chemiosmotic model is based upon the spatial arrangement of membrane proteins.
- Evolution - In an effort to reconstruct the origin of oxidative phosphorylation and the evolution of cells, biologists compare similarities in the chemiosmotic machinery of mitochondria to that of chloroplasts and bacteria.

E. Cellular respiration generates many ATP molecules for each sugar molecule it oxidizes: *a review*

During cellular respiration, *most* energy flows in this sequence:

Glucose NADH electron transport chain proton motive force ATP

The *net* ATP yield from the oxidation of one glucose molecule to six carbon dioxide molecules can be estimated by adding:

1. ATP produced directly by substrate-level phosphorylation during glycolysis and the Krebs cycle.
   - A net of two ATPs is produced during glycolysis. The debit of two ATPs used during the investment phase is subtracted from the four ATPs produced during the energy-yielding phase.

- Two ATPs are produced during the Krebs cycle.

2. ATP produced when chemiosmosis couples electron transport to oxidative phosphorylation.
   - The electron transport chain creates enough proton-motive force to produce a maximum of *three ATPs* for each electron pair that travels from NADH to oxygen. The average yield is actually between two and three ATPs per NADH (2.7).
   - $FADH_2$ produced during the Krebs cycle is worth a maximum of only *two ATPs*, since it donates electrons at a lower energy level to the electron transport chain.
   - In most eukaryotic cells, the ATP yield is lower due to a NADH produced during glycolysis. The mitochondrial membrane is impermeable to NADH, so its electrons must be carried across the membrane in by one of several "shuttle" reactions. Depending on which shuttle is operating, electrons can be transferred to either NAD^+ or FAD^+ . A pair of electrons passed to FAD^+ yields about two ATP, whereas a pair of electrons passed to NAD^+ yields about 13 ATP.
   - Maximum ATP yield for each glucose oxidized during cellular respiration:

Process	ATP Produced Directly by Substrate-level Phosphorylation	Reduced Coenzyme	ATP Produced by Oxidative Phosphorylation	Total
Glycolysis	*Net* 2 ATP	2 NADH	4 to 6 ATP	6-8
Oxidation of Pyruvate	_____	2 NADH	6 ATP	6
Krebs cycle	2 ATP	6 NADH 2 $FADH_2$	18 ATP 4 ATP	24
			Total	36-38

   - This tally only *estimates* the ATP yield from respiration (see Campbell, Figure 9.15). Some variables that affect ATP yield include:
     - The proton-motive force may be used to drive other kinds of cellular work such as active transport.
     - The total ATP yield is inflated (10%) by rounding off the number of ATPs produced per NADH to three.

Cellular respiration is remarkably efficient in the transfer of chemical energy from glucose to ATP.

- Estimated efficiency in eukaryotic cells is about 40%.
- Energy lost in the process is released as heat.

$$\text{Calculated by } \frac{7.3 \text{ kcal/mol ATP} \times 38 \text{ mol ATP/mol glucose}}{686 \text{ kcal/mol glucose}} \times 100$$

III. Related Metabolic Processes

A. Fermentation enables some cells to produce ATP without the help of oxygen

Food can be oxidized under *anaerobic* conditions.

Aerobic = (Aer = air; bios = life); existing in the presence of oxygen

Anaerobic = (An = without; aer = air); existing in the absence of free oxygen

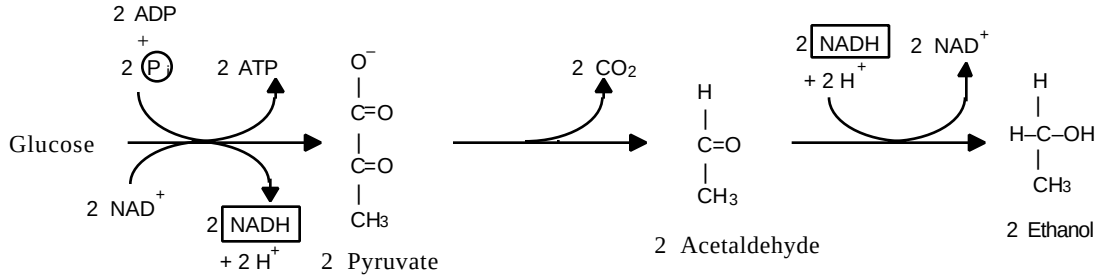
Fermentation = Anaerobic catabolism of organic nutrients

Glycolysis oxidizes glucose to two pyruvate molecules, and the oxidizing agent for this process is NAD^+, *not* oxygen.

- Some energy released from the exergonic process of glycolysis drives the production of two net ATPs by substrate-level phosphorylation.
- Glycolysis produces a net of two ATPs whether conditions are aerobic or anaerobic.
  - *Aerobic conditions*: Pyruvate is *oxidized* further by substrate-level phosphorylation and by oxidative phosphorylation and more ATP is made as NADH passes electrons to the electron transport chain. NAD^+ is regenerated in the process.
  - *Anaerobic conditions*: Pyruvate is *reduced*, and NAD^+ is regenerated. This prevents the cell from depleting the pool of NAD^+, which is the oxidizing agent necessary for glycolysis to continue. No additional ATP is produced.

Fermentation recycles NAD^+ from NADH. This process consists of anaerobic glycolysis plus subsequent reactions that regenerate NAD^+ by reducing pyruvate. Two of the most common types of fermentation are (1) alcohol fermentation and (2) lactic acid fermentation (see Campbell, Figure 9.16).

Alcohol fermentation:

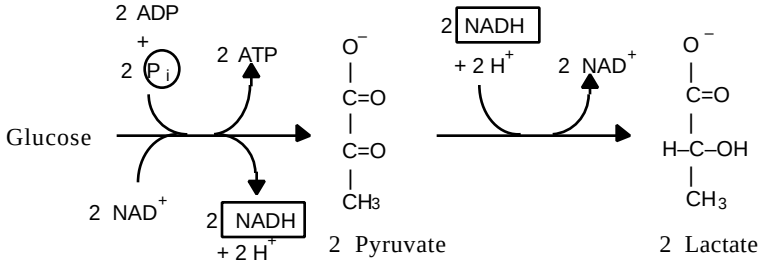


Pyruvate is converted to ethanol in two steps:

a. Pyruvate loses carbon dioxide and is converted to the two-carbon compound acetaldehyde.
b. NADH is oxidized to NAD^+ and acetaldehyde is reduced to ethanol.

Many bacteria and yeast carry out alcohol fermentation under anaerobic conditions.

Lactic acid fermentation:



NADH is oxidized to NAD^+ and pyruvate is reduced to lactate.

- Commercially important products of lactic acid fermentation include cheese and yogurt.
- When oxygen is scarce, human muscle cells switch from aerobic respiration to lactic acid fermentation. Lactate accumulates, but it is gradually carried to the liver where it is converted back to pyruvate when oxygen becomes available.

1. Fermentation and respiration compared

The anaerobic process of fermentation and aerobic process of cellular respiration are similar in that both metabolic pathways:

- Use glycolysis to oxidize glucose and other substrates to pyruvate, producing a net of two ATPs by substrate phosphorylation
- Use NAD^+ as the oxidizing agent that accepts electrons from food during glycolysis

Fermentation and cellular respiration differ in:

- How NADH is oxidized back to NAD^+. Recall that the oxidized form, NAD^+, is necessary for glycolysis to continue.
  - During fermentation, NADH passes electrons to pyruvate or some derivative. As pyruvate is reduced, NADH is oxidized to NAD^+. Electrons transferred from NADH to pyruvate or other substrates are not used to power ATP production.
  - During cellular respiration, the stepwise electron transport from NADH to oxygen not only drives oxidative phosphorylation, but regenerates NAD^+ in the process.
- Final electron acceptor
  - In fermentation, the final electron acceptor is pyruvate (lactic acid fermentation), acetaldehyde (alcohol fermentation), or some other organic molecule.
  - In cellular respiration, the final electron acceptor is oxygen.
- Amount of energy harvested
  - During fermentation, energy stored in pyruvate is unavailable to the cell.
  - Cellular respiration yields 18 times more ATP per glucose molecule than does fermentation. The higher energy yield is a consequence of the Krebs cycle which completes the oxidation of glucose and thus taps the chemical bond energy still stored in pyruvate at the end of glycolysis.
- Requirement for oxygen
  - Fermentation does not require oxygen.
  - Cellular respiration occurs only in the presence of oxygen.

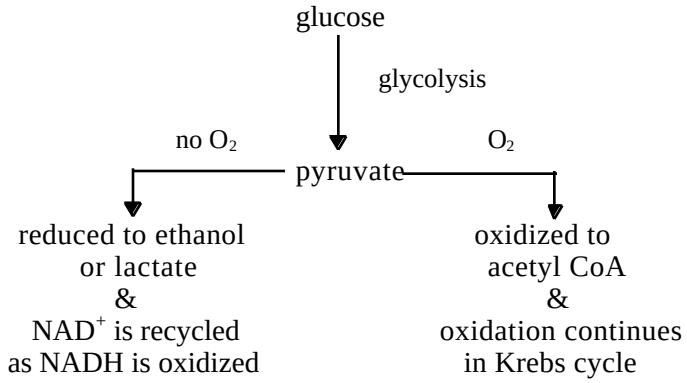
Organisms can be classified based upon the effect oxygen has on growth and metabolism.

Strict (obligate) aerobes = Organisms that require oxygen for growth and as the final electron acceptor for aerobic respiration.

Strict (obligate) anaerobes = Microorganisms that only grow in the absence of oxygen and are, in fact, poisoned by it.

Facultative anaerobes = Organisms capable of growth in either aerobic or anaerobic environments.

- Yeasts, many bacteria, and mammalian muscle cells are facultative anaerobes.
- Can make ATP by fermentation in the absence of oxygen or by respiration in the presence of oxygen.
- Glycolysis is common to both fermentation and respiration, so pyruvate is a key juncture in catabolism (see Campbell, Figure 9.18).



3. The evolutionary significance of glycolysis

The first prokaryotes probably produced ATP by glycolysis. Evidence includes the following:

- Glycolysis does not require oxygen, and the oldest known bacterial fossils date back to 3.5 billion years ago when oxygen was not present in the atmosphere.
- Glycolysis is the most widespread metabolic pathway, so it probably evolved early.
- Glycolysis occurs in the cytosol and does not require membrane-bound organelles. Eukaryotic cells with organelles probably evolved about two billion years after prokaryotic cells.

B. Glycolysis and the Krebs cycle connect to many other metabolic pathways

1. The versatility of catabolism

Respiration can oxidize organic molecules other than glucose to make ATP. Organisms obtain most calories from fats, proteins, disaccharides and polysaccharides. These complex molecules must be enzymatically hydrolyzed into simpler molecules or monomers that can enter an intermediate reaction of glycolysis or the Krebs cycle (see Campbell, Figure 9.19).

Glycolysis can accept a wide range of carbohydrates for catabolism.

- Starch is hydrolyzed to glucose in the digestive tract of animals.
- In between meals, the liver hydrolyzes glycogen to glucose.
- Enzymes in the small intestine break down disaccharides to glucose or other monosaccharides.

Proteins are hydrolyzed to amino acids.

- Organisms synthesize new proteins from some of these amino acids.
- Excess amino acids are enzymatically converted to intermediates of glycolysis and the Krebs cycle. Common intermediates are pyruvate, acetyl CoA, and α-ketoglutarate.
- This conversion process deaminates amino acids, and the resulting nitrogenous wastes are excreted and the carbon skeleton can be oxidized.

Fats are excellent fuels because they are rich in hydrogens with high energy electrons. Oxidation of one gram of fat produces twice as much ATP as a gram of carbohydrate.

- Fat sources may be from the diet or from storage cells in the body.
- Fats are digested into glycerol and fatty acids.
- Glycerol can be converted to glyceraldehyde phosphate, an intermediate of glycolysis.

- Most energy in fats is in fatty acids, which are converted into acetyl CoA by *beta oxidation*. The resulting two-carbon fragments can enter the Krebs cycle.

2. Biosynthesis (anabolic pathways)

Some organic molecules of food provide the carbon skeletons or raw materials for the synthesis of new macromolecules.

- Some organic monomers from digestion can be used *directly* in anabolic pathways.
- Some precursors for biosynthesis do not come directly from digested food, but instead come from glycolysis or Krebs cycle intermediates which are diverted into anabolic pathways.
- These anabolic pathways *require energy (*ATP) produced by catabolic pathways of glycolysis and respiration.
- Glycolysis and the Krebs cycle are metabolic interchanges that can convert one type of macromolecule to another in response to the cell's metabolic demands.

C. Feedback mechanisms control cellular respiration

Cells respond to changing metabolic needs by controlling reaction rates.

- Anabolic pathways are switched off when their products are in ample supply. The most common mechanism of control is *feedback inhibition* (see Campbell, Chapter 6).
- Catabolic pathways, such as glycolysis and Krebs cycle, are controlled by regulating enzyme activity at strategic points.

A key control point of catabolism is the third step of glycolysis, which is catalyzed by an allosteric enzyme, *phosphofructokinase* (see Campbell, Figure 9.20).

- The ratio of ATP to ADP and AMP reflects the energy status of the cell, and phosphofructokinase is sensitive to changes in this ratio.
- Citrate (produced in Krebs cycle) and ATP are *allosteric inhibitors* of phosphofructokinase, so when their concentrations rise, the enzyme slows glycolysis. As the rate of glycolysis slows, Krebs cycle also slows since the supply of acetyl CoA is reduced. This synchronizes the rates of glycolysis and Krebs cycle.
- ADP and AMP are *allosteric activators* for phosphofructokinase, so when their concentrations relative to ATP rise, the enzyme speeds up glycolysis which speeds up the Krebs cycle.
- There are other allosteric enzymes that also control the rates of glycolysis and the Krebs cycle.

REFERENCES

Campbell, N., et al. *Biology*. 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Lehninger, A.L., D.L. Nelson and M.M. Cox. *Principles of Biochemistry.* 2nd ed. New York: Worth, 1993.

Matthews, C.K. and K.E. van Holde. *Biochemistry*. 2nd ed. Redwood City, California: Benjamin/Cummings, 1996.

CHAPTER 10
PHOTOSYNTHESIS

OUTLINE

I. Photosynthesis in Nature
   A. Plants and other autotrophs are the producers of the biosphere
   B. Chloroplasts are the sites of photosynthesis in plants

II. The Pathways of Photosynthesis
   A. Evidence that chloroplasts split water molecules enabled researchers to track atoms through photosynthesis: *science as a process*
   B. The light reactions and the Calvin cycle cooperate in converting light energy to the chemical energy of food: *an overview*
   C. The light reactions transform solar energy to the chemical energy of ATP and NADPH: *a closer look*
   D. The Calvin cycle uses ATP and NADPH to convert CO_2 to sugar: *a closer look*
   E. Alternative mechanisms of carbon fixation have evolved in hot, arid climates
   F. Photosynthesis is the biosphere's metabolic foundation: *a review*

OBJECTIVES

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

1. Distinguish between autotrophic and heterotrophic nutrition.
2. Distinguish between photosynthetic autotrophs and chemosynthetic autotrophs.
3. Describe the location and structure of the chloroplast.
4. Explain how chloroplast structure relates to its function.
5. Write a summary equation for photosynthesis.
6. Explain van Niel's hypothesis and describe how it contributed to our current understanding of photosynthesis.
7. Explain the role of REDOX reactions in photosynthesis.
8. Describe the wavelike and particlelike behaviors of light.
9. Describe the relationship between an action spectrum and an absorption spectrum.
10. Explain why the absorption spectrum for chlorophyll differs from the action spectrum for photosynthesis.
11. List the wavelengths of light that are most effective for photosynthesis.
12. Explain what happens when chlorophyll or accessory pigments absorb photons.
13. List the components of a photosystem and explain their function.
14. Trace electron flow through photosystems II and I.
15. Compare cyclic and noncyclic electron flow and explain the relationship between these components of the light reactions.

16. Summarize the light reactions with an equation and describe where they occur.
17. Describe important differences in chemiosmosis between oxidative phosphorylation in mitochondria and photophosphorylation in chloroplasts.
18. Summarize the carbon-fixing reactions of the Calvin cycle and describe changes that occur in the carbon skeleton of the intermediates.
19. Describe the role of ATP and NADPH in the Calvin cycle.
20. Describe what happens to rubisco when the O_2 concentration is much higher than CO_2.
21. Describe the major consequences of photorespiration.
22. Describe two important photosynthetic adaptations that minimize photorespiration.
23. Describe the fate of photosynthetic products.

KEY TERMS

photosynthesis
autotrophs
heterotrophs
chlorophyll
mesophyll
stomata
stroma
light reactions
Calvin cycle
NADP+
photophosphorylation
carbon fixation
wavelength
electromagnetic spectrum
visible light
photons
spectrophotometer
absorption spectrum
chlorophyll *a*
action spectrum
chlorophyll *b*
carotenoids
photo systems
reaction center
primary electron acceptor
photosystem I
photosystem II
noncyclic electron flow
noncyclic photophosphorylation
cyclic electron flow
cyclic photophosphorylation
glyceraldehyde 3-phophate (G3P)
rubisco
C_3 plants
photorespiration
C_4 plants
bundle-sheath cells
mesophyll cells
PEP carboxylase
crassulacean acid metabolism
CAM plants

LECTURE NOTES

I. Photosynthesis in Nature

Photosynthesis transforms solar light energy trapped by chloroplasts into chemical bond energy stored in sugar and other organic molecules. This process:

- Synthesizes energy-rich organic molecules from the energy-poor molecules, CO_2 and H_2O
- Uses CO_2 as a carbon source and light energy as the energy source
- Directly or indirectly supplies energy to most living organisms

A. Plants and other autotrophs are the producers of the biosphere

Organisms acquire organic molecules used for energy and carbon skeletons by one of two nutritional modes: 1) autotrophic nutrition or 2) heterotrophic nutrition.

Autotrophic nutrition = (Auto = self; trophos = feed); nutritional mode of synthesizing organic molecules from inorganic raw materials

- Examples of autotrophic organisms are plants, which require only CO_2, H_2O and minerals as nutrients.
- Because autotrophic organisms produce organic molecules that enter an ecosystem's food store, autotrophs are also known as *producers*.

- Autotrophic organisms require an energy source to synthesize organic molecules. That energy source may be from light (*photoautotrophic*) or from the oxidation of inorganic substances (*chemoautotrophic*).
  - *Photoautotrophs* = Autotrophic organisms that use light as an energy source to synthesize organic molecules. Examples are photosynthetic organisms such as plants, algae, and some prokaryotes.
  - *Chemoautotrophs* = Autotrophic organisms that use the oxidation of inorganic substances, such as sulfur or ammonia, as an energy source to synthesize organic molecules. Unique to some bacteria, this is a rarer form of autotrophic nutrition.

Heterotrophic nutrition = (Heteros = other; trophos = feed); nutritional mode of acquiring organic molecules from compounds produced by other organisms.
Heterotrophs are unable to synthesize organic molecules from inorganic raw materials.

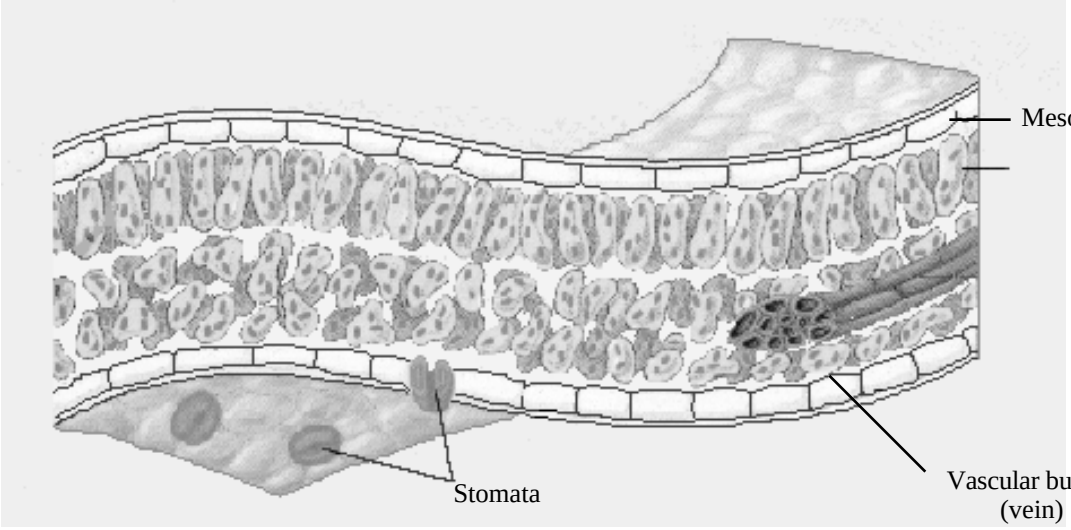
- Heterotrophs are also known as *consumers*.
- Examples are animals that eat plants or other animals.
- Examples also include *decomposers*, heterotrophs that decompose and feed on organic litter. Most fungi and many bacteria are decomposers.
- Most heterotrophs depend on photoautotrophs for food and oxygen (a by-product of photosynthesis).

B. Chloroplasts are the sites of photosynthesis in plants

Although all green plant parts have chloroplasts, leaves are the major sites of photosynthesis in most plants (see Campbell, Figure 10.2).

- *Chlorophyll* is the green pigment in chloroplasts that gives a leaf its color and that absorbs the light energy used to drive photosynthesis.

Leaf cross-section:



- Chloroplasts are primarily in cells of *mesophyll*, green tissue in the leaf's interior.
- CO_2 enters and O_2 exits the leaf through microscopic pores called *stomata*.
- Water absorbed by the roots is transported to leaves through veins or *vascular bundles* which also export sugar from leaves to nonphotosynthetic parts of the plant.

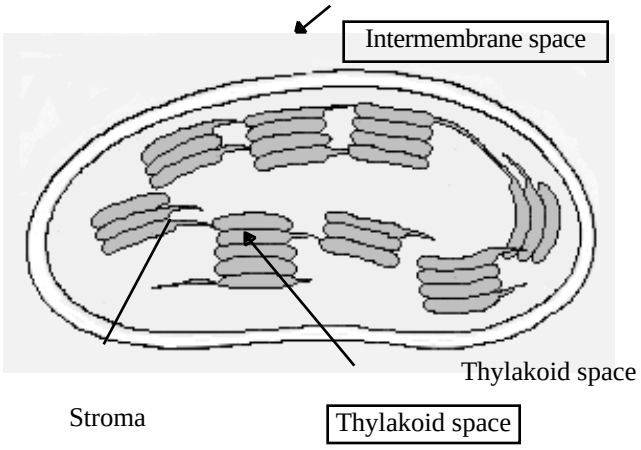
Chloroplasts are lens-shaped organelles measuring about 2 – 4 µm by 4 – 7 µm. These organelles are divided into three functional compartments by a system of membranes:

1. **Intermembrane space**

   The chloroplast is bound by a double membrane which partitions its contents from the cytosol. A narrow *intermembrane space* separates the two membranes.

2. **Thylakoid space**

   Thylakoids form another membranous system within the chloroplast. The thylakoid membrane segregates the interior of the chloroplast into two compartments: *thylakoid space* and *stroma*.



Thylakoids = Flattened membranous sacs inside the chloroplast

- Chlorophyll is found in the thylakoid membranes.
- Thylakoids function in the steps of photosynthesis that initially convert light energy to chemical energy.

Thylakoid space = Space inside the thylakoid

Grana = (Singular, granum); stacks of thylakoids in a chloroplast

3. **Stroma**

   Reactions that use chemical energy to convert carbon dioxide to sugar occur in the *stroma*, viscous fluid outside the thylakoids.

   Photosynthetic prokaryotes lack chloroplasts, but have chlorophyll built into the plasma membrane or membranes of numerous vesicles within the cell.

   - These membranes function in a manner similar to the thylakoid membranes of chloroplasts.
   - Photosynthetic membranes of cyanobacteria are usually arranged in parallel stacks of flattened sacs similar to the thylakoids of chloroplasts.

II. The Pathways of Photosynthesis

A. Evidence that chloroplasts split water molecules enabled researchers to track atoms through photosynthesis: *science as a process*

Some steps in photosynthesis are not yet understood, but the following summary equation has been known since the early 1800s:

$$6\,CO_2 + 12\,H_2O + \text{light energy} \longrightarrow C_6H_{12}O_6 + 6\,O_2 + 6\,H_2O$$

- Glucose ($C_6H_{12}O_6$) is shown in the summary equation, though the main products of photosynthesis are other carbohydrates.
- Water is on both sides of the equation because photosynthesis consumes 12 molecules and forms 6.

Indicating the net consumption of water simplifies the equation:

$$6\,CO_2 + 6\,H_2O + \text{light energy} \longrightarrow C_6H_{12}O_6 + 6\,O_2$$

- In this form, the summary equation for photosynthesis is the reverse of that for cellular respiration.
- Photosynthesis and cellular respiration both occur in plant cells, but plants do not simply reverse the steps of respiration to make food.

The simplest form of the equation is: $CO_2 + H_2O \longrightarrow CH_2O + O_2$

- CH_2O symbolizes the general formula for a carbohydrate.
- In this form, the summary equation emphasizes the production of a sugar molecule, one carbon at a time. Six repetitions produces a glucose molecule.

1. The splitting of water

The discovery that O_2 released by plants is derived from H_2O and not from CO_2, was one of the earliest clues to the mechanism of photosynthesis.

- In the 1930s, C.B. van Niel from Stanford University challenged an early model that predicted that:
  a. O_2 released during photosynthesis came from CO_2.
     $CO_2 \longrightarrow C + O_2$
  b. CO_2 was split and water was added to carbon.
     $C + H_2O \longrightarrow CH_2O$
- Van Niel studied bacteria that use hydrogen sulfide (H_2S) rather than H_2O for photosynthesis and produce yellow sulfur globules as a by-product.
  $CO_2 + 2\,H_2S \longrightarrow CH_2O + H_2O + 2\,S$
- Van Niel deduced that these bacteria split H_2S and used H to make sugar. He generalized that all photosynthetic organisms required hydrogen, but that the source varied:

  general: $CO_2 + 2\,H_2X \longrightarrow CH_2O + H_2O + 2\,X$

  sulfur bacteria: $CO_2 + 2\,H_2S \longrightarrow CH_2O + H_2O + 2\,S$

  plants: $CO_2 + 2\,H_2O \longrightarrow CH_2O + H_2O + O_2$
- Van Niel thus hypothesized that plants split water as a source of hydrogen and release oxygen as a by-product.

Scientists later confirmed van Niel's hypothesis by using a heavy isotope of oxygen (^{18}O) as a tracer to follow oxygen's fate during photosynthesis.

- If water was labeled with tracer, released oxygen was ^{18}O:
  Experiment 1: $CO_2 + 2\,H_2O^* \longrightarrow CH_2O + H_2O + O_2$
- If the ^{18}O was introduced to the plant as CO_2, the tracer did not appear in the released oxygen:
  Experiment 2: $CO_2^* + 2H_2O \longrightarrow CH_2O^* + H_2O^* + O_2$

An important result of photosynthesis is the extraction of hydrogen from water and its incorporation into sugar.

- Electrons associated with hydrogen have more potential energy in organic molecules than they do in water, where the electrons are closer to electronegative oxygen.
- Energy is stored in sugar and other food molecules in the form of these high-energy electrons.

2. Photosynthesis as a redox process

Respiration is an exergonic redox process; energy is *released* from the oxidation of sugar.

- Electrons associated with sugar's hydrogens lose potential energy as carriers transport them to oxygen, forming water.
- Electronegative oxygen pulls electrons down the electron transport chain, and the potential energy released is used by the mitochondrion to produce ATP.

Photosynthesis is an endergonic redox process; energy is *required* to reduce carbon dioxide.

- Light is the energy source that boosts potential energy of electrons as they are moved from water to sugar.
- When water is split, electrons are transferred from the water to carbon dioxide, reducing it to sugar.

B. The light reactions and the Calvin cycle cooperate in transforming light to the chemical energy of food: *an overview*

Photosynthesis occurs in two stages: the *light reactions* and the *Calvin cycle*.

Light reactions = In photosynthesis, the reactions that convert light energy to chemical bond energy in ATP and NADPH. These reactions:

- Occur in the thylakoid membranes of chloroplasts
- Reduce $NADP^+$ to NADPH
  - Light absorbed by chlorophyll provides the energy to reduce $NADP^+$ to NADPH, which temporarily stores the energized electrons transferred from water.
  - $NADP^+$ (nicotinamide adenine dinucleotide phosphate), a coenzyme similar to NAD^+ in respiration, is reduced by adding a pair of electrons along with a hydrogen nucleus, or H^+.
- Give off O_2 as a by-product from the splitting of water
- Generate ATP. The light reactions power the addition of a phosphate group to ADP in a process called *photophosphorylation.*

Calvin cycle = In photosynthesis, the carbon-fixation reactions that assimilate atmospheric CO_2 and then reduce it to a carbohydrate; named for Melvin Calvin. These reactions:

- Occur in the stroma of the chloroplast
- First incorporate atmospheric CO_2 into existing organic molecules by a process called *carbon fixation*, and then reduce fixed carbon to carbohydrate

Carbon fixation = The process of incorporating CO_2 into organic molecules.

The Calvin cycle reactions do not require light directly, but reduction of CO_2 to sugar requires the *products* of the light reactions:

- NADPH provides the reducing power.
- ATP provides the chemical energy.

Chloroplasts thus use light energy to make sugar by coordinating the two stages of photosynthesis (see Campbell, Figure 10.4).

- Light reactions occur in the thylakoids of chloroplasts.
- Calvin cycle reactions occur in the stroma.
- As $NADP^+$ and ADP contact thylakoid membranes, they pick up electrons and phosphate respectively, and then transfer their high-energy cargo to the Calvin cycle.

C. The light reactions transform solar energy to the chemical energy of ATP and NADPH: *a closer look*

To understand how the thylakoids of chloroplasts transform light energy into the chemical energy of ATP and NADPH, it is necessary to know some important properties of light.

1. The nature of sunlight

Sunlight is *electromagnetic energy*. The quantum mechanical model of electromagnetic radiation describes light as having a behavior that is both wavelike and particlelike.

a. Wavelike properties of light

- *Electromagnetic energy* is a form of energy that travels in rhythmic waves which are disturbances of electric and magnetic fields.
- A *wavelength* is the distance between the crests of electromagnetic waves.
- The electromagnetic spectrum ranges from wavelengths that are less than a nanometer (gamma rays) to those that are more than a kilometer (radio waves) (see Campbell, Figure 10.5).
- *Visible light*, which is detectable by the human eye, is only a small portion of the electromagnetic spectrum and ranges from about 380 to 750 nm. The wavelengths most important for photosynthesis are within this range of visible light.

b. Particlelike properties of light

- Light also behaves as if it consists of discrete particles or quanta called *photons*.
- Each photon has a fixed quantity of energy which is *inversely* proportional to the wavelength of light. For example, a photon of violet light has nearly twice as much energy as a photon of red light.

The sun radiates the full spectrum of electromagnetic energy.

- The atmosphere acts as a selective window that allows visible light to pass through while screening out a substantial fraction of other radiation.
- The visible range of light is the radiation that drives photosynthesis.
- Blue and red, the two wavelengths most effectively absorbed by chlorophyll, are the colors most useful as energy for the light reactions.

2. Photosynthetic pigments: the light receptors

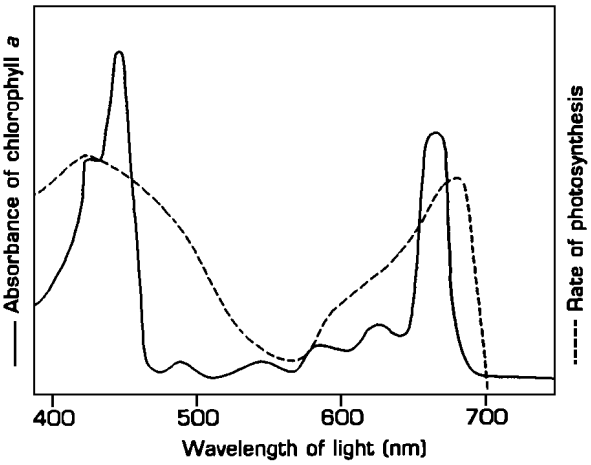
Light may be reflected, transmitted, or absorbed when it contacts matter (see Campbell, Figure 10.6).

Pigments = Substances which absorb visible light

- Different pigments absorb different wavelengths of light.
- Wavelengths that are absorbed disappear, so a pigment that absorbs all wavelengths appears black.
- When white light, which contains all the wavelengths of visible light, illuminates a pigment, the color you see is the color most reflected or transmitted by the pigment. For example, a leaf appears green because chlorophyll absorbs red and blue light but transmits and reflects green light.

Each pigment has a characteristic *absorption spectrum* or pattern of wavelengths that it absorbs. It is expressed as a graph of absorption versus wavelength.

- The absorption spectrum for a pigment in solution can be determined by using a *spectrophotometer*, an instrument used to measure what proportion of a specific wavelength of light is absorbed or transmitted by the pigment (see Campbell Methods Box).
- Since chlorophyll *a* is the light-absorbing pigment that participates directly in the light reactions, the absorption spectrum of chlorophyll *a* provides clues as to which wavelengths of visible light are most effective for photosynthesis (see Campbell, Figure 10.7a).



A graph of wavelength versus rate of photosynthesis is called an *action spectrum* and profiles the relative effectiveness of different wavelengths of visible light for driving photosynthesis (see Campbell, Figure 10.7b).

- The action spectrum of photosynthesis can be determined by illuminating chloroplasts with different wavelengths of light and measuring some indicator of photosynthetic rate, such as oxygen release or carbon dioxide consumption (see Campbell, Figure 10.7c).
- It is apparent from the action spectrum of photosynthesis that blue and red light are the most effective wavelengths for photosynthesis and green light is the least effective.

The *action spectrum* for photosynthesis does not exactly match the *absorption spectrum* for chlorophyll *a*.

- Since chlorophyll *a* is not the only pigment in chloroplasts that absorb light, the absorption spectrum for chlorophyll *a* underestimates the effectiveness of some wavelengths.
- Even though only special chlorophyll *a* molecules can participate directly in the light reactions, other pigments, called *accessory pigments*, can absorb light and transfer the energy to chlorophyll *a*.

The *accessory pigments* expand the range of wavelengths available for photosynthesis. These pigments include:

- *Chlorophyll b*, a yellow-green pigment with a structure similar to chlorophyll *a*. This minor structural difference gives the pigments slightly different absorption spectra (see Campbell, Figure 10.8).
- *Carotenoids*, yellow and orange hydrocarbons that are built into the thylakoid membrane with the two types of chlorophyll (see Campbell, Figure 10.7a).

3. Photoexcitation of chlorophyll

What happens when chlorophyll or accessory pigments absorb photons (see Campbell, Figure 10.9)?

- Colors of absorbed wavelengths disappear from the spectrum of transmitted and reflected light.
- The absorbed photon boosts one of the pigment molecule's electrons in its lowest-energy state (*ground state*) to an orbital of higher potential energy (*excited state*).

The only photons absorbed by a molecule are those with an energy state equal to the difference in energy between the ground state and excited state.

- This energy difference varies from one molecule to another. Pigments have unique absorption spectra because pigments only absorb photons corresponding to specific wavelengths.
- The photon energy absorbed is converted to potential energy of an electron elevated to the excited state.

The excited state is unstable, so excited electrons quickly fall back to the ground state orbital, releasing excess energy in the process. This released energy may be:

- Dissipated as heat
- Reradiated as a photon of lower energy and longer wavelength than the original light that excited the pigment. This afterglow is called *fluorescence.*

Pigment molecules do not fluoresce when in the thylakoid membranes, because nearby *primary electron acceptor* molecules trap excited state electrons that have absorbed photons.

- In this redox reaction, chlorophyll is photo-oxidized by the absorption of light energy and the electron acceptor is reduced.
- Because no primary electron acceptor is present, *isolated* chlorophyll fluoresces in the red part of the spectrum and dissipates heat.

4. Photosystems: light-harvesting complexes of the thylakoid membrane

Chlorophyll *a*, chlorophyll *b* and the carotenoids are assembled into *photosystems* located within the thylakoid membrane. Each photosystem is composed of:

a. Antenna complex

- Several hundred chlorophyll *a*, chlorophyll *b* and carotenoid molecules are light-gathering antennae that absorb photons and pass the energy from molecule to molecule (see Campbell, Figure 10.10). This process of resonance energy transfer is called *inductive resonance*.
- Different pigments within the antennal complex have slightly different absorption spectra, so collectively they can absorb photons from a wider range of the light spectrum than would be possible with only one type of pigment molecule.

b. Reaction-center chlorophyll

Only one of the many chlorophyll *a* molecules in each complex can actually *transfer* an excited electron to initiate the light reactions. This specialized chlorophyll *a* is located in the *reaction center*.

c. Primary electron acceptor

- Located near the reaction center, a primary electron acceptor molecule traps excited state electrons released from the reaction center chlorophyll.
- The transfer of excited state electrons from chlorophyll to primary electron acceptor molecules is the first step of the light reactions. The energy stored in the trapped electrons powers the synthesis of ATP and NADPH in subsequent steps.

Two types of photosystems are located in the thylakoid membranes, *photosystem I* and *photosystem II.*

- The reaction center of photosystem I has a specialized chlorophyll *a* molecule known as *P700,* which absorbs best at 700 nm (the far red portion of the spectrum).
- The reaction center of photosystem II has a specialized chlorophyll *a* molecule known as *P680,* which absorbs best at a wavelength of 680 nm.

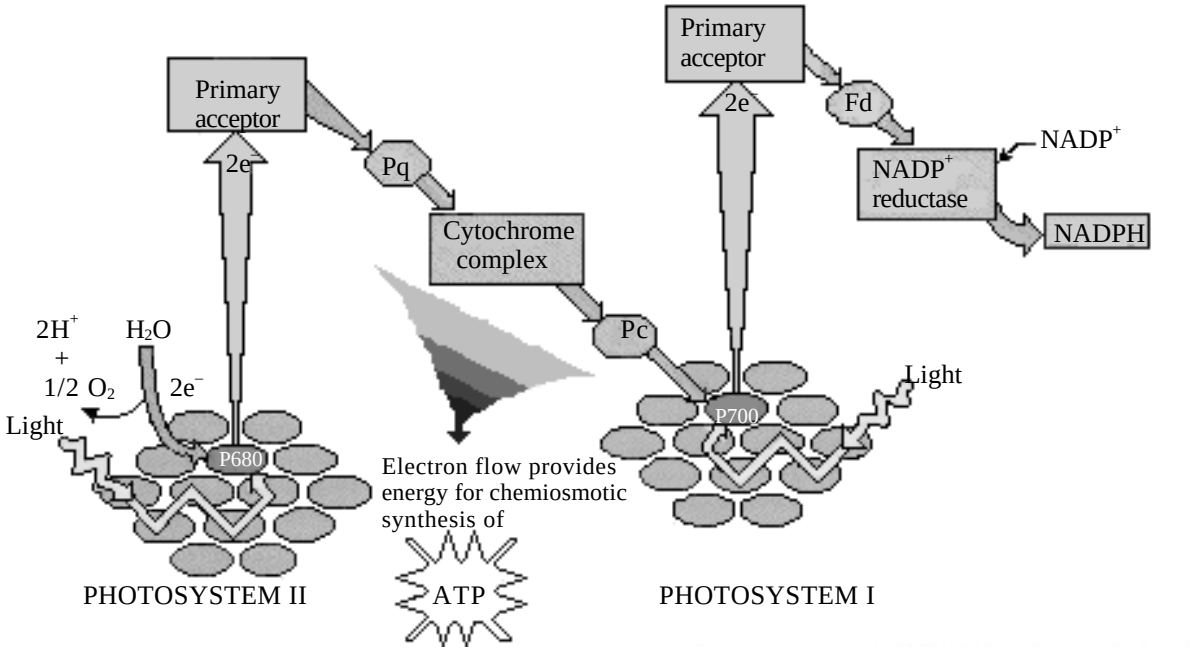
- P700 and P680 are identical chlorophyll *a* molecules, but each is associated with a different protein. This affects their electron distribution and results in slightly different absorption spectra.

5. Noncyclic electron flow

There are two possible routes for electron flow during the light reactions: *noncyclic flow* and *cyclic flow*.

Both photosystem I and photosystem II function and cooperate in noncyclic electron flow, which transforms light energy to chemical energy stored in the bonds of NADPH and ATP (see Campbell, Figure 10.11). This process:

- Occurs in the thylakoid membrane
- Passes electrons continuously from water to $NADP^+$
- Produces ATP by *noncyclic photophosphorylation*
- Produces NADPH.
- Produces O_2



Light excites electrons from P700, the reaction center chlorophyll in photosystem I. These excited state electrons do not return to the reaction center chlorophyll, but are ultimately stored in NADPH, which will later be the electron donor in the Calvin cycle.

- Initially, the excited state electrons are transferred from P700 to the primary electron acceptor for photosystem I.
- The primary electron acceptor passes these excited state electrons to *ferredoxin* (Fd), an iron-containing protein.
- *$NADP^+$ reductase* catalyzes the redox reaction that transfers these electrons from ferredoxin to $NADP^+$, producing reduced coenzyme – NADPH.
- The oxidized P700 chlorophyll becomes an oxidizing agent as its electron "holes" must be filled; photosystem II supplies the electrons to fill these holes.

When the antenna assembly of photosystem II absorbs light, the energy is transferred to the P680 reaction center .

- Electrons ejected from P680 are trapped by the photosystem II primary electron acceptor.
- The electrons are then transferred from this primary electron acceptor to an electron transport chain embedded in the thylakoid membrane. The first carrier in the chain, *plastoquinone* (Pq) receives the electrons from the primary electron acceptor. In a cascade of redox reactions, the electrons travel from Pq to a complex of two cytochromes to plastocyanin (Pc) to P700 of photosystem I.
- As these electrons pass down the electron transport chain, they lose potential energy until they reach the ground state of P700.
- These electrons then fill the electron vacancies left in photosystem I when $NADP^+$ was reduced.

Electrons from P680 flow to P700 during noncyclic electron flow, restoring the missing electrons in P700. This, however, leaves the P680 reaction center of photosystem II with missing electrons; the oxidized P680 chlorophyll thus becomes a strong oxidizing agent.

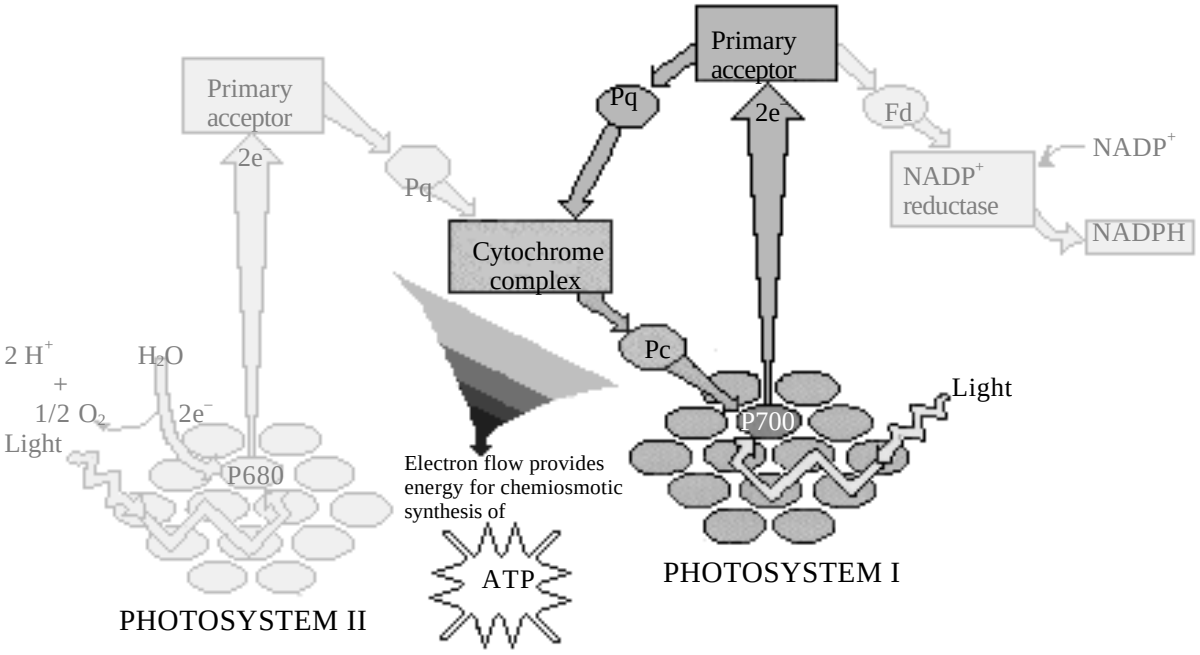
- A water-splitting enzyme extracts electrons from water and passes them to oxidized P680, which has a high affinity for electrons.
- As water is oxidized, the removal of electrons splits water into two hydrogen ions and an oxygen atom.
- The oxygen atom immediately combines with a second oxygen atom to form O_2. It is this water-splitting step of photosynthesis that releases O_2.

As excited electrons give up energy along the transport chain to P700, the thylakoid membrane couples the exergonic flow of electrons to the endergonic reactions that phosphorylate ADP to ATP.

- This coupling mechanism is *chemiosmosis*.
- Some electron carriers can only transport electrons in the company of protons.
- The protons are picked up on one side of the thylakoid membrane and deposited on the opposite side as the electrons move to the next member of the transport chain.
- The electron flow thus stores energy in the form of a proton gradient across the thylakoid membrane – a *proton-motive force*.
- An ATP synthase enzyme in the thylakoid membrane uses the proton-motive force to make ATP. This process is called *photophosphorylation* because the energy required is light.
- This form of ATP production is called *noncyclic photophosphorylation.*

6. Cyclic electron flow

Cyclic electron flow is the simplest pathway, but involves only photosystem I and generates ATP without producing NADPH or evolving oxygen.



- It is cyclic because excited electrons that leave from chlorophyll *a* at the reaction center return to the reaction center.
- As photons are absorbed by Photosystem I, the P700 reaction center chlorophyll releases excited-state electrons to the primary electron acceptor; which, in turn, passes them to ferredoxin. From there the electrons take an alternate path that sends them tumbling down the electron transport chain to P700. This is the same electron transport chain used in noncyclic electron flow.
- With each redox reaction along the electron transport chain, electrons lose potential energy until they return to their ground-state orbital in the P700 reaction center.
- The exergonic flow of electrons is coupled to ATP production by the process of chemiosmosis. This process of ATP production is called *cyclic photophosphorylation.*
- Absorption of another two photons of light by the pigments send a second pair of electrons through the cyclic pathway.

The function of the cyclic pathway is to produce additional ATP.

- It does so *without* the production of NADPH or O_2.
- Cyclic photophosphorylation supplements the ATP supply required for the Calvin cycle and other metabolic pathways. The noncyclic pathway produces approximately equal amounts of ATP and NADPH, which is not enough ATP to meet demand.
- NADPH concentration might influence whether electrons flow through cyclic or noncyclic pathways.

7. A comparison of chemiosmosis in chloroplasts and mitochondria

Chemiosmosis = The coupling of exergonic electron flow down an electron transport chain to endergonic ATP production by the creation of an electrochemical proton gradient across a membrane. The proton gradient drives ATP synthesis as protons diffuse back across the membrane.

Chemiosmosis in chloroplasts and chemiosmosis in mitochondria are similar in several ways:

- An electron transport chain assembled in a membrane translocates protons across the membrane as electrons pass through a series of carriers that are progressively more electronegative.
- An ATP synthase complex built into the same membrane, couples the diffusion of hydrogen ions down their gradient to the phosphorylation of ADP.
- The ATP synthase complexes and some electron carriers (including quinones and cytochromes) are very similar in both chloroplasts and mitochondria.

Oxidative phosphorylation in mitochondria and photophosphorylation in chloroplasts differ in the following ways:

a. Electron transport chain

- Mitochondria transfer chemical energy from food molecules to ATP. The high-energy electrons that pass down the transport chain are extracted by the oxidation of food molecules.
- Chloroplasts transform light energy into chemical energy. Photosystems capture light energy and use it to drive electrons to the top of the transport chain.

b. Spatial organization

- The inner mitochondrial membrane pumps protons from the matrix out to the intermembrane space, which is a reservoir of protons that power ATP synthase.
- The chloroplast's thylakoid membrane pumps protons from the stroma into the thylakoid compartment, which functions as a proton reservoir. ATP is produced as protons diffuse from the thylakoid compartment back to the stroma through ATP synthase complexes that have catalytic heads on the membrane's stroma side. Thus, ATP forms in the stroma where it drives sugar synthesis during the Calvin cycle (see Campbell, Figure 10.14).

There is a large proton or pH gradient across the thylakoid membrane.

- When chloroplasts are illuminated, there is a thousand-fold difference in H^+ concentration. The pH in the thylakoid compartment is reduced to about 5 while the pH in the stroma increases to about 8.
- When chloroplasts are in the dark, the pH gradient disappears, but can be reestablished if chloroplasts are illuminated.
- Andre Jagendorf (1960s) produced compelling evidence for chemiosmosis when he induced chloroplasts to produce ATP in the dark by using artificial means to create a pH gradient. His experiments demonstrated that during photophosphorylation, the function of the photosystems and the electron transport chain is to create a proton-motive force that drives ATP synthesis.

A tentative model for the organization of the thylakoid membrane includes the following:

- Proton pumping by the thylakoid membrane depends on an asymmetric placement of electron carriers that accept and release protons (H^+).
- There are three steps in the light reactions that contribute to the proton gradient across the thylakoid membrane:
  1. Water is split by Photosystem II on the thylakoid side, releasing protons in the process.
  2. As plastoquinone (Pq), a mobile carrier, transfers electrons to the cytochrome complex, it translocates protons from the stroma to the thylakoid space.
  3. Protons in the stroma are removed from solution as $NADP^+$ is reduced to NADPH.
- NADPH and ATP are produced on the side of the membrane facing the stroma where sugar is synthesized by the Calvin cycle.

Students must be able to visualize the spatial arrangement of electron carriers in the membrane, since this arrangement is a crucial component of the chemiosmosis model. Figure 10.15 nicely illustrates this spatial arrangement.

8. Summary of light reactions

During *noncyclic electron flow*, the photosystems of the thylakoid membrane transform light energy to the chemical energy stored in NADPH and ATP. This process:

- Pushes low energy-state electrons from water to NADPH, where they are stored at a higher state of potential energy. NADPH, in turn, is the electron donor used to reduce carbon dioxide to sugar (Calvin cycle).
- Produces ATP from this light driven electron current
- Produces oxygen as a by-product

During *cyclic electron flow*, electrons ejected from P700 reach ferredoxin and flow *back* to P700. This process:

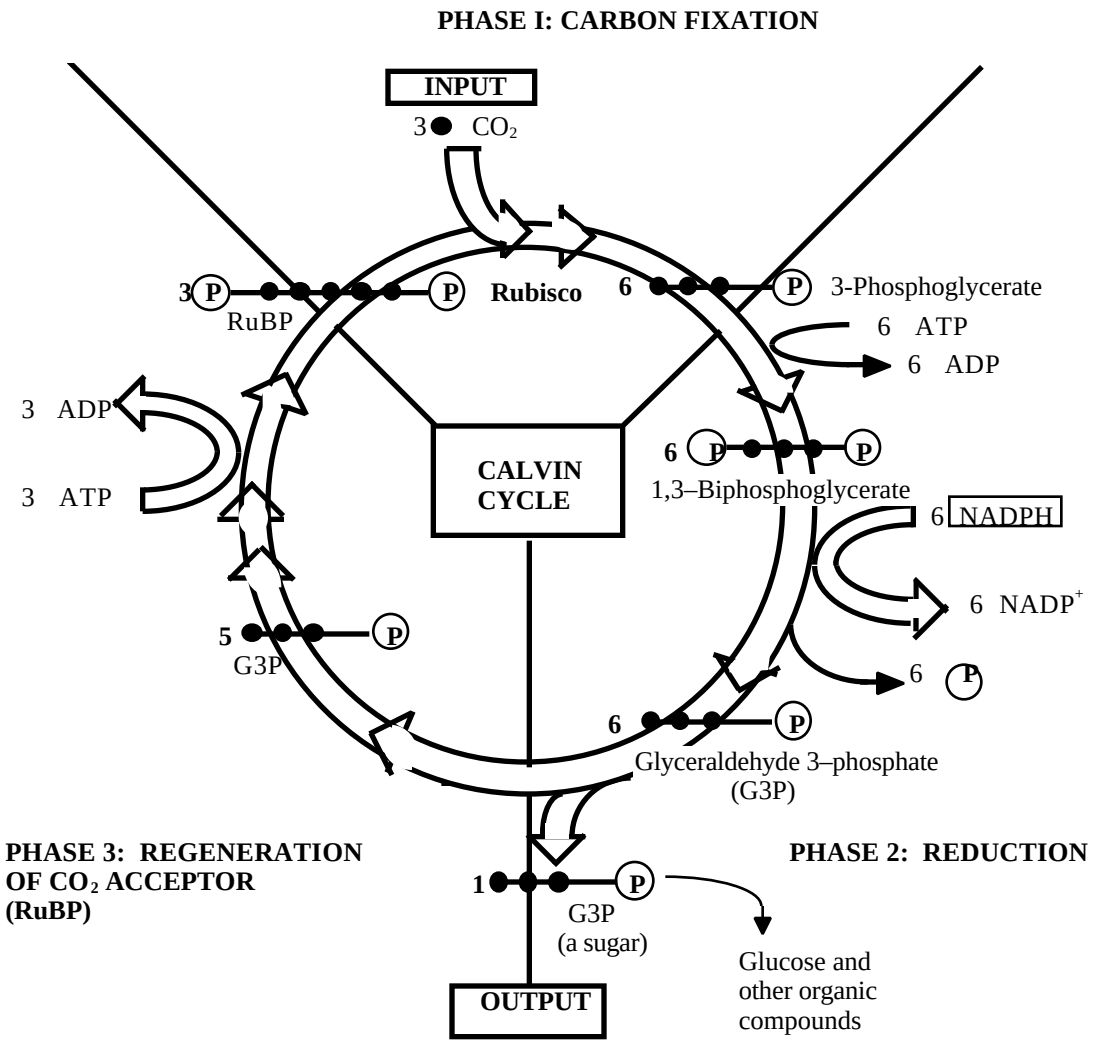
- Produces ATP
- Unlike noncyclic electron flow, does *not* produce NADPH or O_2

D. The Calvin cycle uses ATP and NADPH to convert CO_2 to sugar: *a closer look*

ATP and NADPH produced by the light reactions are used in the Calvin cycle to reduce carbon dioxide to sugar.

- The Calvin cycle is similar to the Krebs cycle in that the starting material is regenerated by the end of the cycle.
- Carbon enters the Calvin cycle as CO_2 and leaves as sugar.
- ATP is the energy source, while NADPH is the reducing agent that adds high-energy electrons to form sugar.
- The Calvin cycle actually produces a three-carbon sugar *glyceraldehyde 3-phosphate* (G3P).

Students can easily follow the Calvin cycle if you use a diagram for reference, such as Figure 10.16. This figure is especially helpful because you can go through the cycle twice; once to count carbons and once to follow the reactions pointing out where ATP and NADPH are used, where glyceraldehyde phosphate is produced and how RuBP is regenerated.

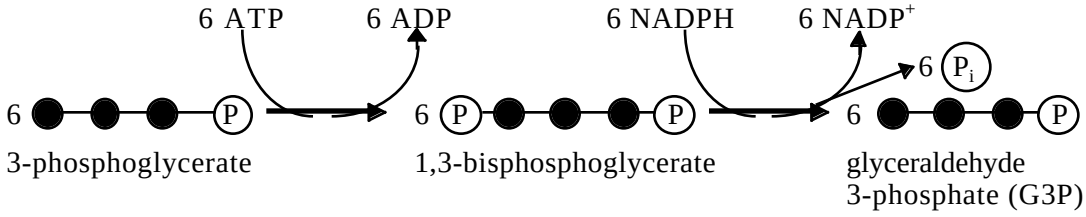


For the Calvin cycle to synthesize one molecule of sugar (G3P), three molecules of CO_2 must enter the cycle. The cycle may be divided into three phases:

Phase 1: Carbon Fixation. The Calvin cycle begins when each molecule of CO_2 is attached to a five-carbon sugar, *ribulose biphosphate* (*RuBP*).

- This reaction is catalyzed by the enzyme *RuBP carboxylase* (*rubisco*) – one of the most abundant proteins on Earth..
- The product of this reaction is an unstable six-carbon intermediate that immediately splits into two molecules of 3-phosphoglycerate.
- For every three CO_2 molecules that enter the Calvin cycle via rubisco, three RuBP molecules are carboxylated forming six molecules of 3-phosphoglycerate.

Phase 2: Reduction. This endergonic reduction phase is a two-step process that couples ATP hydrolysis with the reduction of 3-phosphoglycerate to glyceraldehyde phosphate.



- An enzyme phosphorylates 3-phosphoglycerate by transferring a phosphate group from ATP. This reaction:
  - Produces 1, 3-bisphosphoglycerate
  - Uses six ATP molecules to produce six molecules of 1,3-bisphosphoglycerate.
  - Primes 1,3-bisphosphoglycerate for the addition of high-energy electrons from NADPH.
- Electrons from NADPH reduce the carboxyl group of 1,3-bisphosphoglycerate to the aldehyde group of glyceraldehyde 3-phosphate (G3P).
  - The product, G3P, stores more potential energy than the initial reactant, 3-phosphoglycerate.
  - G3P is the same three-carbon sugar produced when glycolysis splits glucose.
- For every three CO_2 molecules that enter the Calvin cycle, six G3P molecules are produced, only one of which can be counted as net gain.
  - The cycle begins with three five-carbon RuBP molecules – a total of 15 carbons.
  - The six G3P molecules produced contain 18 carbons, a net gain of three carbons from CO_2.
  - One G3P molecule exits the cycle; the other five are recycled to regenerate three molecules of RuBP.

Phase 3: Regeneration of CO_2 acceptor (RuBP). A complex series of reactions rearranges the carbon skeletons of five G3P molecules into three RuBP molecules.

- These reactions require three ATP molecules.
- RuBP is thus regenerated to begin the cycle again.

For the net synthesis of one G3P molecule, the Calvin cycle uses the products of the light reactions:

- 9 ATP molecules
- 6 NADPH molecules

G3P produced by the Calvin cycle is the raw material used to synthesize glucose and other carbohydrates.

- The Calvin cycle uses 18 ATP and 12 NADPH molecules to produce one glucose molecule.

E. Alternative mechanisms of carbon fixation have evolved in hot, arid climates

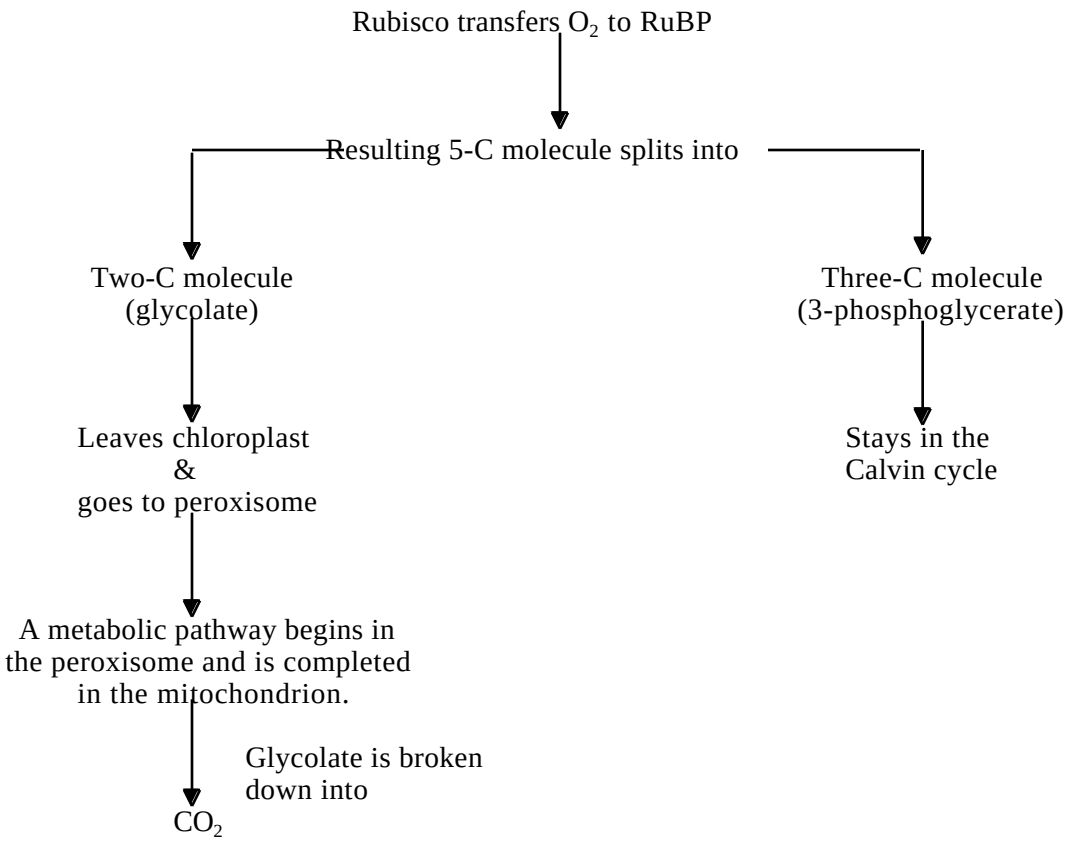
1. Photorespiration: an evolutionary relic?

A metabolic pathway called *photorespiration* reduces the yield of photosynthesis.

Photorespiration = In plants, a metabolic pathway that consumes oxygen, evolves carbon dioxide, produces no ATP and decreases photosynthetic output.

- Occurs because the active site of rubisco can accept O_2 as well as CO_2
- Produces no ATP molecules
- Decreases photosynthetic output by reducing organic molecules used in the Calvin cycle

When the O_2 concentration in the leaf's air spaces is higher than CO_2 concentration, rubisco accepts O_2 and transfers it to RuBP. (The "photo" in photorespiration refers to the fact that this pathway usually occurs in light when photosynthesis reduces CO_2 and raises O_2 in the leaf spaces.)



(The "respiration" in photorespiration refers to the fact that this process uses O_2 and releases CO_2.)

Some scientists believe that photorespiration is a metabolic relic from earlier times when the atmosphere contained less oxygen and more carbon dioxide than is present today.

- Under these conditions, when rubisco evolved, the inability of the enzyme's active site to distinguish carbon dioxide from oxygen would have made little difference.
- This affinity for oxygen has been retained by rubisco and some photorespiration is bound to occur.

Whether photorespiration is beneficial to plants is not known.

- It is known that some crop plants (e.g., soybeans) lose as much as 50% of the carbon fixed by the Calvin cycle to photorespiration.
- If photorespiration could be reduced in some agricultural plants, crop yields and food supplies would increase.

Photorespiration is fostered by hot, dry, bright days.

- Under these conditions, plants close their stomata to prevent dehydration by reducing water loss from the leaf.
- Photosynthesis then depletes available carbon dioxide and increases oxygen within the leaf air spaces. This condition favors photorespiration.

Certain species of plants, which live in hot arid climates, have evolved alternate modes of carbon fixation that minimize photorespiration. C_4 and CAM are the two most important of these photosynthetic adaptations.

2. C_4 plants

The Calvin cycle occurs in most plants and produces 3-phosphoglycerate, a three-carbon compound, as the first stable intermediate.

- These plants are called *C_3 plants* because the first stable intermediate has three carbons.
- Agriculturally important C_3 plants include rice, wheat, and soybeans.

Many plant species preface the Calvin cycle with reactions that incorporate carbon dioxide into four-carbon compounds.

- These plants are called *C_4 plants*.
- The C_4 pathway is used by several thousand species in at least 19 families including corn and sugarcane, important agricultural grasses.
- This pathway is adaptive, because it enhances carbon fixation under conditions that favor photorespiration, such as hot, arid environments.

Leaf anatomy of C_4 plants spatially segregates the Calvin cycle from the initial incorporation of CO_2 into organic compounds. There are two distinct types of photosynthetic cells:

1. Bundle-sheath cells
   - Arranged into tightly packed sheaths around the veins of the leaf
   - Thylakoids in the chloroplasts of bundle-sheath cells are not stacked into grana.
   - The Calvin cycle is confined to the chloroplasts of the bundle sheath.
   2. Mesophyll cells are more loosely arranged in the area between the bundle sheath and the leaf surface.

The Calvin cycle of C4 plants is preceded by incorporation of CO_2 into organic compounds in the mesophyll (see Campbell, Figure 10.18)

Step 1: CO_2 is added to phosphoenolpyruvate (PEP) to form oxaloacetate, a four-carbon product.

- *PEP carboxylase* is the enzyme that adds CO_2 to PEP. Compared to rubisco, it has a much greater affinity for CO_2 and has *no* affinity for O_2.
- Thus, PEP carboxylase can fix CO_2 efficiently when rubisco cannot ¾ under hot, dry conditions that cause stomata to close, CO_2 concentrations to drop and O_2 concentrations to rise.

Step 2: After CO_2 has been fixed by mesophyll cells, they convert oxaloacetate to another four-carbon compound (usually malate).

Step 3: Mesophyll cells then export the four-carbon products (e.g., malate) through plasmodesmata to bundle-sheath cells.

- In the bundle-sheath cells, the four carbon compounds release CO_2, which is then fixed by rubisco in the Calvin cycle.
- Mesophyll cells thus pump CO_2 into bundle-sheath cells, minimizing photorespiration and enhancing sugar production by maintaining a CO_2 concentration sufficient for rubisco to accept CO_2 rather than oxygen.

3. CAM plants

A second photosynthetic adaptation exists in succulent plants adapted to very arid conditions. These plants open their stomata primarily at night and close them during the day (opposite of most plants).

- This conserves water during the day, but prevents CO_2 from entering the leaves.

- When stomata are open at night, CO_2 is taken up and incorporated into a variety of organic acids. This mode of carbon fixation is called *crassulacean acid metabolism (CAM)*.
- The organic acids made at night are stored in vacuoles of mesophyll cells until morning, when the stomata close.
- During daytime, light reactions supply ATP and NADPH for the Calvin cycle. At this time, CO_2 is released from the organic acids made the previous night and is incorporated into sugar in the chloroplasts.

The CAM and C_4 pathways:

- Are similar in that CO_2 is first incorporated into organic intermediates before it enters the Calvin cycle.
- Differ in that the initial steps of carbon fixation in C_4 plants are structurally separate from the Calvin cycle; in CAM plants, the two steps occur at separate times.

Regardless of whether the plant uses a C_3, C_4 or CAM pathway, all plants use the Calvin cycle to produce sugar from CO_2.

F. Photosynthesis is the biosphere's metabolic foundation: *a review*

On a global scale, photosynthesis makes about 160 billion metric tons of carbohydrate per year. No other chemical process on Earth is more productive or is as important to life.

- Light reactions capture solar energy and use it to:
  - Produce ATP
  - Transfer electrons from water to $NADP^+$ to form NADPH
- The Calvin cycle uses ATP and NADPH to fix CO_2 and produce sugar.

Photosynthesis transforms light energy to chemical bond energy in sugar molecules.

- Sugars made in chloroplasts supply the entire plant with chemical energy and carbon skeletons to synthesize organic molecules.
- Nonphotosynthetic parts of a plant depend on organic molecules exported from leaves in veins.
  - The disaccharide *sucrose* is the transport form of carbohydrate in most plants.
  - Sucrose is the raw material for cellular respiration and many anabolic pathways in nonphotosynthetic cells.
- Much of the sugar is *glucose* – the monomer linked to form *cellulose*, the main constituent of plant cell walls.

Most plants make more organic material than needed for respiratory fuel and for precursors of biosynthesis.

- Plants consume about 50% of the photosynthate as fuel for cellular respiration.
- Extra sugars are synthesized into starch and stored in storage cells of roots, tubers, seeds, and fruits.
- Heterotrophs also consume parts of plants as food.

Photorespiration can reduce photosynthetic yield in hot dry climates. Alternate methods of carbon fixation minimize photorespiration.

- C4 plants spatially separate carbon fixation from the Calvin cycle.
- CAM plants temporally separate carbon fixation from the Calvin cycle.

REFERENCES

Atkins, P.W. *Atoms, Electrons, and Change.* New York, Oxford: W.H. Freeman and Company, 1991. Chapter 9, "Light and Life" is a witty, imaginative description of photosynthesis. Though written for a lay audience, it is probably best appreciated by someone already familiar with photosynthesis.

Campbell, N., et al. *Biology.* 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Lehninger, A.L., D.L. Nelson and M.M. Cox. *Principles of Biochemistry.* 2nd ed. New York: Worth, 1993.

Matthews, C.K. and K.E. van Holde. *Biochemistry*. 2nd ed. Redwood City, California: Benjamin/Cummings, 1996.

CHAPTER 11
CELL COMMUNICATION

OUTLINE

I. An Overview of Cell Signaling
   A. Cell signaling evolved early in the history of life
   B. Communicating cells may be close together or far apart
   C. The three stages of cell signaling are reception, transduction, and response

II. Signal Reception and the Initiation of Transduction
   A. A chemical signal binds to a receptor protein, causing the protein to change shape
   B. Most signal receptors are plasma-membrane proteins

III. Signal Transduction Pathways
   A. Pathways relay signals from receptors to cellular responses
   B. Protein phosphorylation, a common mode of regulation in cells, is a major mechanism of signal transduction
   C. Certain small molecules and ions are key components of signaling pathways (second messengers)

IV. Cellular Responses to Signals
   A. In response to a signal, a cell may regulate activities in the cytoplasm or transcription in the nucleus
   B. Elaborate pathways amplify and specify the cell's responses to signals

OBJECTIVES

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

1. Categorize chemical signals in terms of the proximity of the communicating cells.
2. Overview the basic elements of a signaling system of a target cell.
3. Describe the nature of a ligand-receptor interaction and state how such interactions initiate a signal transduction system.
4. Compare and contrast G-protein-linked receptors, tyrosine-kinase receptors, and ligand-gated ion channels.
5. Describe how phosphorylation propagates signal information.
6. Describe how cAMP is formed and how it propagates signal information.
7. Describe how the cytoplasmic concentration of Ca^{2+} can be altered and how this increased pool of Ca^{2+} is involved with signal transduction.
8. Describe how signal information is transduced into cellular responses in the cytoplasm and in the nucleus.
9. Describe how signal amplification is accomplished in target cells.

10. Describe how target cells discriminate among signals and how the same signal can elicit multiple cellular responses.

KEY WORDS

signal transduction pathway	tyrosine kinase	cyclic AMP (cAMP)
local regulator	tyrosine-kinase receptor	adenylyl cyclase
hormone	ligand-gated ion channels	diacylglycerol (DAG)
ligand	protein kinase	inositol triphosphate (IP_3)
G-protein-linked receptor	protein phosphatase	calmodulin
G-protein	second messenger	

LECTURE NOTES

Regulation is an essential feature of life. It unifies the various levels of biological organization by embracing the fields of molecular and cell biology, organismal biology, and population biology and ecology. It provides the necessary coordination for all aspects of life, including metabolism, growth, development, and reproduction.

Chemical substances are the principal agents of biological regulation and they exert their effects on cells through signaling systems. This chapter describes the fundamental components of cell signaling systems.

I. An Overview of Cell Signaling

A. Cell signaling evolved early in the history of life

Yeast mating behavior is coordinated by chemical signaling.

- Yeast (unicellular eukaryotes) have two mating types: *a* and α.
- Type *a* cells secrete an *a*-factor chemical signal; type α cells secrete α-factor.
- The binding of *a*-factor to type α cells and the binding of α-factor to type *a* cells induces type *a* and type α to move toward one another and fuse.

The steps by which yeast mating signals are converted into yeast cell responses are similar to how chemical signals in prokaryotes (bacteria), plants, and animals are converted to specific cell responses.

In general, the steps by which a chemical signal is converted to a specific cell response is called a *signal transduction pathway*.

B. Communicating cells may be close together or far apart

A chemical signal that communicates between two nearby cells is called a *local regulator*. Two types of local signaling have been described in animals: paracrine signaling and synaptic signaling.

- In paracrine signaling, one cell secretes the signal into extracellular fluid and the signal acts on a nearby target cell. Examples of signals which act in a paracrine fashion are growth factors, a group of factors which stimulate cells to divide and grow.
- In synaptic signaling, a nerve cell releases a signal (e.g., neurotransmitter) into a synapse, the narrow space between the transmitting cell and a target cell, such as another nerve cell or muscle cell.

A chemical signal which communicates between cells some distance apart is called a *hormone*.

Hormones have been described in both plants (e.g., ethylene, a gas which promotes growth and fruit ripening) and animals (e.g., insulin, a protein which controls various aspects of metabolism, including the regulation of blood glucose levels).

The distinction between local regulators and hormones is for convenience. A particular chemical signal may act both as a local regulator and as a hormone.

> Insulin, for example, may act in a paracrine fashion on adjacent cells (e.g., other insulin cells in the pancreas, acting to inhibit the further release of insulin in a negative feedback manner) and in a hormonal fashion on distant cells (e.g., liver cells, which store carbohydrate as glycogen).

Cells also may communicate by direct contact. Some plant and animal cell possess junctions though which signals can travel between adjacent cells.

C. The three stages of cell signaling are reception, transduction, and response

In order for a chemical signal to elicit a specific response, the target cell must possess a signaling system for the signal. Cells which do not possess the appropriate signaling system do not respond to the signal.

The signaling system of a target cell consists of the following elements:

- *Signal reception.* The signal binds to a specific cellular protein called a receptor, which is often located on the surface of the cell.
- *Signal transduction.* The binding of the signal changes the receptor in some way, usually a change in conformation or shape. The change in receptor initiates a process of converting the signal into a specific cellular response; this process is called signal transduction. The transduction system may have one or many steps.
- *Cellular response.* The transduction system triggers a specific cellular response. The response can be almost any cellular activity, such as activation of an enzyme or altered gene expression.

The critical features of the target cell signaling system were elucidated by Earl Sutherland (awarded the Nobel Prize in 1971 for his contributions to the understanding of signal transduction) and colleagues who were working on how the hormone, epinephrine, affects carbohydrate metabolism (e.g., glycogen breakdown to glucose-1-phosphate) in liver cells.

- Epinephrine stimulates glycogen breakdown by stimulating the cytosolic enzyme, glycogen phosphorylase (cellular response).
- Epinephrine could only stimulate glycogen phosphorylase activity when presented to intact cells, suggesting that:
  - The plasma membrane is critical for transmitting the signal (reception)
  - Activation of glycogen phosphorylase required the presence of an intermediate step or steps in side the cell (signal transduction)

The mechanisms of the cell signaling process help ensure that important processes occur in the right cells, at the right time, and in proper coordination with other cells of the organism.

II. Signal Reception and the Initiation of Transduction

A. A chemical signal binds to a receptor protein, causing the protein to change shape

Chemical signals bind to specific receptors.

- The signal molecule is complementary to a specific region of the receptor protein; this interaction is similar to that between a substrate and an enzyme.
- The signal behaves as a *ligand*, a term for a small molecule that binds to another, larger molecule.

Binding of the ligand to the receptor can lead to the following events:

- Alteration in receptor conformation or shape; such alterations may lead to the activation of the receptor which enables it to interact with other cellular molecules
- Aggregation of receptor complexes

B. Most signal receptors are plasma-membrane proteins

Many signal molecules cannot pass freely through the plasma membrane. The receptors for such signal molecules are located on the plasma membrane. Three families of plasma-membrane receptors—*G-protein-linked receptors, tyrosine kinase receptors,* and ion channel receptors— will be described.

1. G-protein-linked receptors

The structure of a ***G-protein-linked receptor*** is characterized by a single polypeptide chain that is threaded back and forth through the plasma membrane in such a way as to possess seven transmembrane domains. An example of a G-protein-linked receptor is the epinephrine receptor.

The receptor propagates the signal by interacting with a variety of proteins on the cytoplasmic side of the membrane called G-proteins, so named because they bind guanine nucleotides, GTP and GDP.

- The function of the G-protein is influenced by the nucleotide to which it is bound:
  - G-proteins bound to GDP are inactive.
  - G-proteins bound to GTP are active.
- When a ligand binds to a G-protein-linked receptor, the receptor changes its conformation and interacts with a G-protein. This interaction causes the GDP bound to the inactive G-protein to be displaced by GTP, thereby activating the G-protein.
- The activated G-protein binds to another protein, usually an enzyme, resulting in the activation of a subsequent target protein.
- The activation state of the G-protein is only temporary, because the active G-protein possesses endogenous GTPase activity, which hydrolyzes the bound GTP to GDP.

• G-protein-linked receptors and G-proteins mediate a host of critical metabolic and developmental processes (e.g., blood vessel growth and development). Defects in the G-protein signaling system form the bases of many human disease states (e.g., cholera).

2. Tyrosine-kinase receptors

The structure of a tyrosine-kinase receptor is characterized by an extracellular ligand-binding domain and a cytosolic domain possessing tyrosine kinase enzyme activity. Examples of tyrosine-kinase receptors are the receptors for numerous growth factors, such as PDGFs, the family of factors which serve as external modulators of the cell-cycle control system.

Propagation of the signal involves several steps as follows:

- Ligand binding causes aggregation of two receptor units, forming receptor dimers.
- Aggregation activates the endogenous tyrosine kinase activity on the cytoplasmic domains.
- The endogenous tyrosine kinase catalyzes the transfer of phosphate groups from ATP to the amino acid tyrosine contained in a particular protein. In this case, the tyrosines which are phosphoryled are in the cytoplasmic domain of the tyrosine-kinase receptor itself (thus, this step is an autophosphorylation).

- The phosphorylated domain of the receptor interacts with other cellular proteins, resulting in the activation of a second, or relay, protein. The relay proteins may or may not be phosphorylated by the tyrosine kinase of the receptor. Many different relay proteins may be activated, each leading to the initiation of many, possibly different, transduction systems.
- One of the activated relay proteins may be *protein phosphatase*, an enzyme which hydrolyzes phosphate groups off of proteins. The dephosphorylation of the tyrosines on the tyrosine kinase domain of the receptor results in inactivating the receptor and the termination of the signal process.

3. Ion-channel receptors

Some chemical signals bind to *ligand-gated ion channels*. These are protein pores in the membrane that open or close in response to ligand binding, allowing or blocking the flow of specific ions (e.g., Na^+, Ca^{2+}). An example of an ion-gated channel would be the binding of a neurotransmitter to a neuron, allowing the inward flow of Na^{2+} that leads to the depolarization of the neuron and the propagation of a nervous impulse to adjacent cells.

Not all signal receptors are located on the plasma membrane. Some are proteins located in the cytoplasm or nucleus of target cells.

- In order for a chemical signal to bind to these intracellular receptors, the signal molecule must be able to pass through plasma membrane. Examples of signals which bind to intracellular receptors include the following:
  - Nitric oxide (NO)
  - Steroid (e.g., estradiol, progesterone, testosterone) and thyroid hormones of animals

III. Signal Transduction Pathways

A. Pathways relay signals from receptors to cellular responses

Ligand binding to a receptor triggers the first step in the chain of reactions—the signal transduction pathway— that leads to the conversion of the signal to a specific cellular response.

- The transduction system does not physically pass along the signal molecule, rather the *information* is passed along. At each step of the process, the nature of the information is converted, or transduced, into a different form.

B. Protein phosphorylation, a common mode of regulation in cells, is a major mechanism of signal transduction

The process of phosphorylation, or the transferring a phosphate group from ATP to a protein substrate, which is catalyzed by enzymes called *protein kinases*, is a common cellular mechanism for regulating the functional activity of proteins.

Protein phosphorylation is commonly used in signal pathways in the cytoplasm of cells. Unlike the case with tyrosine-kinase receptors, protein kinases in the cytoplasm do not act on themselves, but rather on other proteins (sometimes enzymes) and attach the phosphate group to serine or threonine residues.

- Some phosphorylations result in activation of the target protein (increased catalytic activity in the case of an enzyme target). An example of a stimulatory phosphorylation cascade is the pathway involved in the breakdown of glycogen as elucidated by Sutherland, et al. (see Campbell, Figures 11.10; 11.15).
- Some phosphorylations result in inactivation (decreased catalytic activity in the case of an enzyme target).

Cells turn off the signal transduction pathway when the initial signal is no longer present. The effects of protein kinases are reversed by another class of enzymes known as ***protein phosphatases.***

C. Certain small molecules and ions are key components of signaling pathways (second messengers)

Not all of the components of a signal transduction pathways are proteins. Some signaling systems rely on small, nonprotein, water soluble molecules or ions. Such signaling components are called *second messengers.* Two second messenger systems are the *cyclic AMP (cAMP)* system and the Ca^{2+}- *inositol triphosphate (IP_3)* system.

1. Cyclic AMP

Sutherland's group ultimately found that the substance mediating the action of epinephrine on liver glycogen breakdown was cAMP (second messenger). Our present understanding of the transduction steps associated with cAMP is as follows:

- Ligand (first message) binds to a receptor.
- Receptor conformation changes; G-protein complex is activated.
- The active G-protein in turn activates the enzyme, adenylyl cyclase, which is associated with the cytoplasmic side of the plasma membrane.
- Adenylyl cyclase converts ATP to cAMP.
- cAMP binds to and activates a cytoplasmic enzyme, protein kinase A.
- Protein kinase A, as was the case for protein kinases mentioned previously, propagates the message by phosphorylating various other proteins that lead to the cellular response (e.g., glycogen breakdown; see Campbell Figure 11.15).

The pool of cAMP in the cytoplasm is transient because of the breakdown of cAMP by another enzyme to an inactive form (AMP). This conversion provides a shut-off mechanism to the cell to ensure that the target responses ceases in the absence of ligand.

A number of hormones in addition to epinephrine (e.g., glucagon) use cAMP as a second messenger.

2. Calcium ions and inositol triphosphate

Many signaling molecules induce their specific responses in target cells by increasing the cytoplasm's concentration of Ca^{2+}. The Ca^{2+} pool can be affected in two ways:

- Ligand binding to a Ca^{2+}-gated ion channel (discussed above)
- Activation of the *inositol triphosphate (IP_3)* signaling pathway

Activation of the IP_3 pathway involves the following steps:

- Ligand binding results in a conformation change in the receptor.
- The altered receptor activates an enzyme associated with the cytoplasmic side of the plasma membrane (phospholipase). The activated enzyme hydrolyzes membrane phospholipids, giving rise to two important second messengers: IP_3 and *diacylglycerol.*
- Diacylglycerol is linked to a signaling pathway that involves another protein kinase.
- IP_3 is linked to a Ca^{2+} signaling pathway. IP_3 binds to Ca^{2+}-gated channels. A large number of such channels are located on the ER, in the lumen of which high amounts of Ca^{2+} are sequestered. IP_3 binding to these receptors and increases the cytoplasmic concentration of Ca^{2+} (in this case Ca^{2+} could be considered a tertiary messenger; however, by convention, all post-receptor small molecules in the transduction system are referred to as second messengers).

Ca^{2+} acts to affect signal transduction in two ways:

- Directly by affecting the activity or function of target proteins
- Indirectly by first binding to a relay protein, *calmodulin*. Calmodulin, in turn, principally affects transduction systems by modulating the activities of protein kinases and protein phosphatases.

IV. Cellular Responses to Signals

A. In response to a signal, a cell may regulate activities in the cytoplasm or transcription in the nucleus

The signal transduction system ultimately brings about the specific cellular response by regulating specific processes in the cytoplasm or in the nucleus.

In the cytoplasm, the signaling can affect the *function or activity* of proteins which carry out various processes, including:

- Rearrangement of the cytoskeleton
- Opening or closing of an ion channel
- Serve at key points in metabolic pathways (e.g., glycogen phosphorylase in the glycogen breakdown scheme; see Campbell Figure 11.15)

In the nucleus, the signaling system affects the *synthesis* of new proteins and enzymes by modulating the expression (turn on or turn off) specific genes. Gene expression involves transcription of DNA into mRNA as well as the translation of mRNA into protein.

- Signal transduction systems can modulate virtually every aspect of gene expression. One example is the regulation of the activity of transcription factors, proteins required for appropriate transcription.
- Dysfunction of signaling pathways that affect gene regulation (e.g., pathways that transduce growth factor action) can have serious consequences and may even lead to cancer.

B. Elaborate pathways amplify and specify the cell's responses to signals

The elaborate nature of cellular signal transduction systems functions to: amplify signal (and, thus, response) and contribute to the specificity of the response.

1. Signal amplification

The production of second messengers such cAMP provides a built in means of signal amplification in that the binding of one ligand (first message) can lead to the production of many second messages. The degree of amplification is heightened when the second messenger system is linked to a phosphorylation cascade as in the case of the process of glycogen breakdown. As a result of this inherent amplification, the binding of very few epinephrine molecules to the surface of a liver cell can result in the release of millions of glucose molecules resulting from glycogen breakdown (see Campbell Figure 11.15).

2. Signal specificity

Only target cells with the appropriate receptor bind to a particular signaling molecule to initiate the transduction of a signal into a specific cellular response.

A particular signal can bind to different cell types and result in different responses in each of the cell types. This is possible because each of the different cell types can express a unique collection of proteins. As a result, the receptor on (or in) each of the different cell types can be linked to variant signal transduction pathways, each leading to a different response. An example is epinephrine action on vertebrate liver and cardiac muscle cells. In liver cells, the principal response is glycogen breakdown (see Campbell Figure 11.15); whereas, in cardiac muscle cells, epinephrine stimulates contraction.

A single cell type may possess divergent and/or convergent ("cross-talk") signal transduction pathways. Such schemes facilitate coordination of cellular responses and economize on the number of required transduction elements (see Campbell Figure 11.17). The diverse symptoms of the human inherited disorder Wiscott-Aldrich syndrome stem from a single defect in a relay protein of a transduction system.

An important feature of cell signaling systems is that there exists mechanisms to both turn-on and turn-off the system. The turn-off mechanisms ensure that cells respond appropriately to changing conditions.

REFERENCES

Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts, and J. Watson. *Molecular Biology of the Cell*, 3rd ed. New York: Garland, 1994.

Bolander, F. *Molecular Endocrinology*, 2nd ed. New York: Academic Press, 1994.

Hadley, M. *Endocrinology*, 3rd ed. Englewood Cliffs: Prentice-Hall, 1992..

Norman, A. and G. Litwack. *Hormones*, 2nd ed. New York: Academic Press, 1997

Norris, D. *Vertebrate Endocrinology*, 3rd ed. New York: Academic Press, 1997.

CHAPTER 12
THE CELL CYCLE

OUTLINE

I. The Key Roles of Cell Division
   A. Cell division functions in reproduction, growth, and repair
   B. Cell division distributes identical sets of chromosomes to daughter cells

II. The Mitotic Cell Cycle
   A. The mitotic phase alternates with interphase in the cell cycle: *an overview*
   B. The mitotic spindle distributes chromosomes to daughter cells: *a closer look*
   C. Cytokinesis divides the cytoplasm: *a closer look*
   D. Mitosis in eukaryotes may have evolved from binary fission in bacteria

III. Regulation of the Cell Cycle
   A. A molecular control system drives the cell cycle
   B. Internal and external cues help regulate the cell cycle
   C. Cancer cells have escaped from cell-cycle controls

OBJECTIVES

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

1. Describe the structural organization of the genome.
2. Overview the major events of cell division that enable the genome of one cell to be passed on to two daughter cells.
3. Describe how chromosome number changes throughout the human life cycle.
4. List the phases of the cell cycle and describe the sequence of events that occurs during each phase.
5. List the phases of mitosis and describe the events characteristic of each phase.
6. Recognize the phases of mitosis from diagrams or micrographs.
7. Draw or describe the spindle apparatus including centrosomes, nonkinetochore microtubules, kinetochore microtubules, asters, and centrioles (in animal cells).
8. Describe what characteristic changes occur in the spindle apparatus during each phase of mitosis.
9. Explain the current models for poleward chromosomal movement and elongation of the cell's polar axis.
10. Compare cytokinesis in animals and plants.
11. Describe the process of binary fission in bacteria and how this process may have evolved to mitosis in eukaryotes.
12. Describe the roles of checkpoints, cyclin, Cdk, and MPF, in the cell-cycle control system.

13. Describe the internal and external factors which influence the cell-cycle control system.
14. Explain how abnormal cell division of cancerous cells differs from normal cell division.

KEY TERMS

cell cycle	chromosomes	kinetochore	growth factor
cell division	interphase	metaphase plate	density-dependent inhibition
genome	G_1 phase	cleavage furrow	anchorage dependence
somatic cell	S phase	cell plate	transformation
gametes	G_2 phase	binary fission	tumor
chromatin	prophase	cell-cycle control system	benign tumor
sister chromatids	prometaphase	checkpoint	malignant tumor
centromere	metaphase	G_0 phase	metastasis
mitosis	anaphase	cyclin	
cytokinesis	telophase	cyclin-dependent kinase	
mitotic (M) phase	mitotic spindle	MPF	

LECTURE NOTES

The ability to reproduce distinguishes living organisms from nonliving objects; this ability has a cellular basis.'

All cells arise from preexisting cells. This fundamental principle, known as the cell doctrine, was originally postulated by Rudolf Virchow in 1858, and it provides the basis for the continuity of life.

A cell reproduces by undergoing a coordinated sequence of events in which it duplicates its contents and then divides in two. This cycle of duplication and division, known as the *cell cycle*, is the means by which all living things reproduce.

I. The Key Roles of Cell Division

A. Cell division functions in reproduction, growth, and repair

Cells reproduce for many reasons.

- In unicellular organisms, the division of one cell to form two reproduces an entire organism (e.g., bacteria, yeast, *Amoeba*) (see Campbell, Figure 12.1a).
- In multicellular organisms, cell division allows:
  - Growth and development from the fertilized egg (see Campbell, Figure 12.1b)
  - Replacement of damaged or dead cells

Cell division is a finely controlled process that results in the distribution of identical hereditary material—DNA—to two daughter cells. A dividing cell:

- Precisely replicates its DNA
- Allocates the two copies of DNA to opposite ends of the cell
- Separates into two daughter cells containing identical hereditary information

B. Cell division distributes identical sets of chromosomes to daughter cells

The total hereditary endowment of a cell of a particular species is called its genome.

The genomes of some species are quite small (e.g., prokaryotes), while the genomes of other species are quite large (e.g., eukaryotes).

The replication, division, and distribution of the large genomes of eukaryotes is possible because the genomes are organized into multiple functional units called *chromosomes* (see Campbell, Figure 12.2).

Eukaryotic chromosomes have the following characteristics:

- They are supercoils of a DNA-protein complex called *chromatin*. Each chromosome consists of the following:
  - A single, long, double-stranded molecule of DNA, segments of which are called *genes*
  - Various proteins which serve to maintain the structure of the chromosome or are involved with the expression of genes, DNA replication, and DNA repair
- They exist in a characteristic number in different species (e.g., human somatic cells have 46); gamete cells (sperm or ova) possess half the number of chromosomes of somatic cells (e.g., human gametes have 23)
- The exist in different states at different stages of the cell cycle.
  - During interphase, the chromosomes are loosely folded; cannot be seen with a light microscope
  - During the mitotic phase, chromosomes are highly folded and condensed; can be seen with a light microscope

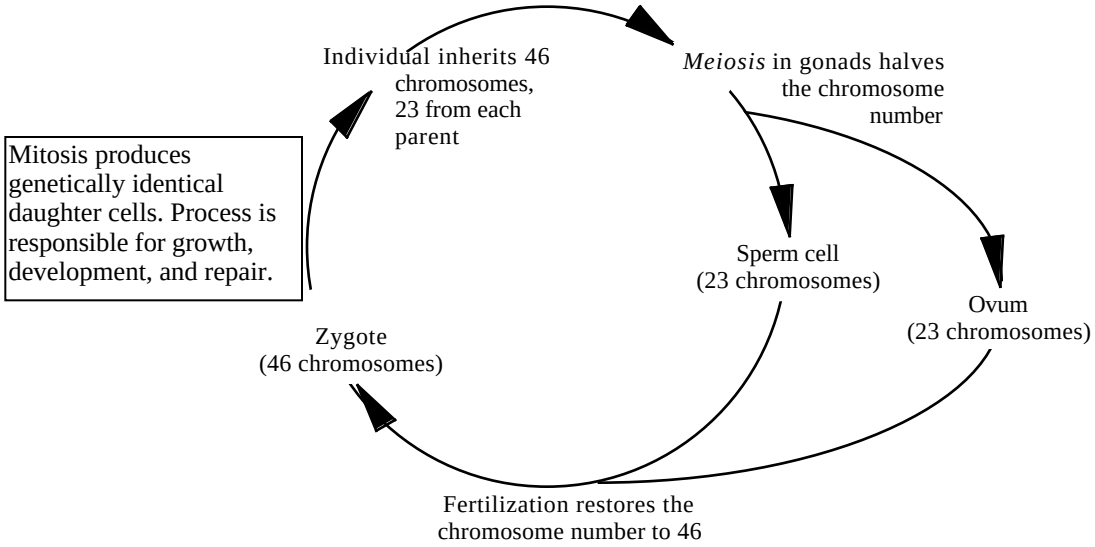
In preparation for eukaryotic cell division, the complete genome is duplicated. As a result of this duplication, each chromosome consists of two *sister chromatids.* The two chromatids possess identical copies of the chromosome's DNA and are initially attached to each other at a specialized region called the *centromere* (see Campbell, Figure 12.3).

Cell division usually proceeds in two sequential steps: nuclear division (*mitosis*) and division of the cytoplasm (*cytokinesis*). Not all cells undergo cytokinesis following mitosis.

In mitosis, the sister chromatids are pulled apart, and this results in the segregation of two sets of chromosomes, one set at each end of the cell.

In cytokinesis, the cytoplasm is divided and two separate daughter cells are formed, each containing a single nucleus with one set of chromosomes.

HUMAN LIFE CYCLE



In plant cells, cytokinesis occurs by *cell plate* formation across the parent cell's midline (old metaphase plate).

- Golgi-derived vesicles move along microtubules to the cell's center, where they fuse into a disc-like cell plate.

- Additional vesicles fuse around the edge of the plate, expanding it laterally until its membranes touch and fuse with the existing parent cell's plasma membrane.
- A new cell wall forms as cellulose is deposited between the two membranes of the cell plate.

II. The Mitotic Cell Cycle

A. The mitotic phase alternates with interphase in the cell cycle: *an overview*

Cell division is just a portion of the life, or *cell cycle*, of a cell (see Campbell, Figure 12.4).

The cell cycle is a well-ordered sequence of events in which a cell duplicates its contents and then divides in two.

- Some cells go through repeated cell cycles.
- Other cells never or rarely divide once they are formed (e.g., vertebrate nerve and muscle cells).

The cell cycle alternates between the *mitotic* (*M) phase*, or dividing phase, and *interphase*, the nondividing phase:

- M phase, the shortest part of the cell cycle and the phase during which the cell divides, includes:
  1. *Mitosis* - Division of the nucleus
  2. *Cytokinesis* - Division of the cytoplasm
- *Interphase*, the nondividing phase, includes most of a cell's growth and metabolic activities.
  - Is about 90% of the cell cycle
  - Is a period of intense biochemical activity during which the cell grows and copies its chromosomes in preparation for cell division
  - Consists of three periods:
    1. *G_1 phase* - First growth phase (G stands for "gap")
    2. *S phase* - Synthesis phase occurs when DNA is synthesized as chromosomes are duplicated (S stands for "synthesis")
    3. *G_2 phase* - Second growth phase

Mitosis is unique to eukaryotes and may be an evolutionary adaptation for distributing a large amount of genetic material.

- Details may vary, but overall process is similar in most eukaryotes.
- It is a reliable process with only one error per 100,000 cell divisions.

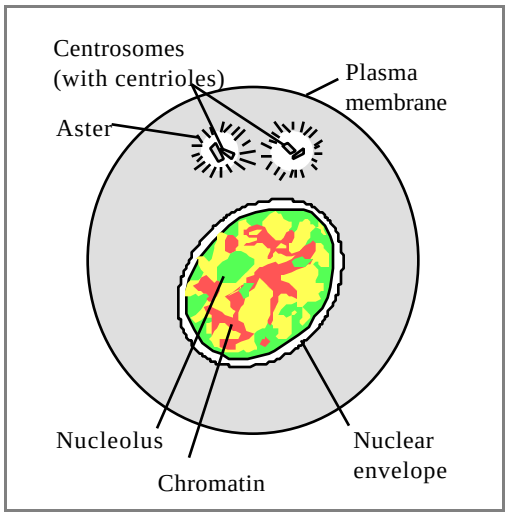
Mitosis is a continuous process, but for ease of description, mitosis is usually divided into five stages: *prophase*, *prometaphase*, *metaphase*, *anaphase*, and *telophase* (see Campbell, Figures 12.5 and 12.9):

When cytokinesis occurs, it usually is concomitant with telophase of mitosis. The details of mitosis and cytokinesis follow (as exemplified by the pattern of cell division displayed by animal cells):

G_2 of interphase

A G_2 cell is characterized by:

- A well-defined nucleus bounded by a nuclear envelope
- One or more nucleoli
- Two centrosomes adjacent to the nucleus (formed earlier by replication of a single centrosome)
- In animals, a pair of centrioles in each centrosome
- In animals, a radial microtubular array (*aster*) around each pair of centrioles
- Duplicated chromosomes that cannot be distinguished individually due to loosely packed chromatin fibers. (Chromosomes were duplicated earlier in S phase.)
- See also Campbell, Figure 12.5



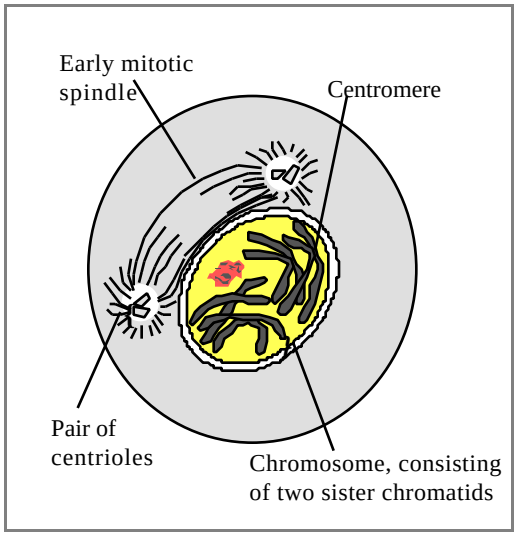
Prophase

In the nucleus:

- Nucleoli disappear
- Chromatin fibers condense into discrete, observable chromosomes, composed of two identical sister chromatids joined at the centromere.

In the cytoplasm:

- Mitotic spindle forms. It is composed of microtubules between the two *centrosomes* or microtubule-organizing centers.
- Centrosomes move apart, apparently propelled along the nuclear surface by lengthening of the microtubule bundles between them.
- See also Campbell, Figure 12.5



Prometaphase

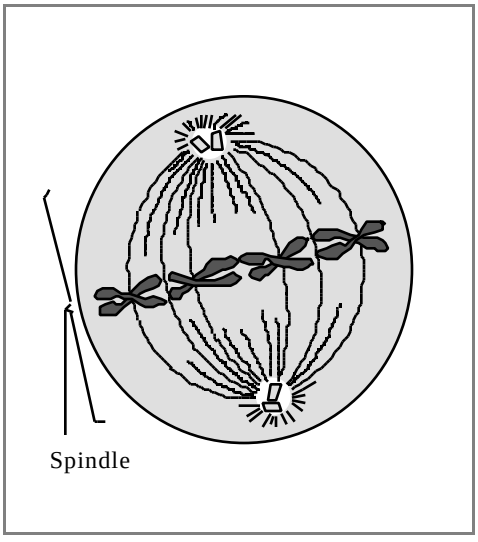
During prometaphase:

- Nuclear envelope fragments, which allows microtubules to interact with the highly condensed chromosomes.
- *Spindle fibers* (bundles of microtubules) extend from each pole toward the cell's equator.
- Each chromatid now has a specialized structure, the *kinetochore,* located at the centromere region.
- *Kinetochore microtubules* become attached to the kinetochores and put the chromosomes into agitated motion.
- *Nonkinetochore microtubules* radiate from each centrosome toward the metaphase plate without attaching to chromosomes. Nonkinetochore microtubules radiating from one pole overlap with those from the opposite pole.
- See also Campbell, Figure 12.5

Metaphase

During metaphase:

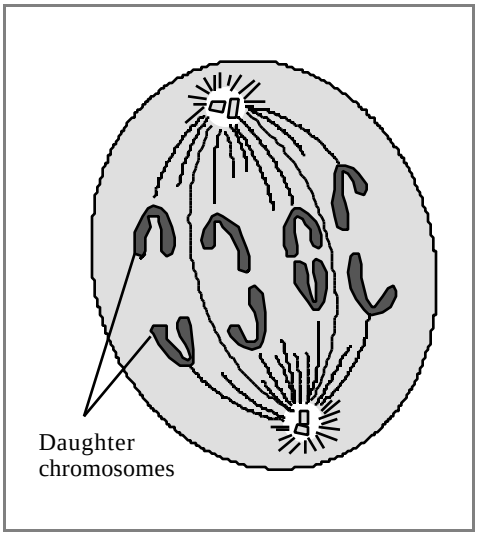
- Centrosomes are positioned at opposite poles of the cell.
- Chromosomes move to the *metaphase plate,* the plane equidistant between the spindle poles.
- Centromeres of all chromosomes are aligned on the metaphase plate.
- The long axis of each chromosome is roughly at a right angle to the spindle axis.
- Kinetochores of sister chromatids face opposite poles, so identical chromatids are attached to kinetochore fibers radiating from opposite ends of the parent cell.
- Entire structure formed by nonkinetochore microtubules plus kinetochore microtubules is called the *spindle*.
- See also Campbell, Figure 12.6



Anaphase

Anaphase is characterized by movement. It begins when paired centromeres of each chromosome move apart.

- Sister chromatids split apart into separate chromosomes and move towards opposite poles of the cell.
- Because kinetochore fibers are attached to the centromeres, the chromosomes move centromere first in a "V" shape.
- Kinetochore microtubules shorten at the kinetochore end as chromosomes approach the poles (see Campbell, Figure 12.7).
- Simultaneously, the poles of the cell move farther apart, elongating the cell.



At the end of anaphase, the two poles have identical collections of chromosomes.

Telophase and Cytokinesis

During telophase:

- Nonkinetochore microtubules further elongate the cell.
- Daughter nuclei begin to form at the two poles.
- Nuclear envelopes form around the chromosomes from fragments of the parent cell's nuclear envelope and portions of the endomembrane system.
- Nucleoli reappear.
- Chromatin fiber of each chromosome uncoils and the chromosomes become less distinct.

By the end of telophase:

- Mitosis, the equal division of one nucleus into two genetically identical nuclei, is complete.
- Cytokinesis has begun and the appearance of two separate daughter cells occurs shortly after mitosis is completed.

A lecture on mitosis may not last the entire period if it is limited to just a description of mitotic stages. Though it may be tempting to continue with meiosis during the same class period, it is not recommended. Students easily confuse the two processes because they are somewhat similar, so it helps to allow some time for students to assimilate the mitosis material, before discussing meiosis. It is effective to summarize with a comparison of the two processes after the topic of meiosis has been discussed.

B. The mitotic spindle distributes chromosomes to daughter cells: *a closer look*

Many of the events of mitosis depend on the formation of a *mitotic spindle*. The mitotic spindle forms in the cytoplasm from *microtubules* and associated proteins.

- Microtubules of the cytoskeleton are partially disassembled during spindle formation.
  - Spindle microtubules are aggregates of two proteins, α- and β-tubulin.
  - Spindle microtubules elongate by the adding tubulin subunits at one end.
- The assembly of spindle microtubules begins in the *centrosome* or microtubule organizing center.

- In animal cells, a pair of centrioles is in the center of the centrosome, but there is evidence that centrioles are not essential for cell division:
  - If the centrioles of animal cells are destroyed with a laser microbeam, spindles still form and function during mitosis.
  - Plant centrosomes generally lack centrioles.

The chronology of mitotic spindle formation is as follows:

Interphase. The centrosome replicates to form two centrosomes located just outside the nucleus.

Prophase. The two centrosomes move farther apart.

- Spindle microtubules radiate from the centrosomes, elongating at the end away from their centrosome.

Prometaphase. By the end of prometaphase, the two centrosomes are at opposite poles and the chromosomes have moved to the cell's midline.

- Each chromatid of a replicated chromosome develops its own *kinetochore*, a structure of proteins and chromosomal DNA on the centromere. The chromosome's two distinct kinetochores face opposite directions.
- Some spindle microtubules attach to the kinetochores and are called kinetochore microtubules.
- Some spindle microtubules extend from the centrosomes and overlap with those radiating from the cell's opposite pole. These are called nonkinetochore microtubules.

Kinetochore microtubules interact to: (1) arrange the chromosomes so kinetochores face the poles and (2) align the chromosomes at the cell's midline.

The most stable arrangement occurs when sister kinetochores are attached by microtubules to opposite spindle poles.

- Initially, kinetochore microtubules from one pole may attach to a kinetochore, moving the chromosome toward that pole. This movement is checked when microtubules from the opposite pole attach to the chromosome's other kinetochore.
- The chromosome oscillates back and forth until it stabilizes and aligns at the cell's midline.
- Microtubules can remain attached to a kinetochore only if there is opposing tension from the other side. It is this opposing tension that stabilizes the microtubule-kinetochore connection and allows the proper alignment and movement of chromosomes at the cell's midline.

Metaphase. All the duplicated chromosomes align on the cell's midline, or *metaphase plate*.

Anaphase. The chromosome's centromeres split and the sister chromatids move as separate chromosomes toward opposite ends of the cell. The kinetochore and nonkinetochore microtubules direct the segregation of the chromosomes (see Campbell, Figure 12.7).

The kinetochore microtubules function in the poleward movement of chromosomes. Based on experimental evidence, the current model is that:

- Kinetochore microtubules shorten during anaphase by depolymerizing at their kinetochore ends; pulling the chromosomes poleward.
- The mechanism of this interaction between kinetochores and microtubules may involve microtubule-walking proteins similar to dynein that "walk" a chromosome along the shortening microtubules.

The function of the nonkinetochore microtubules:

- Nonkinetochore tubules elongate the whole cell along the polar axis during anaphase.

- These tubules overlap at the middle of the cell and slide past each other away from the cell's equator, reducing the degree of overlap.
- It is hypothesized that dynein cross-bridges may form between overlapping tubules to slide them past one another. Alternatively, motor molecules may link the microtubules to other cytoskeletal elements to drive the sliding.
- ATP provides the energy for this endergonic process.

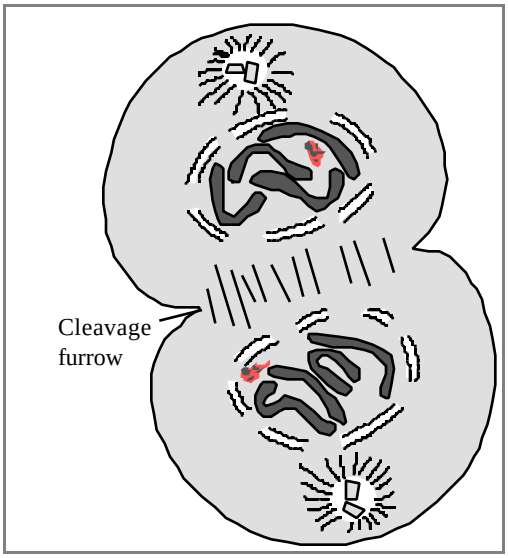
Telophase. At the end of anaphase, the duplicate sets of chromosomes are clustered at opposite ends of the elongated parent cell.

- Nuclei reform during telophase.
- Cytokinesis usually divides the cell's cytoplasm and is coincident with telophase of mitosis. In some exceptional cases, mitosis is not followed by cytokinesis (e.g., certain slime molds form multinucleated masses called *plasmodia*).

C. Cytokinesis divides the cytoplasm: *a closer look*

Cytokinesis, the process of cytoplasmic division, begins during telophase of mitosis. The process by which cytokinesis is accomplished differs in animal and plant cells. In animal cells, cytokinesis occurs by a process called *cleavage*:

- First, a *cleavage furrow* forms as a shallow groove in the cell surface near the old metaphase plate (see Campbell, Figure 12.8).
- A contractile ring of actin microfilaments forms on the cytoplasmic side of the furrow; this ring contracts until it pinches the parent cell in two.
- Finally, the remaining mitotic spindle breaks, and the two cells become completely separate.



Campbell, Figure 12.9 shows mitosis in a plant cell.

D. Mitosis in eukaryotes may have evolved from binary fission in bacteria

Because prokaryotes (bacteria) are smaller and simpler than eukaryotes and because they preceded eukaryotes on Earth by billions of years, it is reasonable to suggest that the carefully orchestrated process of mitosis had its origins in prokaryotes. Prokaryotes contain:

- Most genes in a single circular chromosome composed of a double-stranded DNA molecule and associated proteins.
- Only about 1/1000 the DNA of eukaryotes, but prokaryotic chromosomes still contain a large amount of DNA relative to the small prokaryotic cell. Consequently, bacterial chromosomes are highly folded and packed within the cell.

Prokaryotes reproduce by *binary fission*, a process during which bacteria replicate their chromosomes and equally distribute copies between the two daughter cells (see Campbell, Figure 12.10).

- The chromosome is replicated; each copy remains attached to the plasma membrane at adjacent sites.
- Between the attachment sites the membrane grows and separates the two copies of the chromosome.
- The bacterium grows to about twice its initial size, and the plasma membrane pinches inward.
- A cell wall forms across the bacterium between the two chromosomes, dividing the original cell into two daughter cells.

Certain modern algae display unusual patterns of nuclear division which may represent intermediate stages between bacterial binary fission and eukaryotic mitosis (see Campbell, Figure 12.11).

III. Regulation of the Cell Cycle

A. A molecular control system drives the cell cycle

Normal growth, development and maintenance depend on the timing and rate of mitosis. Various cell types differ in their pattern of cell division; for example:

- Human skin cells divide frequently.
- Liver cells only divide in appropriate situations, such as wound repair.
- Nerve, muscle and other specialized cells do not divide in mature humans.

The cell cycle is coordinated by the *cell-cycle control system*, a molecular signaling system which cyclically switches on the appropriate parts of the cell-cycle machinery and then switches them off (see Campbell, Figure 12.13).

The cell-cycle control system consists of a cell-cycle molecular clock and a set of *checkpoints*, or switches, that ensure that appropriate conditions have been met before the cycle advances. When the control system malfunctions, as will be seen later, cancer may result.

The cell-cycle control system has checkpoints in the G_1, G_2, and M phases of the cell cycle.

- Signals registered at the checkpoints report the status of various cellular conditions (e.g., Is the environment favorable? Is the cell big enough? Are all DNA replicated?
- Checkpoints integrate a variety of internal (intracellular) and external (extracellular) information.
- For many cells, the G_1 checkpoint (known as the "restriction point" in mammalian cells) is the most important.
  - A go-ahead signal usually indicates that the cell will complete the cycle and divide.
  - In the absence of a go-ahead signal, the cell may exit the cell cycle, switching to the nondividing state called *G_0 phase.*
  - Many cells of the human body are in the G_0 phase. Muscle and nerve cells will remain in G_0 until they die. Liver cells may be recruited back to the cell cycle under certain cues, such as growth factors.

The ordered sequence of cell cycle events is synchronized by rhythmic changes in the activity of certain *protein kinases.*

- Protein kinases are enzymes that catalyze the transfer of a phosphate group from ATP to a target protein.

- Phosphorylation, in turn, induces a conformational change that either activates or inactivates a target protein.
- Changes in target proteins affect the progression through the cell cycle.

Cyclical changes in kinase activity are controlled by another class of regulatory proteins called *cyclins*.

- These regulatory proteins are named cyclins, because their concentrations change cyclically during the cell cycle.
- Protein kinases that regulate cell cycles are *cyclin-dependent kinases* (*Cdks*); they are active only when attached to a particular cyclin.
- Even though Cdk concentration stays the same throughout the cell cycle, its activity changes in response to the changes in cyclin concentration (see Campbell, Figure 12.14a).

An example of a cyclin-Cdk complex is *MPF (maturation promoting factor)*, which controls the cell's progress through the G_2 checkpoint to mitosis (see Campbell, Figure 12.14b).

Cyclin's rhythmic changes in concentration regulate MPF activity, and thus acts as a mitotic clock that regulates the sequential changes in a dividing cell.

- Cyclin is produced at a uniform rate throughout the cell cycle, and it accumulates during interphase.
- Cyclin combines with Cdk to form active MPF, so as cyclin concentration rises and falls, the amount of active MPF changes in a similar way.
- MPF phosphorylates proteins that participate in mitosis and initiates the following process:
  - Chromosome condensation during prophase
  - Nuclear envelope dispersion during prometaphase
- In the latter half of mitosis, MPF activates proteolytic enzymes.
  - The proteolytic enzymes destroy cyclin which leads to the reduction of MPF activity (the Cdk portion of MPF is not degraded).
  - The proteolytic enzymes also are involved in driving the cell cycle past the M-phase checkpoint, which controls the onset of anaphase.
- Continuing cyclin synthesis raises the concentration again during interphase. This newly synthesized cyclin binds to Cdk to form MPF, and mitosis begins again.

Rhythmic changes in different cyclin-Cdk complexes regulate other cell cycle stages.

B. Internal and external cues help regulate the cell cycle

The cell-cycle control system integrates a variety of internal (intracellular) and external (extracellular) information. Knowledge of the chemical signaling pathways that transduce this information into modulation of the cell-cycle machinery is just emerging.

The kinetochores provide internal cues that signal the M-phase checkpoint about the status of chromosome-spindle interactions. All chromosomes must be attached to spindle microtubules before the M-phase checkpoint allows the cycles to proceed to anaphase. This ensures that daughter cells do not end up with missing or extra chromosomes.

- Kinetochores not attached to spindles trigger a signaling pathway that keeps the anaphase promoting complex (APC) in an inactive state.
- Once all kinetochores are attached, the wait signal stops, and the APC complex becomes active. The APC complex contains proteolytic enzymes which break down cyclin.

Using tissue culture, researchers have identified several external factors, both chemical and physical, that can influence cell division:

1. Chemical factors

- If essential nutrients are left out of the culture medium, cells will not divide.
- Specific regulatory substances called *growth factors* are necessary for most cultured mammalian cells to divide, even if all other conditions are favorable. For example:
  - Binding of platelet-derived growth factor (PDGF) to cell membrane receptors, stimulates cell division in fibroblasts. This regulation probably occurs not only in cell culture, but in the animal's body as well—a response that helps heal wounds.
  - Other cell types may have membrane receptors for different growth factors or for different combinations of several growth factors.

2. Physical factors

- Crowding inhibits cell division in a phenomenon called *density-dependent inhibition.* Cultured cells stop dividing when they form a single layer on the container's inner surface. If some cells are removed, those bordering the open space divide again until the vacancy is filled (see Campbell, Figure 12.15a).
  - Density-dependent inhibition is apparently a consequence of the fact that quantities of nutrients and growth regulators may be insufficient to support cell division, if cell density is too high.
  - Most animal cells also exhibit anchorage dependence. To divide, normal cells must adhere to a substratum, such as the surface of a culture dish or the extracellular matrix of a tissue. anchorage is signaled to the cell-cycle control system via pathways involving membrane proteins and elements of the cytoskeleton that are linked to them.
- Density-dependent and anchorage-dependent inhibition probably occur in the body's tissues as well as in cell culture. Cancer cells are abnormal and do not exhibit density-dependent or anchorage-dependent inhibition.

C. Cancer cells have escaped from cell-cycle controls

Cancer cells do not respond normally to the body's control mechanisms. They divide excessively, invade other tissues and, if unchecked, can kill the whole organism.

- Cancer cells in culture do not stop growing in response to cell density (density-dependent inhibition); they do not stop dividing when growth factors are depleted (see Campbell, Figure 12.15b).
- Cancer cells may make growth factors themselves.
- Cancer cells may have an abnormal growth factor signaling system.
- Cancer cells in culture are immortal in that they continue to divide indefinitely, as long as nutrients are available. Normal mammalian cells in culture divide only about 20 to 50 times before they stop.
- Cancer cells that stop dividing do so at random points in the cycle instead of at checkpoints.

Abnormal cells which have escaped normal cell-cycle controls are the products of mutate or *transformed* normal cells.

The immune system normally recognizes and destroys transformed cells that have converted from normal to cancer cells.

- If abnormal cells evade destruction, they may proliferate to form a *tumor,* an unregulated growing mass of cells within otherwise normal tissue.

- If the cells remain at this original site, the mass is called a *benign tumor* and can be completely removed by surgery.
- A *malignant tumor* is invasive enough to impair normal function of one or more organs of the body. Only an individual with a malignant tumor is said to have cancer (see Campbell, Figure 12.16).

Properties of malignant (cancerous) tumors include:

- Anomalous cell cycle; excessive proliferation
- May have unusual numbers of chromosomes
- May have aberrant metabolism
- Lost attachments to neighboring cells and extracellular matrix—usually a consequence of abnormal cell surface changes.

Cancer cells also may separate from the original tumor and spread into other tissues, possibly entering the blood and lymph vessels of the circulatory system.

- Migrating cancer cells can invade other parts of the body and proliferate to form more tumors.
- This spread of cancer cells beyond their original sites is called *metastasis*.
- If a tumor metastasizes, it is usually treated with radiation and chemotherapy, which is especially harmful to actively dividing cells.

Researchers are beginning to understand how a normal cell is transformed into a cancerous one. Although the causes of cancer may be diverse, cellular transformation always involves the alteration of genes that somehow influence the cell-cycle control system.

REFERENCES

Alberts, B., D. Bray, A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter. *Essential Cell Biology: An Introduction to the Molecular Biology of the Cell.* New York: Garland, 1997.

Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts and J.D. Watson. *Molecular Biology of the Cell.* 2nd ed. New York: Garland, 1989.

Becker, W.M., J.B. Reece and M.F. Puente. *The World of the Cell.* 3rd ed. Redwood City, California: Benjamin/Cummings, 1996.

Campbell, N., et al. *Biology.* 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Kleinsmith, L.J. and V.M. Kish. *Principles of Cell Biology.* New York: Harper and Row, Publ., 1988.

Varmus, H. and R.A. Weinberg. *Genes and the Biology of Cancer*. New York: Scientific American Books, 1993.

CHAPTER 13

MEIOSIS AND SEXUAL LIFE CYCLES

OUTLINE

I. An Introduction to Heredity
   A. Offspring acquire genes from parents by inheriting chromosomes
   B. Like begets like, more or less: a comparison of asexual versus sexual reproduction
II. The Role of Meiosis in Sexual Life Cycles
   A. Fertilization and meiosis alternate in sexual life cycles
   B. Meiosis reduces chromosome number from diploid to haploid: *a closer look*
III. Origins of Genetic Variation
   A. Sexual life cycles produce genetic variation among offspring
   B. Evolutionary adaptation depends on a population's genetic variation

OBJECTIVES

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

1. Explain why organisms only reproduce their own kind, and why offspring more closely resemble their parents than unrelated individuals of the same species.
2. Explain what makes heredity possible.
3. Distinguish between asexual and sexual reproduction.
4. Diagram the human life cycle and indicate where in the human body that mitosis and meiosis occur; which cells are the result of meiosis and mitosis; and which cells are haploid.
5. Distinguish among the life cycle patterns of animals, fungi, and plants.
6. List the phases of meiosis I and meiosis II and describe the events characteristic of each phase.
7. Recognize the phases of meiosis from diagrams or micrographs.
9. Describe the process of synapsis during prophase I, and explain how genetic recombination occurs.
10. Describe key differences between mitosis and meiosis; explain how the end result of meiosis differs from that of mitosis.
11. Explain how independent assortment, crossing over, and random fertilization contribute to genetic variation in sexually reproducing organisms.
12. Explain why inheritable variation was crucial to Darwin's theory of evolution.
13. List the sources of genetic variation.

KEY TERMS

heredity	karyotype	zygote	meiosis II
variation	homologous	diploid cells	synapsis
genetics	chromosomes	meiosis	tetrad
gene	sex chromosomes	alternation of	chiasmata
asexual reproduction	autosome	generations	chiasma
clone	gamete	sporophyte	crossing over
sexual reproduction	haploid cell	spores	
life cycle	fertilization	gametophyte	
somatic cell	syngamy	meiosis I	

LECTURE NOTES

Reproduction is an emergent property associated with life. The fact that organisms reproduce their own kind is a consequence of heredity.

Heredity = Continuity of biological traits from one generation to the next

- Results from the transmission of hereditary units, or *genes*, from parents to offspring.
- Because they share similar genes, offspring more closely resemble their parents or close relatives than unrelated individuals of the same species.

Variation = Inherited differences among individuals of the same species

- Though offspring resemble their parents and siblings, they also diverge somewhat as a consequence of inherited differences among them.
- The development of *genetics* in this century has increased our understanding about the mechanisms of variation and heredity.

Genetics = The scientific study of heredity and hereditary variation.

> Beginning students often compartmentalize their knowledge, which makes it difficult to transfer and apply information learned in one context to a new situation. Be forewarned that unless you point it out, some students will never make the connection that meiosis, sexual reproduction, and heredity are all aspects of the same process.

I. An Introduction to Heredity

A. Offspring acquire genes from parents by inheriting chromosomes

DNA = Type of nucleic acid that is a polymer of four different kinds of nucleotides.

Genes = Units of hereditary information that are made of *DNA* and are located on *chromosomes*.

- Have specific sequences of nucleotides, the monomers of DNA
- Most genes program cells to synthesize specific proteins; the action of these proteins produce an organism's inherited traits.

Inheritance is possible because:

- DNA is precisely replicated producing copies of genes that can be passed along from parents to offspring.
- Sperm and ova carrying each parent's genes are combined in the nucleus of the fertilized egg.

The actual transmission of genes from parents to offspring depends on the behavior of *chromosomes*.

Chromosomes = Organizational unit of heredity material in the nucleus of eukaryotic organisms

- Consist of a single long DNA molecule (double helix) that is highly folded and coiled along with proteins
- Contain genetic information arranged in a linear sequence
- Contain hundreds or thousands of genes, each of which is a specific region of the DNA molecule, or *locus*

Locus = Specific location on a chromosome that contains a gene

- Each species has a characteristic number of chromosomes; humans have 46 (except for their reproductive cells).

B. Like begets like, more or less: a comparison of asexual versus sexual reproduction

Asexual Reproduction	Sexual Reproduction
Single individual is the sole parent.	Two parents give rise to offspring.
Single parent passes on *all* its genes to its offspring.	Each parent passes on *half* its genes, to its offspring.
Offspring are genetically identical to the parent.	Offspring have a unique combination of genes inherited from both parents.
Results in a *clone*, or genetically identical individual. Rarely, genetic differences occur as a result of *mutation*, a change in DNA (see Campbell, Figure 13.1).	Results in greater genetic variation; offspring vary genetically from their siblings and parents (see Campbell, Figure 13.2).

What generates this genetic variation during sexual reproduction? The answer lies in the process of meiosis.

III. The Role of Meiosis in Sexual Life Cycles

A. Fertilization and meiosis alternate in sexual life cycles

1. The human life cycle

Follows the same basic pattern found in all sexually reproducing organisms; *meiosis* and *fertilization* result in alternation between the haploid and diploid condition (see Campbell, Figure 13.3).

Life cycle = Sequence of stages in an organism's reproductive history, from conception to production of its own offspring

Somatic cell = Any cell other than a sperm or egg cell

- Human somatic cells contain 46 chromosomes distinguishable by differences in size, position of the centromere, and staining or banding pattern.
- Using these criteria, chromosomes from a photomicrograph can be matched into *homologous* pairs and arranged in a standard sequence to produce a karyotype.

Karyotype = A display or photomicrograph of an individual's somatic-cell metaphase chromosomes that are arranged in a standard sequence. (See Campbell, Methods Box: *Preparation of a Karyotype*)

- Human karyotypes are often made with lymphocytes.
- Can be used to screen for chromosomal abnormalities

Homologous chromosomes (homologues) = A pair of chromosomes that have the same size, centromere position, and staining pattern.

- With one exception, homologues carry the same genetic loci.

- Homologous *autosomes* carry the same genetic loci; however, human *sex chromosomes* carry different loci even though they pair during prophase of meiosis I.

Autosome = A chromosome that is not a sex chromosome.

Sex chromosome = Dissimilar chromosomes that determine an individual's sex

- Females have a homologous pair of X chromosomes.
- Males have one X and one Y chromosome.
- Thus, humans have 22 pairs of autosomes and one pair of sex chromosomes.

Chromosomal pairs in the human karyotype are a result of our sexual origins.

- One homologue is inherited from each parent.
- Thus, the 46 somatic cell chromosomes are actually two sets of 23 chromosomes; one a maternal set and the other a paternal set.
- Somatic cells in humans and most other animals are *diploid.*

Diploid = Condition in which cells contain two sets of chromosomes; abbreviated as 2*n*

Haploid = Condition in which cells contain one set of chromosomes; it is the chromosome number of *gametes* and is abbreviated as *n*

Gamete = A haploid reproductive cell

- *Sperm cells* and *ova* are gametes, and they differ from somatic cells in their chromosome number. Gametes only have one set of chromosomes.
- Human gametes contain a single set of 22 autosomes and one sex chromosome (either an X or a Y).
- Thus, the haploid number of humans is 23.

The diploid number is restored when two haploid gametes unite in the process of *fertilization*. Sexual intercourse allows a haploid sperm cell from the father to reach and fuse with an ovum from the mother.

Fertilization = The union of two gametes to form a *zygote*

Zygote = A diploid cell that results from the union of two haploid gametes

- Contains the maternal and parental haploid sets of chromosomes from the gametes and is diploid (2*n*)
- As humans develop from a zygote to sexually mature adults, the zygote's genetic information is passed with precision to all somatic cells by mitosis.

Gametes are the only cells in the body that are *not* produced by mitosis.

- Gametes are produced in the ovaries or testes by the process of *meiosis*.
- *Meiosis* is a special type of cell division that produces haploid cells and compensates for the doubling of chromosome number that occurs at fertilization.
- Meiosis in humans produces sperm cells and ova which contain 23 chromosomes.
- When fertilization occurs, the diploid condition (2*n*=46) is restored in the zygote.

2. The variety of sexual life cycles

Alternation of meiosis and fertilization is common to all sexually reproducing organisms; however, the timing of these two events in the life cycle varies among species. There are three basic patterns of sexual life cycles (see Campbell, Figure 13.4):

a. Animal

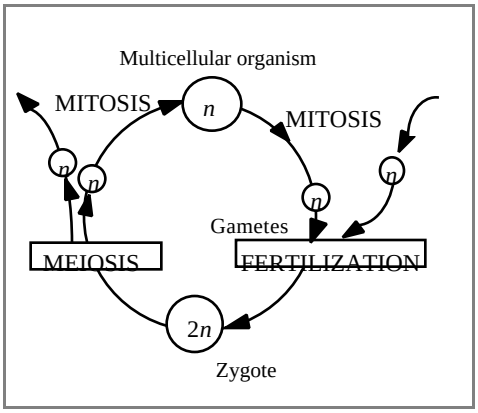
In animals, including humans,

gametes are the only haploid cells.

- Meiosis occurs during gamete production. The resulting gametes undergo no further cell division before fertilization.
- Fertilization produces a diploid zygote that divides by mitosis to produce a diploid multicellular animal.

b. Fungi and some protists

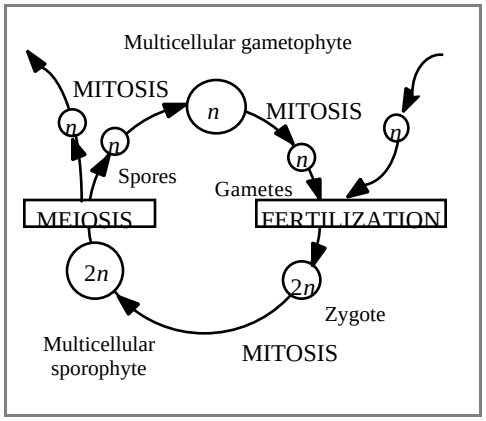
In many fungi and some protists, the only diploid stage is the zygote.



- Meiosis occurs immediately after the zygote forms.
- Resulting haploid cells divide by *mitosis* to produce a haploid multicellular organism.
- Gametes are produced by *mitosis* from the already haploid organism.

c. Plants and some algae

Plants and some species of algae alternate between multicellular haploid and diploid generations.



- This type of life cycle is called an *alternation of generations*.
- The multicellular diploid stage is called a *sporophyte*, or spore-producing plant. Meiosis in this stage produces haploid cells called *spores*.
- Haploid spores divide mitotically to generate a multicellular haploid stage called a *gametophyte*, or gamete-producing plant.
- Haploid gametophytes produce gametes by mitosis.
- Fertilization produces a diploid zygote which develops into the next sporophyte generation.

B. Meiosis reduces chromosome number from diploid to haploid: *a closer look*

Meiosis and sexual reproduction significantly contribute to genetic variation among offspring.

Meiosis includes steps that closely resemble corresponding steps in mitosis (see Campbell, Figure 13.5).

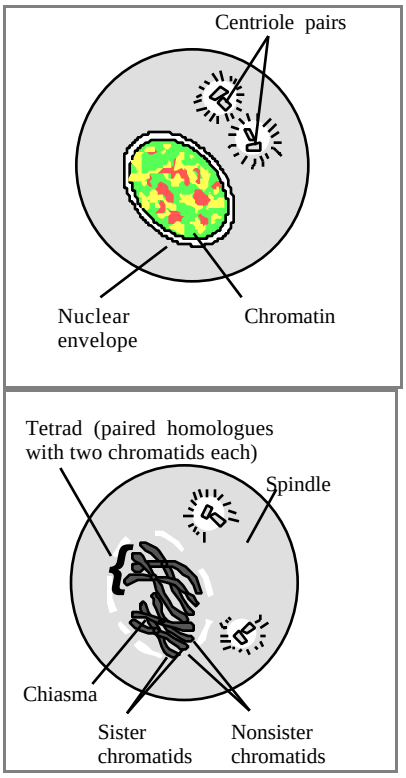
- Like mitosis, meiosis is preceded by replication of the chromosomes.
- Meiosis differs from mitosis in that this single replication is followed by two consecutive cell divisions: *meiosis I* and *meiosis II*.

- These cell divisions produce *four* daughter cells instead of two as in mitosis.
- The resulting daughter cells have *half* the number of chromosomes as the original cell; whereas, daughter cells of mitosis have the same number of chromosomes as the parent cell.
- Campbell, Figure 13.6 shows mitosis and meiosis in animals.

The stages of meiotic cell division:

Interphase I. Interphase I precedes meiosis.

- Chromosomes replicate as in mitosis.
- Each duplicated chromosome consists of two identical sister chromatids attached at their centromeres.
- Centriole pairs in animal cells also replicate into two pairs.



Meiosis I. This cell division segregates the two chromosomes of each homologous pair and reduces the chromosome number by one-half. It includes the following four phases:

Prophase I. This is a longer and more complex process than prophase of mitosis.

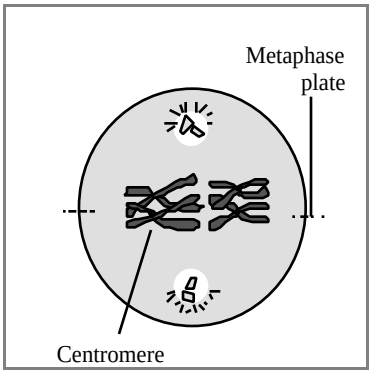
- Chromosomes condense.
- *Synapsis* occurs. During this process, homologous chromosomes come together as pairs.
- Chromosomes condense further until they are distinct structures that can be seen with a microscope. Since each chromosome has two chromatids, each homologous pair in synapsis appears as a complex of four chromatids or a *tetrad*.
- In each tetrad, sister chromatids of the same chromosome are attached at their centromeres. Nonsister chromatids are linked by X-shaped *chiasmata*, sites where homologous strand exchange or *crossing-over* occurs.
- Chromosomes thicken further and detach from the nuclear envelope.

As prophase I continues, the cell prepares for nuclear division.

- Centriole pairs move apart and spindle microtubules form between them.
- Nuclear envelope and nucleoli disperse.
- Chromosomes begin moving to the metaphase plate, midway between the two poles of the spindle apparatus.
- Prophase I typically occupies more than 90% of the time required for meiosis.

Metaphase I. Tetrads are aligned on the metaphase plate.

- Each synaptic pair is aligned so that centromeres of homologues point toward opposite poles.
- Each homologue is thus attached to kinetochore microtubules emerging from the pole it faces, so that the two homologues are destined to separate in anaphase and move toward opposite poles.



Anaphase I. Homologues separate and are moved toward the poles by the spindle apparatus.

- Sister chromatids remain attached at their centromeres and move as a unit toward the same pole, while the homologue moves toward the opposite pole.
- This differs from mitosis during which chromosomes line up individually on the metaphase plate (rather than in pairs) and sister chromatids are moved apart toward opposite poles of the cell.

Telophase I and Cytokinesis. The spindle apparatus continues to separate homologous chromosome pairs until the chromosomes reach the poles.

- Each pole now has a haploid set of chromosomes that are each still composed of two sister chromatids attached at the centromere.
- Usually, cytokinesis occurs simultaneously with telophase I, forming two haploid daughter cells. *Cleavage furrows* form in animal cells, and cell plates form in plant cells.
- In some species, nuclear membranes and nucleoli reappear, and the cell enters a period of *interkinesis* before meiosis II. In other species, the daughter cells immediately prepare for meiosis II.
- Regardless of whether a cell enters interkinesis, *no DNA replication occurs before meiosis II.*

Meiosis II. This second meiotic division separates sister chromatids of each chromosome.

Prophase II

- If the cell entered interkinesis, the nuclear envelope and nucleoli disperse.
- Spindle apparatus forms and chromosomes move toward the metaphase II plate.

Metaphase II

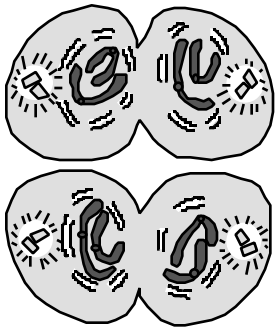
- Chromosomes align singly on the metaphase plate.
- Kinetochores of sister chromatid*s point toward opposite poles.*

Anaphase II

- Centromeres of sister chromatids separate.
- Sister chromatids of each pair (now individual chromosomes) move toward opposite poles of the cell.

Telophase II and Cytokinesis

- Nuclei form at opposite poles of the cell.
- Cytokinesis occurs producing four haploid daughter cells.



Haploid daughter cells

1. Mitosis and meiosis compared

Spending class time on a comparison of mitosis and meiosis is really worth the effort. It not only brings closure to the topic, but also provides an opportunity to check for understanding. One check is to ask students to identify unlabeled diagrams of various stages in mitosis and meiosis. The ability to distinguish metaphase of mitosis from metaphase of meiosis I, is particularly diagnostic of student understanding.

If you are fortunate enough to have video capability in your classroom, you can show moving sequences of mitosis and meiosis. The fact that these are dynamic processes involving chromosomal movement is not a trivial point, but is often lost in the course of a lecture where the only visuals are drawings or micrographs.

Though the processes of mitosis and meiosis are similar in some ways, there are some key differences (see Campbell, Figure 13.7):

- *Meiosis is a reduction division.* Cells produced by mitosis have the same number of chromosomes as the original cell, whereas cells produced by meiosis have *half* the number of chromosomes as the parent cell.
- *Meiosis creates genetic variation.* Mitosis produces *two* daughter cells genetically identical to the parent cell and to each other. Meiosis produces *four* daughter cells genetically different from the parent cell and from each other.
- *Meiosis is two successive nuclear divisions.* Mitosis, on the other hand, is characterized by just one nuclear division.

Comparison of Meiosis I and Mitosis

	Meiosis I	Mitosis
Prophase	*Synapsis* occurs to form tetrads. *Chiasmata* appear as evidence that crossing over has occurred.	Neither synapsis nor crossing over occurs.
Metaphase	Homologous pairs (tetrads) align on the metaphase plate.	Individual chromosomes align on the metaphase plate.
Anaphase	Meiosis I separates pairs of chromosomes. Centromeres do *not* divide and sister chromatids stay together. Sister chromatids of each chromosome move to the *same* pole of the cell; only the homologues separate.	Mitosis separates sister chromatids of individual chromosomes. Centromeres divide and sister chromatids move to *opposite* poles of the cell.

Meiosis II is virtually identical in mechanism to mitosis, separating sister chromatids.

III. Origins of Genetic Variation

A. Sexual life cycles produce genetic variation among offspring

Meiosis and fertilization are the primary sources of genetic variation in sexually reproducing organisms. Sexual reproduction contributes to genetic variation by:

- Independent assortment
- Crossing over during prophase I of meiosis

- Random fusion of gametes during fertilization

1. Independent assortment of chromosomes

At metaphase I, each homologous pair of chromosomes aligns on the metaphase plate. Each pair consists of one maternal and one paternal chromosome.

- The orientation of the homologous pair to the poles is random, so there is a 50-50 chance that a particular daughter cell produced by meiosis I will receive the maternal chromosome of a homologous pair, and a 50-50 chance that it will receive the paternal chromosome.
- Each homologous pair of chromosomes orients independently of the other pairs at metaphase I; thus, the first meiotic division results in *independent assortment* of maternal and paternal chromosomes (see Campbell, Figure 13.8)
- A gamete produced by meiosis contains just one of all the possible combinations of maternal and paternal chromosomes.

Independent assortment = The random distribution of maternal and paternal homologues to the gametes. (In a more specific sense, assortment refers to the random distribution of genes located on different chromosomes.)

- Since each homologous pair assorts independently from all the others, the process produces 2^n possible combinations of maternal and paternal chromosomes in gametes, where *n* is the haploid number.
- In humans, the possible combinations would be 2^{23}, or about eight million.
- Thus, each human gamete contains one of eight million possible assortments of chromosomes inherited from that person's mother and father.
- Genetic variation results from this reshuffling of chromosomes, because the maternal and paternal homologues will carry different genetic information at many of their corresponding loci.

2. Crossing over

Another mechanism that increases genetic variation is the process of *crossing over*, during which homologous chromosomes exchange genes.

Crossing over = The exchange of genetic material between homologues; occurs during prophase of meiosis I. This process:

- Occurs when homologous portions of two nonsister chromatids trade places. During prophase I, X-shaped *chiasmata* become visible at places where this homologous strand exchange occurs.
- Produces chromosomes that contain genes from *both* parents.
- In humans, there is an average of two or three crossovers per chromosome pair.
- Synapsis during prophase I is precise, so that homologues align gene by gene. The exact mechanism of synapsis is still unknown, but involves a protein apparatus, the *synaptonemal complex,* that joins the chromosomes closely together.
- Campbell, Figure 13.9 shows the results of crossing over during meiosis.

3. Random fertilization

Random fertilization is another source of genetic variation in offspring.

- In humans, when individual ovum representative of one of eight million possible chromosome combinations is fertilized by a sperm cell, which also represents one of eight million possibilities, the resulting zygote can have one of 64 trillion possible diploid combinations (without considering variations from crossing over!).

B. Evolutionary adaptation depends on a population's genetic variation

Heritable variation is the basis for Charles Darwin's theory that natural selection is the mechanism for evolutionary change. Natural selection:

- Increases the frequency of heritable variations that favor the reproductive success of some individuals over others
- Results in *adaptation*, the accumulation of heritable variations that are favored by the environment
- In the face of environmental change, genetic variation increases the likelihood that some individuals in a population will have heritable variations that help them cope with the new conditions.

There are two sources of genetic variation:

1. Sexual reproduction. Results from independent assortment in meiosis I, crossing over in prophase of meiosis I, and random fusion of gametes during fertilization.
2. Mutation, which are random and relatively rare structural changes made during DNA replication in a gene could result from mistakes.

REFERENCES

Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts and J.D. Watson. *Molecular Biology of the Cell.* 3rd ed. New York: Garland, 1994.

Becker, W.M., J.B. Reece, and M.F. Puente. The World of the Cell. 3rd. ed. Redwood City, California: Benjamin/Cummings, 1996.

Campbell, N, et al. *Biology*. 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Kleinsmith, L.J. and V.M. Kish. *Principles of Cell Biology*. New York: Harper and Row, Publ., 1988.

CHAPTER 14
MENDEL AND THE GENE IDEA

OUTLINE

I. Gregor Mendel's Discoveries
   A. Mendel brought an experimental and quantitative approach to genetics: *science as a process*
   B. By the law of segregation, the two alleles for a character are packaged into separate gametes
   C. By the law of independent assortment, each pair of alleles segregates into gametes independently
   D. Mendelian inheritance reflects rules of probability
   E. Mendel discovered the particulate behavior of genes: *a review*

II. Extending Mendelian Genetics
   A. The relationship between genotype and phenotype is rarely simple

III. Mendelian Inheritance in Humans
   A. Pedigree analysis reveals Mendelian patterns in human inheritance
   B. Many human disorders follow Mendelian patterns of inheritance
   C. Technology is providing new tools for genetic testing and counseling

OBJECTIVES

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

1. Describe the favored model of heredity in the 19th century prior to Mendel, and explain how this model was inconsistent with observations.
2. Explain how Mendel's hypothesis of inheritance differed from the blending theory of inheritance.
3. List several features of Mendel's methods that contributed to his success.
4. List four components of Mendel's hypothesis that led him to deduce the law of segregation.
5. State, in their own words, Mendel's law of segregation.
6. Use a Punnett square to predict the results of a monohybrid cross and state the phenotypic and genotypic ratios of the F_2 generation.
7. Distinguish between genotype and phenotype; heterozygous and homozygous; dominant and recessive.
8. Explain how a testcross can be used to determine if a dominant phenotype is homozygous or heterozygous.
9. Define random event, and explain why it is significant that allele segregation during meiosis and fusion of gametes at fertilization are random events.
10. Use the rule of multiplication to calculate the probability that a particular F_2 individual will be homozygous recessive or dominant.

11. Given a Mendelian cross, use the rule of addition to calculate the probability that a particular F_2 individual will be heterozygous.
12. Describe two alternate hypotheses that Mendel considered for how two characters might segregate during gamete formation, and explain how he tested those hypotheses.
13. State, in their own words, Mendel's law of independent assortment.
14. Use a Punnett square to predict the results of a dihybrid cross and state the phenotypic and genotypic ratios of the F_2 generation.
15. Using the laws of probability, predict from a trihybrid cross between two individuals that are heterozygous for all three traits, what expected proportion of the offspring would be:
    a. Homozygous for the three dominant traits
    b. Heterozygous for all three traits
    c. Homozygous recessive for two specific traits and heterozygous for the third
16. Give an example of incomplete dominance and explain why it is not evidence for the blending theory of inheritance.
17. Explain how the phenotypic expression of the heterozygote is affected by complete dominance, incomplete dominance and codominance.
18. Describe the inheritance of the ABO blood system and explain why the I^A and I^B alleles are said to be *codominant.*
19. Define and give examples of pleiotropy.
20. Explain, in their own words, what is meant by "one gene is epistatic to another."
21. Explain how epistasis affects the phenotypic ratio for a dihybrid cross.
22. Describe a simple model for polygenic inheritance, and explain why most polygenic characters are described in quantitative terms.
23. Describe how environmental conditions can influence the phenotypic expression of a character.
24. Given a simple family pedigree, deduce the genotypes for some of the family members.
25. Describe the inheritance and expression of cystic fibrosis, Tay-Sachs disease, and sickle-cell disease.
26. Explain how a lethal recessive gene can be maintained in a population.
27. Explain why consanguinity increases the probability of homozygosity in offspring.
28. Explain why lethal dominant genes are much more rare than lethal recessive genes.
29. Give an example of a late-acting lethal dominant in humans and explain how it can escape elimination.
30. Explain how carrier recognition, fetal testing and newborn screening can be used in genetic screening and counseling.

KEY TERMS

character
trait
true-breeding
hybridization
monohybrid cross
P generation
F_1 generation
F_2 generation
alleles
dominant allele
recessive allele
law of segregation
homozygous
heterozygous
phenotype
genotype
testcross
dihybrid cross
law of independent assortment
incomplete dominance
complete dominance
codominance
multiple alleles
pleiotropy
epistasis
quantitative character
polygenic inheritance
norm of reaction
multifactorial
carriers
cystic fibrosis
Tay-Sachs disease
sickle-cell disease
Huntington's disease

LECTURE NOTES

Listed below are a few suggestions for teaching Mendelian genetics:

1. There is a certain baseline working vocabulary that students need in order to follow your lecture, understand the text and solve problems. It is more economical to recognize this fact and just begin with some "definitions you should know." Once that is done, you can use the terms in context during the lecture and focus attention on the major points rather than on defining terms.
2. Demonstrating how to work a Punnett square and how to solve genetics problems is obviously necessary. But your students will learn best if they actively participate in the process. You can structure opportunities for students to solve problems during lecture and then let them participate in the explanation. If time does not allow this, it is highly recommended that there be an additional recitation or problem-solving session outside of class.
3. After Mendel's laws of segregation and independent assortment have been introduced, it is extremely useful to put up a transparency of meiosis and ask students to identify where in meiosis that segregation and assortment occur. Many students will not make this connection on their own.

I. Gregor Mendel's Discoveries

Based upon their observations from ornamental plant breeding, biologists in the 19th century realized that both parents contribute to the characteristics of offspring. Before Mendel, the favored explanation of heredity was the *blending theory*.

Blending theory of heredity = Pre-Mendelian theory of heredity proposing that hereditary material from each parent mixes in the offspring; once blended like two liquids in solution, the hereditary material is inseparable and the offspring's traits are some intermediate between the parental types. According to this theory:

- Individuals of a population should reach a uniform appearance after many generations.
- Once hereditary traits are blended, they can no longer be separated out to appear again in later generations.

This blending theory of heredity was inconsistent with the observations that:

- Individuals in a population do not reach a uniform appearance; inheritable variation among individuals is generally preserved.
- Some inheritable traits skip one generation only to reappear in the next.

Modern genetics began in the 1860s when Gregor Mendel, an Augustinian monk, discovered the fundamental principles of heredity. Mendel's great contribution to modern genetics was to replace the blending theory of heredity with the *particulate theory of heredity*.

Particulate theory of heredity = Gregor Mendel's theory that parents transmit to their offspring discrete inheritable factors (now called genes) that remain as separate factors from one generation to the next.

A. Mendel brought an experimental and quantitative approach to genetics: *science as a process*

While attending the University of Vienna from 1851-1853, Mendel was influenced by two professors:

- Doppler, a physicist, trained Mendel to apply a *quantitative experimental* approach to the study of natural phenomena.
- Unger, a botanist, interested Mendel in the causes of inheritable variation in plants.

These experiences inspired Mendel to use key elements of the scientific process in the study of heredity. Unlike most nineteenth century biologists, he used a quantitative approach to his experimentation.

In 1857, Mendel was living in an Augustinian monastery, where he bred garden peas in the abbey garden. He probably chose garden peas as his experimental organisms because:

- They were available in many easily distinguishable varieties.
- Strict control over mating was possible to ensure the parentage of new seeds. Petals of the pea flower enclose the pistil and stamens, which prevents cross-pollination. Immature stamens can be removed to prevent self-pollination. Mendel hybridized pea plants by transferring pollen from one flower to another with an artist's brush (see Campbell, Figure 14.1).

Character = Detectable inheritable feature of an organism

Trait = Variant of an inheritable character

Mendel chose characters in pea plants that differed in a relatively clear-cut manner. He chose seven characters, each of which occurred in two alternative forms:

1. Flower color (purple or white)
2. Flower position (axial or terminal)
3. Seed color (yellow or green)
4. Seed shape (round or wrinkled)
5. Pod shape (inflated or constricted)
6. Pod color (green or yellow)
7. Stem length (tall or dwarf)

True breeding = Always producing offspring with the same *traits* as the parents when the parents are self-fertilized

Mendel started his experiments with true-breeding plant varieties, which he hybridized (cross-pollinated) in experimental crosses.

- The true-breeding parental plants of such a *cross* are called the *P generation* (parental).
- The hybrid offspring of the P generation are the F_1 *generation* (first filial).
- Allowing F_1 generation plants to self-pollinate, produces the next generation, the F_2 *generation* (second filial).

Mendel observed the transmission of selected traits for at least three generations and arrived at two principles of heredity that are now known as the *law of segregation* and the *law of independent assortment.*

B. By the law of segregation, the two alleles for a character are packaged into separate gametes

When Mendel crossed true-breeding plants with different character traits, he found that the traits did not blend.

- Using the scientific process, Mendel designed experiments in which he used large sample sizes and kept accurate quantitative records of the results.
- For example, a cross between true-breeding varieties, one with purple flowers and one with white flowers, produced F_1 *progeny* (offspring) with only purple flowers.

Hypothesis: Mendel hypothesized that if the inheritable factor for white flowers had been lost, then a cross between F_1 plants should produce only purple-flowered plants.

Experiment: Mendel allowed the F_1 plants to self-pollinate.

Results: There were 705 purple-flowered and 224 white-flowered plants in the F_2 generation—a ratio of 3:1. The inheritable factor for white flowers was not lost, so the hypothesis was rejected (see Campbell, Figure 14.2).

Conclusions: From these types of experiments and observations, Mendel concluded that since the inheritable factor for white flowers was not lost in the F_1 generation, it must have been masked by the presence of the purple-flower factor. Mendel's factors are now called *genes*; and in Mendel's terms, purple flower is the *dominant trait* and white flower is the *recessive trait.*

Mendel repeated these experiments with the other six characters and found similar 3:1 ratios in the F_2 generations (see Campbell, Table 14.1). From these observations he developed a hypothesis that can be divided into four parts:

1. Alternative forms of genes are responsible for variations in inherited characters.
   - For example, the gene for flower color in pea plants exists in two alternative forms; one for purple color and one for white color.
   - Alternative forms for a gene are now called *alleles* (see Campbell, Figure 14.3).
2. For each character, an organism inherits two alleles, one from each parent.
   - Mendel deduced that each parent contributes one "factor," even though he did not know about chromosomes or meiosis.
   - We now know that Mendel's factors are genes. Each genetic locus is represented twice in diploid organisms, which have homologous pairs of chromosomes, one set for each parent. Homologous loci may have identical alleles as in Mendel's true-breeding organisms, or the two alleles may differ, as in the F_1 hybrids.\
3. If the two alleles differ, one is fully expressed (dominant allele); the other is completely masked (recessive allele).
   - Dominant alleles are designated by a capital letter: P = purple flower color.
   - Recessive alleles are designated by a lowercase letter: p = white flower color.
4. The two alleles for each character segregate during gamete production.
   - Without any knowledge of meiosis, Mendel deduced that a sperm cell or ovum carries only one allele for each inherited characteristic, because allele pairs separate (segregate) from each other during gamete production.
   - Gametes of true-breeding plants will all carry the same allele. If different alleles are present in the parent, there is a 50% chance that a gamete will receive the dominant allele, and a 50% chance that it will receive the recessive allele.
   - This sorting of alleles into separate gametes is known as Mendel's law of segregation.

Mendel's law of segregation = Allele pairs segregate during gamete formation (meiosis), and the paired condition is restored by the random fusion of gametes at fertilization (see Campbell, Figure 14.4).

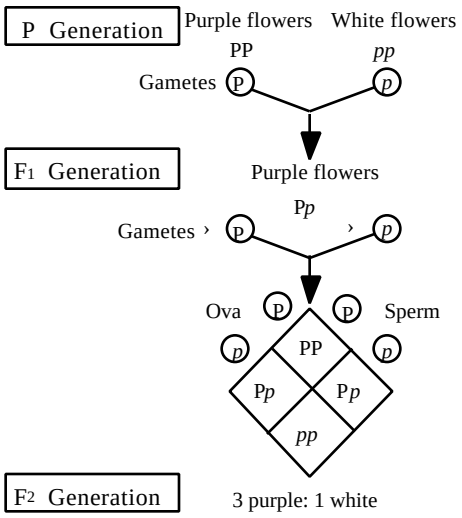
This law predicts the 3:1 ratio observed in the F_2 generation of a monohybrid cross.

- F_1 hybrids (Pp) produce two classes of gametes when allele pairs segregate during gamete formation. Half receive a purple-flower allele (P) and the other half the white-flower allele (p).
- During self-pollination, these two classes of gametes unite randomly. Ova containing purple-flower alleles have equal chances of being fertilized by sperm carrying purple-flower alleles or sperm carrying white-flower alleles.
- Since the same is true for ova containing white-flower alleles, there are four equally likely combinations of sperm and ova.

The combinations resulting from a genetic cross may be predicted by using a *Punnett square*.

The F_2 progeny would include:

- One-fourth of the plants with two alleles for purple flowers.
- One-half of the plants with one allele for purple flowers and one allele for white flowers. Since the purple-flower allele is dominant, these plants have purple flowers.
- One-fourth of the plants with two alleles for white flower color, which will have white flowers since no dominant allele is present.



The pattern of inheritance for all seven of the characteristics studied by Mendel was the same: one parental trait disappeared in the F_1 generation and reappeared in one-fourth of the F_2 generation.

1. Some useful genetic vocabulary

Homozygous = Having two identical alleles for a given trait (e.g., PP or pp).

- All gametes carry that allele.
- Homozygotes are *true-breeding.*

Heterozygous = Having two different alleles for a trait (e.g., Pp).

- Half of the gametes carries one allele (P) and the remaining half carries the other (p).
- Heterozygotes are not true-breeding.

Phenotype = An organism's expressed traits (e.g., purple or white flowers).

- In Mendel's experiment above, the F_2 generation had a 3:1 *phenotypic ratio* of plants with purple flowers to plants with white flowers.

Genotype = An organism's genetic makeup (e.g., PP, Pp, or pp).

- The *genotypic ratio* of the F_2 generation was 1:2:1 (1 PP:2 Pp:1 pp).
- Campbell, Figure 14.5 compares genotype to phenotype.

2. The testcross

Because some alleles are dominant over others, the genotype of an organism may not be apparent. For example:

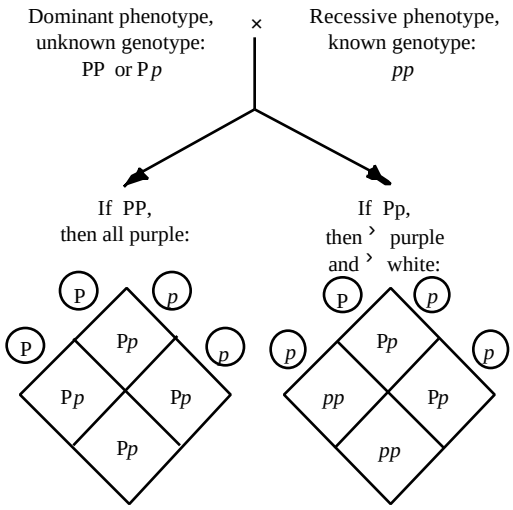
- A pea plant with purple flowers may be either homozygous dominant (PP) or heterozygous (Pp).

To determine whether an organism with a dominant phenotype (e.g., purple flower color) is homozygous dominant or heterozygous, you use a *testcross.*

Testcross = The breeding of an organism of unknown genotype with a homozygous recessive (see also Campbell, Figure 14.6).

- For example, if a cross between a purple-flowered plant of unknown genotype (P___) produced only purple-flowered plants, the parent was probably homozygous dominant since a PP × pp cross produces all purple-flowered progeny that are heterozygous (Pp).

- If the progeny of the testcross contains both purple and white phenotypes, then the purple-flowered parent was heterozygous since a Pp × pp cross produces Pp and pp progeny in a 1:1 ratio.



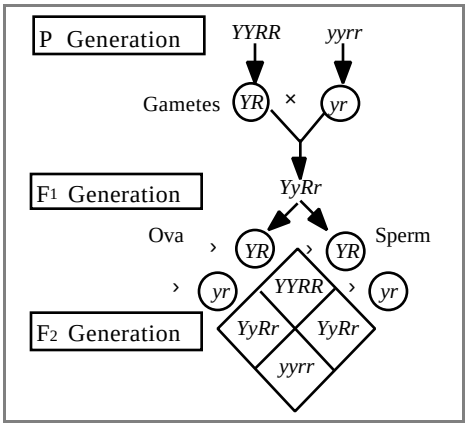
C. By the law of independent assortment, each pair of alleles segregates into gametes independently

Mendel deduced the law of segregation from experiments with *monohybrid crosses*, breeding experiments that used parental varieties differing in a single trait. He then performed crosses between parental varieties that differed in two characters or *dihybrid crosses*.

Dihybrid cross = A mating between parents that are heterozygous for two characters (dihybrids).

- Mendel began his experiments by crossing true-breeding parent plants that differed in two characters such as seed color (yellow or green) and seed shape (round or wrinkled). From previous monohybrid crosses, Mendel knew that yellow seed (Y) was dominant to green (y), and that round (R) was dominant to wrinkled (r).
- Plants homozygous for round yellow seeds (RRYY) were crossed with plants homozygous for wrinkled green seeds (rryy).
- The resulting F_1 dihybrid progeny were heterozygous for both traits (RrYy) and had round yellow seeds, the dominant phenotypes.
- From the F_1 generation, Mendel could not tell if the two characters were inherited independently or not, so he allowed the F_1 progeny to self-pollinate. In the following experiment, Mendel considered two alternate hypotheses (see also Campbell, Figure 14.7):

Hypothesis 1: If the two characters segregate *together*, the F_1 hybrids can only produce the same two classes of gametes (RY and ry) that they received from the parents, and the F_2 progeny will show a 3:1 phenotypic ratio.

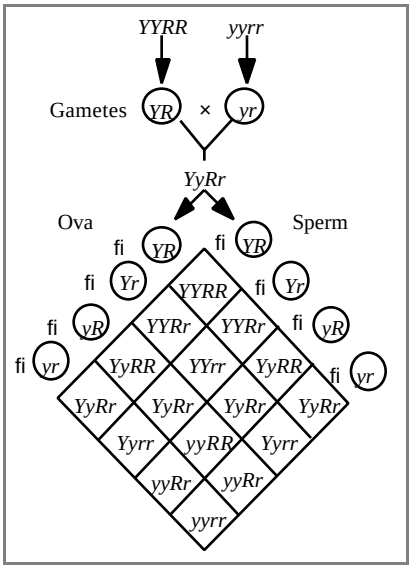


Hypothesis 2: If the two characters segregate *independently*, the F_1 hybrids will produce four classes of gametes (RY, Ry, rY, ry), and the F_2 progeny will show a 9:3:3:1 ratio.

Experiment: Mendel performed a dihybrid cross by allowing self-pollination of the F_1 plants (RrYy × RrYy).

Results: Mendel categorized the F_2 progeny and determined a ratio of 315:108:101:32, which approximates 9:3:3:1.

- These results were repeatable. Mendel performed similar dihybrid crosses with all seven characters in various combinations and found the same 9:3:3:1 ratio in each case.
- He also noted that the ratio for each individual gene pair was 3:1, the same as that for a monohybrid cross.



Conclusions: The experimental results supported the hypothesis that *each allele pair segregates independently during gamete formation.*

This behavior of genes during gamete formation is referred to as *Mendel's law of independent assortment.*

Mendel's law of independent assortment = Each allele pair segregates independently of other gene pairs during gamete formation.

D. Mendelian inheritance reflects rules of probability

Segregation and independent assortment of alleles during gamete formation and fusion of gametes at fertilization are random events. Thus, if we know the genotypes of the parents, we can predict the most likely genotypes of their offspring by using the simple *laws of probability*:

- The probability scale ranges from 0 to 1; an event that is certain to occur has a probability of 1, and an event that is certain *not* to occur has a probability of 0.
- The probabilities of all possible outcomes for an event must add up to 1.
- For example, when tossing a coin or rolling a six-sided die:

Event		Probability
Tossing heads with a two-headed coin	1	1 + 0 = 1
Tossing tails with a two-headed coin	0	
Tossing heads with a normal coin	1/2	1/2 + 1/2 = 1
Tossing tails with a normal coin	1/2	
Rolling 3 on a six-sided die	1/6	1/6 + 5/6 = 1
Rolling a number other than 3	5/6	

Random events are *independent* of one another.

- The outcome of a random event is unaffected by the outcome of previous such events.
- For example, it is possible that five successive tosses of a normal coin will produce five heads; however, the probability of heads on the sixth toss is still 1/2.

Two basic rules of probability are helpful in solving genetics problems: the *rule of multiplication* and the *rule of addition.*

1. Rule of multiplication

Rule of multiplication = The probability that independent events will occur simultaneously is the product of their individual probabilities (see Campbell, Figure 14.8). For example:

Question: In a Mendelian cross between pea plants that are heterozygous for flower color (Pp), what is the probability that the offspring will be homozygous recessive?

Answer:

Probability that an egg from the F1 (Pp) will receive a p allele = 1/2.

Probability that a sperm from the F1 will receive a p allele = 1/2.

The overall probability that two recessive alleles will unite at fertilization: $1/2 \times 1/2 = 1/4$.

This rule also applies to dihybrid crosses. For example:

Question: For a dihybrid cross, YyRr × YyRr, what is the probability of an F_2 plant having the genotype YYRR?

Answer:

Probability that an egg from a YyRr parent will receive the Y and R alleles = $1/2 \times 1/2 = 1/4$.

Probability that a sperm from a YyRr parent will receive a the Y and R alleles = $1/2 \times 1/2 = 1/4$.

The overall probability of an F_2 plant having the genotype YYRR: $1/4 \times 1/4 = \underline{\underline{1/16}}$.

2. Rule of addition

Rule of addition = The probability of an event that can occur in two or more independent ways is the sum of the separate probabilities of the different ways. For example:

Question: In a Mendelian cross between pea plants that are heterozygous for flower color (Pp), what is the probability of the offspring being a heterozygote?

Answer: There are two ways in which a heterozygote may be produced: the dominant allele (P) may be in the egg and the recessive allele (p) in the sperm, or the dominant allele may be in the sperm and the recessive in the egg. Consequently, the probability that the offspring will be heterozygous is the sum of the probabilities of those two possible ways:

Probability that the dominant allele will be in the egg with the recessive in the sperm is $1/2 \times 1/2 = 1/4$.

Probability that the dominant allele will be in the sperm and the recessive in the egg is $1/2 \times 1/2 = 1/4$.

Therefore, the probability that a heterozygous offspring will be produced is $1/4 + 1/4 = \underline{\underline{1/2}}$.

3. Using rules of probability to solve genetics problems

The rules of probability can be used to solve complex genetics problems. For example, Mendel crossed pea varieties that differed in three characters (*trihybrid crosses*).

Question: What is the probability that a trihybrid cross between two organisms with the genotypes AaBbCc and AaBbCc will produce an offspring with the genotype aabbcc?

Answer: Because segregation of each allele pair is an independent event, we can treat this as three separate monohybrid crosses:

Aa × Aa:	probability for aa offspring	=	1/4
Bb × Bb:	probability for bb offspring	=	1/4
Cc × Cc:	probability for cc offspring	=	1/4

The probability that these independent events will occur simultaneously is the product of their independent probabilities (rule of multiplication). So the probability that the offspring will be aabbcc is:

1/4 aa × 1/4 bb × 1/4 cc = 1/64

For another example, consider a trihybrid cross of garden peas, where:

Character	Trait & Genotype
Flower Color	Purple: PP, Pp
	White: pp
Seed Color	Yellow: YY, Yy
	Green: yy
Seed Shape	Round: RR, Rr
	Wrinkled: rr

Question: phenotypes for *at least* two of the three traits?

PpYyRr × Ppyyrr

Answer: First list those genotypes that are homozygous recessive for at least two traits, (note that this includes the homozygous recessive for all three traits). Use the *rule of multiplication* to calculate the probability that offspring would be one of these genotypes. Then use the *rule of addition* to calculate the probability that two of the three traits would be homozygous recessive.

Genotypes with at least two homozygous recessives			Probability of genotype
ppyyRr	1/4 × 1/2 × 1/2	=	1/16
ppYyrr	1/4 × 1/2 × 1/2	=	1/16
Ppyyrr	1/2 × 1/2 × 1/2	=	2/16
PPyyrr	1/4 × 1/2 × 1/2	=	1/16
ppyyrr	1/4 × 1/2 × 1/2	=	1/16
		=	6/16 or 3/8 chance of two recessive traits

D. Mendel discovered the particulate behavior of genes: *a review*

If a seed is planted from the F_2 generation of a monohybrid cross, we cannot predict with absolute certainty that the plant will grow to produce white flowers (pp). We *can* say that there is a 1/4 chance that the plant will have white flowers.

- Stated in statistical terms: among a large sample of F_2 plants, 25% will have white flowers.
- The larger the sample size, the closer the results will conform to predictions.

Mendel's quantitative methods reflect his understanding of this statistical feature of inheritance. Mendel's laws of segregation and independent assortment are based on the premise that:

- Inheritance is a consequence of discrete factors (genes) that are passed on from generation to generation.
- Segregation and assortment are random events and thus obey the simple laws of probability.

II. Extending Mendelian Genetics

A. The relationship between genotype and phenotype is rarely simple

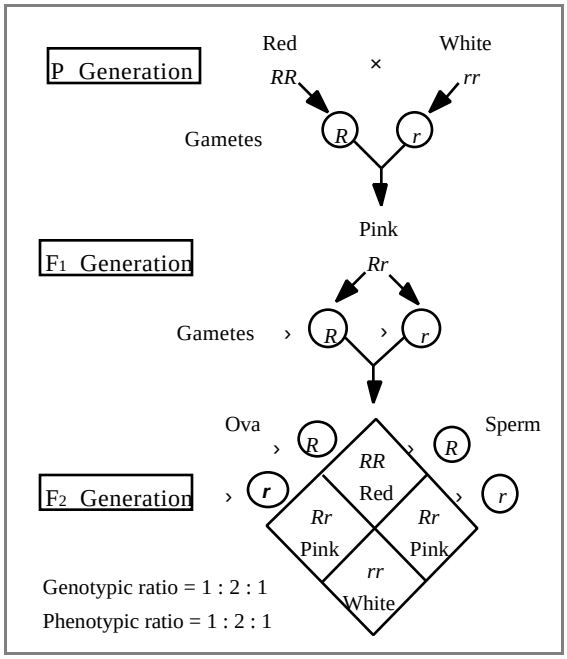
As Mendel described it, characters are determined by one gene with two alleles; one allele completely dominant over the other. There are other patterns of inheritance not described by Mendel, but his laws of segregation and independent assortment can be extended to these more complex cases.

1. Incomplete dominance

In cases of *incomplete dominance*, one allele is not completely dominant over the other, so the heterozygote has a phenotype that is intermediate between the phenotypes of the two homozygotes (see Campbell, Figure 14.9).

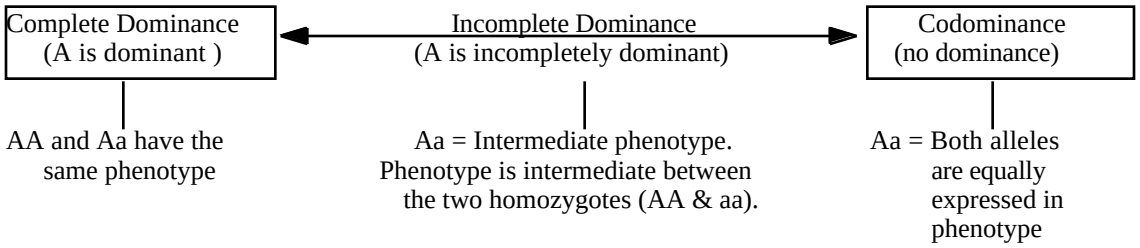
Incomplete dominance = Pattern of inheritance in which the dominant phenotype is not fully expressed in the heterozygote, resulting in a phenotype intermediate between the homozygous dominant and homozygous recessive.

- For example, when red snapdragons (RR) are crossed with white snapdragons (rr), all F_1 hybrids (Rr) have pink flowers. (The heterozygote produces half as much red pigment as the homozygous red-flowered plant.)
- Since the heterozygotes can be distinguished from homozygotes by their phenotypes, the phenotypic and genotypic ratios from a monohybrid cross are the same—1:2:1.
- Incomplete dominance is *not* support for the blending theory of inheritance, because alleles maintain their integrity in the heterozygote and segregate during gamete formation. Red and white phenotypes reappear in the F_2 generation.



2. What is a dominant allele?

Dominance/recessiveness relationships among alleles vary in a continuum from *complete dominance* on one end of the spectrum to *codominance* on the other, with various degrees of incomplete dominance in between these extremes.



Complete dominance = Inheritance characterized by an allele that is fully expressed in the phenotype of a heterozygote and that masks the phenotypic expression of the recessive allele; state in which the phenotypes of the heterozygote and dominant homozygote are indistinguishable.

Codominance = Inheritance characterized by full expression of both alleles in the heterozygote.

- For example, the MN blood-group locus codes for the production of surface glycoproteins on the red blood cell. In this system, there are three blood types: M, N and MN.

Blood Type	**Genotype**
M	MM
N	NN
MN	MN

- The MN blood type is the result of full phenotypic expression of *both* alleles in the heterozygote; both molecules, M and N, are produced on the red blood cell.

Apparent dominance/recessiveness relationships among alleles reflect the level at which the phenotype is studied. For example:

- *Tay-Sachs disease* is a recessively inherited disease in humans; only children who are homozygous recessive for the Tay-Sachs allele have the disease.
- Brain cells of Tay-Sachs babies lack a crucial lipid-metabolizing enzyme. Thus, lipids accumulate in the brain, causing the disease symptoms and ultimately leading to death.
- At the *organismal level*, since heterozygotes are symptom free, it appears that the normal allele is completely dominant and the Tay-Sachs allele is recessive.
- At the *biochemical level*, inheritance of Tay-Sachs seems to be incomplete dominance of the normal allele, since there is an intermediate phenotype. Heterozygotes have an enzyme activity level that is intermediate between individuals homozygous for the normal allele and individuals with Tay-Sachs disease.
- At the *molecular level*, the normal allele and the Tay-Sachs allele are actually codominant. Heterozygotes produce equal numbers of normal and dysfunctional enzymes. They lack disease symptoms, because half the normal amount of functional enzyme is sufficient to prevent lipid accumulation in the brain.

Dominance/recessiveness relationships among alleles:

- Are a consequence of the mechanism that determines phenotypic expression, not the ability of one allele to subdue another at the level of the DNA
- Do not determine the relative abundance of alleles in a population
    - In other words, dominant alleles are not necessarily more common and recessive alleles more rare.

- For example, the allele for polydactyly is quite rare in the U.S. (1 in 400 births), yet it is caused by a dominant allele. (Polydactyly is the condition of having extra fingers or toes.)

3. Multiple alleles

Some genes may have *multiple alleles*; that is, more than just two alternative forms of a gene. The inheritance of the ABO blood group is an example of a locus with three alleles (see Campbell, Figure 14.10).

Paired combinations of three alleles produce four possible phenotypes:

- Blood type A, B, AB, or O.
- A and B refer to two genetically determined polysaccharides (A and B antigens) which are found on the surface of red blood cells different from the (different from the MN characters).

There are three alleles for this gene: I^A, I^B, and i.

- The I^A allele codes for the production of A antigen, the I^B allele codes for the production of B antigen, and the i allele codes for *no* antigen production on the red blood cell (neither A or B).
- Alleles I^A and I^B are *codominant* since both are expressed in heterozygotes.
- Alleles I^A and I^B are dominant to allele i, which is recessive.
- Even though there are three possible alleles, every person carries only two alleles which specify their ABO blood type; one allele is inherited from each parent.

Since there are three alleles, there are six possible genotypes:

Blood Type	Possible Genotypes	Antigens on the Red Blood Cell	Antibodies in the Serum
A	I^AI^A I^Ai	A	anti-B
B	I^BI^B I^Bi	B	anti-A
AB	I^AI^B	A, B	----
O	ii	----	anti-A anti-B

Foreign antigens usually cause the immune system to respond by producing *antibodies*, globular proteins that bind to the foreign molecules causing a reaction that destroys or inactivates it. In the ABO blood system:

- The antigens are located on the red blood cell and the antibodies are in the serum.
- A person produces antibodies against foreign blood antigens (those not possessed by the individual). These antibodies react with the foreign antigens causing the blood cells to clump or *agglutinate*, which may be lethal.
- For a blood transfusion to be successful, the red blood cell *antigens of the donor* must be compatible with the *antibodies of the recipient.*

4. Pleiotropy

Pleiotropy = The ability of a single gene to have multiple phenotypic effects.

- There are many hereditary diseases in which a single defective gene causes complex sets of symptoms (e.g., sickle-cell anemia).

- One gene can also influence a combination of seemingly unrelated characteristics. For example, in tigers and Siamese cats, the gene that controls fur pigmentation also influences the connections between a cat's eyes and the brain. A defective gene causes both abnormal pigmentation and cross-eye condition.

5. Epistasis

Different genes can interact to control the phenotypic expression of a single trait. In some cases, a gene at one locus alters the phenotypic expression of a second gene, a condition known as *epistasis* (see Campbell, Figure 14.12).

Epistasis = (Epi=upon; stasis=standing) Interaction between two nonallelic genes in which one modifies the phenotypic expression of the other.

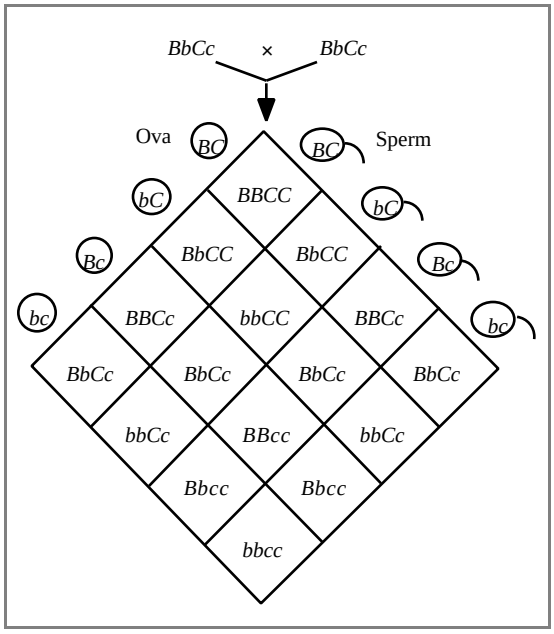
- If one gene suppresses the phenotypic expression of another, the first gene is said to be *epistatic* to the second.
- If epistasis occurs between two nonallelic genes, the phenotypic ratio resulting from a dihybrid cross will deviate from the 9:3:3:1 Mendelian ratio.
- For example, in mice and other rodents, the gene for pigment deposition (C) is epistatic to the gene for pigment (melanin) production. In other words, whether the pigment can be deposited in the fur determines whether the coat color can be expressed. Homozygous recessive for pigment deposition (cc) will result in an albino mouse regardless of the genotype at the black/brown locus (BB, Bb or bb):

  CC, Cc = Melanin deposition

  cc = Albino

  BB, Bb = Black coat color

  bb = Brown coat color



- Even though both genes affect the same character (coat color), they are inherited separately and will assort independently during gamete formation. A cross between black mice that are heterozygous for the two genes results in a 9:3:4 phenotypic ratio:

  9 Black (B_C_)

  3 Brown (bbC_)

  4 Albino (_cc)

6. Polygenic inheritance

Mendel's characters could be classified on an either-or basis, such as purple versus white flower. Many characters, however, are *quantitative characters* that vary in a continuum within a population.

Quantitative characters = Characters that vary by degree in a continuous distribution rather than by discrete (either-or) qualitative differences.

- Usually, continuous variation is determined not by one, but by many segregating loci or *polygenic inheritance*.

Polygenic inheritance = Mode of inheritance in which the additive effect of two or more genes determines a single phenotypic character.

For example, skin pigmentation in humans appears to be controlled by at least three separately inherited genes. The following is a simplified model for the polygenic inheritance of skin color:

- Three genes with the dark-skin allele (*A, B, C*) contribute one "unit" of darkness to the phenotype. These alleles are incompletely dominant over the other alleles (*a, b, c*).
- An *AABBCC* person would be very dark and an aabbcc person would be very light.
- An *AaBbCc* person would have skin of an intermediate shade.
- Because the alleles have a cumulative effect, genotypes *AaBbCc* and *AABbcc* make the same genetic contribution (three "units") to skin darkness. (See Campbell, Figure 14.12)
- Environmental factors, such as sun exposure, could also affect the phenotype.

7. Nature versus nurture: the environmental impact on phenotype

Environmental conditions can influence the phenotypic expression of a gene, so that a single genotype may produce a range of phenotypes. This environmentally-induced phenotypic range is the *norm of reaction* for the genotype.

Norm of reaction = Range of phenotypic variability produced by a single genotype under various environmental conditions (see Campbell, Figure 14.13). Norms of reaction for a genotype:

- May be quite limited, so that a genotype only produces a specific phenotype, such as the blood group locus that determines ABO blood type.
- May also include a wide range of possibilities. For example, an individual's blood cell count varies with environmental factors such as altitude, activity level or infection.
- Are generally broadest for polygenic characters, including behavioral traits.

The expression of most polygenic traits, such as skin color, is *multifactorial*; that is, it depends upon many factors - a variety of possible genotypes, as well as a variety of environmental influences.

8. Integrating a Mendelian view of heredity and variation

These patterns of inheritance that are departures from Mendel's original description, can be integrated into a comprehensive theory of Mendelian genetics.

- Taking a holistic view, an organism's entire phenotype reflects its overall genotype and unique environmental history.
- Mendelism has broad applications beyond its original scope; extending the principles of segregation and independent assortment helps explain more complex hereditary patterns such as epistasis and quantitative characters.

III. Mendalian Inheritance in Humans

A. Pedigree analysis reveals Mendelian patterns in human inheritance

Mendelian inheritance in humans is difficult to study because:

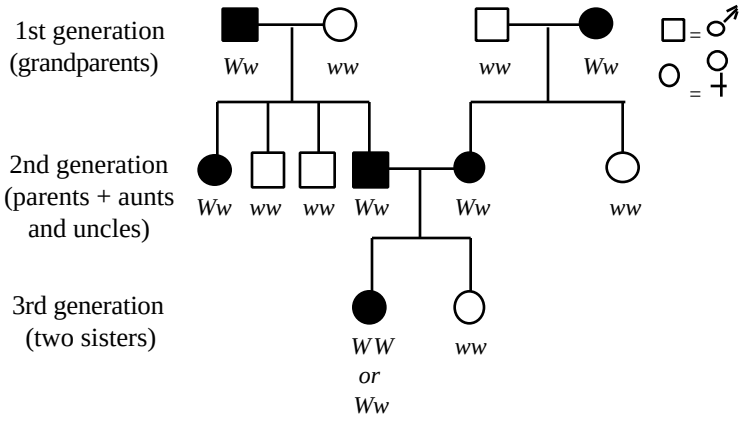
- The human generation time is about 20 years.
- Humans produce relatively few offspring compared to most other species.
- Well-planned breeding experiments are impossible.

Our understanding of Mendelian inheritance in humans is based on the analysis of family pedigrees or the results of matings that have already occurred.

Pedigree = A family tree that diagrams the relationships among parents and children across generations and that shows the inheritance pattern of a particular phenotypic character (see Campbell, Figure 14.14). By convention:

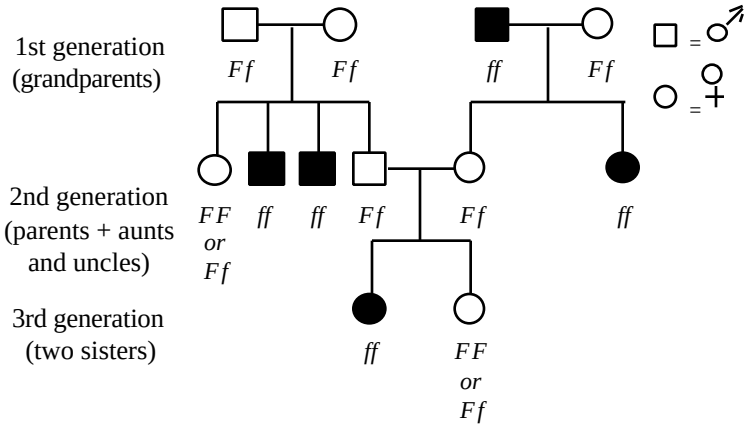
- Squares symbolize males and circles represent females.
- A horizontal line connecting a male and female indicates a mating; offspring are listed below in birth order, from left to right.
- Shaded symbols indicate individuals showing the trait being traced.

Following a dominant trait. For example, family members' genotypes can be deduced from a pedigree that traces the occurrence of widow's peak, the expression of a dominant allele.



- If a widow's peak results from a dominant allele, *W*, then all individuals that do not have a widow's peak hairline must be homozygous recessive (*ww*). The genotypes of all recessives can be written on the pedigree.
- If widow's peak results from a dominant allele, W, then individuals that have a widow's peak hairline must be either homozygous dominant (*WW*) or heterozygous (*Ww*).
- If only some of the second generation offspring have a widow's peak, then the grandparents that show the trait must be heterozygous (*Ww*). (Note: if the grandparents with widow's peak were homozygous dominant, then all their respective offspring would show the trait.)
- Second generation offspring with widow's peaks must be heterozygous, because they are the result of *Ww* × *ww* matings.
- The third generation sister with widow's peak may be either homozygous dominant (*WW*) or heterozygous (*Ww*), because her parents are both heterozygous.

Following a recessive trait. For example, the same family can be used to trace a recessive trait such as attached ear lobes.



- If attached earlobes is due to a recessive allele (*f*), then all individuals with attached earlobes must be homozygous recessive (*ff*).
- Since attached earlobes appears in second generation offspring, the grandparents with free earlobes are heterozygous (*Ff*) since they must be capable of passing on a recessive allele (*f*).
- Since one of the third generation sisters has attached earlobes (ff), her parents are heterozygous; they have free earlobes (dominant trait) and yet must be able to contribute a recessive allele to their daughter. The other sister shows the dominant trait, so her genotype is unknown; it is possible that she may be either homozygous dominant or heterozygous.

Pedigree analysis can also be used to:

- Deduce whether a trait is determined by a recessive or dominant allele. Using the example above:
  - The first-born third generation daughter has attached earlobes. Since both parents *lack* the trait, it must not be determined by a dominant allele.
- Predict the occurrence of a trait in future generations. For example, if the second generation couple decide to have another child,
  - What is the probability the child will have a widow's peak? From a mating of *Ww* × *Ww*:

    Probability of a child being *WW* = 1/4
    Probability of a child being *Ww* = 2/4
    Probability of widow's peak = 3/4

  - What is the probability the child will have attached earlobes? From a mating of *Ff* × *Ff*:

    probability of a child being *ff* = 1/4

  - What is the probability the child will have a widow's peak and attached earlobes? From a cross of *WwFf* × *WwFf*, use the rule of multiplication:

    3/4 (probability of widow's peak) × 1/4 (probability of attached earlobes) = 3/16

This type of analysis is important to geneticists and physicians, especially when the trait being analyzed can lead to a disabling or lethal disorder.

B. Many human disorders follow Mendelian patterns of inheritance

1. Recessively inherited disorders

Recessive alleles that cause human disorders are usually defective versions of normal alleles.

- Defective alleles code for either a malfunctional protein or no protein at all.
- Heterozygotes can be phenotypically normal, if one copy of the normal allele is all that is needed to produce sufficient quantities of the specific protein.

Recessively inherited disorders range in severity from nonlethal traits (e.g., albinism) to lethal diseases (e.g., cystic fibrosis). Since these disorders are caused by recessive alleles:

- The phenotypes are expressed only in homozygotes (aa) who inherit one recessive allele from each parent.
- Heterozygotes (Aa) can be phenotypically normal and act as *carriers*, possibly transmitting the recessive allele to their offspring.

Most people with recessive disorders are born to normal parents, both of whom are carriers.

- The probability is 1/4 that a mating of two carriers (Aa × Aa) will produce a homozygous recessive zygote.
- The probability is 2/3 that a normal child from such a mating will be a heterozygote, or a carrier.

Human genetic disorders are not usually evenly distributed among all racial and cultural groups due to the different genetic histories of the world's people. Three examples of such recessively inherited disorders are *cystic fibrosis*, *Tay-Sachs disease* and *sickle-cell disease*.

Cystic fibrosis, the most common lethal genetic disease in the United States, strikes 1 in every 2,500 Caucasians (it is much rarer in other races).

- Four percent of the Caucasian population are carriers.
- The dominant allele codes for a membrane protein that controls chloride traffic across the cell membrane. Chloride channels are defective or absent in individuals that are homozygous recessive for the cystic fibrosis allele.
- Disease symptoms result from the accumulation of thickened mucus in the pancreas, intestinal tract and lungs, a condition that favors bacterial infections.

Tay-Sachs disease occurs in 1 out of 3,600 births. The incidence is about 100 times higher among Ashkenazic (central European) Jews than among Sephardic (Mediterranean) Jews and non-Jews.

- Brain cells of babies with this disease are unable to metabolize gangliosides (a type of lipid), because a crucial enzyme does not function properly.
- As lipids accumulate in the brain, the infant begins to suffer seizures, blindness and degeneration of motor and mental performance. The child usually dies after a few years.

Sickle-cell disease is the most common inherited disease among African Americans. It affects 1 in 400 African Americans born in the United States (see Campbell, Figure 14.15).

- The disease is caused by a single amino acid substitution in hemoglobin.
- The abnormal hemoglobin molecules tend to link together and crystallize, especially when blood oxygen content is lower than normal. This causes red blood cells to deform from the normal disk-shape to a sickle-shape.

- The sickled cells clog tiny blood vessels, causing the pain and fever characteristic of a sickle-cell crisis.

About 1 in 10 African Americans are heterozygous for the sickle-cell allele and are said to have the *sickle-cell trait*.

- These carriers are usually healthy, although some suffer symptoms after an extended period of low blood oxygen levels.
- Carriers can function normally because the two alleles are codominant (heterozygotes produce not only the abnormal hemoglobin but also normal hemoglobin).
- The high incidence of heterozygotes is related to the fact that in tropical Africa where malaria is endemic, heterozygotes have enhanced resistance to malaria compared to normal homozygotes. Thus, heterozygotes have an advantage over both homozygotesÑthose who have sickle cell disease and those who have normal hemoglobin.

The probability of inheriting the same rare harmful allele from both parents, is greater if the parents are closely related.

Consanguinity = A genetic relationship that results from shared ancestry

- The probability is higher that consanguinous matings will result in homozygotes for harmful recessives, since parents with recently shared ancestry are more likely to inherit the same recessive alleles than unrelated persons.
- It is difficult to accurately assess the extent to which human consanguinity increases the incidence of inherited diseases, because embryos homozygous for deleterious mutations are affected so severely that most are spontaneously aborted before birth.
- Most cultures forbid marriage between closely related adults. This may be the result of observations that stillbirths and birth defects are more common when parents are closely related.

2. Dominantly inherited disorders

Some human disorders are dominantly inherited.

- For example, *achondroplasia* (a type of dwarfism) affects 1 in 10,000 people who are heterozygous for this gene.
- Homozygous dominant condition results in spontaneous abortion of the fetus, and homozygous recessives are of normal phenotype (99.9% of the population).

Lethal dominant alleles are much rarer than lethal recessives, because they:

- Are always expressed, so their effects are not masked in heterozygotes.
- Usually result from new genetic mutations that occur in gametes and later kill the developing embryo.

Late-acting lethal dominants can escape elimination if the disorder does not appear until an advanced age after afflicted individuals may have transmitted the lethal gene to their children. For example,

- *Huntington's disease*, a degenerative disease of the nervous system, is caused by a late-acting lethal dominant allele. The phenotypic effects do not appear until 35 to 40 years of age. It is irreversible and lethal once the deterioration of the nervous system begins.
- Molecular geneticists have recently located the gene for Huntington’s near the tip of chromosome #4.
- Children of an afflicted parent have a 50% chance of inheriting the lethal dominant allele. A newly developed test can detect the Huntington’s allele before disease symptoms appear.

3. Multifactorial disorders

Not all hereditary diseases are simple Mendelian disorders; that is, diseases caused by the inheritance of certain alleles at a single locus. More commonly, people are afflicted by *multifactorial* disorders, diseases that have both genetic and environmental influences.

- Examples include heart disease, diabetes, cancer, alcoholism and some forms of mental illness.
- The hereditary component is often polygenic and poorly understood.
- The best public-health strategy is to educate people about the role of environmental and behavioral factors that influence the development of these diseases.

C. Technology is providing new tools for genetic testing and counseling

Genetic counselors in many hospitals can provide information to prospective parents concerned about a family history for a genetic disorder.

- This preventative approach involves assessing the risk that a particular genetic disorder will occur.
- Risk assessment includes studying the family history for the disease using Mendel's law of segregation to deduce the risk.

For example, a couple is planning to have a child, and both the man and woman had siblings who died from the same recessively inherited disorder. A genetic counselor could deduce the risk of their first child inheriting the disease by using the laws of probability:

Question: What is the probability that the husband and wife are each carriers?

Answer: The genotypic ratio from an Aa × Aa cross is 1 AA:2 Aa:1 aa. Since the parents are normal, they have a $\underline{\underline{2/3}}$ of being carriers.

Question: What is the chance of two carriers having a child with the disease?

Answer: 1/2 (mother's chance of passing on the gene) × 1/2 (father's chance of passing on the gene) = $\underline{\underline{1/4}}$

Question: What is the probability that their firstborn will have the disorder?

Answer: (Chance that the father is a carrier) × (chance that mother is a carrier) × (chance of two carriers having a child with the disease).

$2/3 \times 2/3 \times 1/4 = \underline{\underline{1/9}}$

If the first child is born with the disease, what is the probability that the second child will inherit the disease?

- If the first child is born with the disease, then it is certain that both the man and the woman are carriers. Thus, the probability that other children produced by this couple will have the disease is $\underline{\underline{1/4}}$.
- The conception of each child is an independent event, because the genotype of one child does not influence the genotype of the other children. So there is a 1/4 chance that any additional child will inherit the disease.

1. Carrier recognition

Several tests are available to determine if prospective parents are carriers of genetic disorders.

- Tests are currently available that can determine heterozygous carriers for the Tay-Sachs allele, cystic fibrosis, and sickle-cell disease.
- Tests such as these enable people to make informed decisions about having children, but they could also be abused. Ethical dilemmas about how this information should be used points to the immense social implications of such technological advances.

2. **Fetal testing**

   A couple that learns they are both carriers for a genetic disease and decide to have a child can determine if the fetus has the disease. Between the fourteenth and sixteenth weeks of pregnancy, *amniocentesis*. can be done to remove amniotic fluid for testing (see Campbell, Figure 14.17).

   - During amniocentesis, a physician inserts a needle into the uterus and extracts about ten milliliters of amniotic fluid.
   - The presence of certain chemicals in amniotic fluid indicate some genetic disorders.
   - Some tests (including one for Tay-Sachs) are performed on cells grown in culture from fetal cells sloughed off in the amniotic fluid. These cells can also be karyotyped to identify chromosomal defects.

   Chorionic villus sampling (CVS) is a newer technique during which a physician suctions off a small amount of fetal tissue from the chorionic villi of the placenta.

   - These rapidly dividing embryonic cells can be karyotyped immediately, usually providing results in 24 hoursÑa major advantage over amniocentesis which may take several weeks. (Amniocentesis requires that the cells must first be cultured before karyotyping can be done.)
   - Another advantage of CVS is that it can be performed at only eight to ten weeks of pregnancy.

   Other techniques such as *ultrasound* and *fetoscopy* allow physicians to examine a fetus for major abnormalities.

   - Ultrasound is a non-invasive procedure which uses sound waves to create an image of the fetus.
   - Fetoscopy involves inserting a thin fiber-optic scope into the uterus.

   Amniocentesis and fetoscopy have a 1% risk of complication such as maternal bleeding or fetal death. Thus, they are used only when risk of genetic disorder or birth defect is relatively high.

3. **Newborn screening**

   In most U.S. hospitals, simple tests are routinely performed at birth, to detect genetic disorders such as *phenylketonuria* (PKU).

   - PKU is recessively inherited and occurs in about 1 in 15,000 births in the United States.
   - Children with this disease cannot properly break down the amino acid phenylalanine.
   - Phenylalanine and its by-product (phenylpyruvic acid) can accumulate in the blood to toxic levels, causing mental retardation.
   - Fetal screening for PKU can detect the deficiency in a newborn and retardation can be prevented with a special diet (low in phenylalanine) that allows normal development.

REFERENCES

Campbell, N, et al. *Biology*. 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Griffith, A.J.E., J.H. Miller, D.T. Suzuki, R.C. Lewontin, and W.M. Gelbart. *An Introduction to Genetic Analysis*. 5th ed. New York: W.H. Freeman, 1993.

Kowles, R.V. *Genetics, Society and Decisions*. 1st ed. Columbus, Ohio: Charles E. Merrill, 1985. Though the target audience for this book is non-majors, it can be a useful lecture supplement for contrasting controversial social issues.

Russell, P.J. *Genetics*. 2nd ed. Glenview, Illinois: Scott, Foresman, 1990.