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## CHAPTER 9

### IMMUNOGLOBULIN BIOSYNTHESIS

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Although the process by which a functional gene for immunoglobulin HEAVY and LIGHT CHAINS is formed is highly unusual, the SYNTHESIS, POST-TRANSLATIONAL PROCESSING and SECRETION of these glycoproteins all occur by conventional pathways. The J-CHAIN is required for polymeric Ig and the SECRETORY PIECE for the unique process by which secretory IgA is transported into exocrine fluids. Knowing the molecular and cellular mechanisms involved in Ig production, we can understand the importance of ALLELIC EXCLUSION and the structural differences between SECRETED *versus* MEMBRANE-BOUND FORMS of Ig, and provide a rational basis for the existence of CLASS SWITCHING.

Most circulating immunoglobulin is produced by plasma cells, which are highly specialized and efficient antibody "factories". The biosynthesis of immunoglobulins has much in common with the biosynthesis of other secreted proteins. Several aspects of this process, however, are unusual, and some features are quite unique to immunoglobulins.

The structural components of Ig molecules we need to account for are as follows:

- Light Chains
- Heavy Chains
- Carbohydrate (on H-chain only)
- J-Chain (attached to H-chain of IgM and polymeric IgA)
- Secretory piece (attached to H-chain of secretory IgA)

Let's follow the process of synthesis and assembly of an immunoglobulin molecule in a plasma cell, referring to the diagram (Fig. 9-1) below.

#### FEATURES OF IMMUNOGLOBULIN SYNTHESIS

- 1) *Conventional mRNA molecules* are produced separately for the light and heavy chains, each containing uninterrupted coding sequences for V and C-regions. While the structure of the mRNA is conventional, some of the mechanisms involved in its synthesis (the DNA rearrangements discussed earlier) are quite unique to immunoglobulins. *Translation* of the message occurs on the ribosomes of the *rough endoplasmic reticulum (RER)*.

# IMMUNOGLOBULIN BIOSYNTHESIS

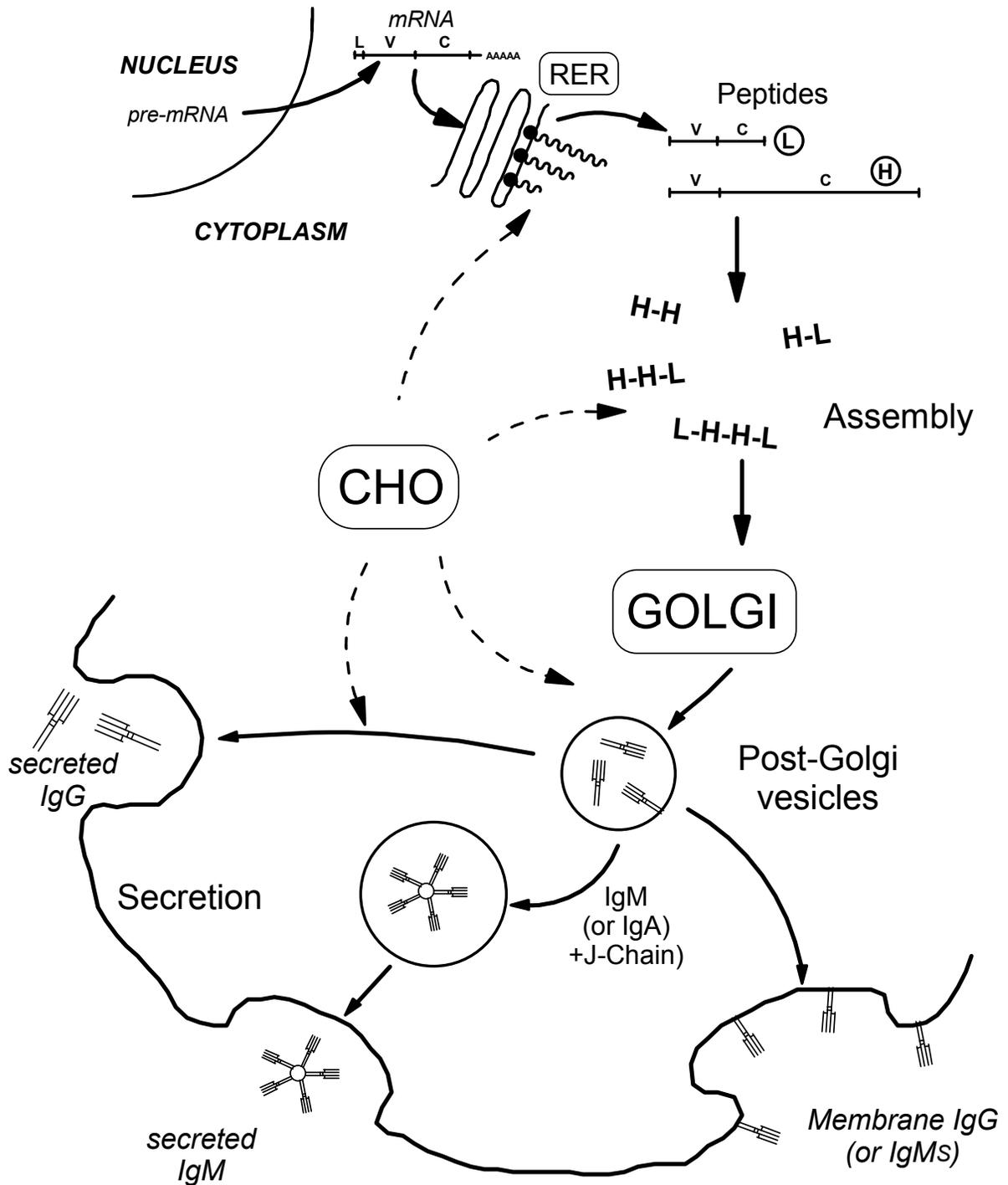
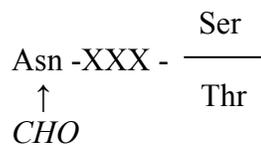


Figure 9-1

- 2) Nascent polypeptide chains are *translocated* across the membrane into the cisternae of the RER. As is the case for any secreted protein, this process requires the presence of the *leader polypeptide* or "signal sequence" at the amino terminus, which is enzymatically cleaved immediately after translocation.
- 3) *Assembly* of the heavy and light chains into a typical IgG-like subunit (H<sub>2</sub>L<sub>2</sub>) by formation of disulfide bonds is a *spontaneous* process - no specific enzymes are thought to be required, although a member of the HSP70 class of molecular "chaperonins" (the Heavy Chain Binding Protein, or BiP) is known to facilitate the proper folding of the heavy chains prior to their association with light chains. In normal plasma cells one sees balanced synthesis of H and L-chains, or a slight excess of L-chains (which are degraded intracellularly). Myeloma cells, on the other hand, often show markedly unbalanced synthesis with a large excess of L-chains secreted into the circulation which are then passed into the urine as Bence-Jones proteins. Some myelomas produce *only* light chains or *only* heavy chains; in the latter case (Heavy-Chain Disease) the H-chains are often defective, and typically have a deleted hinge region.
- 4) Assembled H<sub>2</sub>L<sub>2</sub> molecules *move through the RER to the Golgi apparatus, and into the post-Golgi vesicles*. These vesicles then fuse with the external cell membrane and release their contents, resulting in *secretion of Ig from the plasma cell*.
- 5) Carbohydrate is added to H-chains in a well-defined order, beginning on nascent chains while they are still attached to ribosomes, and continuing throughout the process of movement through the cell right up to the point of secretion. Carbohydrate is added to asparagine residues which are part of a *specific recognition sequence*, namely an asparagine separated by one amino acid residue from a serine or threonine:



In normal antibody molecules carbohydrate is attached *only to H-chain*, and *only in the constant region*, because this recognition sequence is normally present only in C<sub>H</sub>. Carbohydrate is a universal part of Ig heavy chains, and is thought to stabilize the three-dimensional structure of the Fc. [Note: In some myeloma proteins and other monoclonal immunoglobulins one sometimes finds V<sub>H</sub> or V<sub>L</sub>-associated carbohydrate in those cases where, through mutation, a recognition sequence happens to be present in the V-region. Carbohydrate will be attached to *any* accessible recognition sequence by the responsible enzyme system.]

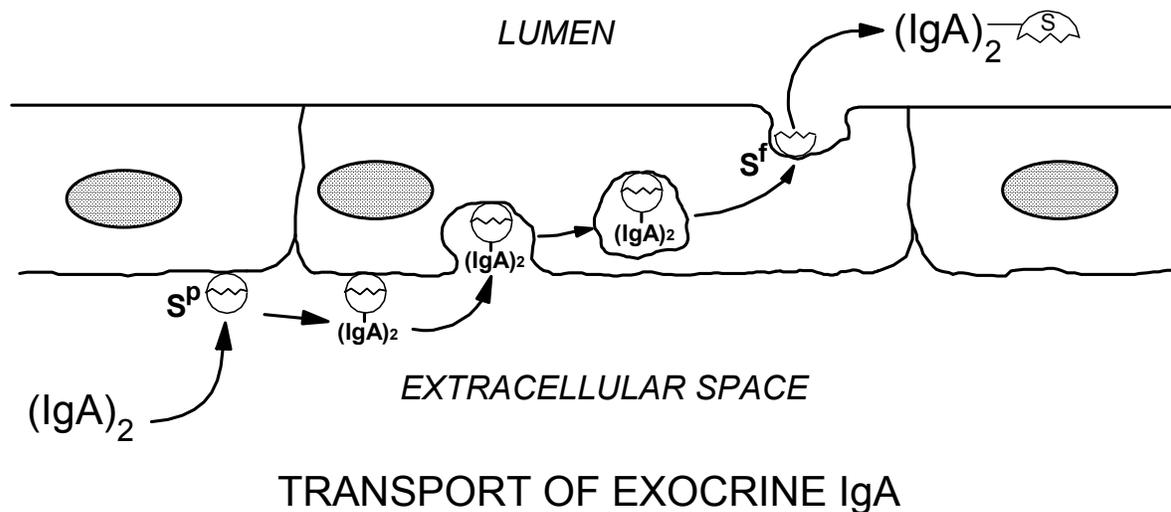
- 6) Just prior to release of the immunoglobulin from the cell, two more events occur:
  - a) The addition of a large final lump of carbohydrate. (The function of H-chain associated carbohydrate is still not fully understood, although it has been shown that its absence may affect the binding of Ig to Fc-receptors.)

- b) In the case of IgM and polymeric IgA, the addition of the *J-chain*. The J-chain is appears to be required for the process of polymerization, although it can be experimentally removed from polymeric Ig without disrupting its structure.

NOTE: Not all Ig produced by a cell is necessarily secreted - some may be retained in the cell membrane and function as the B-cell's antigen receptor. The structural differences between secreted and membrane-bound forms of Ig are discussed below.

## SECRETION OF IgA INTO EXOCRINE FLUIDS

We have accounted for all of the structural components of immunoglobulins except for the S-piece (*secretory piece*) associated with secretory IgA. The S-piece is *not* synthesized by the plasma cell which produced the immunoglobulin, but is added during the process of secretion into exocrine fluids. Let's look at the example of secretion of an IgA molecule into the gut in Figure 9-2 (although secretory IgA also appears in other exocrine secretions such as saliva and bile).



**Figure 9-2**

- 1) Polymeric IgA [mostly  $(\text{IgA})_2$ , with a small amount of  $(\text{IgA})_3$ ], is secreted by plasma cells into the extracellular fluids of the lamina propria of the gut.
- 2) Epithelial cells lining the gut have *S-piece precursor* (labeled  $S^p$  in the figure above) in their abluminal membranes. This  $S^p$  behaves as a membrane receptor and *binds polymeric IgA*.
- 3) Polymeric IgA thus bound is internalized, transported to the luminal side of the cell, and is released into the lumen of the gut following proteolytic cleavage of the  $S^p$  into two fragments.

- 4) *Secretory IgA* now has conventional S-piece (one portion of the S<sup>p</sup>) covalently linked to its H-chain; the rest of the S<sup>p</sup> molecule remains in the epithelial cell membrane (as Sf, or S-fragment) where it is internalized and degraded.
- 5) *Only polymeric IgA* (and to a slight extent IgM) is transported in this manner, presumably because other Ig molecules simply do not bind to the S<sup>p</sup> receptor on the epithelial cell. Monomeric IgA, the major serum form of IgA, is *not* secreted.
- 6) The presence of IgA in exocrine secretions can be of considerable importance in protection against infectious organisms. The normal route of entry of polio virus, for example, is the intestinal tract, and secretory IgA in this location confers a high degree of protection.

We've now seen two processes involving immunoglobulins which are both referred to as "secretion", and it is important not to confuse them.

- *Secretion of Ig from plasma cells*: Mature immunoglobulin molecules are "secreted" from the plasma cell into the extracellular space by the conventional process of exocytosis of post-Golgi vesicles. This same process is responsible for the release from cells of all "secreted" proteins.
- *Exocrine secretion of IgA*: Secretion of polymeric IgA into the mucus of the gut and other exocrine fluids is a distinct process of "secretion". This process, as we have seen, depends on the participation of the "secretory piece", or "S-piece", which is added to IgA by secretory epithelial cells. This process is unique to immunoglobulins, and IgA in particular.

## KEY FEATURES OF ANTIBODY PRODUCTION

- 1) **One antibody-forming cell (AFC) cell produces only one kind of antibody.**
  - a) only *one* light chain isotype ( $\kappa$  or  $\lambda 1$  or  $\lambda 2$ , etc).
  - b) only *one* heavy chain isotype.
  - c) only *one* kind of V<sub>H</sub> and one kind of V<sub>L</sub>; therefore, only *a single idio*type.

One consequence of these features is that *antibody molecules are always symmetrical* with respect to their light and heavy chains.

(NOTE: There are some exceptions to the strict *one-cell-one-antibody* rule. One is the transient simultaneous synthesis by virgin B-cells of both IgM and IgD - the mechanism of this dual synthesis has been discussed in Chapter 8. Another situation which hardly qualifies as an "exception" is discussed under point [4], below. *Neither exception violates the basic principle of the symmetry of Ig molecules, or rules [a] and [c] above.*)

- 2) **Allelic exclusion.** While diploid cells have two copies of every immunoglobulin gene, only *one* of the two is expressed in a given B-cell or plasma cell for each of the light and heavy chain (the other allele is *either rearranged aberrantly* and cannot be expressed, *or is not rearranged* at all). This is an unusual and important feature of immunoglobulins (and the TCR); although recent work has shown that there exist a number of other genes that also exhibit this behavior.

One of the consequences of allelic exclusion is that it ensures the *symmetry* of antibody molecules. Expression of *both* copies of the kappa locus (for example) would result in production of Ig molecules with *two* different V $\kappa$  regions, and therefore two different combining sites (remembering that the assembly of light and heavy chains is a random process). Such asymmetric molecules would have impaired biological activity (see the earlier discussion of affinity and avidity in Chapter 3), and would substantially dilute the pool of active bivalent molecules.

- 3) **Heavy chain class switching.** A particular antibody-forming cell can switch from production of IgM to IgG, from IgG to IgA, etc. The light chain does not change, nor does the V $_H$ ; *only the C $_H$  changes*, so that the *original combining site* (and therefore *idiotype*) is now associated with a molecule of a *different class* or subclass.

**Switching is unidirectional**--an IgG-producing cell *cannot* go back to production of IgM, for instance. The molecular basis of this irreversibility was discussed in the Chapter 8.

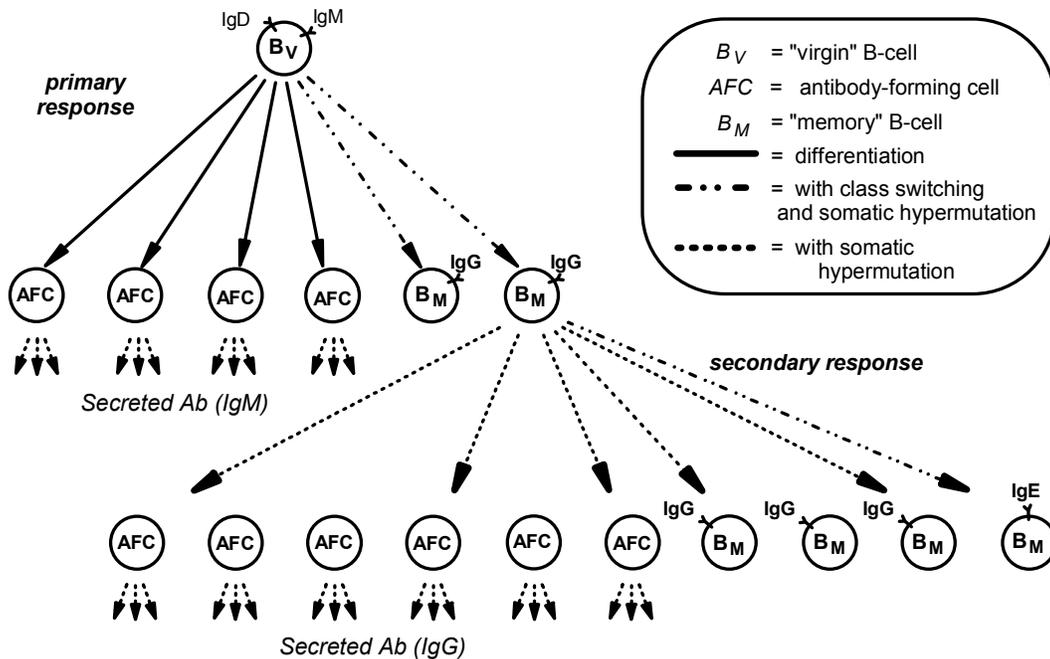
Very rare myelomas have been found which produce two or more classes of immunoglobulin ("*double producers*"). These proteins generally differ *only* in their C $_H$  and are considered to represent the results of class switching in these cells, the myeloma cells having gotten "stuck" during this process.

- 4) **Membrane-bound versus secreted immunoglobulins.** A virgin B-cell bears IgM (and possibly IgD) in its *membrane*; following stimulation it begins to *secrete* IgM into its surrounding environment. *These two forms of IgM are structurally different.* The mu heavy chain of membrane-bound IgM has an amino acid sequence at its carboxy-terminal end which anchors the molecule into the membrane; the secreted form of IgM has a *different* C-terminal sequence which lacks a membrane anchoring region. The membrane-bound form of IgM is also incapable of associating with J-chain and forming its normal pentameric structure; *membrane IgM, therefore, exists exclusively in monomeric form* (H $_2$ L $_2$ ), also known as IgM $_S$  or "sub-unit" IgM.

The two forms of *mu* chain are synthesized via an alternative splicing scheme analogous to that which allows simultaneous IgM and IgD synthesis (as was discussed in Chapter 8). *Similar differences exist for secreted versus membrane-bound forms of IgG, IgA and IgE heavy chains.*

A virgin B-cell produces *only* the membrane-bound form of IgM. As it develops into an antibody-secreting plasma cell it may transiently produce *both* membrane and secreted IgM, but it eventually produces only the secreted form. Likewise, a *memory B-cell* which synthesizes only membrane-bound IgG will shift to producing exclusively the secreted form of IgG as it develops into a plasma cell following secondary antigen stimulation.

Figure 9-3 shows the relationships between "virgin" B-cells ( $B_V$ , IgM-bearing), "memory" B-cells ( $B_M$ , shown as IgG-bearing, but can also be IgA or IgE-bearing), and antibody-forming cells (AFC, typically with little or no membrane-bound Ig). This illustrates some of the features of primary and secondary immune responses we have already discussed in Chapter 7 (e.g., more rapid and efficient generation of AFC's from memory cells than from virgin B-cells), but also adds those membrane phenotypes of the participating cells which are a consequence of the molecular processes we have just covered (class switching from IgM to IgG, A or E). Note that the membrane-bound IgM (and IgD) which characterizes virgin B-cells is lost during the generation of *either* AFCs or memory cells.



### IMMUNOGLOBULIN EXPRESSION BY B-CELLS AND THEIR PROGENY

**Figure 9-3**

- 5) **Somatic Hypermutation.** The V-regions of membrane Ig on memory cells typically express sequences *different* from those of the naïve B-cell from which they were derived, resulting in a marked increase in overall diversity which is reflected in the serum antibody response. This is the result of the accumulation of point mutations through a process called *somatic hypermutation*. As indicated by the broken lines in the figure above, this process occurs only during the generation of B-memory cells following isotype switching, and therefore affects only IgG, IgA and IgE.

The mechanism involves the inactivation of the normal process of DNA editing, which exists to maintain sequence integrity during DNA replication. This inactivation, however, takes place only over a limited region *within and near the V-regions of both heavy and light chains*, excluding the constant regions. The point mutations which occur are, of course, random, but the only ones we will observe are the minority which happen to increase antibody affinity, thereby allowing the variant B-cells to be selected by antigen for continued proliferation and differentiation.

## WHY CLASS SWITCHING?

B-cells start out with IgM and IgD on their surface; after antigenic triggering they lose their IgD and become IgM secretors. Most of them will subsequently switch from IgM to production of some other class of immunoglobulin during the course of an immune response. Why, then, do immune responses not start immediately with the final mixture of immunoglobulin isotypes? Why start every clone with IgM and go through the complex process of class switching?

There is clearly an advantage to ending up with a variety of isotypes represented in serum, because each will have its distinct set of biological activities. But the question still remains, why start everything off with IgM and only switch afterwards?

*We can better understand this by recalling the phenomenon of **affinity maturation**:*

- a) As a consequence of clonal selection, the affinity of antibodies produced early in a response will always be lower than those produced later on (remember that the generation of the antibody repertoire is *random*).
- b) Low affinity antibodies will tend to be less biologically effective than higher affinity ones because they bind less well to their target antigens.
- c) The immune response can compensate for the *low affinity* of early antibodies by making *polymeric IgM* which will have a very *high avidity*, even with very low affinity combining sites.
- d) However, IgM is very expensive for a cell to produce, it requires six times more raw materials and energy per molecule than IgG. Once the immune response has had time to become established using IgM antibodies, the process of affinity maturation can allow the expensive IgM to be replaced with more economical IgG-like antibodies, also gaining the additional benefit of a wide diversity of biological effector functions.

### CHAPTER 9, STUDY QUESTIONS:

1. List each of the structural components which make up the various isotypes of human immunoglobulins. Describe the processes by which each is incorporated into a mature immunoglobulin.
2. What are the two distinct processes which are referred to as immunoglobulin "secretion"?
3. Why is allelic exclusion such a critically important feature of AFC's?