Kurdistan region - Iraq
Cihan University - Erbil
College of Science
Department of Biology

A seminar on

Antibody Diversity

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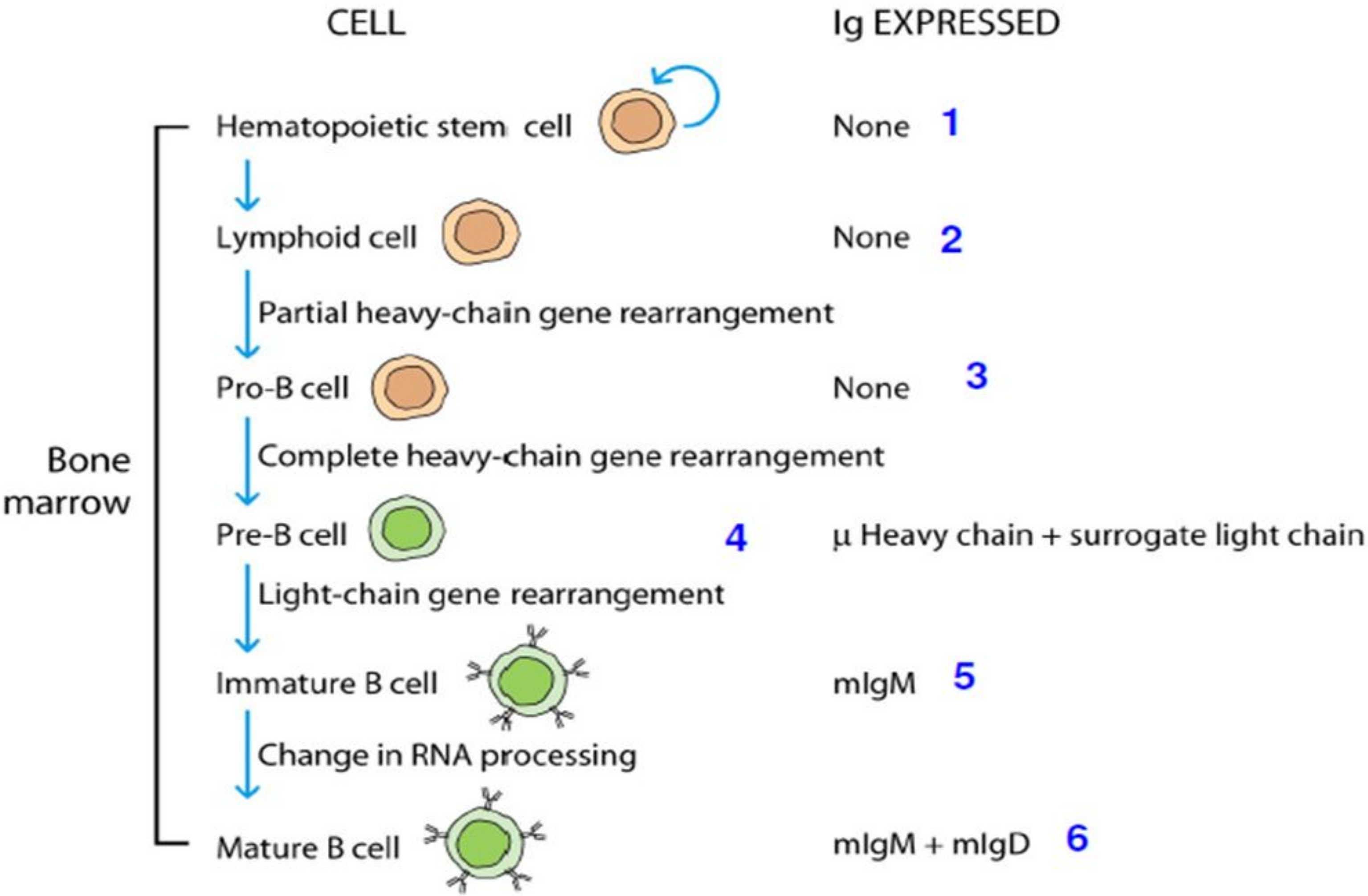


CELL	Ig EXPRESSED
Hematopoietic stem cell	None 1
Lymphoid cell	None 2
Pro-B cell	None 3
Pre-B cell 4	µ Heavy chain + surrogate light chain
Immature B cell	mIgM 5
Mature B cell	mIgM + mIgD 6

Bone marrow

Hematopoietic stem cell → Lymphoid cell

Lymphoid cell → Partial heavy-chain gene rearrangement → Pro-B cell

Pro-B cell → Complete heavy-chain gene rearrangement → Pre-B cell

Pre-B cell → Light-chain gene rearrangement → Immature B cell

Immature B cell → Change in RNA processing → Mature B cell

Hi, I'm Captain MAGED.

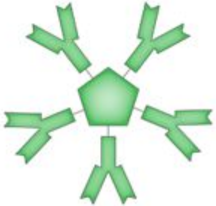
M

A

G
E
D

Secretory Igs

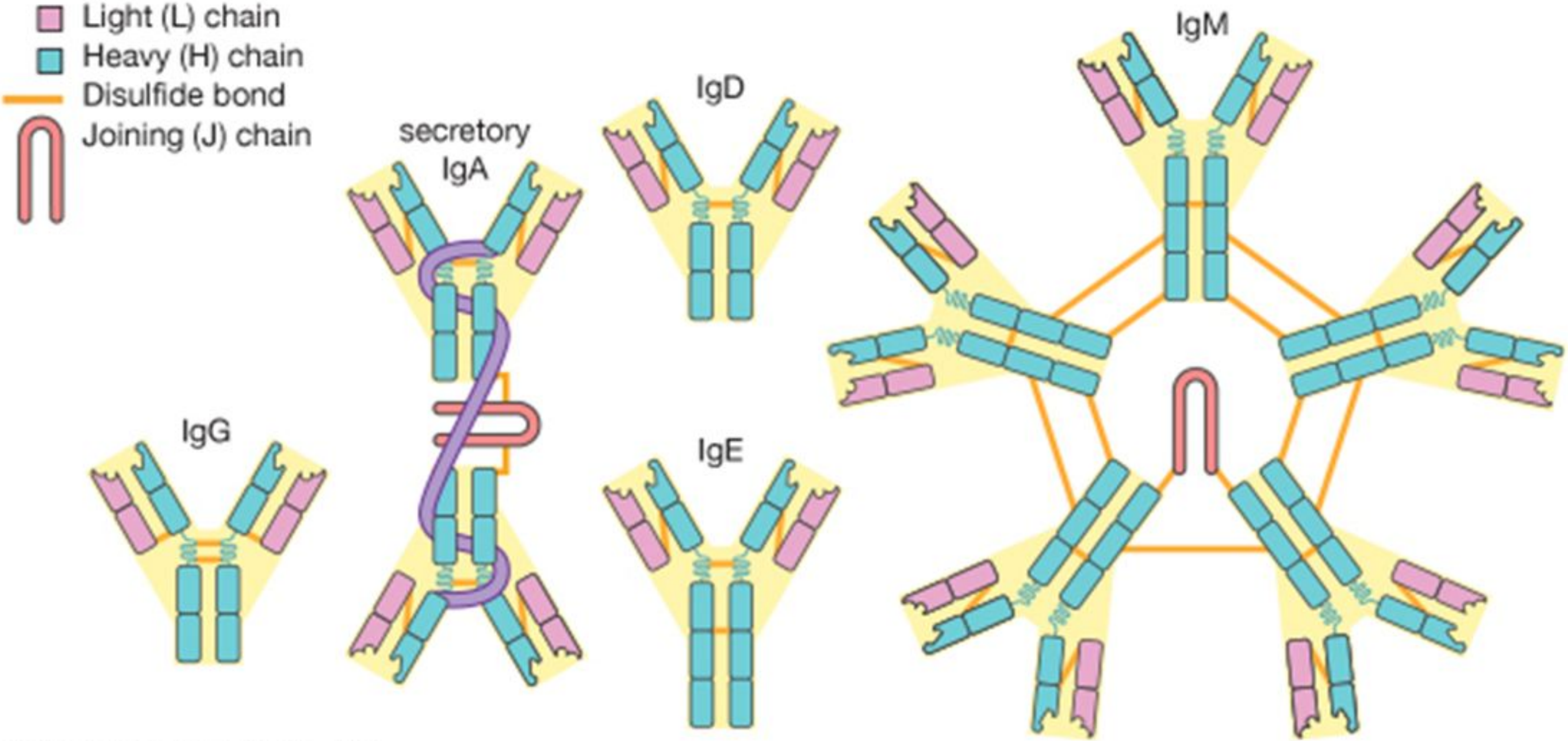
Pentamer
IgM



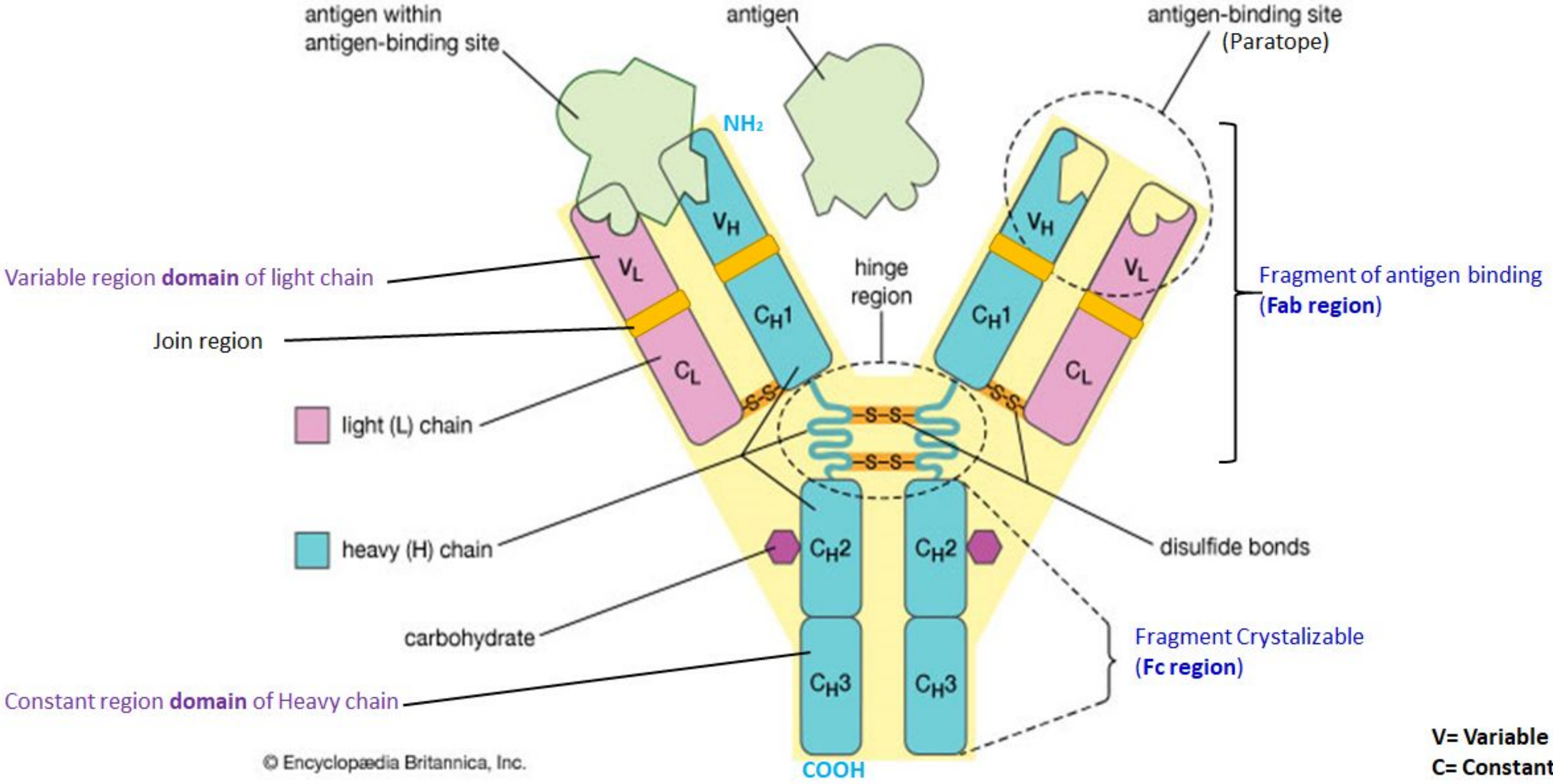
Dimer
IgA

Monomer
IgG
IgE
IgD

Immunoglobulins structure



Immunoglobulin Structure



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V= Variable region
C= Constant region

Mechanisms that B cells use to produce antibodies diversity

Heavy chain chain have a M.W of 50-75 kDa, approximately 400 amino acid on chromosome 14,
which is twice the number in Light chain with a M.W of 25 kDa.

L-chain are two types: kappa (κ) chain on chromosome 2

and lambda (λ) chain on chromosome 22

Each Ig must include one type of L-chain.
Either kappa (κ) or lambda (λ) chain.

Each B-cell needs 3 gene segment pools for producing of antibody

Even in the absence of antigen stimulation, a human can probably make more than 10^{12} different antibody molecules

called *pre-immune antibody repertoire*.

antibodies can cross-react with a variety of related but different antigenic determinants, making the antibody defense force even more formidable (enormous).

The preimmune repertoire is apparently large enough to ensure that there will be an antigen-binding site to fit almost any potential antigenic determinant, albeit with low affinity.

After repeated stimulation by antigen, B cells can make antibodies that bind their antigen with much higher affinity a process called *affinity maturation*.

Thus, antigen stimulation greatly increases the antibody arsenal (armory).

Antibodies are proteins, and proteins are encoded by genes. Antibody diversity therefore poses a special genetic problem: how can an animal make more antibodies than there are genes in its genome? (The human genome, for example, contains fewer than 50,000 genes.) This problem is not quite as formidable as it might first appear. Recall that the variable regions of both the light and heavy chains of antibodies usually form the antigen-binding site. Thus, an animal with 1000 genes encoding light chains and 1000 genes encoding heavy chains could, in principle, combine their products in 1000 × 1000 different ways to make 10^6 different antigen-binding sites (although, in reality, not every light chain can combine with every heavy chain to make an antigen-binding site).

We now know that each type of antibody chain—κ light chains, λ light chains, and heavy chains—has a separate pool of **gene segments** and exons from which a single polypeptide chain is eventually synthesized. Each pool is on a different chromosome and contains a large number of gene segments encoding the V region of an antibody chain and, as we saw in Figure 4, a smaller number of exons encoding the C region. During the development of a B cell, a complete coding sequence for each of the two antibody chains to be synthesized is assembled by site-specific genetic recombination.

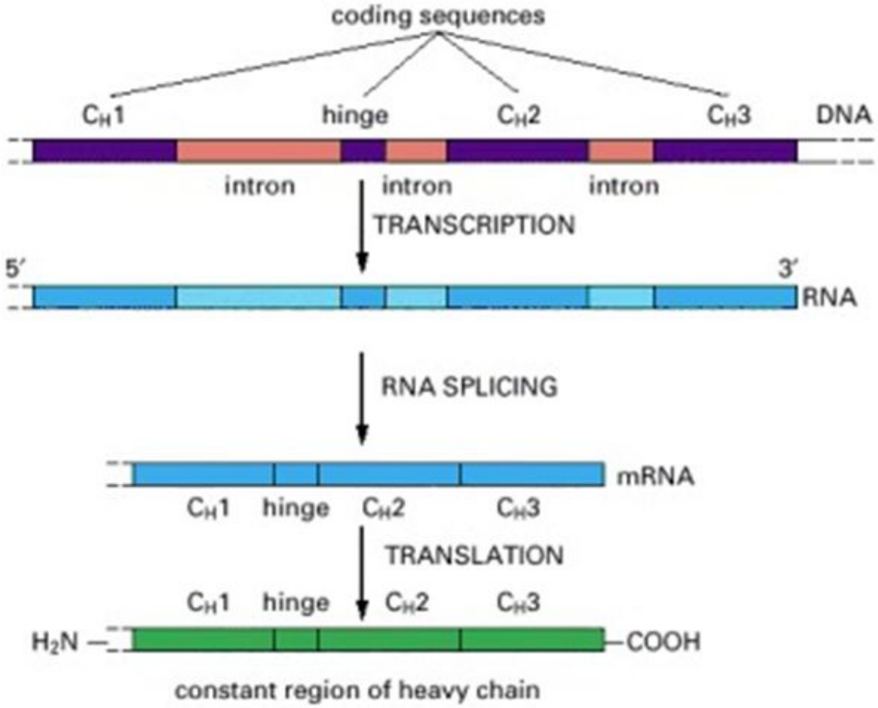
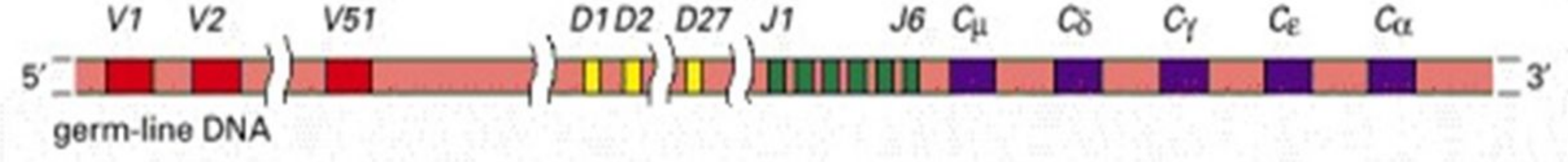


Figure 4: The organization of the DNA sequences that encode the constant region of an antibody heavy chain

The coding sequences (exons) for each domain and for the hinge region are separated by noncoding sequences (introns). The intron sequences are removed by splicing the primary RNA transcripts to form mRNA. The presence of introns in the DNA is thought to have facilitated accidental duplications of DNA segments that gave rise to the antibody genes during evolution. The DNA and RNA sequences that encode the variable region of the heavy chain are not shown.

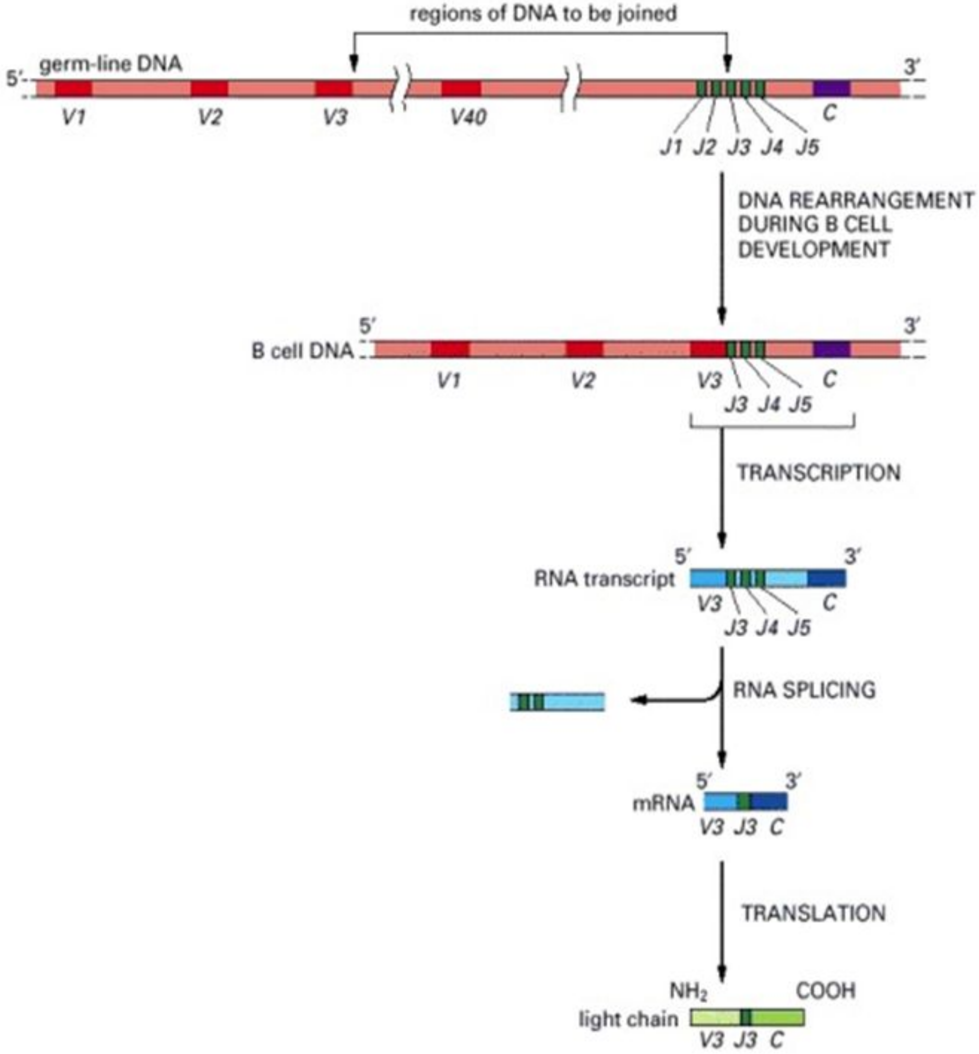
In addition to bringing together the separate gene segments and the C-region exons of the antibody gene, these rearrangements also activate transcription from the gene promoter through changes in the relative positions of the enhancers and silencers acting on the promoter. Thus, a complete antibody chain can be synthesized only after the DNA has been rearranged. As we shall see, the process of joining gene segments contributes to the diversity of antigen-binding sites in several ways.



The human heavy-chain gene-segment pool

There are **51** *V* segments, **27** ***D*** segments, **6** ***J*** segments, and an ordered cluster of *C*-region exons, each cluster encoding a different class of heavy chain. The *D* segment (and part of the *J* segment) encodes amino acids in the third hypervariable region, which is the most variable part of the V region. The figure is not drawn to scale: the total length of the heavy chain locus is over 2 mega-bases (2,000,000 bases).

Moreover, many details are omitted. For instance, each *C* region is encoded by multiple exons (see Figure 4); there are four clusters of C_{γ}-region exons ($C_{\gamma 1}$, $C_{\gamma 2}$, $C_{\gamma 3}$, and $C_{\gamma 4}$), and the V_H gene segments are clustered on the chromosome in groups of homologous families. The genetic mechanisms involved in producing a heavy chain are the same as those shown before, for light chains except that two DNA rearrangement steps are required instead of one. First a *D* segment joins to a *J* segment, and then a *V* segment joins to the rearranged *DJ* segment.



The (*Variable & joining region)* joining process involved in making a human κ light chain

In the "germ-line" DNA (where the antibody genes are not being expressed and are therefore not rearranged), the cluster of five *J* gene segments is separated from the *C*-region exon by a short intron and from the 40 *V* gene segments by thousands of nucleotide pairs. During the development of a B cell, the randomly chosen *V* gene segment *(V3* in this case) is moved to lie precisely next to one of the *J* gene segments *(J3* in this case). The "extra" *J* gene segments *(J4* and *J5)* and the intron sequence are transcribed (along with the joined *V3* and *J3* gene segments and the C-region exon) and then removed by RNA splicing to generate mRNA molecules in which the *V3, J3,* and *C* sequences are contiguous. These mRNAs are then translated into κ light chains. A *J* gene segment encodes the C-terminal 15 or so amino acids of the V region, and the *V-J* segment junction coincides with the third hypervariable region of the light chain, which is the most variable part of the V region.

The Result

The large number of inherited *V*, *J*, and *D* gene segments available for encoding antibody chains makes a substantial contribution on its own to antibody diversity, but the combinatorial joining of these segments (called *combinatorial diversification*) greatly increases this contribution.

40 *V* segments **X** **5** *J* segments in the human κ light-chain **=** **200** different **κ-chain** V regions

40 * 5 = **200**

30 *V* segments **X** **4** *J* segments in the human κ light-chain **=** **200** different **λ-chain** V regions

30 * 4 = **120**

51 *V* segments **X** 6 *J* segments **X** 27 *D* segments in the human heavy-chain = 8262 different **heavy-chain** V regions.

51 * 6 * 27 = **8262**

The combinatorial diversification resulting from the assembly of different combinations of inherited *V*, *J*, and *D* gene segments just discussed is an important mechanism for diversifying the antigen-binding sites of antibodies.

By this mechanism alone, a human can make about 2.6×10^6 (**$320_{(200+120)}$ × 8262= 2,643,840**) different antigen-binding sites.

Human immune system makes (about 10^{12})

=

combinatorial diversification increase the number of possibilities 10^6

+

In addition, the joining mechanism itself greatly increases this number of possibilities (probably more than 10^8-fold), making it much greater than the total number of B cells (about 10^{12}) in a human.

During B cell development, the *V* and *J* gene segments (for the light chain) and the *V*, *D*, and *J* gene segments (for the heavy chain) are joined together to form a functional V_L- or V_H-region coding sequence by a process of site-specific recombination called **V(D)J joining**.

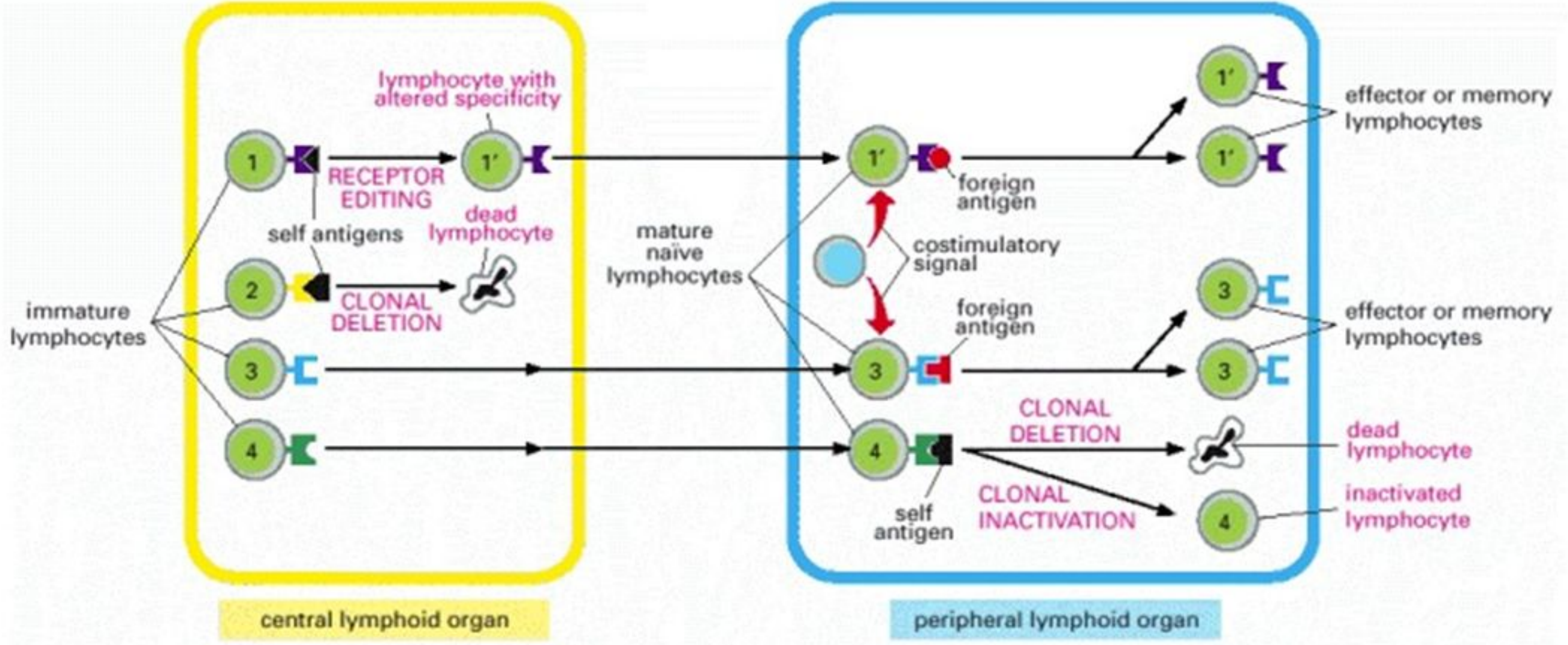
Conserved DNA sequences flank each gene segment and serve as recognition sites for the joining process, ensuring that only appropriate gene segments recombine. Thus, for example, a *V* segment will always join to a *J* or *D* segment but not to another *V* segment. Joining is mediated by an enzyme complex called the **V(D)J recombinase**. This complex contains two proteins that are specific to developing lymphocytes, as well as enzymes that help repair damaged DNA in all our cells.

The lymphocyte-specific proteins of the V(D)J recombinase are encoded by two closely linked genes called *rag-1* and *rag-2 (rag = r*ecombination *a*ctivating *g*enes).

The **RAG proteins** introduce double-strand breaks at the flanking DNA sequences, and this is followed by a rejoining process that is mediated by both the RAG proteins and the enzymes involved in general DNA double-strand repair (discussed in Chapter 5). Thus, if both *rag* genes are artificially expressed in a fibroblast, the fibroblast is now able to rearrange experimentally introduced antibody gene segments just as a developing B cell normally does. Moreover, individuals who are deficient in either *rag* gene or in one of the general repair enzymes are highly susceptible to infection because they are unable to carry out *V(D)J* joining and consequently do not have functional B or T cells. (T cells use the same recombinase to assemble the gene segments that encode their antigen-specific receptors.)

In most cases of site-specific recombination, DNA joining is precise. But during the joining of antibody (and T cell receptor) gene segments, a variable number of nucleotides are often lost from the ends of the recombining gene segments, and one or more randomly chosen nucleotides may also be inserted. This random loss and gain of nucleotides at joining sites is called **junctional diversification,** and it enormously increases the diversity of V-region coding sequences created by recombination, specifically in the third hypervariable region.

In many cases, it will result in a shift in the reading frame that produces a **nonfunctional gene**. Because roughly two in every three rearrangements are “nonproductive” in this way, many developing B cells never make a functional antibody molecule and consequently die in the bone marrow. B cells making functional antibody molecules that bind strongly to self antigens in the bone marrow are stimulated to re-express the RAG proteins and undergo a second round of *V(D)J* rearrangements, thereby changing the specificity of the cell-surface antibody they make—a process referred to as **receptor editing**. Self-reactive B cells that fail to change their specificity in this way are eliminated through the process of clonal deletion.



As mentioned earlier, with the passage of time after immunization, there is usually a progressive **increase in the affinity** of the antibodies produced against the immunizing antigen.

This phenomenon, known as affinity maturation,

is due to the accumulation of point mutations specifically in both heavy-chain and light-chain V-region coding sequences. The mutations occur long after the coding regions have been assembled, when B cells are stimulated by antigen and helper T cells to generate memory cells in a lymphoid follicle in a peripheral lymphoid organ. They occur at the rate of about one per V-region coding sequence per cell generation. Because this is about a million times greater than the spontaneous mutation rate in other genes, the process is called **somatic hypermutation**.

Thank You!

STAY
HOME, STAY
Safe