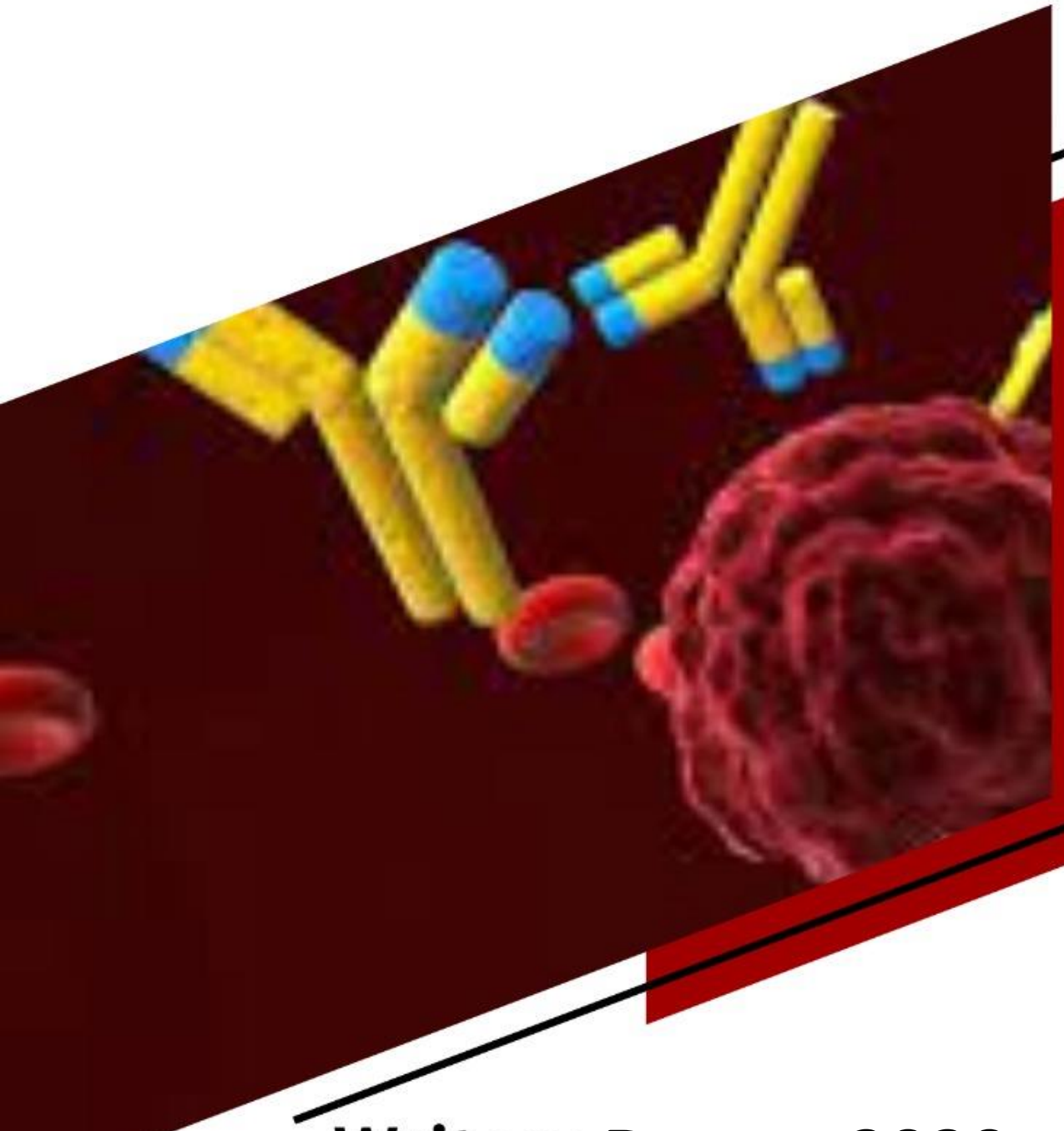
Doctor 021

IMMUNOLOGY

Sheet no. 14



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ANTIGEN RECEPTOR GENE REARRANGEMENT

-Note: we will talk about B-cells, but it is the same process for T-cells except that we have alpha and beta chains instead of heavy and light chains.

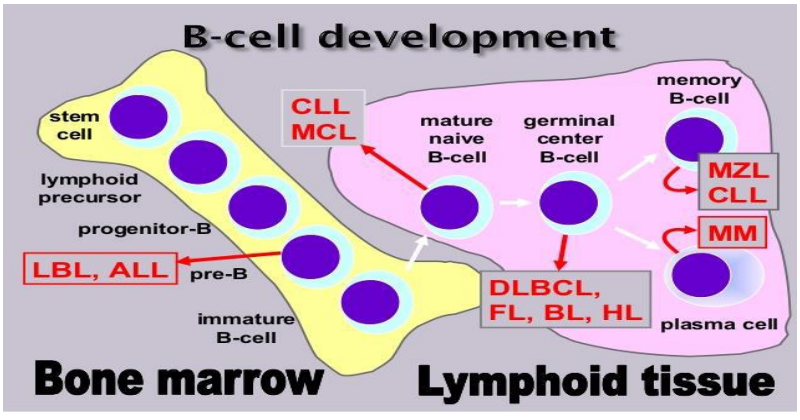
• This is a unique phenomenon that occurs only in a limited population of cells in a certain sequence of DNA.

• Remember, the main goal of mitosis is to produce genetically identical daughter cells, and this means if you compare the DNA sequence of both daughter cells with each other and with the parent cell, they will be identical. However, if you compare the cytoplasm of both daughter cells, it won't be identical, because the process of cytokinesis doesn't equally distribute the cytoplasm between daughter cells.

• All of the human body cells (except eggs and sperm) are genetically identical. This means if you sequence human cells from the liver, skin, hematopoietic stem cells, or any other cell, the DNA will be identical. However, not all the DNA will be transcribed and translated, the DNA will rather undergo a selection process for certain genes to be expressed depending on the need of the organ and tissue for a certain protein.For example: the protein Crystallin gene is present in all cells, and it is expressed in certain tissues like the lens of the eyes, but not expressed in other tissue like the liver. On the other hand, Albumin protein is present in the liver but not in the lens.

-A progenitor lymphoid cell can become a progenitor T cell or progenitor B cell.

SOMATIC RECOMBINATION (VDJ RECOMBINATION)



-The bone marrow is a heterogeneous population of cells containing osteoblasts, osteoclasts, mesenchymal stem cells, and hematopoietic stem cells. Hematopoietic stem cells are dedifferentiated with no distinct structure which means no distinct function.

• Lymphoid cells are produced in the bone marrow through the process of mitosis of hematopoietic stem cells. This will produce precursor lymphoid cells, which will differentiate into immature T or B lymphocytes then they leave the bone marrow and maturation occurs.

• A unique phenomenon occurs in the mitosis of hematopoietic stem cells to give rise to B or T cells.

• B cell development begins in Progenitor Lymphoid Cell through somatic recombination will make an immature B cell with a unique Ab that binds to a specific antigen.

REARRANGEMENT OF ANTIGEN RECEPTOR GENES LYMPHOCYTES

-Once it becomes a progenitor B cell, it will go through somatic recombination of the heavy chain (heavy chain rearrangement)

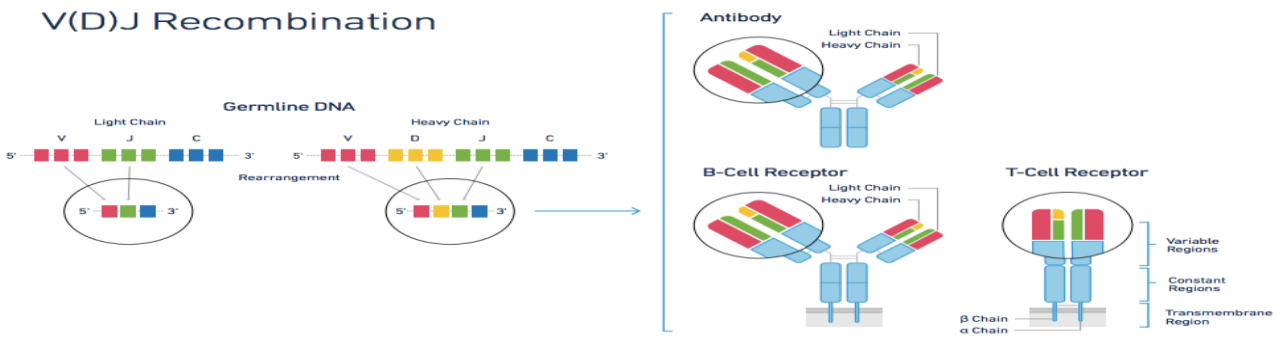
-The genes that encode diverse antigen receptors of B and T lymphocytes are generated by the rearrangement in individual lymphocytes of different:

-variable (V) region gene

-segments with diversity (D)

-and joining (J) gene segment

-Notice that there is no D segment in the light chain sequence



There are millions of antibodies and T cell receptors, if each one of them has it is own gene, this means we have millions of genes, but this is not the case as we only have 22000 genes. Through alternative splicing (skipping exons) on the RNA level, you can make 100000 proteins out of these 22000 genes. But this is not enough as we are talking about millions of proteins only in our immune cells.

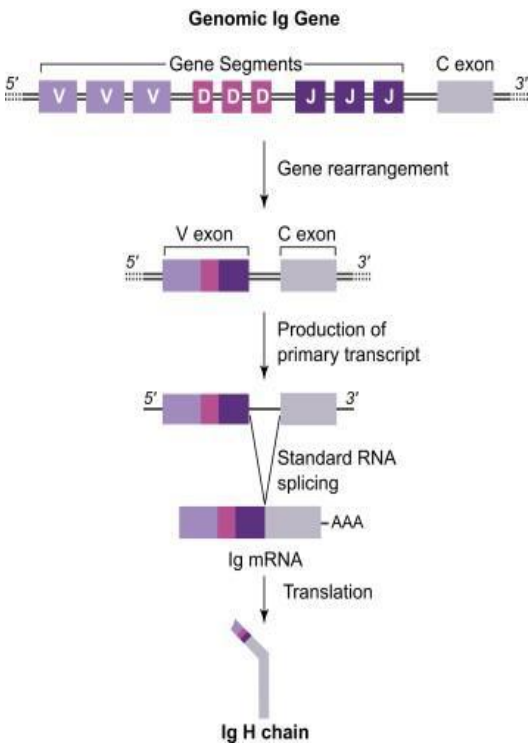
• For this reason, immune cells use somatic rearrangement (or recombination) approach rather than single gene single protein approach.

• In developing B cells, the first antigen receptor gene to be completely rearranged is the Ig heavy chain or Ig H gene.

• Only one segment of each group will appear in the daughter cell, these will undergo transcription to produce pre-mRNA which will undergo processing to produce heavy chain immunoglobulin as a pre-antigen (before the appearance of the immature lymphocytes, the precursor cells will only carry heavy chains).

• Typically, the progenitor B cell will make the IgM Ab, so this progenitor B cell will become a precursor B cell after somatic recombination of the heavy chain.

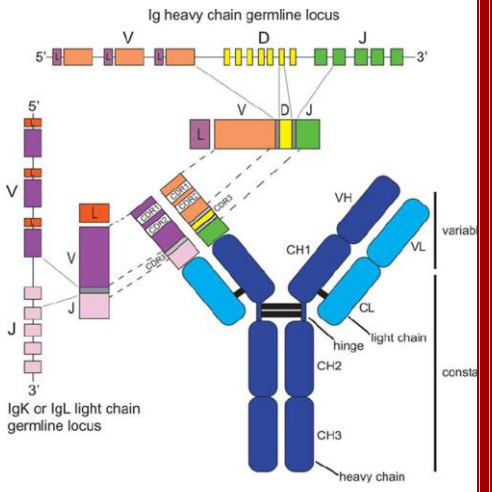
• When it becomes a precursor B cell, this precursor B cell will undergo another somatic recombination but this time of the light chain, this will give rise to immature B cells.



-Cells of the B lymphocyte lineage that successfully rearrange their Ig heavy chain genes express the Ig H chain protein and assemble a pre-antigen receptor known as the pre-BCR

- There are two somatic recombinations that are occurring:

1) in the heavy chain **2) in the light chain**



-Somatic B cell first recombination: in the germline DNA of progenitor B cell, some genes for the heavy chains. Those will make the heavy chains of the Ab

-VDJ recombination involves the VDJ segments.

-There are many V segments, many D segments, and many J segments.

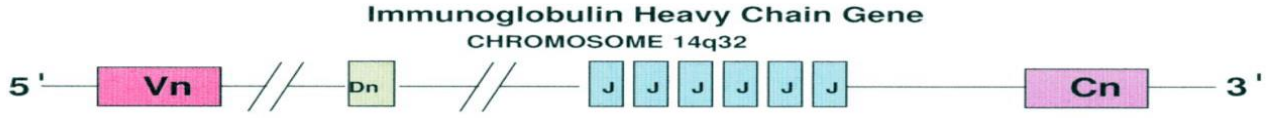
-We will only look at one to understand the process here

In the heavy chain gene, we have: a leader segment, V (variable) region, D (Diversity) Segment, J (Joining) segment, and we have the constant region.

-On the heavy chain gene constant region is known as Constant u (Cu) (Constant μ (Cμ))which will essentially make the Ab for IgM. Other constant regions needed for class switching have other symbols.

• In all cells the VDJ sequence is the same Except for the mature T and B cells were the process of somatic rearrangement of heavy and light chains occurred.

-J and C and in close proximity However the V region and D region are far away from each other:



-During the mitotic divisions of the hematopoietic stem cell, at first, the D segment needs to get closer to the J segments by cutting pieces between the D and J (even pieces from D and J can be cut) and joining them together, this is called DJ rearrangement

• With the same concept, VDJ rearrangement takes place to get the V segment closer.

• Eventually, you will have a daughter B lymphocyte with only 1 V, 1 D, AND 1 J.

• This opposes the definition of mitotic division as the daughter cell misses the DNA segment from the original cell. This is an exception that only happens in the bone marrow hematopoietic stem cells.

❖ **Further explanation:**

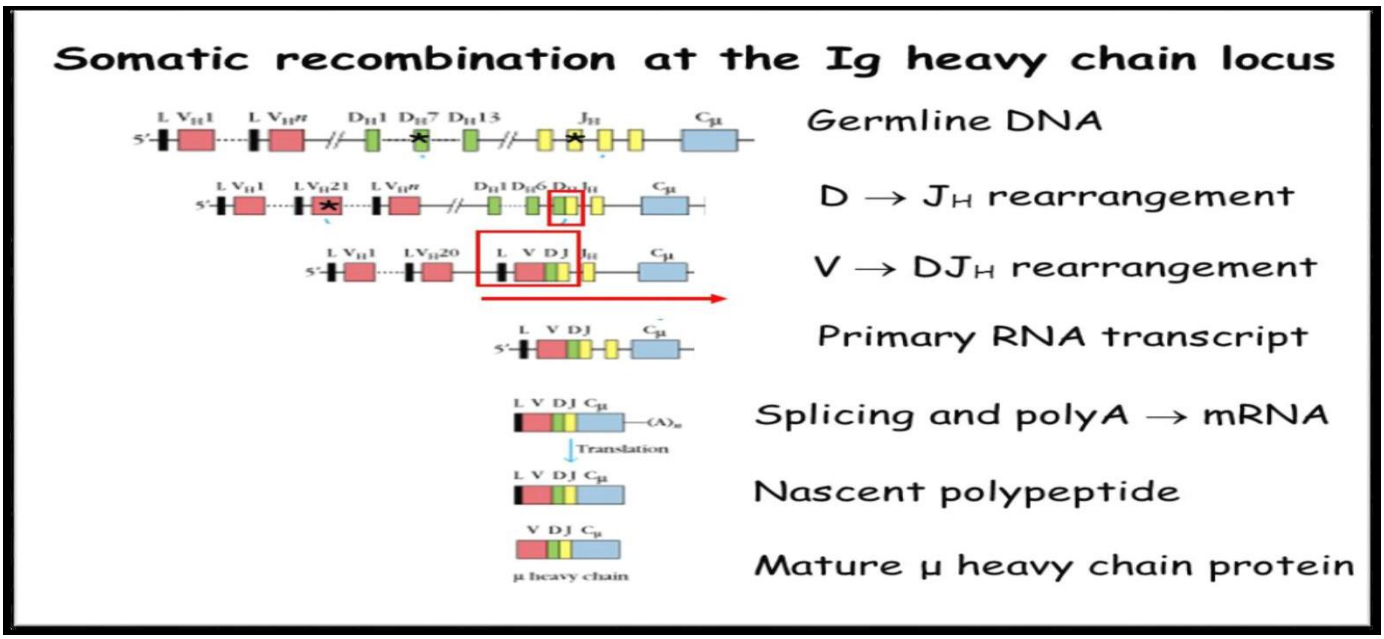
-The first process that occurs is J and D recombination This will cause J and D regions to bind.

-This will bring the C in close proximity. Next we have VDJ recombination with the D and J segment will bind to the variable region bringing the Cu (Constant mu) to everything else.

-Cu consists of many segments.

-For IgM there are four constant regions, because the heavy chain consists of four constant regions. -Following VDJ recombination, this will actually proceed to transcribe RNA so this whole sequence now of VDJC is an RNA.

-Splicing will remove the introns. mRNA will be translated to the heavy chain of the IgM Ab in the immature B cell.

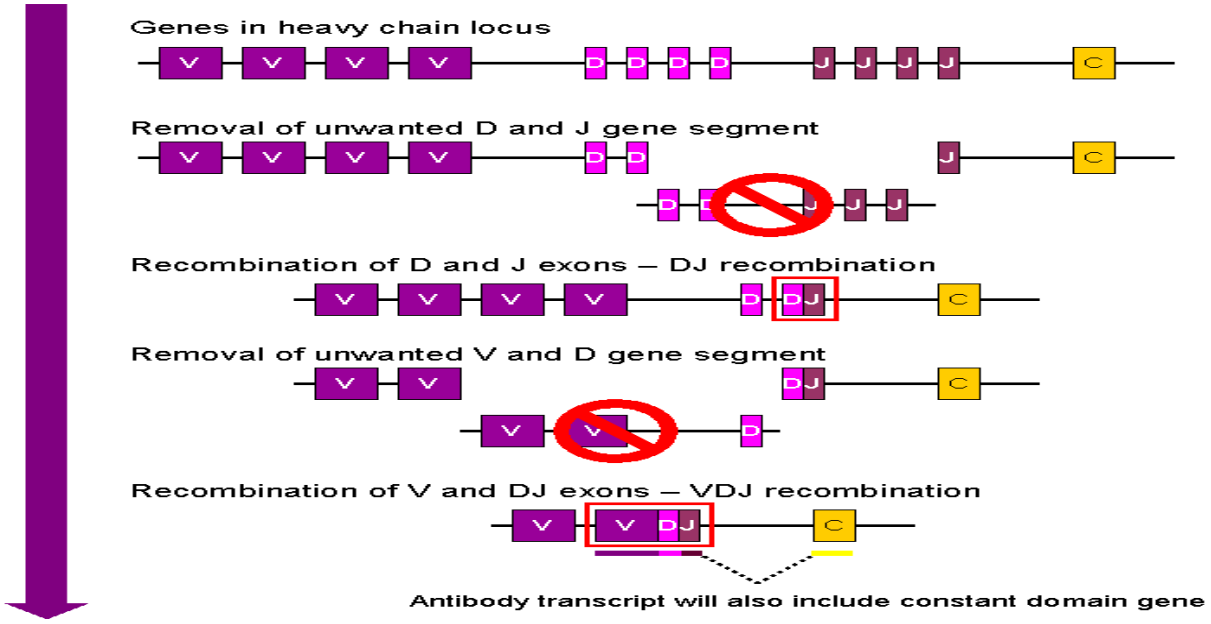


-There are many kinds of V, D, and J segments for the heavy chain gene - The heavy chain gene has:

***1-40 V segments *1-23 D segments, and *1-6 joining segments**

-The first thing, the heavy chain gene will undergo DJ recombination (D and J segments will join together) Next, VDJ recombination, which essentially previously bound D and J will bind to one of the variable segments, and then you transcribe.

-Eventually, you will have one V, one D, and one J segment and four Constant regions (Cu) Introns will be spliced out in the RNA and the mRNA will be translated to protein -> The heavy chain part of the Ig.



-The main idea is that you chose 1 segment out of the different 40 V segments, 1 segment out of the different 23 D segments, and 1 out of the 6 different J segments. This gives rise to millions of possibilities of different combinations giving rise to millions of different variable regions.

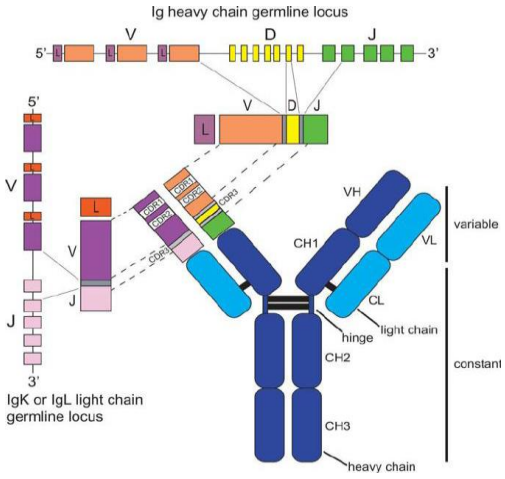
• After the heavy chain, the light chain gene segment undergoes somatic recombination.

• There are two types of light chains: **kappa** and **lambda**.

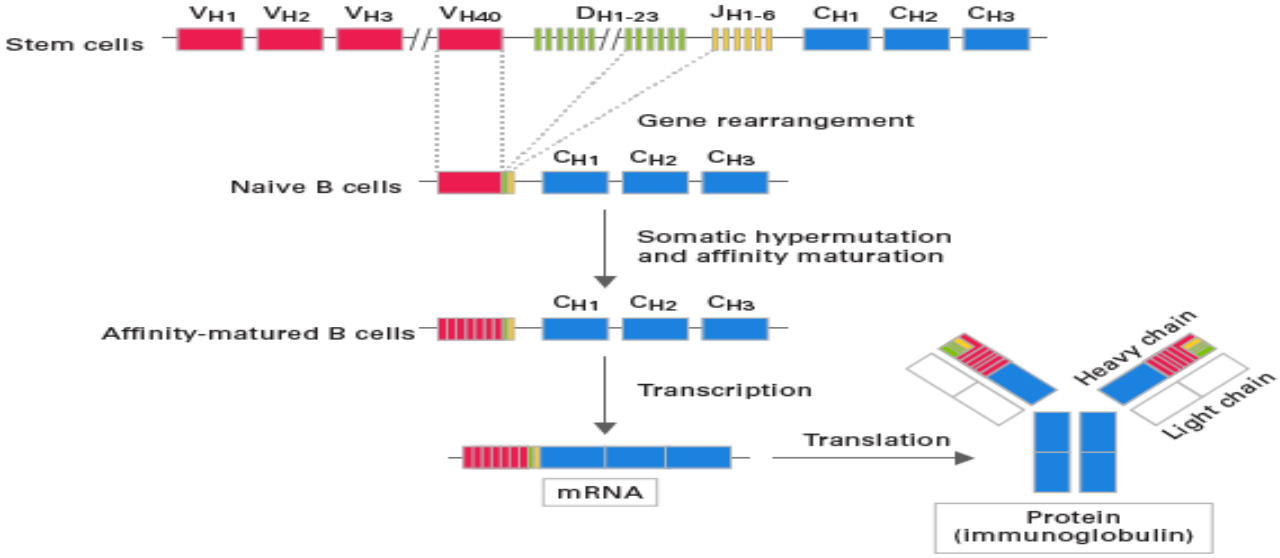
***After the heavy chain has occurred we get a precursor B cell.**

-In the precursor B cell, we have a gene for the light chain which will undergo somatically (VJ) recombination.

***Light chain gene does not contain a D region.**

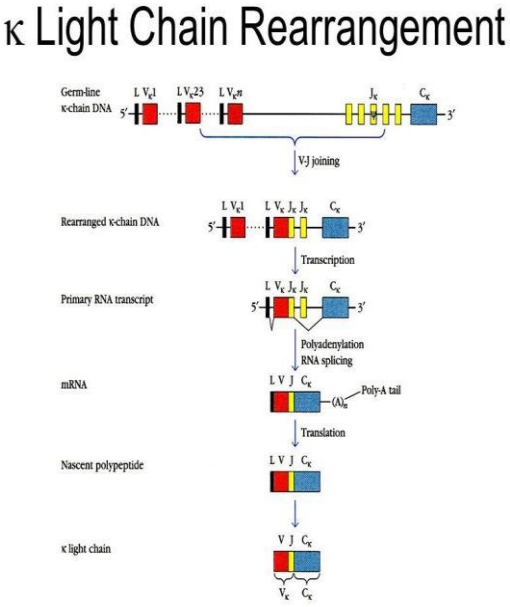


*the following figure shows the exact location of the V segment (red), D segment (green), and J segment (yellow) in the variable region of the immunoglobulin.



❖ **<u>Kappa (K) light chain rearrangement:</u>**

-Both K and L don't have a Diversity segment



-K light chain region consists of:

***1-38 V regions**

***1-5 J segments**

***1 Ck (kappa constant)**

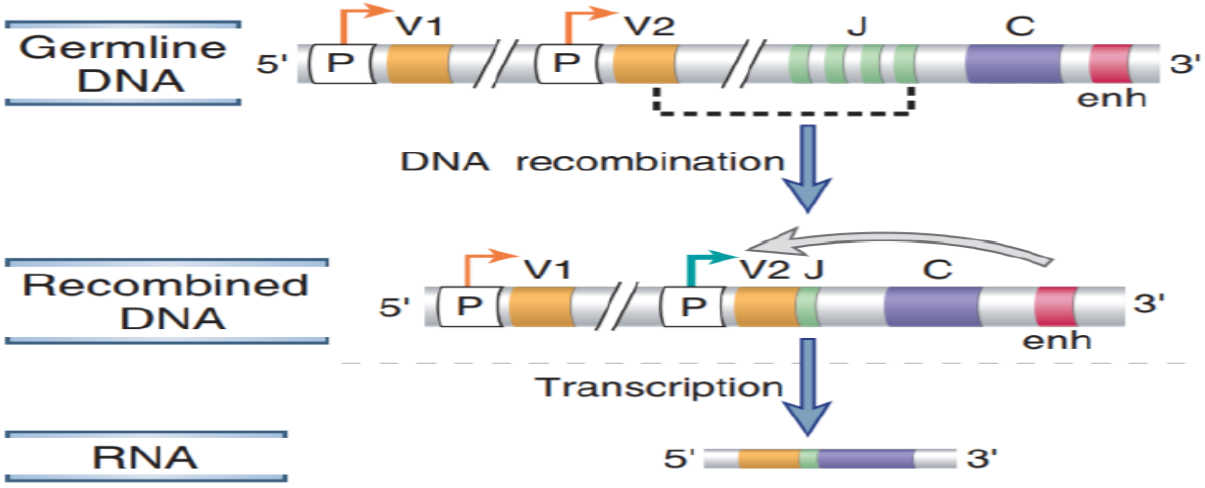
-J and C regions are in close proximity but the V is far away.

The first thing that happens is the J and V recombination with the J and V regions bound together to bring the constant region close proximity.

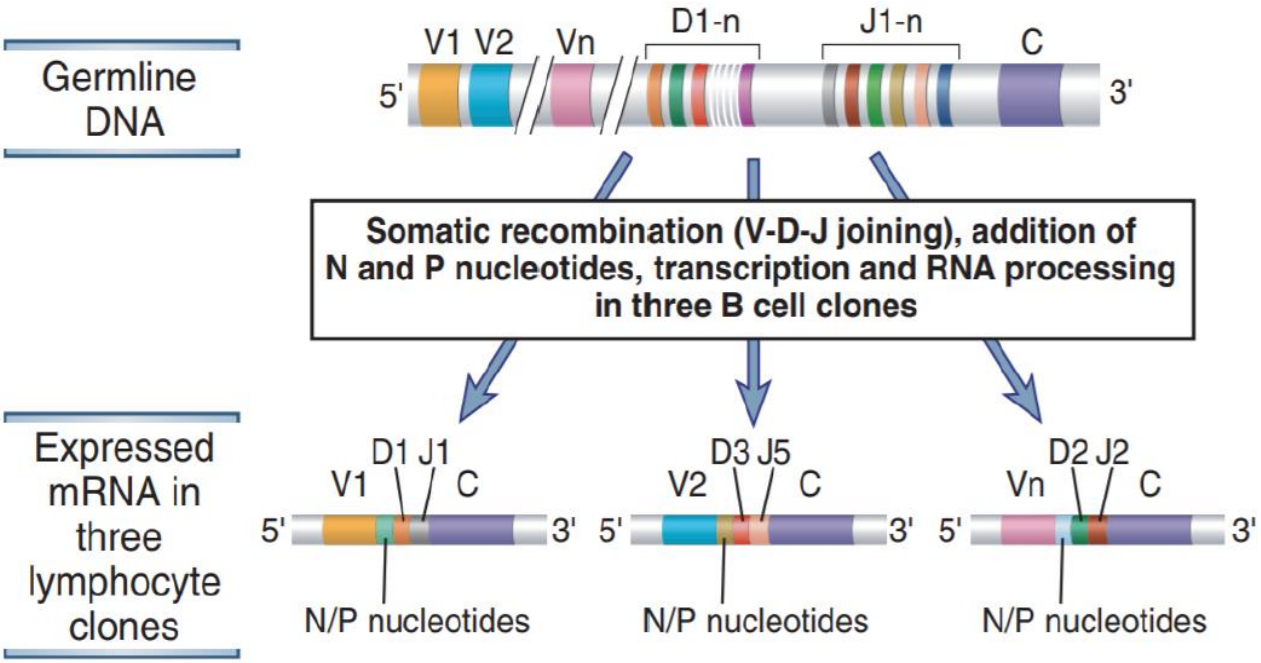
-This is transcribed to RNA which will be Spliced.

-The recombined light chain DNA only shows 1 j segment, 1 v segment and 1 c segment driven by a promoter for the expression of RNA.

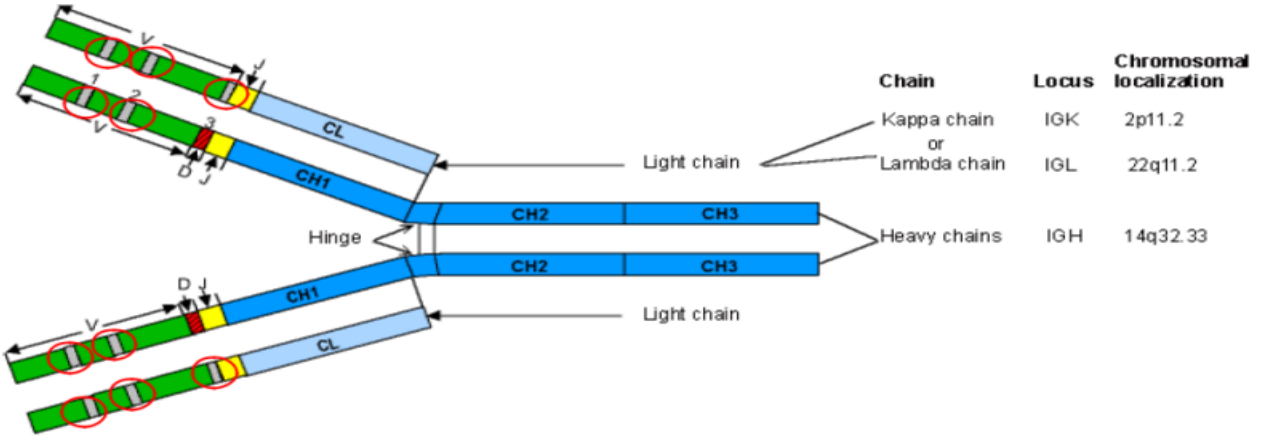
• Other promoters of other segments may not be active (the yellow arrow on the first promoter)



Look at the different probabilities which make different unique antibodies:



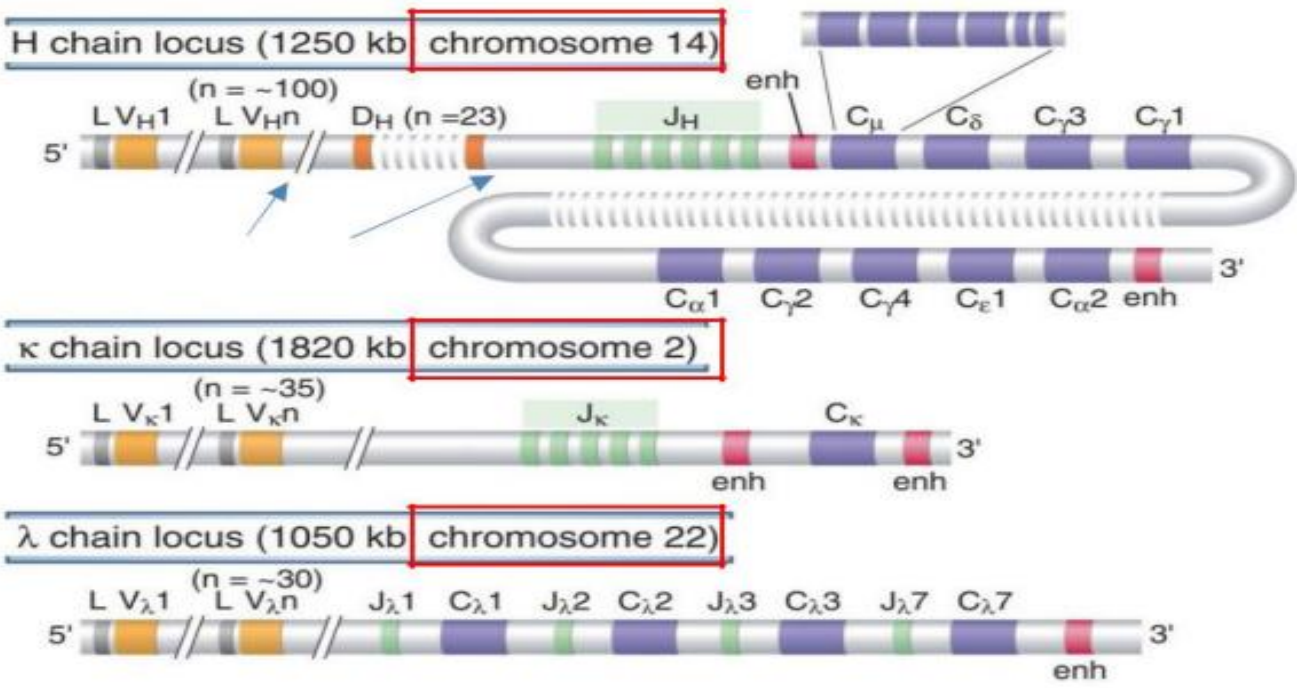
the red circles represent the hypervariable region found on the V segments:



Not only that, during somatic recombination new nucleotides can also be added to increase the diversity and specificity of the Abs.

-You need to remember that the **heavy chain** locus is on **chromosome 14** and **kappa chain** locus is on **chromosome 2** and **lambda chain** locus is on **chromosome 22** but don't memorize other numbers in the figure.

-if the needed immunoglobulin is IgM then the expressed region will be Cu region, if you want to switch to another constant region then you don't express Cu instead you express another one: Cgamma, Clambda…



Different constant regions for class switching. Cμ represents IgM the first one to appear, then C , C ϒ, for different classes.

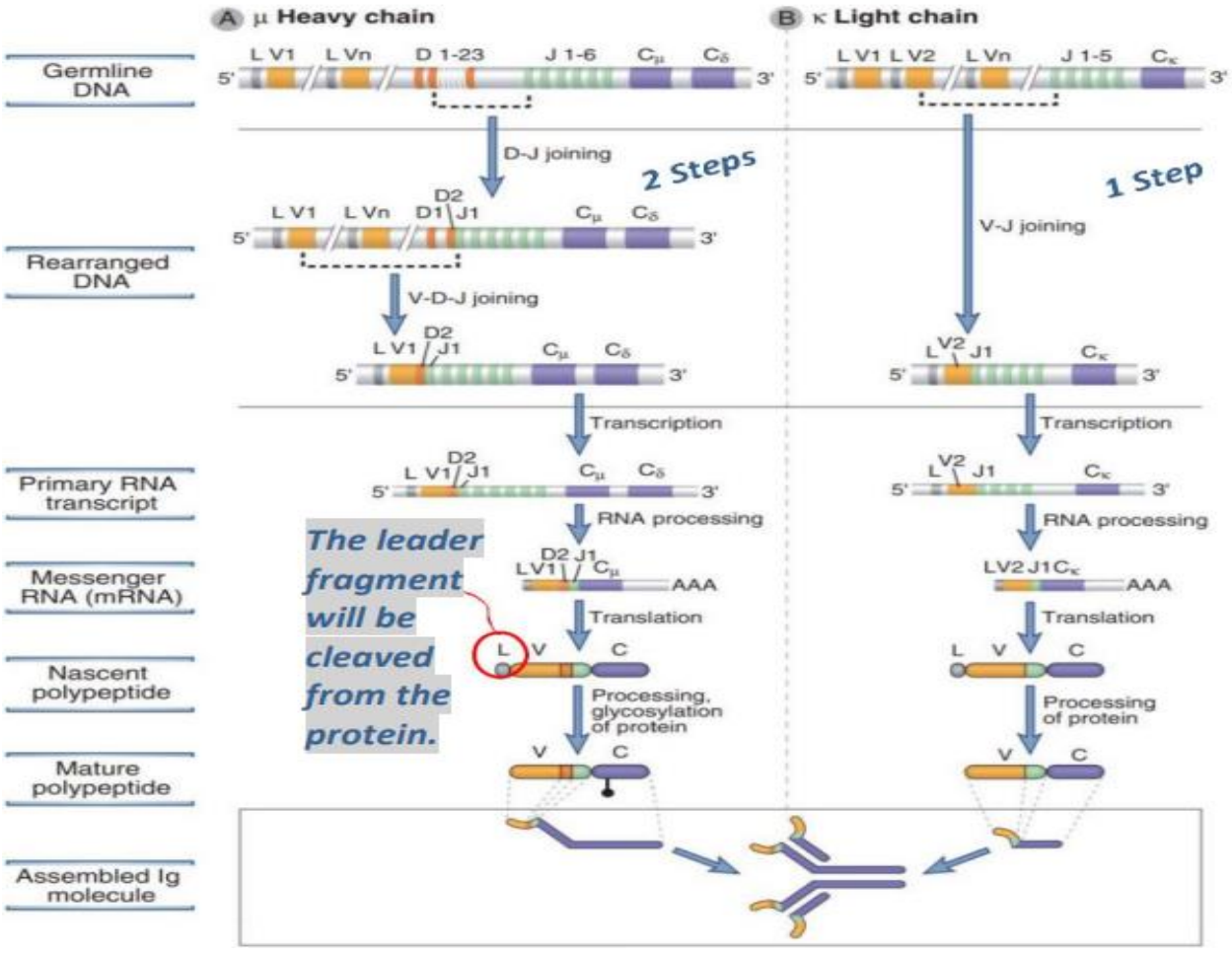
❖ **Comparison between heavy chain and light chain:**

-IN THE FIGURE BELOW Ig heavy and light chain gene recombination and expression.

The sequence of DNA recombination and gene expression events is shown for the Ig μ heavy chain (A) and the Ig κ light chain (B).

In the example shown in A, the V region of the μ heavy chain is encoded by the exons V1, D2, and J1.

In the example shown in B, the V region of the κ chain is encoded by the exons V2 and J1.



-Note that Germline DNA is the top line presented in all our cells, During mitosis in the bone marrow -> somatic recombination occurs

-RNA is transcribed and processed to a messenger RNA which will be translated into protein That will undergo primary, secondary and tertiary post-translational modifications like glycosylation.

COMPLEMENTARITY-DETERMINING REGIONS (CDRS)/ HYPERVARIABLE REGIONS

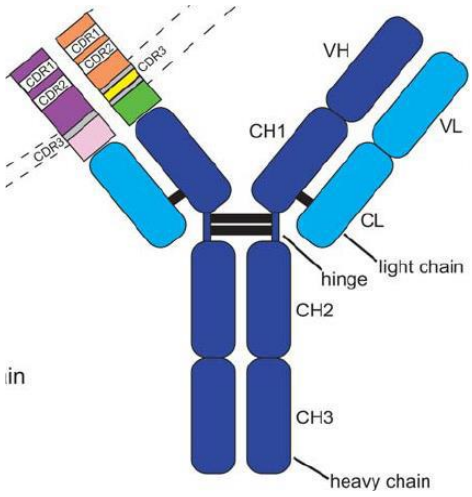
-new nucleotides will be added during the VJ recombination process, to increase the diversity and specificity process.

- if you compare different immunoglobulins, they will only differ in the variable region not the constant.

• New nucleotides will be added during the VJ recombination process, to increase the diversity and specificity process.

• The regions (CDR1, CDR2 AND CDR3) on the light and heavy chain represent the hypervariable region.

• **The following graph shows 3 spikes representing hypervariability in amino acids' position between different immunoglobulins**.



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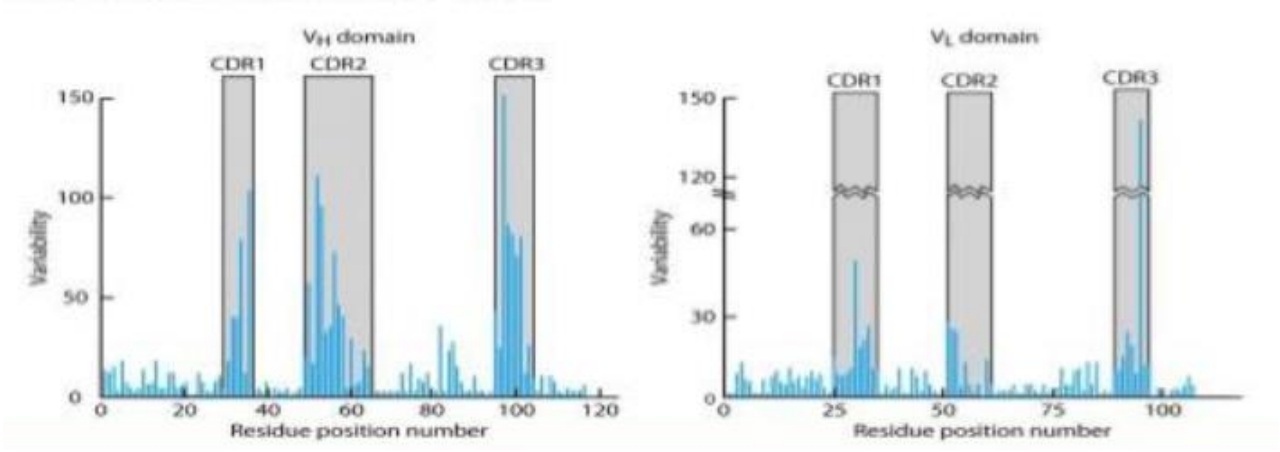
--Hypervariable region (HVR) , complementarity-determining region(CDR):

Within the variable regions of both heavy and light chains, some polypeptide segments show exceptional variability and are termed Hypervariable regions or complementarity-determining regions(CDs).

There are 3 complementarity-determining regions(CDs) on both L and H chains.



-To understand hypervariability, we need to go back to the germline DNA of the variable region (on the hematopoietic stem cells before recombination):

• We said that there are sequences present between the V(D)J sequences, like the recombination signal sequence (RSS).

• V(D)J DNA Recombination Uses RSS and Occurs by Deletion or Inversion.

• RSS is composed of a heptamer (7 nucleotides), a spacer (12 or 23 base pairs), and a nonameric (9 nucleotides).

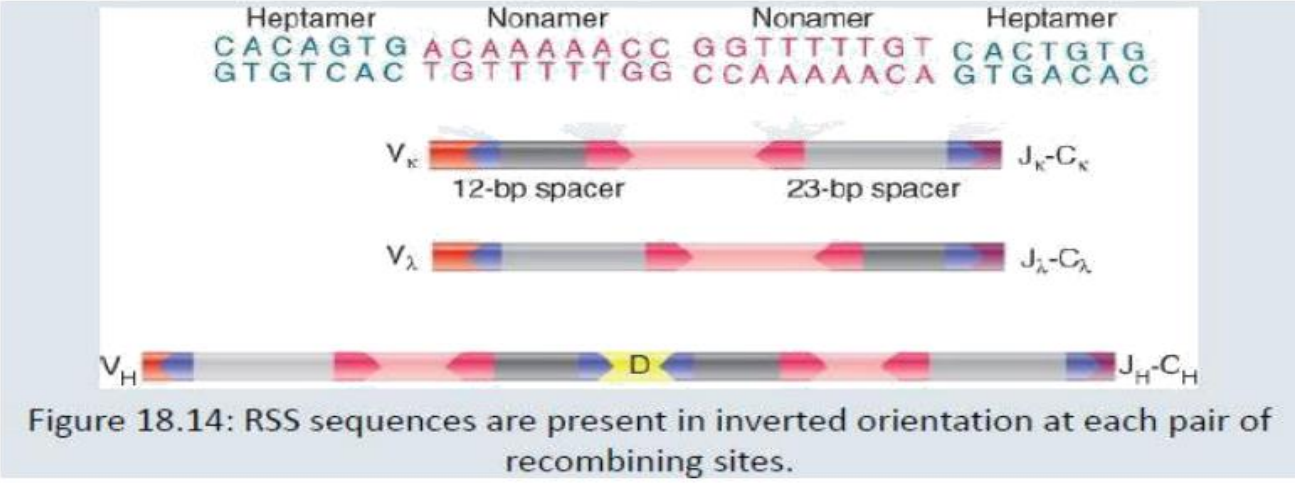
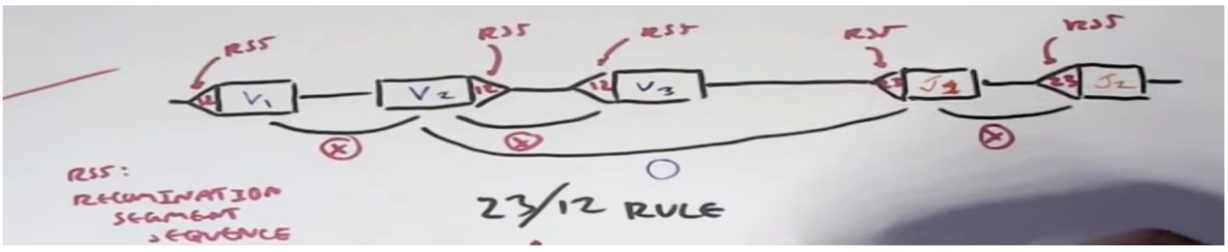


Figure 18.14: RSS sequences are present in inverted orientation at each pair of recombining sites.

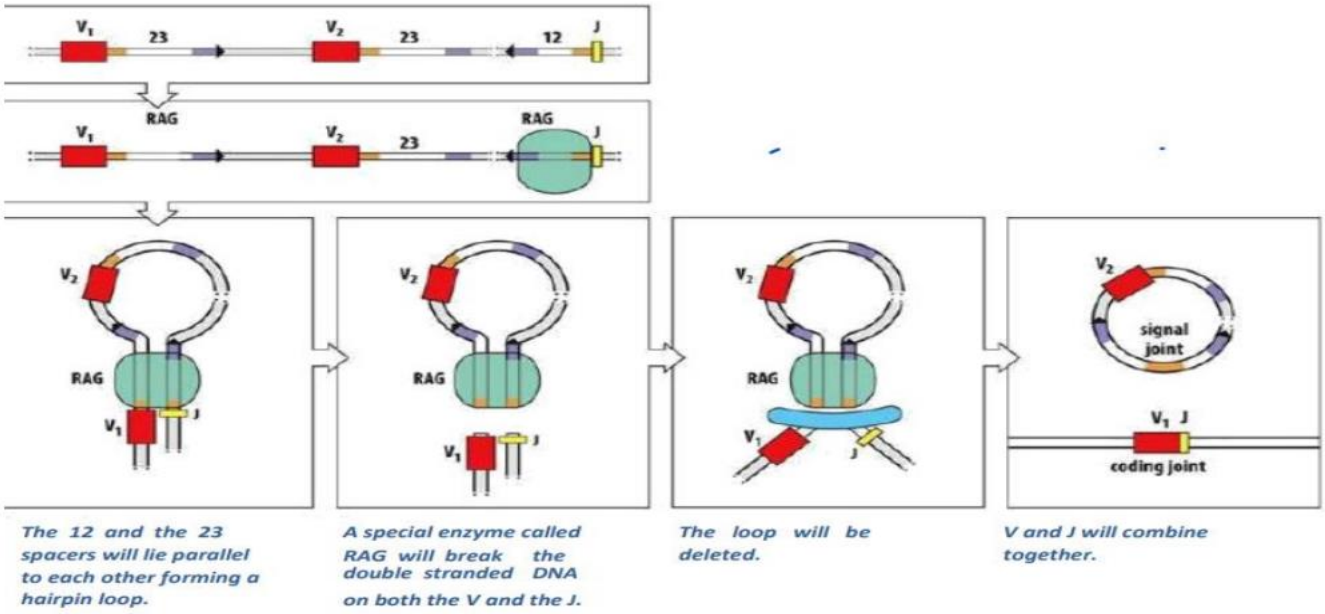
<u>***The RSS motifs have a special rule called 23/12 rule where essentially a 23 and 12 can bind together. This means 23 can't recombine with 23 and 12 can't recombine with 12.</u>

-For example, V1 and V2 cannot bind together because they both have 12 bp sequences, the same as V2 and V3, J1 and J2 (because they have 23 bp spacers). BUT V2 and J1 can



-If you take part in the K light chain gene we have 2 Variable segments (V1, V2) and 1 Joining (J) segment Those segments can undergo recombination. They have a specific RSS motif, it can be either a 23 or 12-bp spacer.

-There are 2 ways to initiate recombination. First, deletion (hairpin loop configuration),,, second: Inversion (Tangled configuration)



-There are two ways to initiate the recombination:

First: Deletion (hairpin loop configuration)

1- the gene creates a hair pen loop, here you can see V and J parallel to each other. The 12 and 23 bp sequences with the heptamer and nanomer sequences on the side, can undergo recombination.

Through recombination, proteins will cut off these bp sequences and we are left with V and J bound together

Through VJ recombination we get DNA with the joining V and J which will then get transcribed into RNA, which will then go through splicing to remove introns producing mRNA and essentially the protein -> which is the Kappa light Chain .

It is important to know that actually during the recombinational process, to increase the specificity and diversity of the light chain new nucleotides are added.

Some pictures related to deletion :

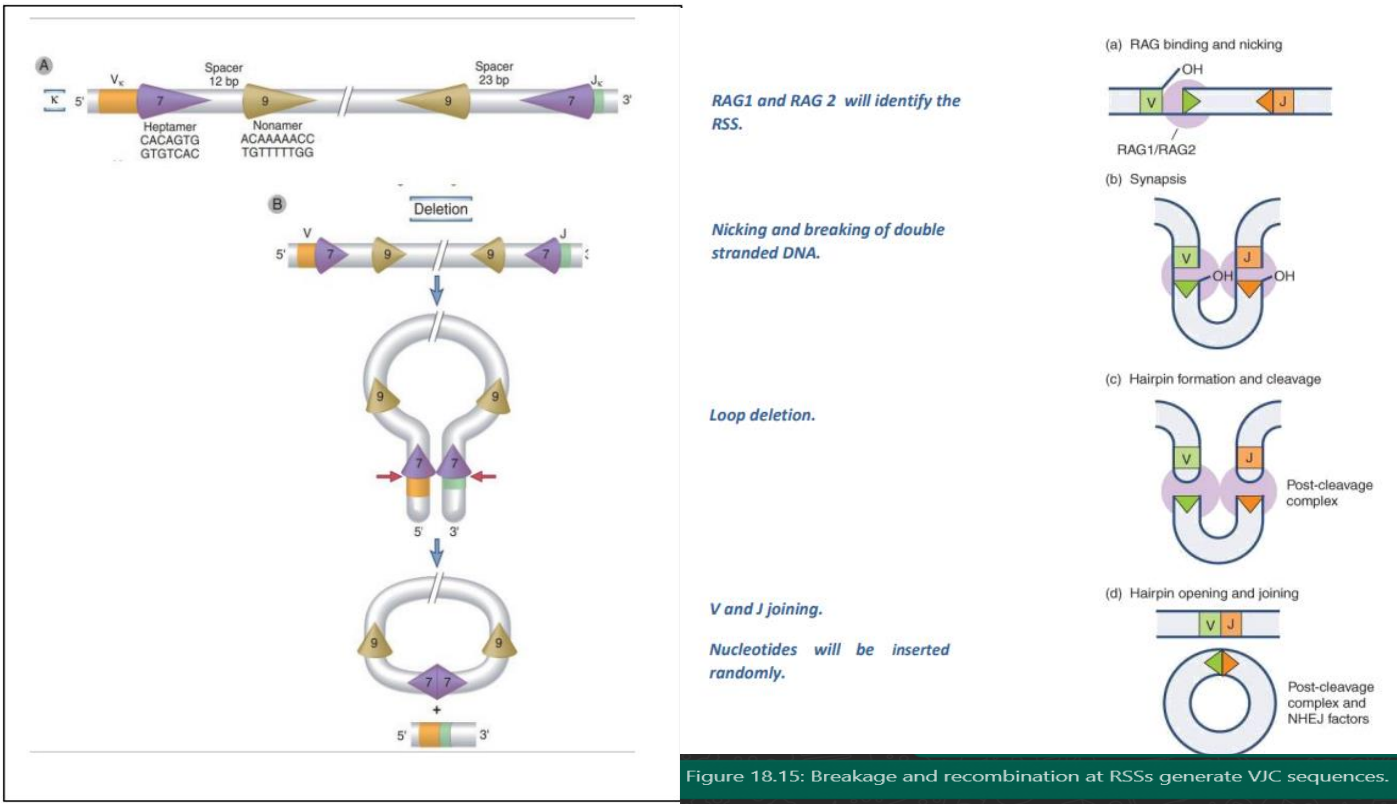
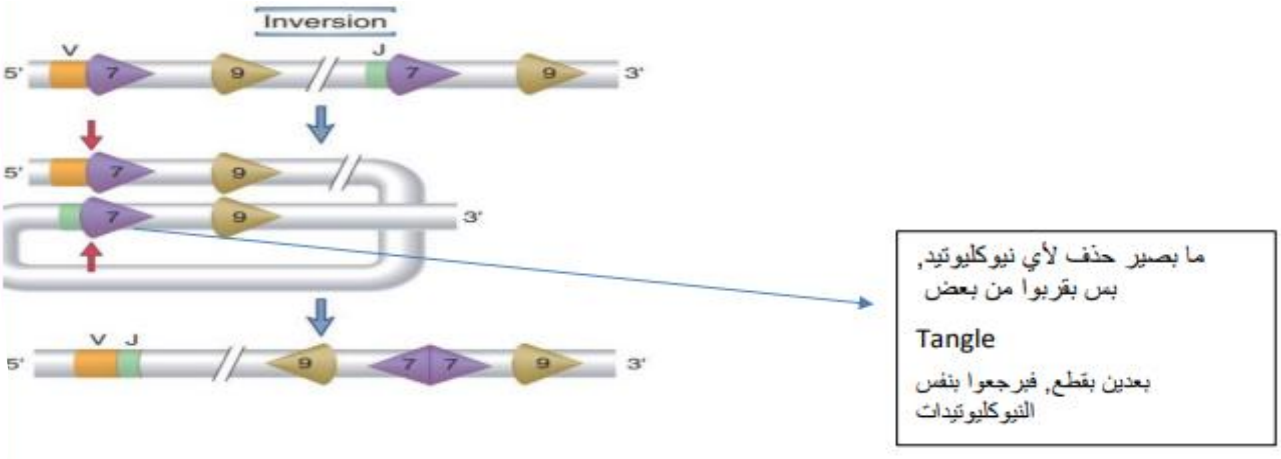


Figure 18.15: Breakage and recombination at RSSs generate VJC sequences.

Second: Inversion (Tangled configuration):

-In this other type of 23/12 rule recombination we have a tangled configuration. Through recombination, V and J can bind together.

-New nucleotides randomly can be added in during recombination which will increase the diversity and specificity



-Proteins involved in random nucleotides addition:

In both the Heavy chain and the Light chain genes the process is the same

-Lets look Kappa light chain region again: we have V and J Essentially what happens in recombination is that RAG1 and RAG2 proteins will bind the motifs of the RSS.

-This will cause RAG1 and 2 to bind together because they have affinity to each other.

-When they bind together they will form a hairpin loop with V and J parallel to each other

-Next, RAG1 and 2 will cleave off this RSS motif

-Following this, other proteins such as Ku70 and Ku80 will bind to the Variable and Joining segments

-Ku proteins initiate repair by forming a hairpin loop where RAG1/2 has broken the RSS

-After forming the hairpin loop other proteins will come into the system.

-A DNA protein kinase (Artemis) will open the hairpin loop which was formed by the Ku proteins.

-Following this, another protein will come in called terminal deoxynucleotidyl transferase (TDT) which will add in nucleotides into the separated variable and joining segments

-The TDT adds nucleotides randomly Because they are separated, DNA ligase and XRCC4 will ligate the ends together. Which will essentially form a repaired and unique V and J recombinant segment

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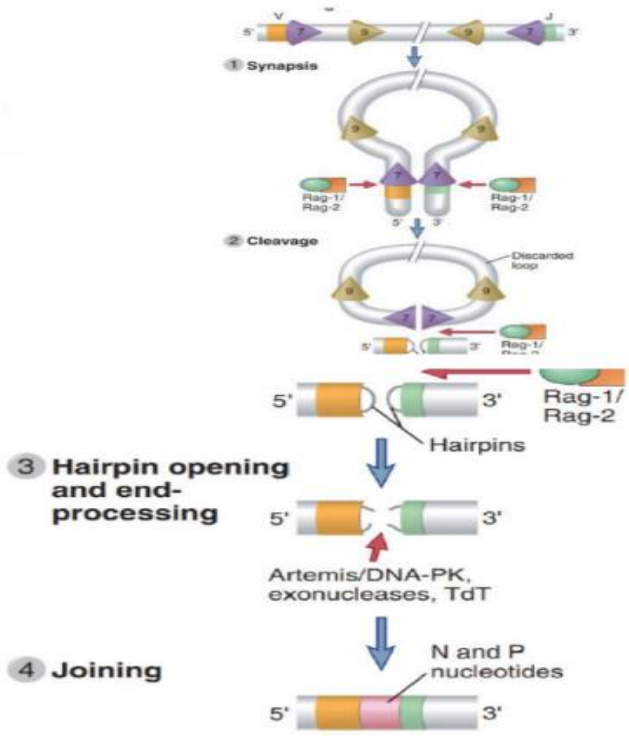
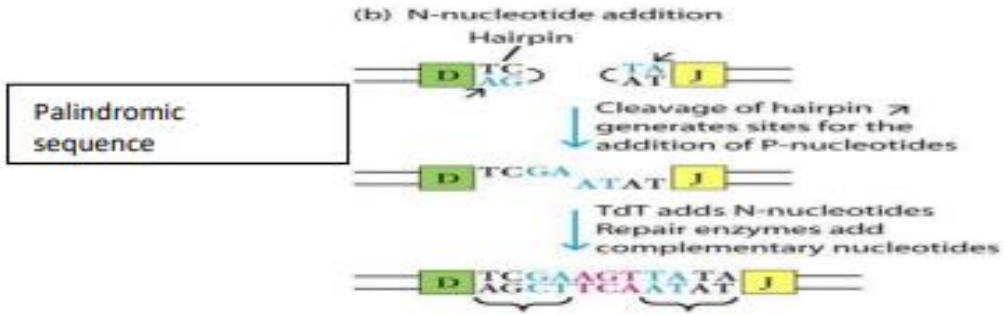
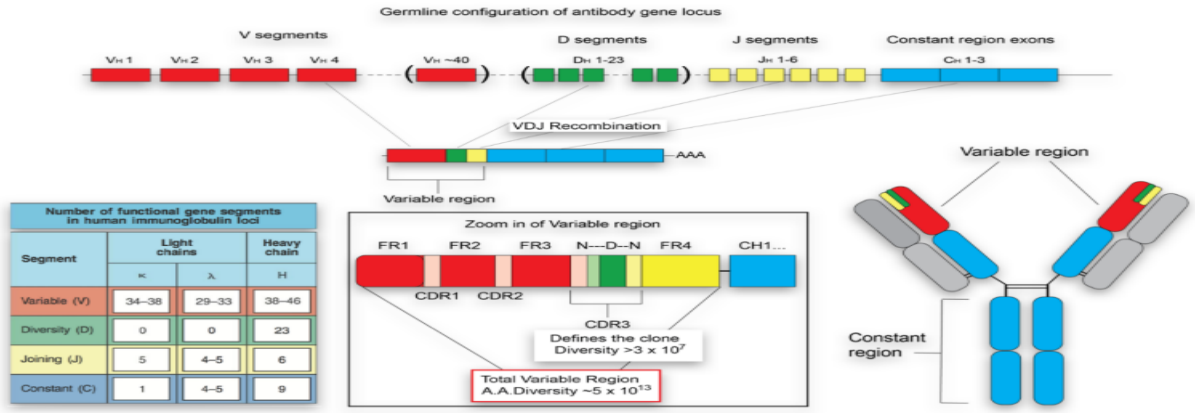


N nucleotide addition at joining segments: the addition of random bases



-Calculating the probabilities of recombination between VDJ segments and the addition of random nucleotides will give a huge number of different immunoglobulins:



Number of functional gene segments in human immunoglobulin loci

Segment	Light chains κ	Light chains λ	Heavy chain H
Variable (V)	34–38	29–33	38–46
Diversity (D)	0	0	23
Joining (J)	5	4–5	6
Constant (C)	1	4–5	9

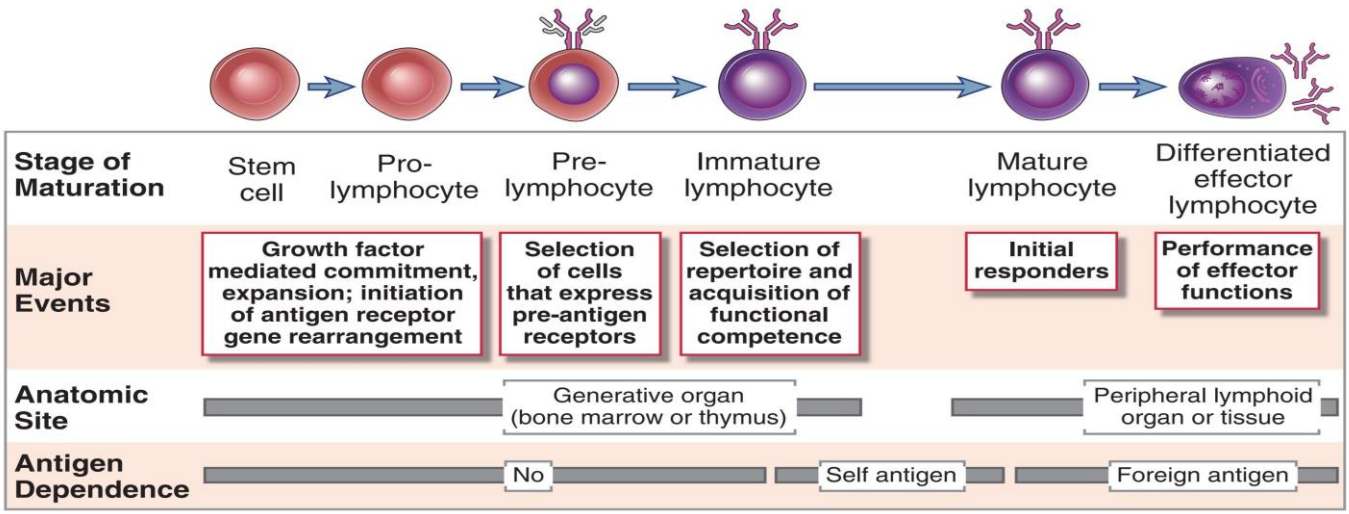


FIGURE 8–1 Stages of lymphocyte maturation. Development of both B and T lymphocytes involves the sequence of maturational stages shown. B cell maturation is illustrated, but the basic stages of T cell maturation are similar.

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