

COURSE: Medical Microbiology, MBIM 650/720 - Fall 2009

TOPIC: Immunoglobulins: Genetics

Lecture # 7

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TEACHING OBJECTIVES:

1. To describe the organization and expression of the immunoglobulin gene families.
2. To explain the origins of antibody diversity.

REQUIRED READING:

Male *et al.* Immunology, 7th Ed., pp 80-85
Murray *et al.* Medical Microbiology, 6th Ed., pp 103 - 104

KEY WORDS:

V gene, C gene, J region, D region, Leader, Enhancer, Promotor, Antibody diversity, Germ line theory, Somatic mutation theory, N region insertions, Junctional diversity, Combinatorial association, Multispecificity, Clonal selection

IMMUNOGLOBULINS: GENETICS

LECTURE NOTES IMMUNOGLOBULINS: GENETICS

I. **History**

1. Amino acid sequencing data revealed that a single C region could be associated with many different V regions. Also, it was shown that a single idiotype could be associated with different C regions (eg. IgM and IgG). To explain these data it was suggested that perhaps the two regions of the Ig molecule were coded for by separate genes and that the V and C region genes were somehow joined before an Ig molecule was made (i.e. there were two genes for one polypeptide). This was a revolutionary concept but with the advent of recombinant DNA technology, it has been shown to be the correct. The Ig heavy and light chains are coded for by three separate gene families each one on a separate chromosome - one for the heavy chain and one for each of the light chain types. Each of these gene families has several V region genes and one or more C region genes. The V and C regions genes are not however immediately adjacent to each other.

II. Light chain gene families

1. Germ line gene organization - The organization of the κ and λ light chain genes in the germ line or undifferentiated cells is depicted in Figure 1.

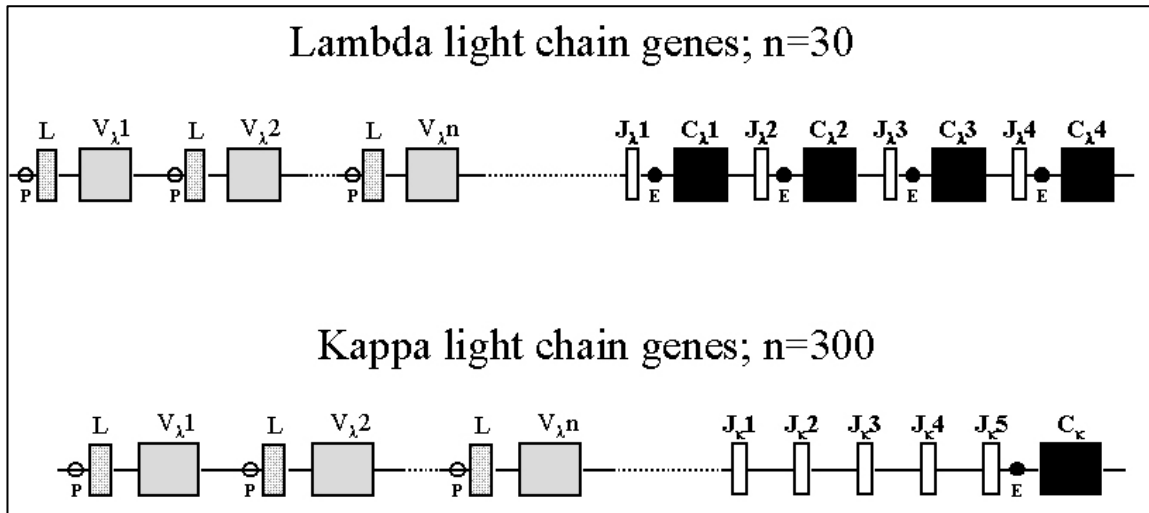


Figure 1

- a. Lambda light chains - The λ gene family is composed of 4 C region genes, one for each subtype of λ chain, and approximately 30 V region genes. Each of the V region genes is composed of two exons, one (L) that codes for a leader region and the other (V) that codes for most of the variable region. Upstream of each of the C genes there is an additional exon called J (joining). The L, V, J and C exons are separated by introns (intervening non-coding sequences).
- b. Kappa light chains - The κ light chain gene family contains only one C region gene, since there is only one type of κ light chain. There are many V region genes (approximately 250) each of which has a leader exon and a V exon. In the κ gene family there are several J exons located between the V and C genes. All of the exons are separated by introns.
2. Gene rearrangement and Expression - As a cell differentiates into a mature B cell that will make a light chain, there is a rearrangement of the various genes (exons) and the gene begins to be expressed as depicted in Figure 2. As a cell commits to become a B cell making a light chain, there is a rearrangement of the genes at the DNA level such that one of the V genes is brought next to one of the J regions. This occurs by a recombination event which removes the intron between the V and J regions. The selection of which V gene is used is not totally random; there is some preference for

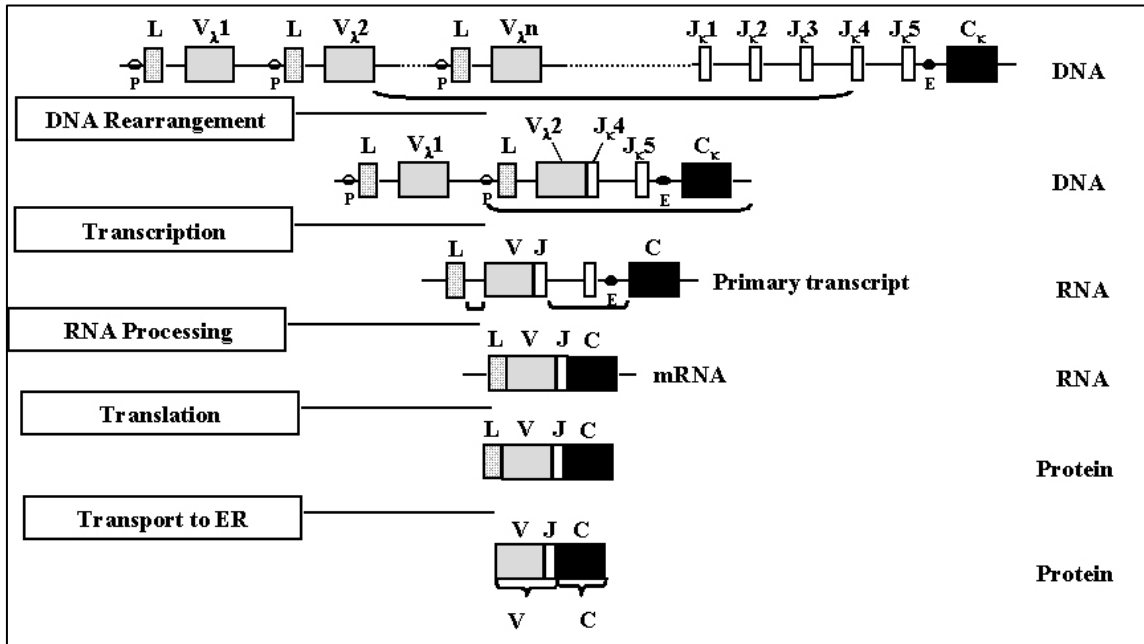


Figure 2

the use of V genes nearest to the J regions. However, with time all V genes can be used so that all combinations of V genes and J regions can be generated.

A consequence of this DNA rearrangement is that the gene becomes transcriptionally active because a promoter (P), which is associated with the V gene, is brought close to an enhancer (E), which is located in the intron between the J and C regions. As transcription initiates from the promoter a pre-mRNA is made which contains sequences from the L, V J and C regions as well as sequences for the introns between L and V and between J and C (See Figure 2). This pre-mRNA is processed (spliced) in the nucleus and the remaining introns are removed. The resulting mRNA has the L, V J and C exons contiguous.

The mRNA is translated in the cytoplasm and the leader is removed as the protein is transported into the lumen of the endoplasmic reticulum. The light chain is assembled with a heavy chain in the endoplasmic reticulum and the Ig is secreted via the normal route of secretory proteins. The region V region of the mature light chain is coded for by sequences in the V gene and J region and the C region by sequences in the C gene.

III. Heavy chain gene family

1. Germ line gene organization - The organization of the heavy chain genes is depicted in Figure 3.

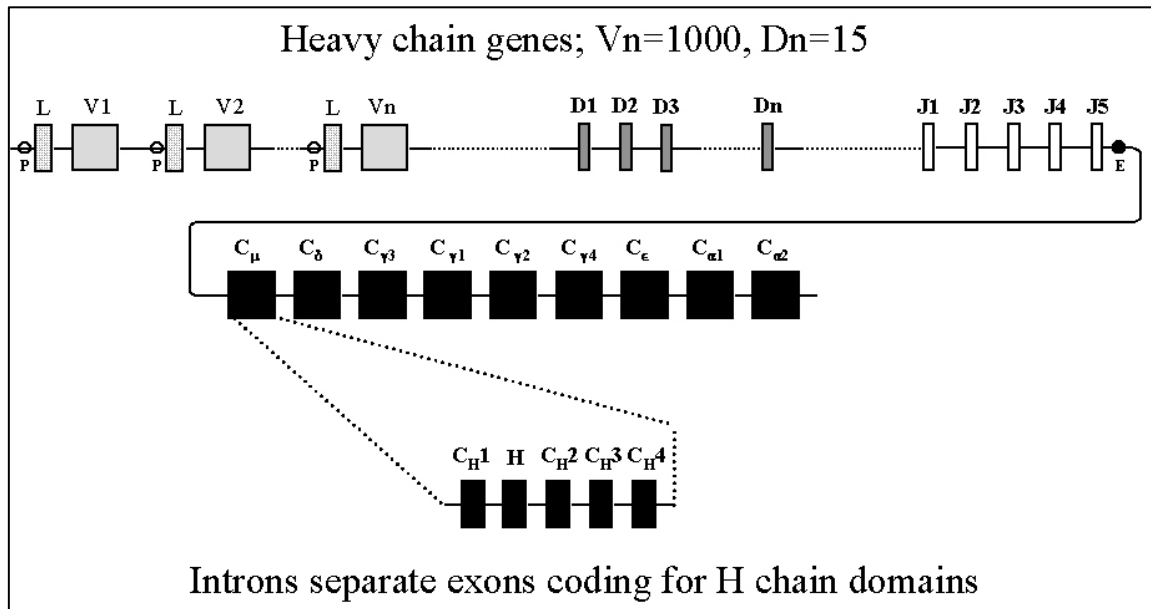


Figure 3

In the heavy chain gene family there are many C genes, one for each class and subclass of Ig. Each of the C genes is actually composed of several exons, one for each domain and another for the hinge region. In the heavy chain gene family there are many V region genes, each composed of a leader and V exon. In addition to several J exons, the heavy chain gene family also contains several additional exons called the D (diversity) exons. All of the exons are separated by introns as depicted in Figure 3.

2. Gene rearrangements and expression - As a cell differentiates into a mature B cell that will make a heavy chain, there is a rearrangement of the various gene segments (exons) and the gene begins to be expressed as depicted in Figures 4 and 5.

As a cell commits to become a B cell making a heavy chain, there are two rearrangements at the DNA level. First, one of the D regions is brought next to one of the J regions and then one of the V genes is brought next to the rearranged DJ region. This occurs by two recombination events which remove the introns between the V, D and J regions. As with the light chains the selection of the heavy chain V gene is not totally random but eventually all of the V genes can be used.

A consequence of these DNA rearrangements is that the gene becomes transcriptionally active because a promoter (P), which is associated with the V gene, is brought close to an enhancer (E), which is located in the intron between the J and C_μ regions. As transcription initiates from the promoter a pre-mRNA is made which contains sequences from the L, V, D, J, C_μ and C_δ regions as well as sequences for the introns between L and V, between J and C_μ , and between C_μ and C_δ (Figure 4).

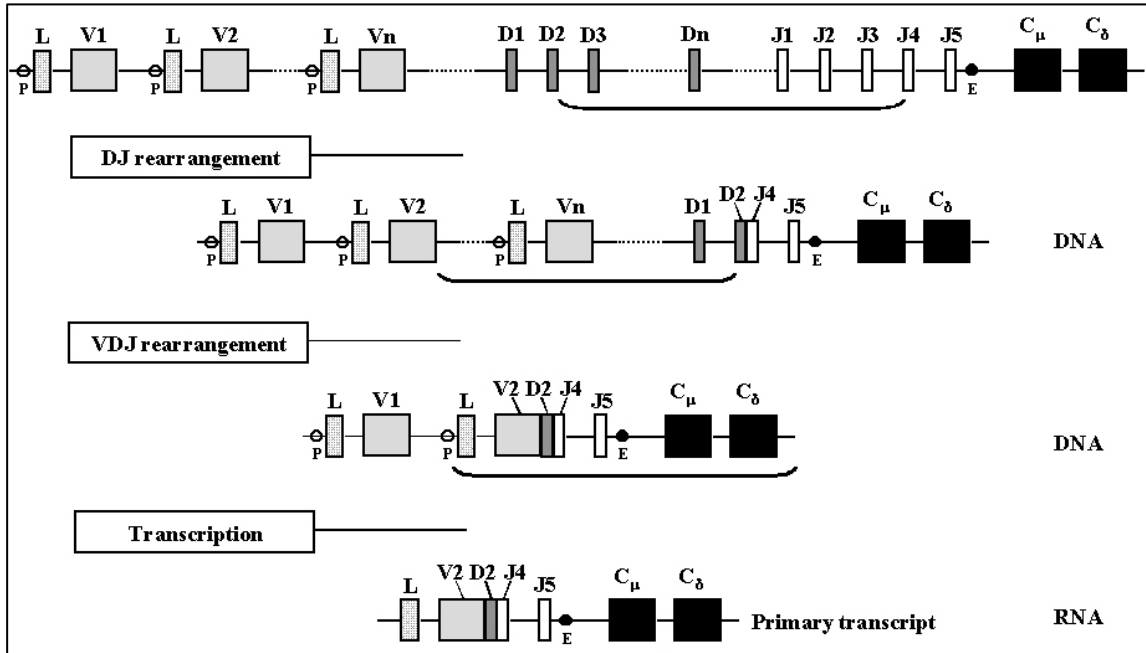


Figure 4

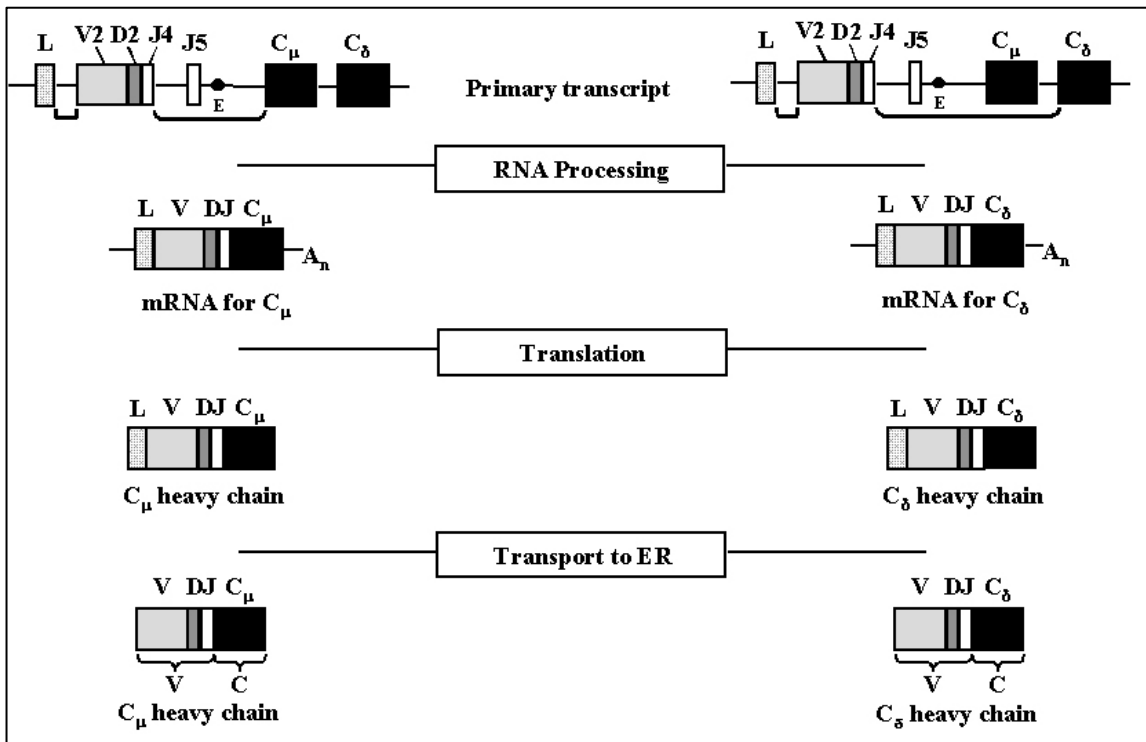


Figure 5

The pre-mRNA is processed (spliced) in the nucleus and the remaining introns, including those between the exons in the C genes, are removed (See Figure 5). The

pre-mRNA can be processed in two ways, one to bring the VDJ next to the C_μ gene and the other to bring the VDJ next to the C_δ gene. The resulting mRNAs have the L, V, D, J and C_μ or C_δ exons contiguous and will code for a μ and a δ chain, respectively.

The mRNAs are translated in the cytoplasm and the leader is removed as the protein is transported into the lumen of the endoplasmic reticulum. The heavy chain is assembled with a light chain in the endoplasmic reticulum and the Ig is secreted via the normal route of secretory proteins. The region V region of the mature heavy chain is coded for by sequences in the V gene, D region and J region and the C region by sequences in the C gene.

IV. Mechanism of DNA rearrangements

Flanking the V, J and D exons there are unique sequences referred to as recombination signal sequences (RSS), which function in recombination. Each RSS consists of a conserved nonamer and a conserved heptamer that are separated by either 12 or 23 base pairs as illustrated in Figure 6. The 12bp and 23 bp spaces correspond to one or two turns of the DNA helix.

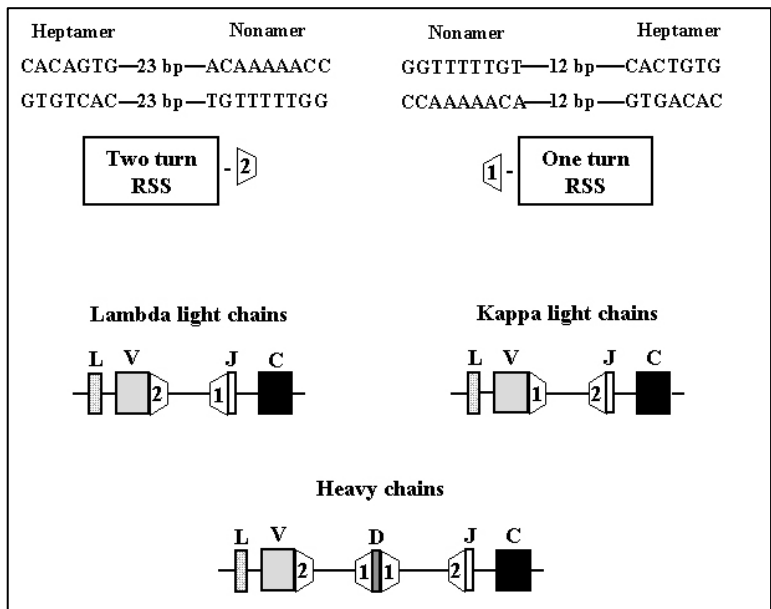


Figure 6

Recombination only occurs between a 1 turn and a 2 turn signal. In the case of the λ light chains there is a 1 turn signal upstream of the J exon and a 2 turn signal downstream of V_λ . In the case of the κ light chains there is a 1 turn signal downstream of the V_κ gene and a 2 turn signal upstream of the J exon..

In the case of the heavy chains there are 1 turn signals on each side of the D exon and a 2 turn signal downstream of the V gene and a 2 turn signal upstream of the J exon. Thus, this ensures that the correct recombination events will occur.

The recombination event results in the removal of the introns between V and J in the case of the light chains or between the V, D, and J in the case of the heavy chains. The recombination event is catalyzed by two proteins, Rag-1 and Rag-2. Mutations in the genes for these proteins results in a severe combined immunodeficiency disease (both T and B cells are deficient), since these proteins and the RSS are involved in generating both the B and T cell receptors for antigen.

V. Order of gene expression in Ig gene families

An individual B cell only produces one type of light chain and one class of heavy chain. (*N.B.* The one exception is that a mature B cell can produce both μ and δ heavy chains but the antibody specificity is the same since the same VDJ region is found on the μ and δ chains). Since any B cell has both maternal and paternal chromosomes which code for the Ig genes there must be some orderly way in which a cell expresses its Ig genes so as to ensure that only one type of light chain and one class of heavy chain is produced.

The order in which the Ig genes are expressed in a B cell is depicted in Figure 7 and 8.

Heavy chain (Figure 7) - A cell first attempts to rearrange one of its heavy chain genes; in some cells the maternal chromosome is selected and in others the paternal chromosome is selected. If the rearrangement is successful so that a heavy chain is made, then no further rearrangements occur in the heavy chain genes. If, on the other hand, the first attempt to rearrange the heavy chain genes is unsuccessful (*i.e.* no heavy chain is made), then the cell attempts to rearrange the heavy chain genes on its other chromosome. If the cell is unsuccessful in rearranging the heavy chain genes the second time, it is destined to be eliminated.

Kappa light chain (Figure 8) - When a cell successfully rearranges a heavy chain gene, it then begins to rearrange one of its κ light chain genes. It is a random event whether the maternal or paternal κ light chain genes are selected. If the rearrangement is unsuccessful (*i.e.* it does not produce a functional κ light chain), then it attempts to rearrange the κ genes on the other chromosome. If a cell successfully rearranges a κ light chain gene, it will be a B cell that makes an Ig with a κ light chain.

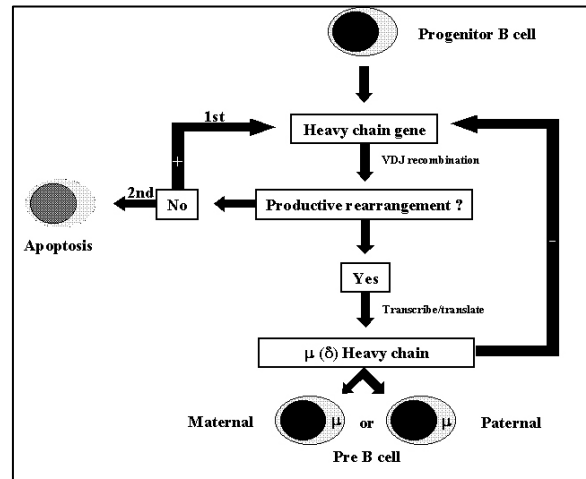


Figure 7

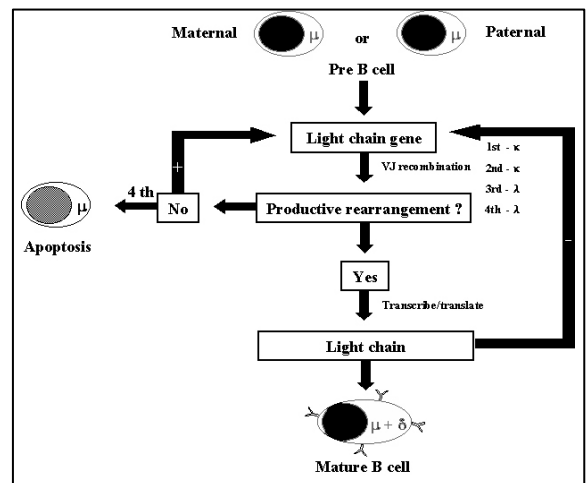


Figure 8

Lambda light chain (Figure 8) - If a cell is unsuccessful in rearranging both of its κ light chain genes, it then attempts to make a λ light chain. It is a random event whether the maternal or paternal λ light chain genes are selected. If the rearrangement is unsuccessful (*i.e.* it does not produce a functional λ light chain), then it attempts to rearrange the λ genes on the other chromosome. If a cell successfully rearranges a λ light chain gene, it will be a B cell that makes an Ig with a λ light chain.

The orderly sequence of rearrangements in the Ig gene families explains:

- 1) Why an individual B cell can only produce one kind of immunoglobulin with one kind of heavy and one kind of light chain.
- 2) Why a individual B cell can only make antibodies of one specificity.
- 3) Why there is allelic exclusion in Ig allotypes at the level of an individual Ig molecule but co-dominant expression of allotypes in the organism as a whole.

VI ORIGIN OF ANTIBODY DIVERSITY

A. **Background** - Antibody diversity refers to the sum total of all the possible Ab specificities that an organism can make. It is estimated that we can make 10^7 - 10^8 different Ab molecules. One of the major questions in immunology has been how can we make so many different antibody molecules. Theories which have attempted to explain the origin of antibody diversity fall into two major categories.

1. Germ line theory - This theory states that we have a different V region gene for each possible antibody we can make.
2. Somatic mutation theory - This theory state that we have only one or a few V region genes and the diversity is generated by somatic mutations which occur in these genes.

B. **Current Concepts** - Our current thinking is that both the germ line and somatic mutation theories have some merit. It is thought that antibody diversity is generated by the following mechanisms.

1. Large number of V genes
 - a) 30 lambda V genes
 - b) 300 kappa V genes
 - c) 1000 heavy chain V genes
2. V-J and V-D-J joining - The region where the light chain V gene and J region or the heavy chain V gene and D and J regions come together is in the 3rd hypervariable

region. Since it is random which V and which J or D regions come together, there is a lot of diversity that can be generated by V-J and V-D-J joining.

3. Junctional diversity (Inaccuracies in V-J and V-D and D-J recombination) - (Figure 9)

Recombination between V-J and V-D-J is not always perfect and additional diversity can arise by errors that occur in the recombination event that brings the V region next to the J or D regions or the D region next to the J region. It is estimated that these inaccuracies can triple the diversity generated by V-J and V-D-J joining. The diversity generated by this mechanisms is occurring in the 3rd hypervariable region and thus, is directly affecting the combining site of the Ab.

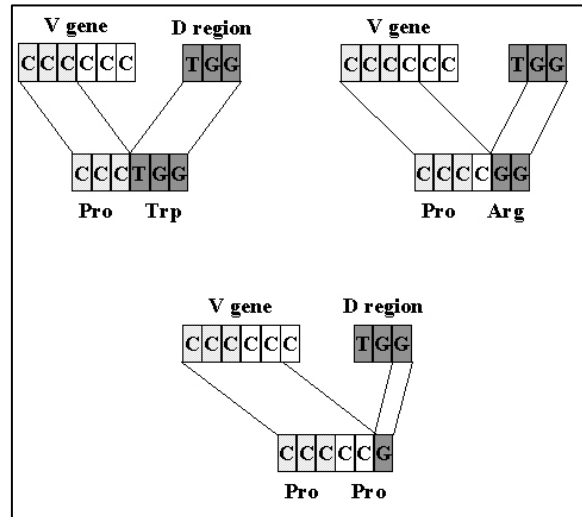


Figure 9

4. N region insertion - At the junction between D and J segments there is often an insertion of a series of nucleotides which is catalyzed by the enzyme terminal transferase. (Terminal transferase catalyzes the random polymerization of nucleotides into DNA without the need for a template. This leads to further diversity in the 3rd hypervariable region.
5. Somatic Mutation - There is evidence that somatic mutations are occurring in the V gene, particularly in the place that codes for the 2nd hypervariable region. Thus, somatic mutation probably contributes to Ab diversity to some extent.
6. Combinatorial Association - Any individual B cell has the potential to make any one of the possible heavy chains and any one of the possible light chains. Thus, different combinations of heavy and light chains within an individual B cell adds further diversity.
7. Multispecificity - Due to cross reactions between antigenic determinants of similar structure an antibody can often react with more than one antigenic determinant. This is termed multispecificity. Multispecificity also contributes to Ab diversity.

An example of how these mechanisms can generate a great deal of diversity is illustrated below:

	B Cell Receptor (Immunoglobulin)	
	Heavy	Kappa
V gene segments	1000	300
D gene segments	15	-
J gene segments	4	4
N region insertion	++	-
Junctional diversity	+++	+
Somatic mutation	+	+
Combinatorial association	V x D x J 1000 X 15 X 4	V x J 300 x 4
Total	6×10^4	1.2×10^3
	x	
Combinatorial association	7.2×10^7	

These calculations do not take into consideration the contributions of lambda light chains, somatic mutation junctional diversity, N region insertions or multispecificity.

The process of gene rearrangement of the heavy and light chains and the combinatorial association of these chains occurs during B cell development and is **independent of antigen**. Clones of B cells expressing all of the possible antibody specificities are produced during development and antigen simply selects those clones which have the appropriate receptor. The selected clones are then activated, proliferate and differentiate into antibody secreting plasma cells.

VII. T CELL RECEPTOR FOR ANTIGEN

T cells also have a receptor for antigen on their surfaces. This receptor is not an immunoglobulin molecule but it is composed of two different polypeptide chains which have constant and variable regions analogous to the immunoglobulins. Diversity in the T cell receptor is also generated in the same way as described for antibody diversity (e.g. by VJ and VDJ joining of gene segments and combinatorial association). However, no somatic mutation has been observed in T cells.

Adapted from Dr. E.P.Mayer