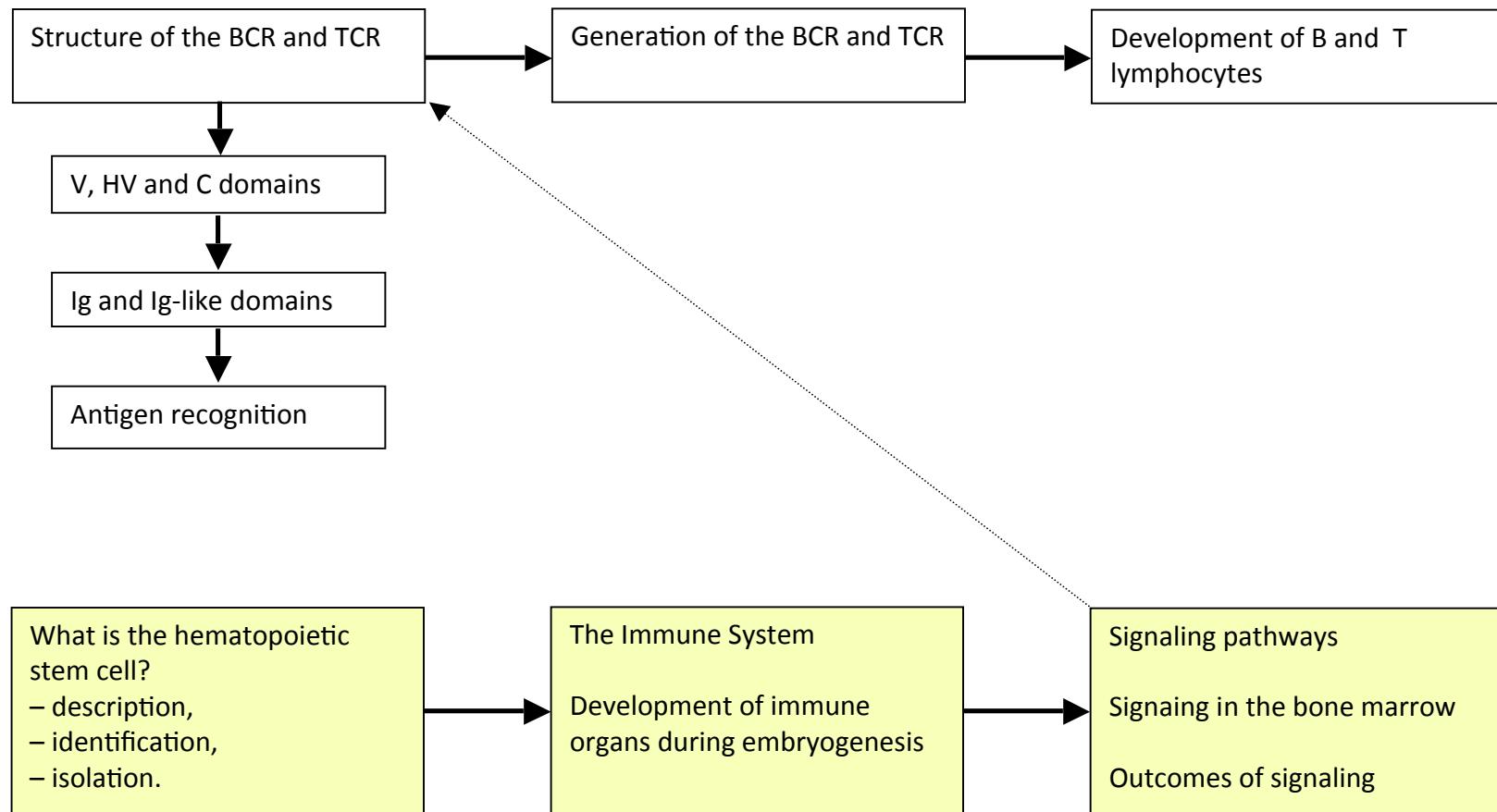


Module 2: B cell and T cell receptors



Module 2: B cell and T cell receptors

Learning Objectives

After completing the relevant sections in Chapters 3, 4 and 6, students should be able to:

- describe the three dimensional structures of the B cell and T cell receptors,
- explain the structure of Ig and Ig-like domains,
- explain the terms variable, hypervariable and constant regions,
- relate the structure of the B cell receptor (BCR) to the T cell receptor (TCR),
- explain the differences in the types of antigen recognized by BCRs and TCRs, and relate it to the structure of the BCR and TCR.

Antigen recognition in the immune system



Antigen recognition is critical for proper functioning of the immune system:

- for the cells of the innate responses, there are germ-line encoded receptors recognizing common structures on classes of microbes,
- for the lymphocytes of the adaptive responses, the receptors are specific for each antigen and involves the random selection of gene segments during the development of the cell.

BCR/Antibody structure

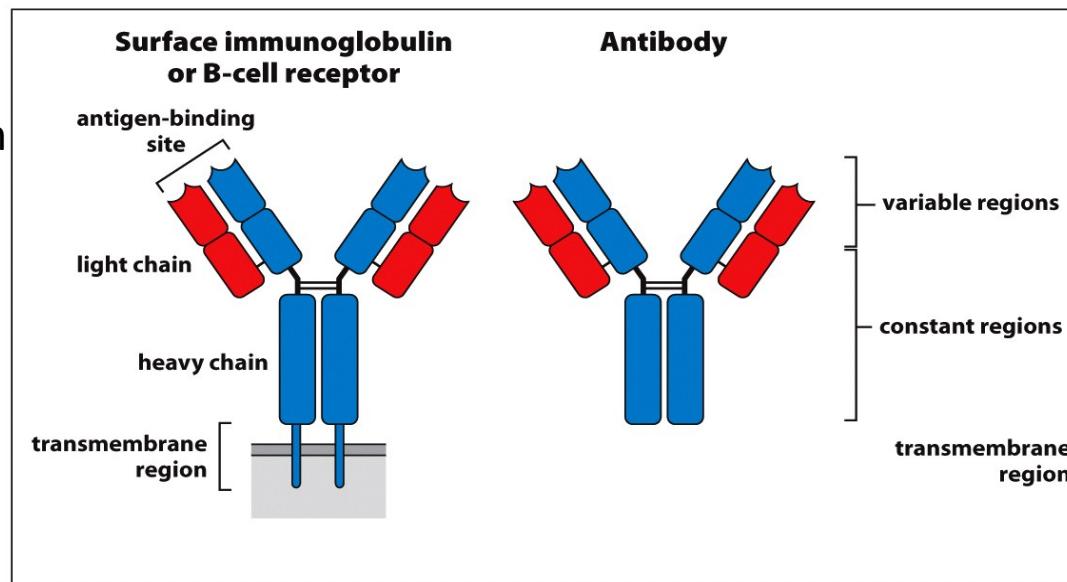
The whole point of B cell activation is the secretion of antibodies.

Antibodies are the secreted form of the BCR.

They are proteins with 4^o structure, assembled from:

- two identical copies of the heavy (H) chain,
- two identical copies of the light (L) chain,
- disulfide bonds hold the chains together.

mlg: bind antigen so that the B cell can be activated.



Ab: bind antigen so that the effector function of the Ab can be engaged.

BCR/Antibody structure

An individual B cell will have thousands of copies of the BCR on its cell surface - each BCR has the same antigen specificity.

Each BCR has the same variable region on both the H and L chain.

The constant region of the H chain might be different.

The BCR is associated with two proteins called Ig- α and Ig- β .

When the B cell is activated by the cross-linking of BCR by antigen, receptor-associated kinases phosphorylate specific tyrosines on the Ig- α and Ig- β .

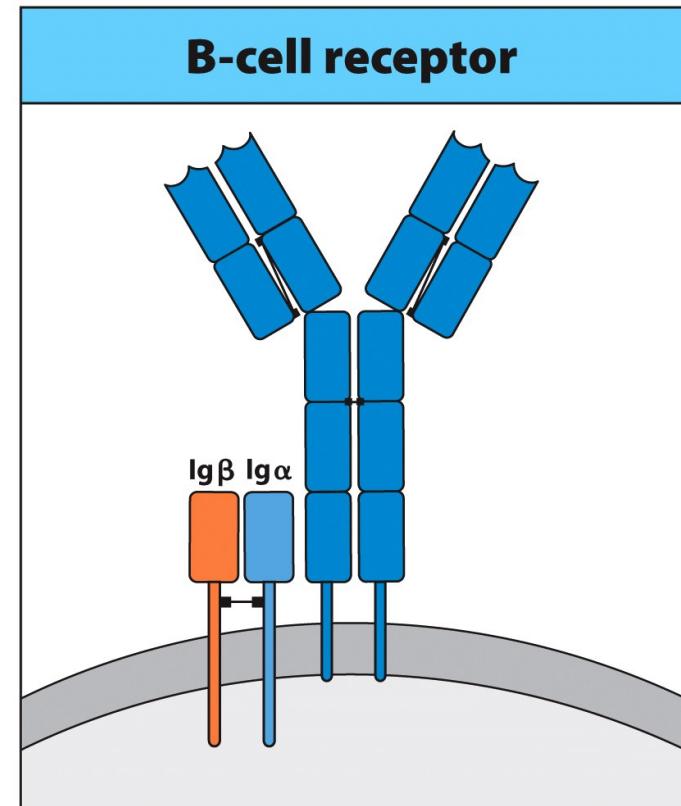


Figure 4.25 The Immune System, 3ed. (© Garland Science 2009)

BCR/Antibody structure

When we talk about BCR or Ab having variable (V) and constant (C) regions, we are talking about their 1^o structure (linear amino acid sequence).

The V_H and V_L regions combine to form the Ag-binding site.

Because the two H and two L chains are identical, it means that the two Ag-binding sites are identical.

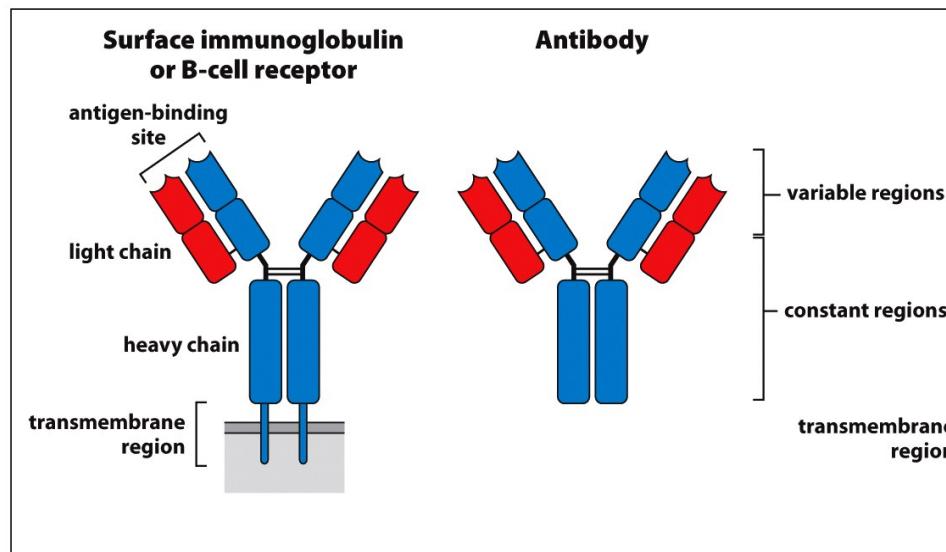


Figure 3.1 The Immune System, 3ed. (© Garland Science 2009)

3-dimensional structure of the BCR/Ab

The BCR and Ab can be described as Y-shaped consisting of three roughly equal sized portions connected by a “flexible tether”.

The BCR and Ab are composed of a series of globular domains (called Ig domains) – each of the domains is approximately the same size.

The L chains have 2 globular Ig domains - the V_L region and the C_L region.

The H chains have 1 V_H Ig domain and 3 or 4 Ig domains in the C_H region.

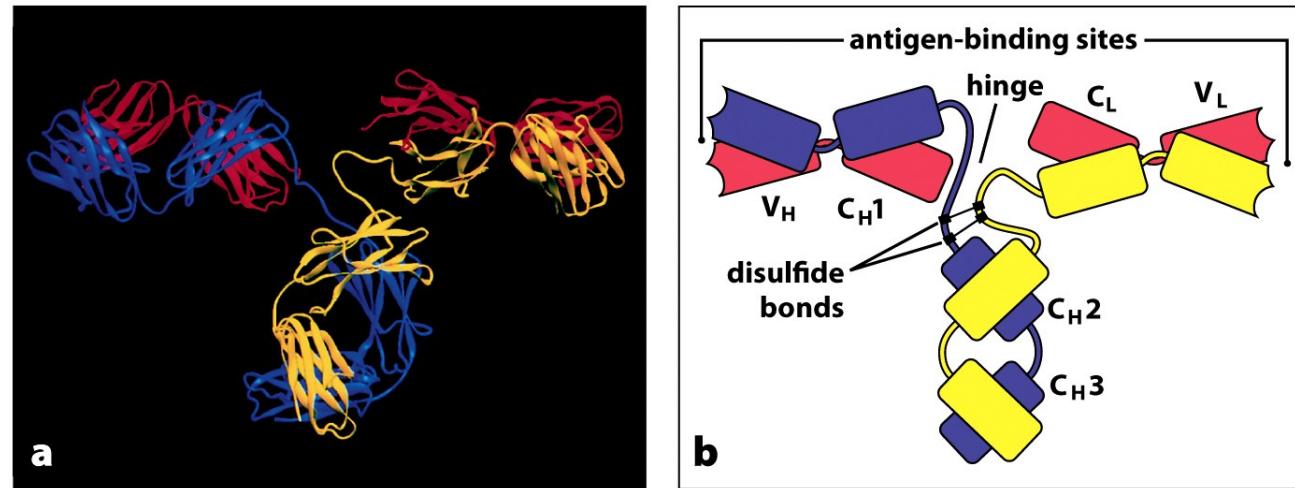


figure 4.6 The Immune System, 3ed. (© Garland Science 2009)

Ig and Ig-like domains

Each globular domain of the Ab or mIg consists of a β barrel made from two β anti-parallel sheets – each globular domain is about 110 amino acids in size. These are called Ig domains.

Ig-like domains are a common structural feature in many proteins involved in immune responses as well as proteins involved in cell-cell recognition in the immune system..

Proteins that contain Ig or Ig-like domains include:

BCR, Abs, TCR, MHC I and II proteins, CD4, CD8, and various cell adhesion molecules like ICAM-1.

Ig domains

The Ig domain - a barrel-shaped structure (called a β barrel) of two anti-parallel β sheets folded over each other and held together by a disulfide bond.

Proteins often have one or more domains linked by a region of the polypeptide chain that is relatively unstructured.

See the supplemental slides for a review of protein structure.



Ig domains

β barrels make up the globular structure of the Ig domain (or Ig-like domain).

The L chains have 2 globular Ig domains - the V_L region and the C_L region.

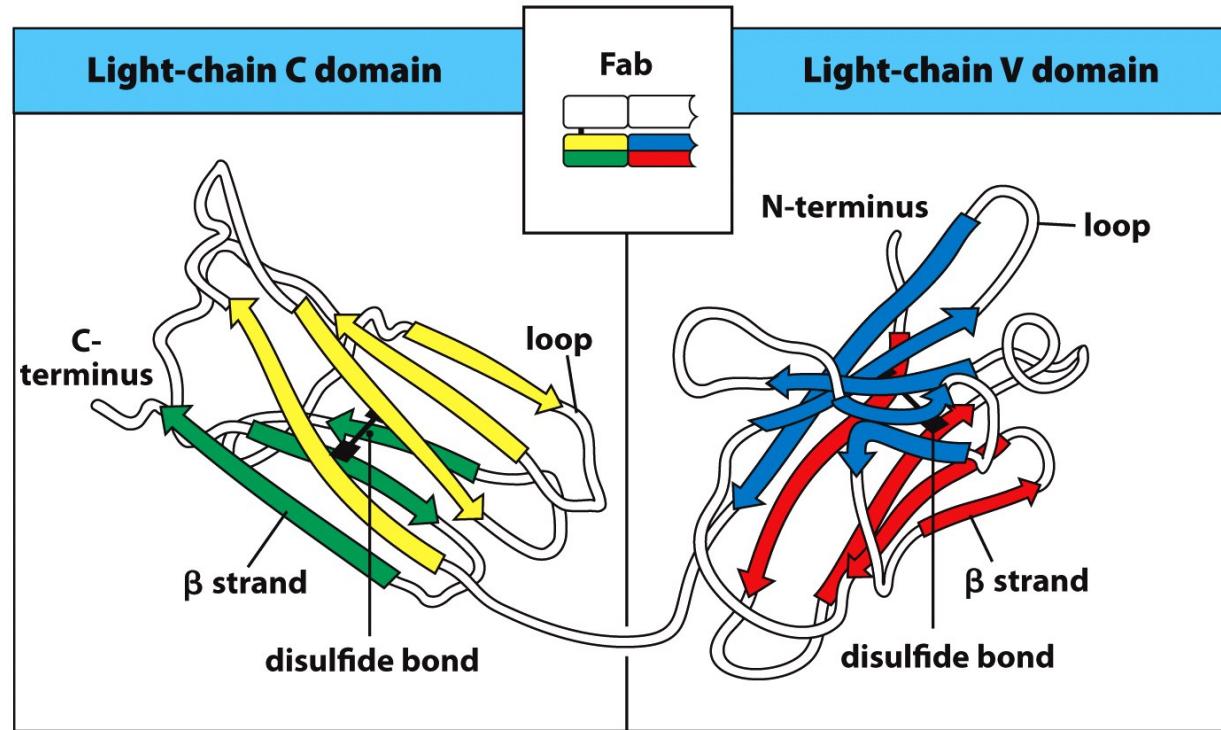


Figure 4.7 The Immune System, 3ed. (© Garland Science 2009)

T cell receptor

The T cell receptor (TCR) and BCR have similar structures but there are differences.

The TCR kind of looks like an “arm” of the BCR with a cytoplasmic tail.

TCRs have an α and β chain, both with 2 Ig-like domains, but only one Ag-binding site.

There is no secreted form of the TCR.

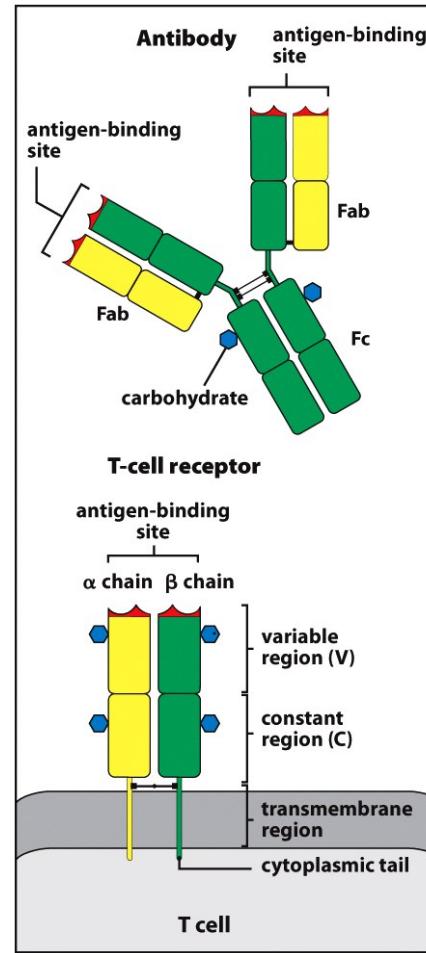


Figure 5.1 The Immune System, 3ed. (© Garland Science 2009)

T cell receptor

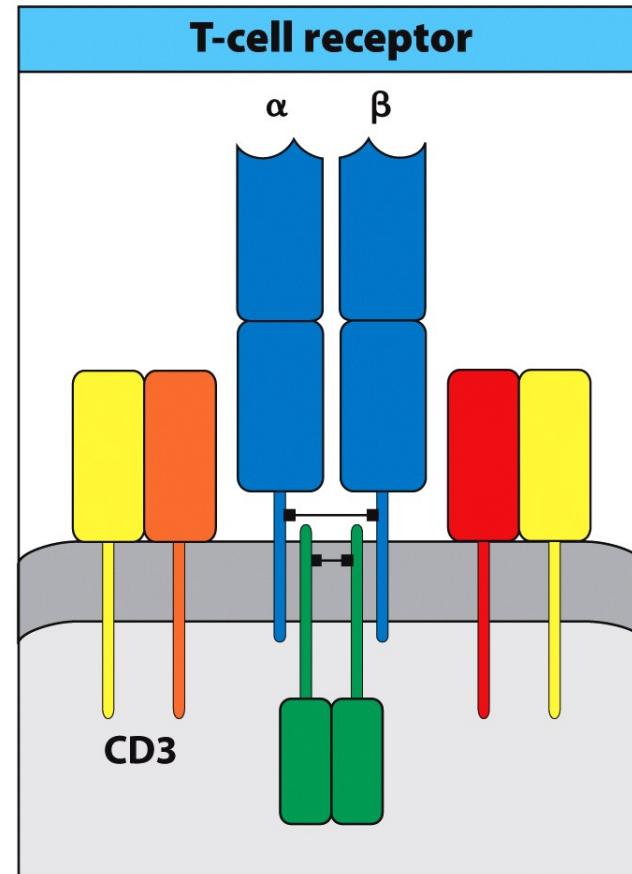
Just like the BCR, the TCR is associated with proteins that are involved in signaling.

This is the CD3 signaling complex.

When the TCR is engaged, receptor-associated kinases phosphorylate specific tyrosines on the CD3 complex.

A functional TCR also has a co-receptor associated with it:

- CD4 for T helper cells,
- CD8 for cytotoxic T cells.



What do we mean by Constant, Variable and Hypervariable?

Constant region - little to no variation in C regions (e.g., of a specific class of BCR or Ab, or the C region of TCRs). 

Variable region - some variation in the amino acid sequence is expected - but there are areas that are relatively conserved (i.e., not much variation) - these regions make up the “framework” structure of the BCR/TCR.

These amino acids would play important roles in the folding of the polypeptide to make the Ig-domain.

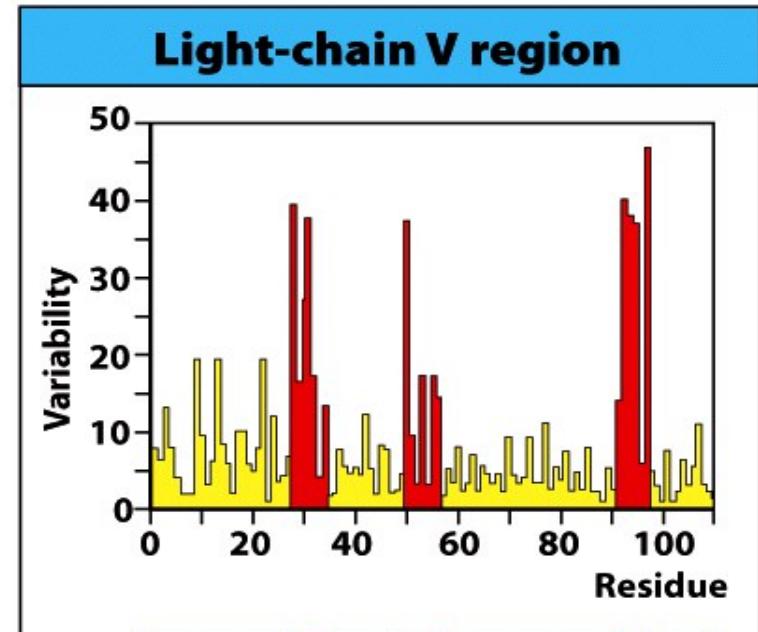
Hyper-variable region - a lot of variation in the amino acid sequence is expected - these stretches of amino acids form part of the antigen-binding pocket of the BCR/TCR, so diversity is required in order for the repertoire of BCR/TCR to recognize many antigens.

What do we mean by Constant, Variable and Hypervariable?

In the 70's and early 80's, the amino acid sequence of the V regions of many Abs were determined by the Edman degradation reaction.

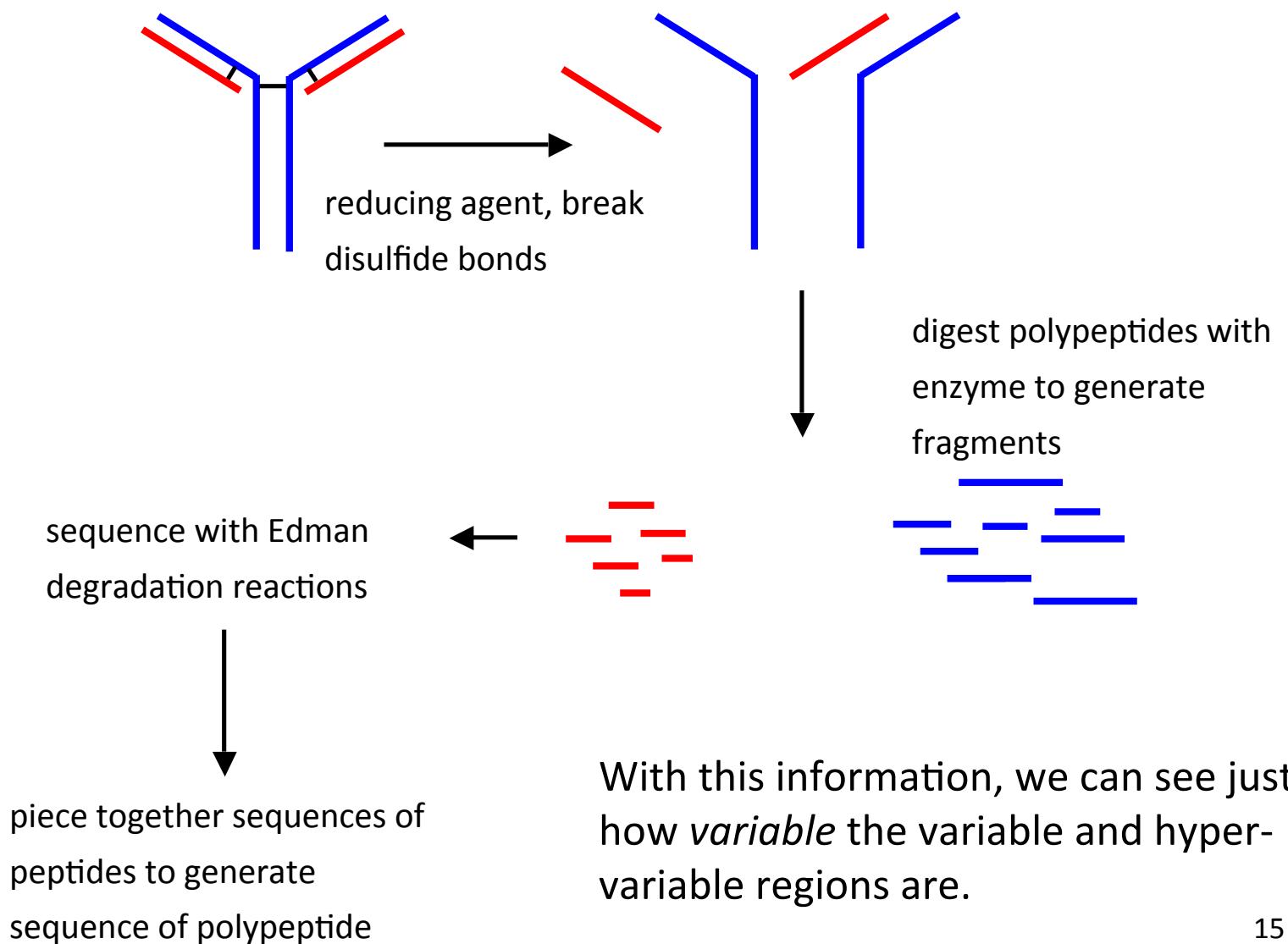
In the variability plots, it was observed that the sequence variability of the amino acids used in the different Ab was not evenly distributed throughout the V region.

The next few slides show the BCR's light chain, but the same concepts apply to the H chain and both chains of the TCR.



An Ig domain (or Ig-like domain) is about 110 amino acid in size.

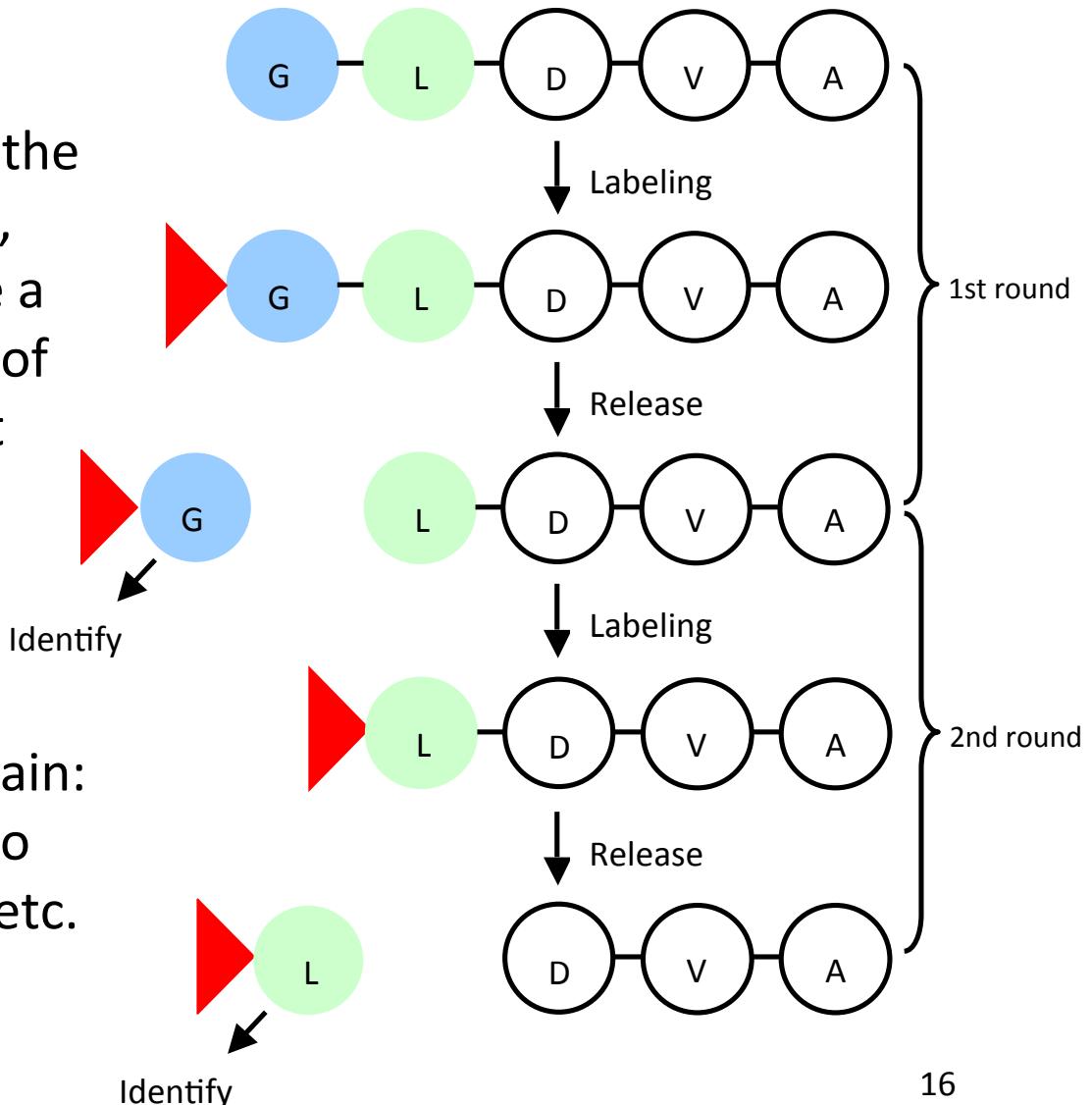
Determining the sequence of amino acids in a polypeptide



Determining the sequence of amino acids in a polypeptide

Edman degradation:

The reaction would break off the "last" amino acid in the chain, and then scientists would use a machine to determine which of the 20 possible amino acids it was.



Then the cycle would start again: break off the new "last" amino acid on the chain, identify it, etc.

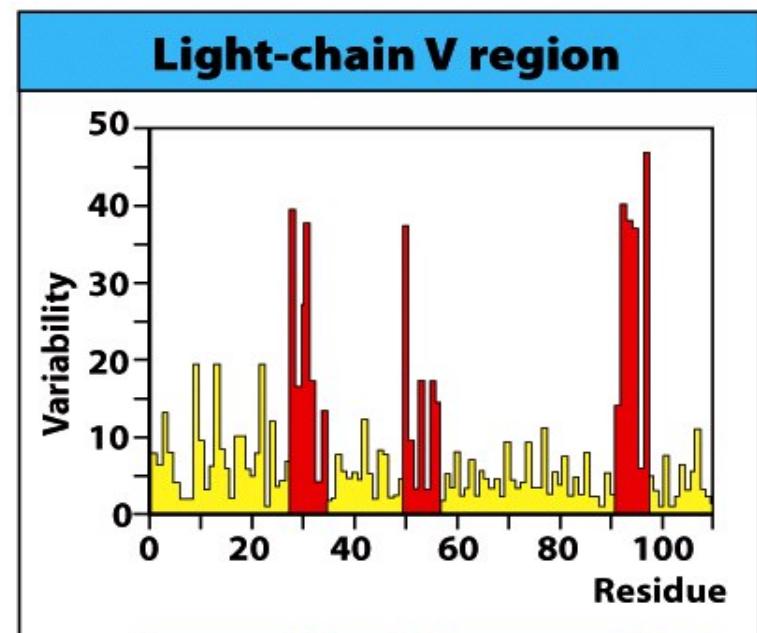
What do we mean by Constant, Variable and Hypervariable?

It was found that both the H and L chains have 3 Hypervariable (HV) regions called HV1, HV2, HV3 that are separated by relatively conserved regions that do not vary very much from one Ab to another.

The HV regions form the Ag-binding site of the Ab or mIg, so it makes sense that we might see a lot of variability in the amino acids that make up this region.

The relatively conserved regions that don't vary much are called framework regions.

These would be important in the forming of the β strands and β sheets.



What do we mean by Constant, Variable and Hypervariable?

When you look at the 3D structure of the V_H or V_L , the HV regions are in the loops between the β strands. They are located close to each other so they form a continuous surface at tip of the H or L chain.

Another name for the HV region is the complementarity-determining regions (CDR). So for the tip at each arm of the Ab or mIg, there are six CDRs – three from each chain.

It is the combination of the CDRs that determine the antigen specificity of the Ab or mIg.

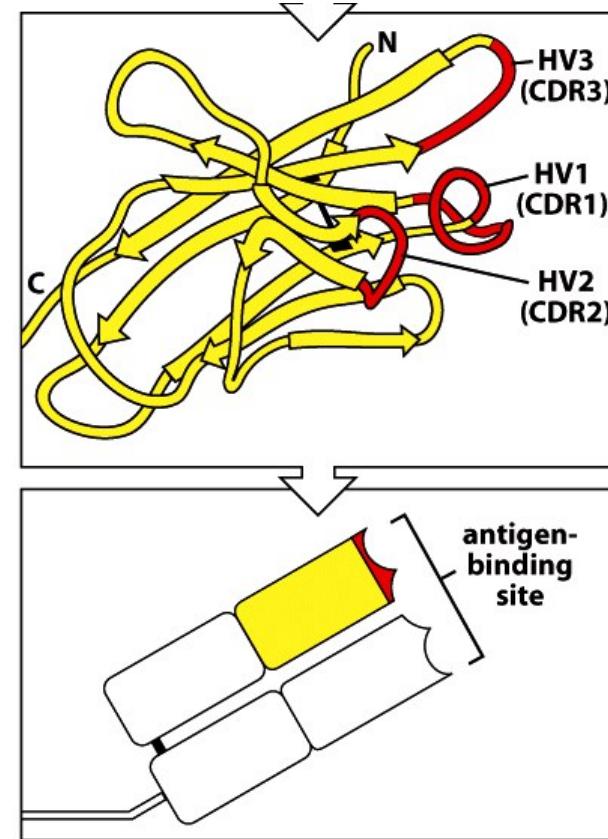


Figure 4.8 The Immune System, 3ed. (© Garland Science 2009)

Antigen recognition by BCRs

BCRs (or Ab) recognize (bind to) three dimensional epitopes of intact, unprocessed antigen.

The molecules can be protein, carbohydrate, lipids, synthetic chemicals etc.

Close contact between surfaces of epitope and Ag-binding site allow formation of n

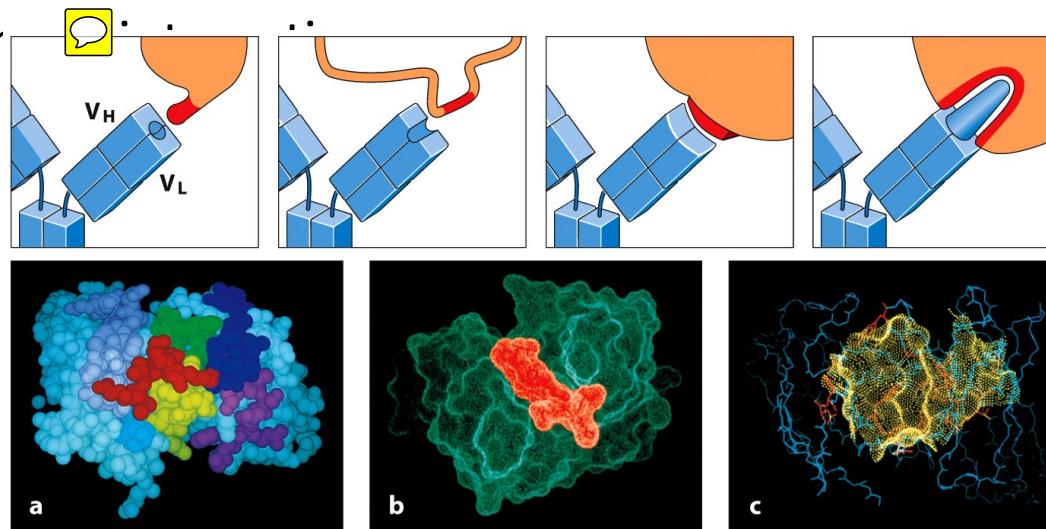


Figure 4.11 The Immune System, 3ed. (© Garland Science 2009)



Antigen recognition by BCRs

The BCR (or Ab) bind epitopes whose 3D shape is complementary to that of its Ag-binding site.

The closer the contact and the more bonds that are formed, the stronger the interaction (higher the affinity).

In most cases, all or most HV regions of both the H and L chain contact the epitope.

The size of the antigen-binding site on BCRs (or Ab) is such that it can bind haptens (small molecules), peptides, or exposed portions of larger molecules.

Antigen recognition by BCRs (or Ab)

The epitope that a given Ab recognizes is a unique 3 dimensional spatial arrangement of atoms.

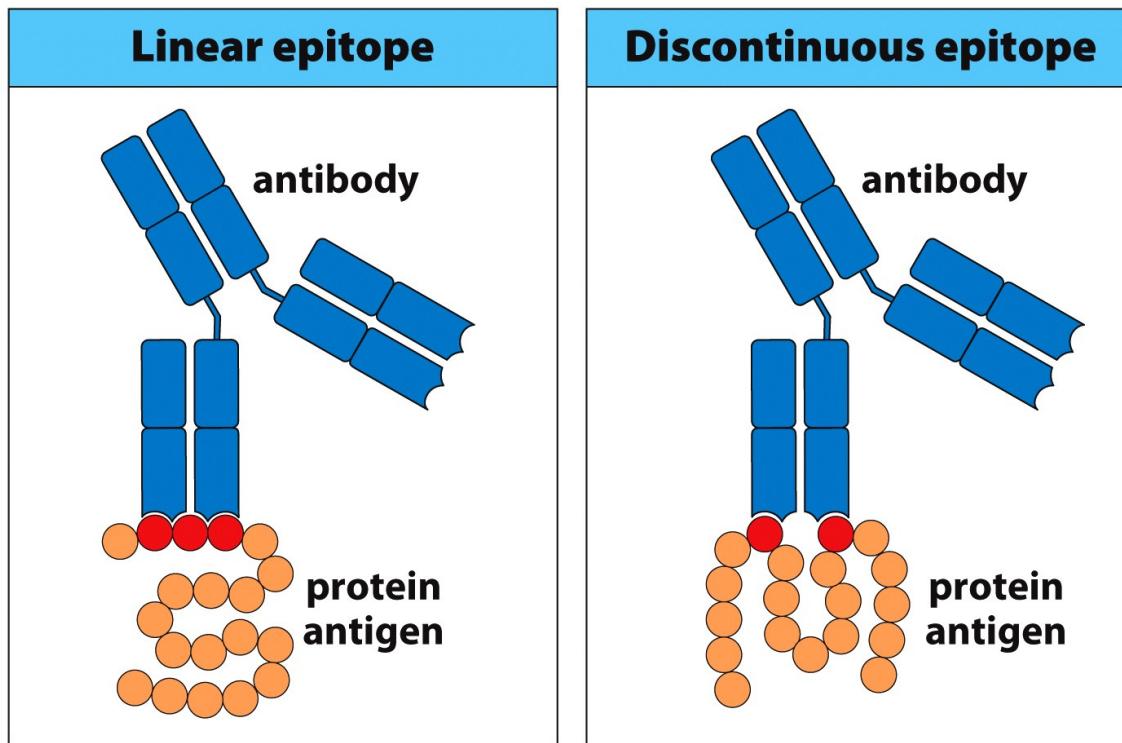
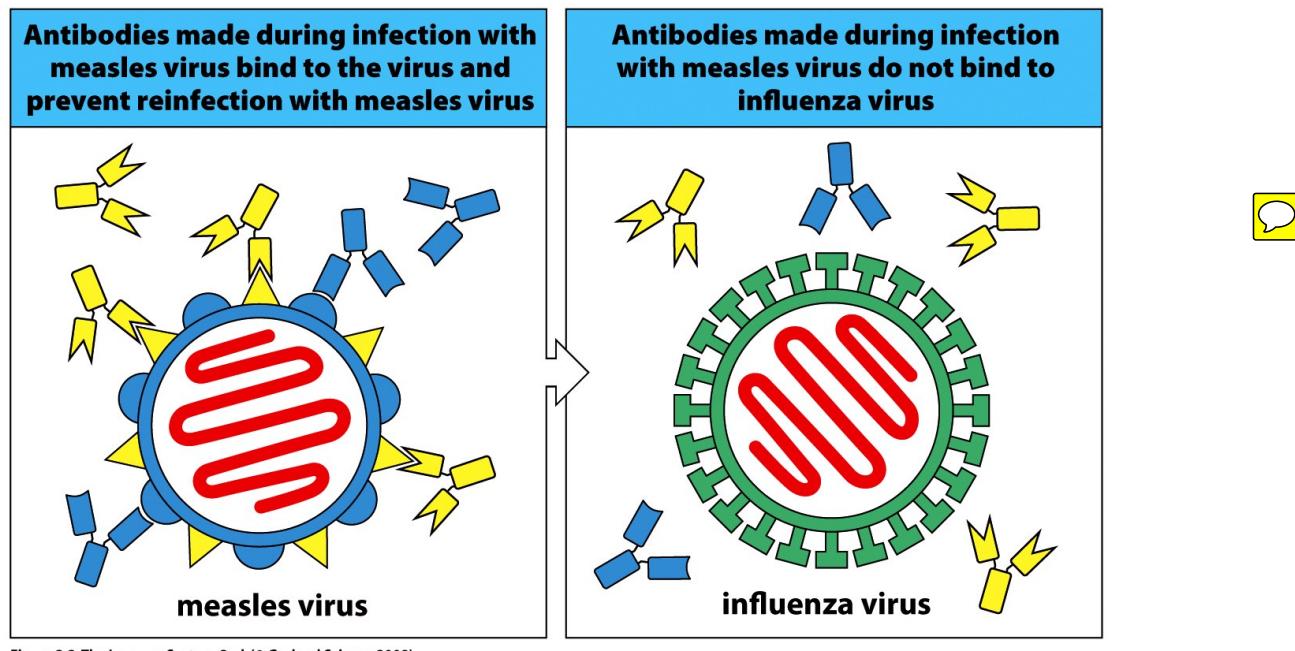


Figure 4.12 The Immune System, 3ed. (© Garland Science 2009)

The amino acids that recognize the epitope are close together when the Ig is folded.

The epitope could be a linear sequence, but it doesn't have to be. It can be a conformational structure.

BCRs (or Ab) are very specific for the molecular details.



The binding of Ab can be very specific. Abs can even sometimes distinguish between closely related molecules (e.g., Y vs. pY).

For example, one amino acid change in the Ab or protein antigen can greatly reduce the binding affinity.



Antigen recognition by TCRs

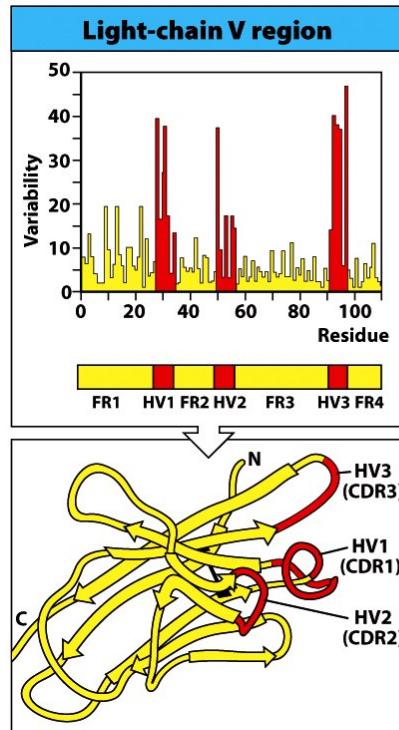
In comparison, TCRs recognize linear peptide epitopes that are associated in the cleft of an MHC protein.

The peptides can be derived from extracellular sources (bacteria, virus, foreign proteins, blood proteins) or cytoplasmic sources (viral proteins, cell proteins).

Two of the HV regions in both the α and β chain interact with the MHC protein and only one HV region in both the α and β chain interact with the peptide.

Antigen recognition by TCRs

The V regions of TCRs α and β chains are very similar to the V regions of Ig heavy and light chains. Note the framework and loop structures that make up the HV regions (same concept as L chain for Ig).



TCR binding site for MHC:peptide complexes

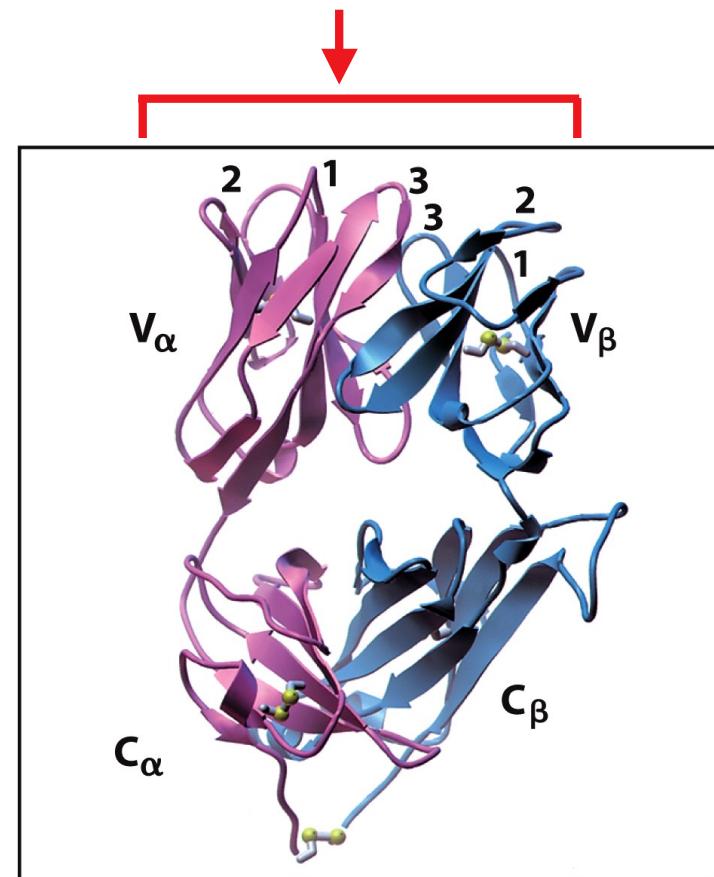
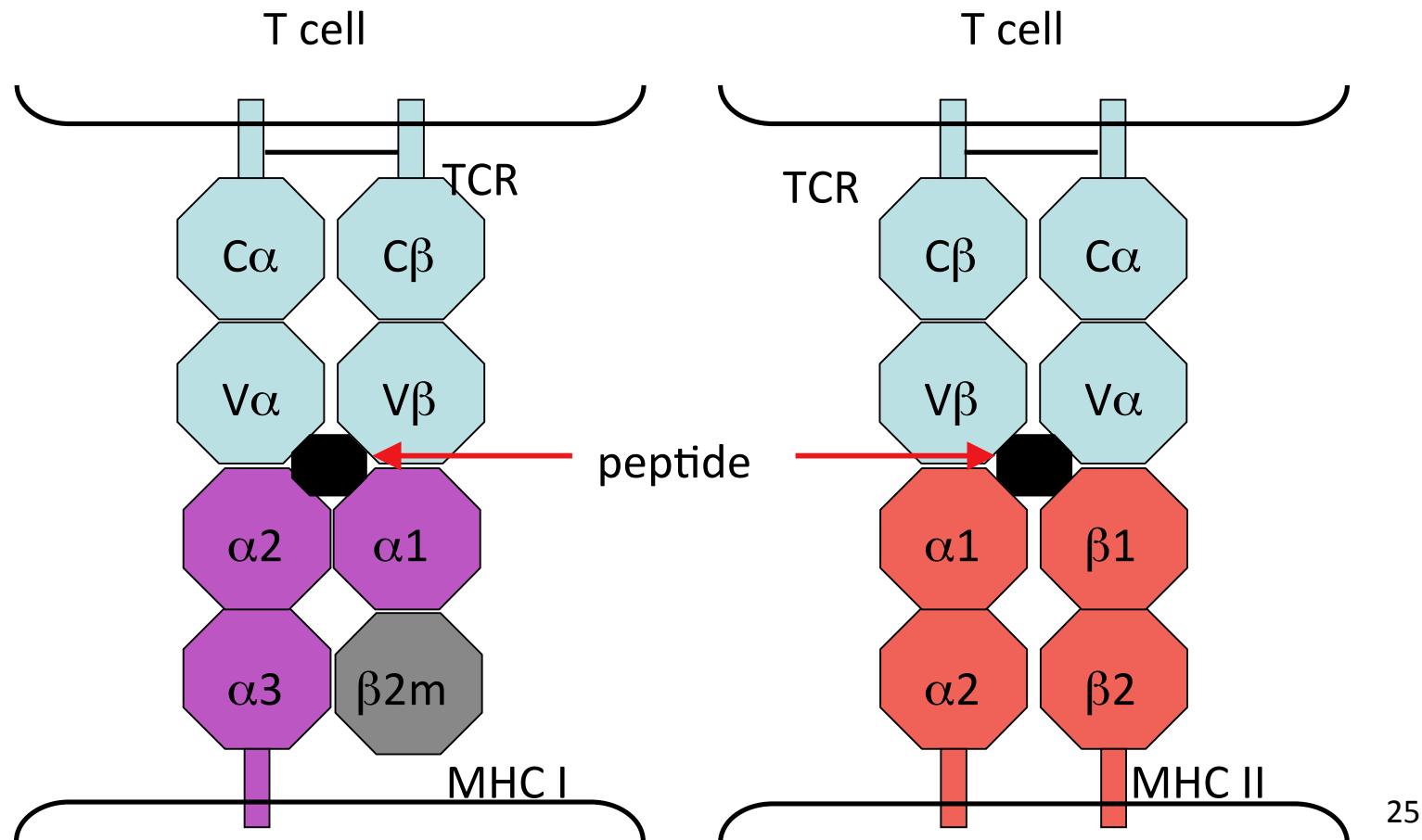


Figure 5.2 The Immune System, 3ed. (© Garland Science 2009)

Antigen recognition by TCRs

The TCR V α and V β are both involved in antigen recognition.

The TCR recognizes the combination of MHC and peptide. The CDR1 and CDR2 regions bind to the MHC, the CDR3 binds the peptide.



Antigen recognition by TCRs

Binding of the TCR to MHC I:peptide complexes

For the $V\alpha$ chain, the 3 CDRs are known as 1α , 2α and 3α .

1α and 2α bind to the MHC, 3α binds the peptide.

For the $V\beta$ chain, the 3 CDRs are known as 1β , 2β and 3β .

1β and 2β bind the MHC, 3β binds the peptide.

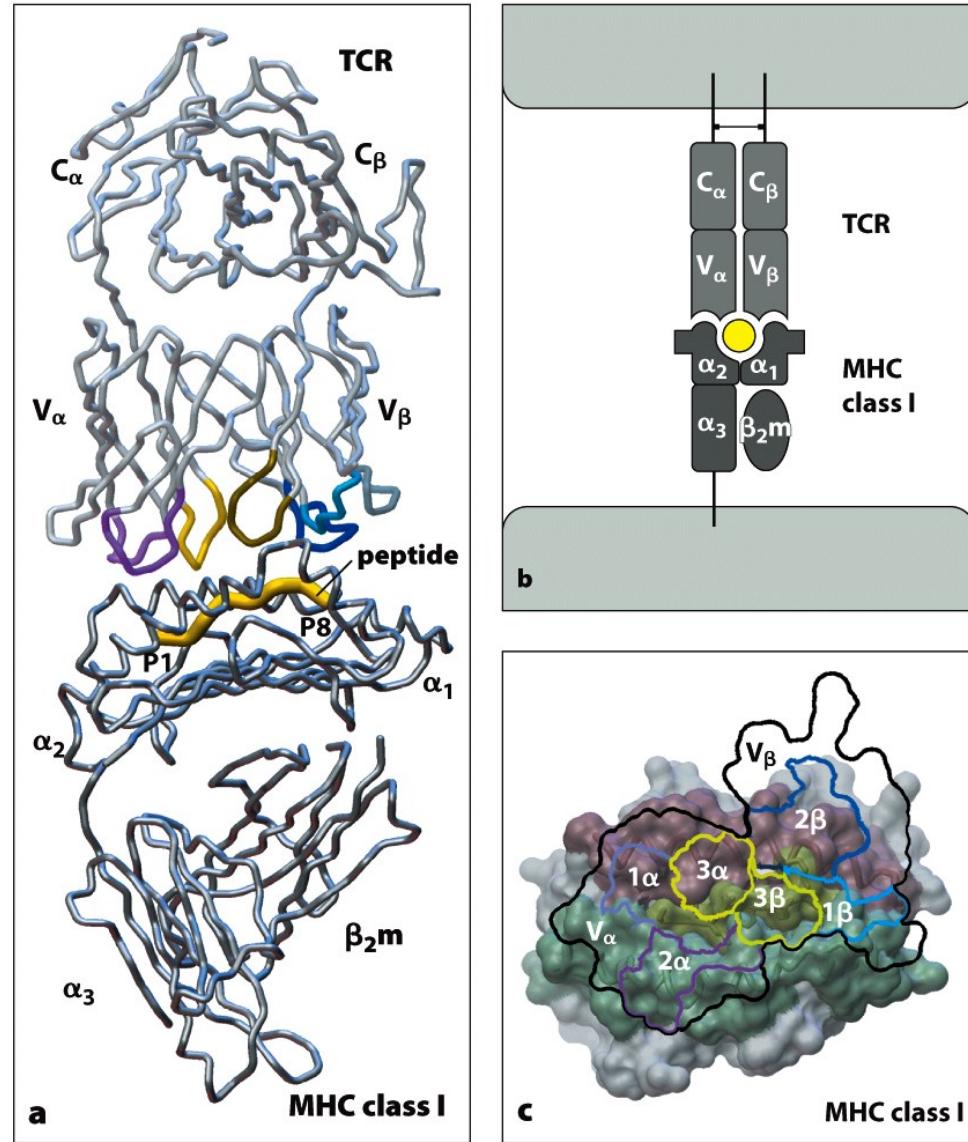
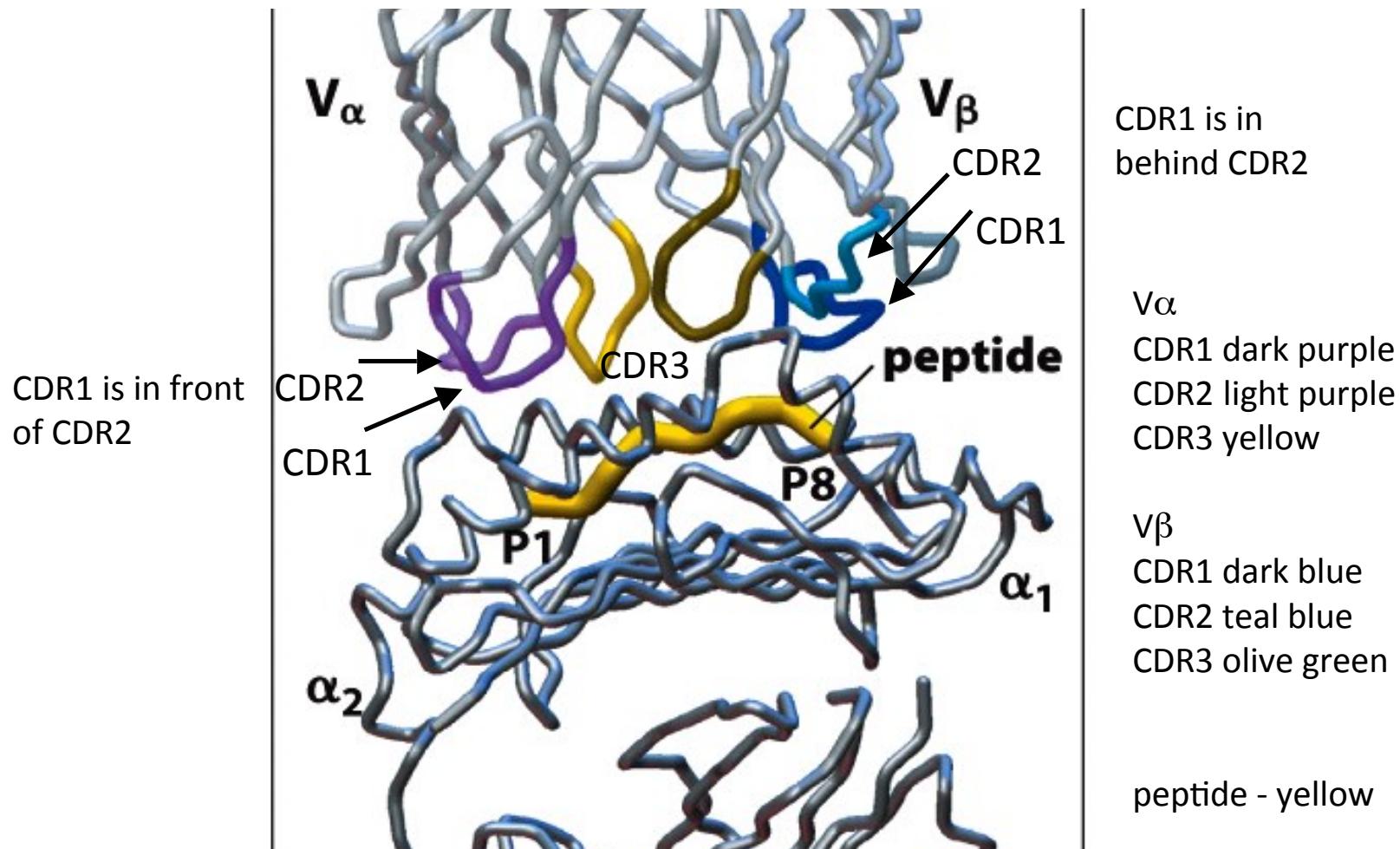


Figure 5.22 The Immune System, 3ed. (© Garland Science 2009)

Antigen recognition by TCRs



Note: error in the figure caption for panel (a), not consistent with panel (c).

From BCR to secreted Ab

As mentioned earlier, the whole point of B cell activation is the secretion of antibodies.

Antibodies are the secreted form of the BCR.

Production of secreted antibodies requires transcription of the DNA and translation of the mRNAs on ribosomes associated with the E.R.

The mRNA that results in a secreted antibody is the result of different splicing patterns in the primary RNA.



From BCR to secreted Ab

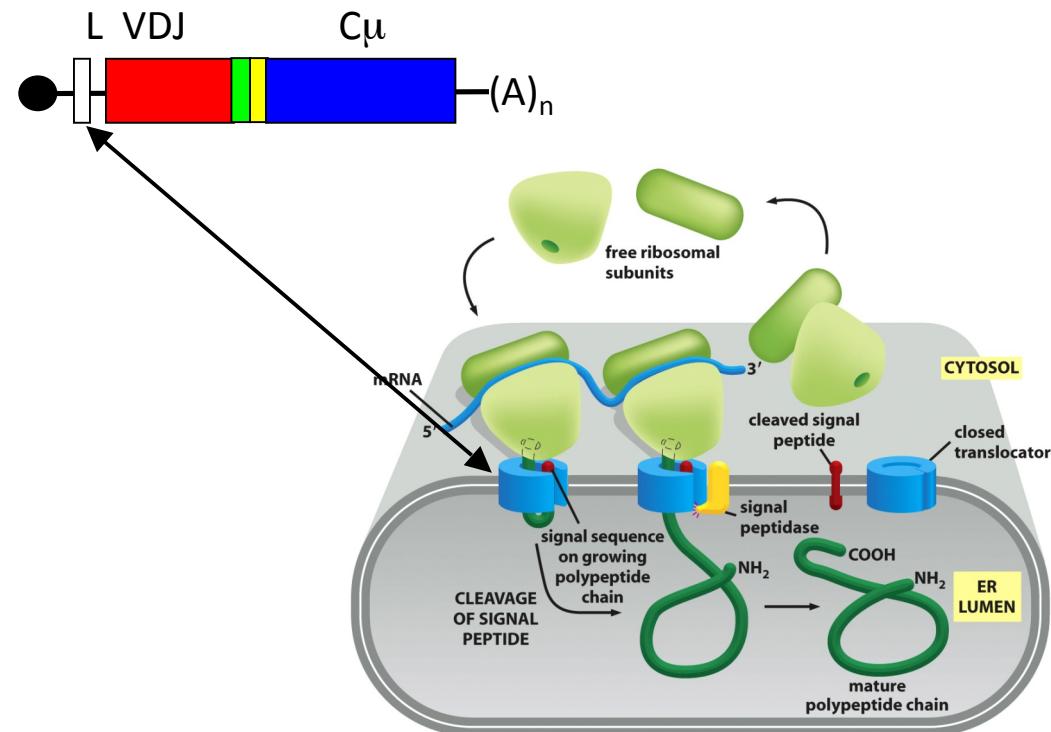
The mRNAs for the H chain, L chain, Ig- α , and Ig- β are translated by membrane bound ribosomes and the nascent polypeptides are co-translationally translocated into the ER (endoplasmic reticulum).

The H chain for the BCR and the Ig- α and Ig- β would have transmembrane regions.

The H chain for the secreted Ab and the L chains do not have a transmembrane region.

BCR and secreted Ab assembly

As the mRNA is being translated on the ribosome, the ER signal sequence emerges from the ribosome and directs the ribosome to a translocator on the ER that forms a pore in the ER membrane through which the polypeptide is translocated.



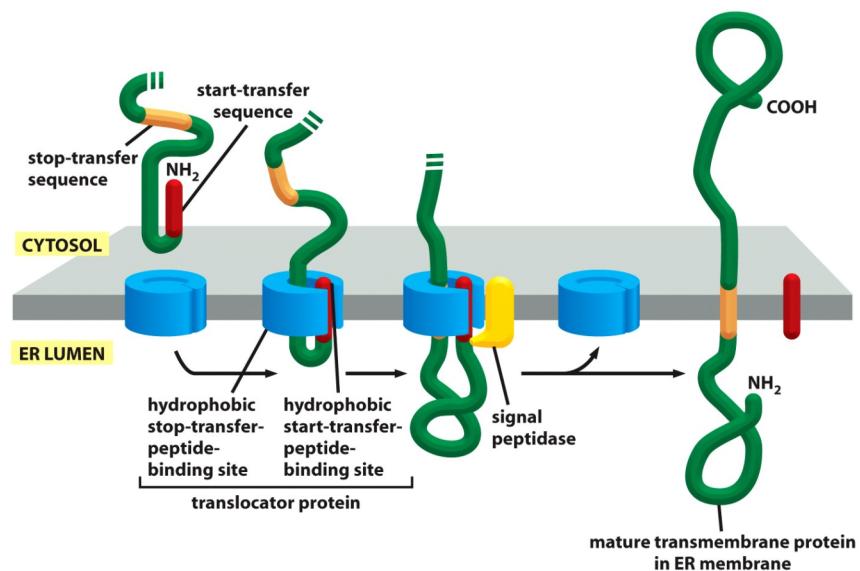
30

Figure 12-38 *Molecular Biology of the Cell* (© Garland Science 2008)

BCR and secreted Ab assembly

A signal peptidase is closely associated with the translocator and clips off the signal sequence during translation, and the mature protein is released into the lumen of the ER immediately after synthesis.

If the protein is to be a transmembrane protein, a portion will be retained in the ER membrane.

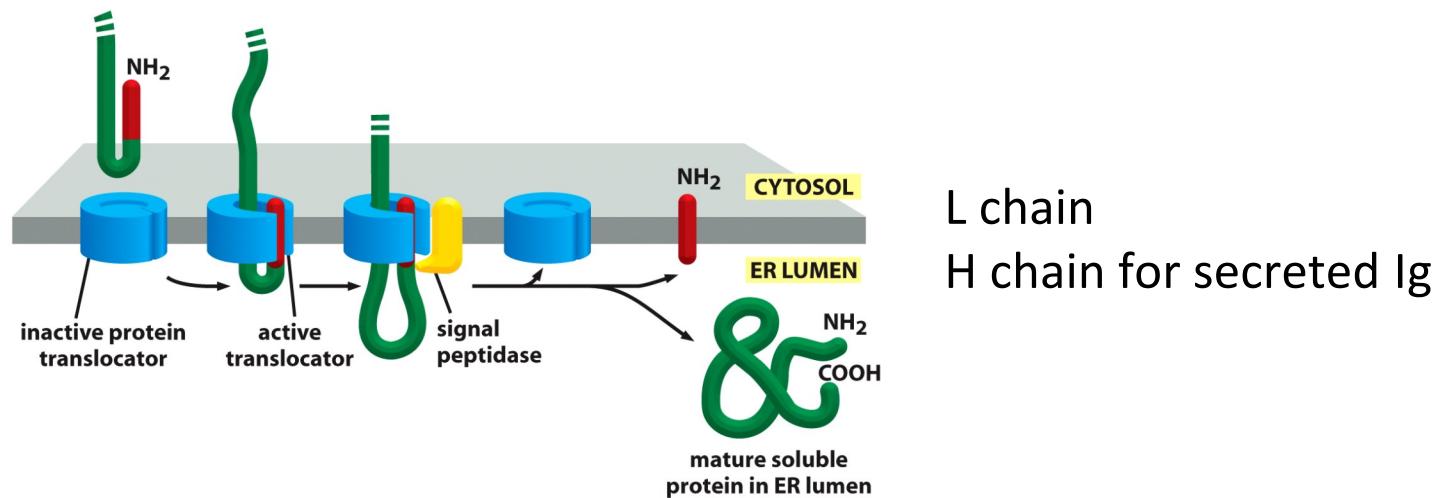


Heavy chain for membrane Ig,
Ig α / β signaling proteins

BCR and secreted Ab assembly

A signal peptidase is closely associated with the translocator and clips off the signal sequence during translation, and the mature protein is released into the lumen of the ER immediately after synthesis.

Proteins that are not membrane bound do not have the retention sequence.



BCR and secreted Ab assembly

The translocator is closed until the ribosome has bound, so that the permeability barrier of the ER membrane is maintained at all times.

The protein is not released into the cytosol so it doesn't fold up and lose the ability to bind to the translocator.

Chaperone proteins assist the individual chains in folding and make sure that none of these polypeptides can exit the ER until a complete BCR complex has been formed.

The H and L chains are linked to each other by disulfide bonds to form membrane-bound Abs.

The Ig- α and Ig- β chains are linked by disulfide bonds to each other.

BCR and secreted Ab assembly

The BCR then associates with the Ig- α/β subunit to form a BCR complex.

The complete BCR complexes then move to the Golgi (where it is glycosylated) and then are transported in vesicles to the cell surface where it may stay on the surface for several days, before being internalized and degraded.

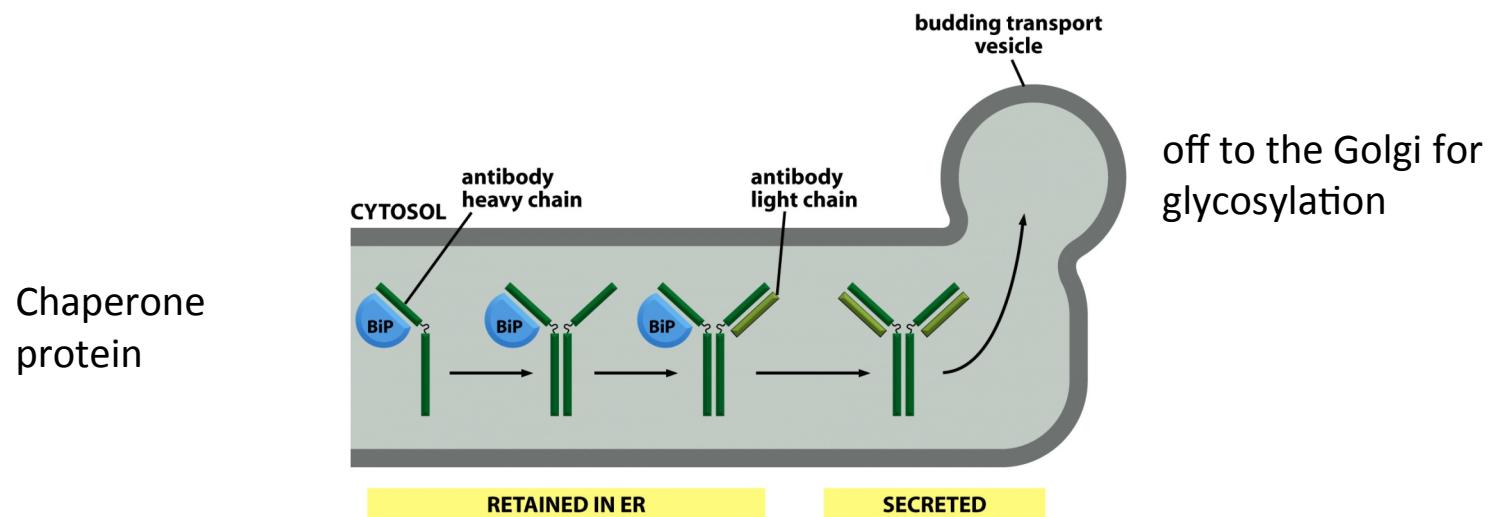


Figure 13-21 *Molecular Biology of the Cell* (© Garland Science 2008)

BCR and secreted Ab assembly

The addition of carbohydrate (glycosylation) in the Golgi protects the polypeptides and increases their stability) and then are transported in vesicles to the cell surface where it may stay on the surface for several days, before being internalized and degraded.

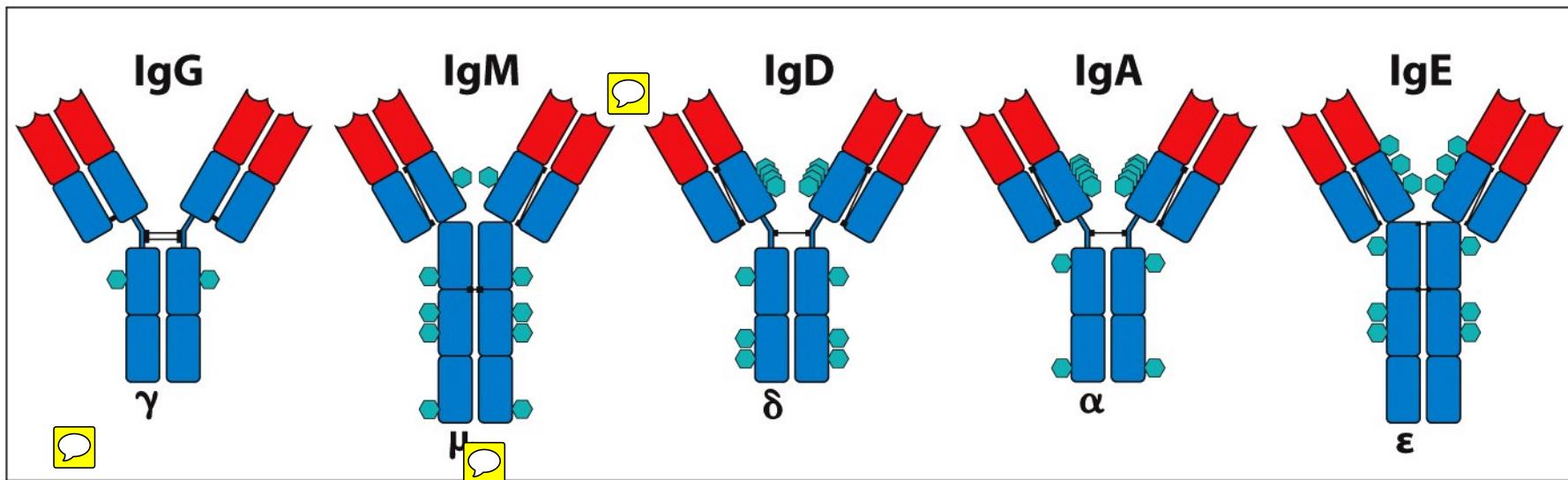


Figure 4.5 The Immune System, 3ed. (© Garland Science 2009)

Some Ig classes have a stretch of amino acids between two Ig domains of the heavy chain called a hinge (provides “flexibility” in how the Ab bind to Ag).



BCR and secreted Ab assembly

Some Ig's are secreted as polymers.

IgM is a pentamer where the monomeric units are joined together with a “J chain”. The monomeric units are cross-linked to the J-chain and each other by di-sulphide bonds.

IgA is a dimer where the monomeric units are joined together with a “J chain”. The monomeric units are cross-linked to the J-chain by di-sulphide bonds.

