Sheet 14

Immunology

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Corrected by | ...

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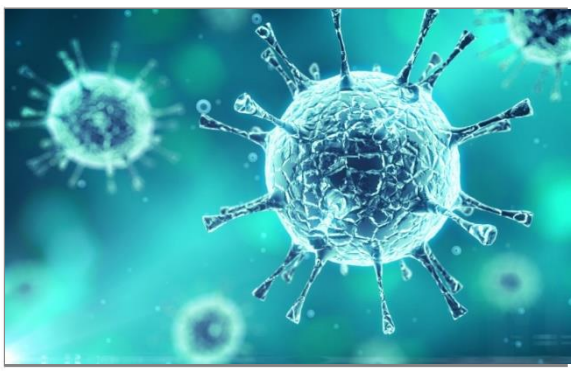


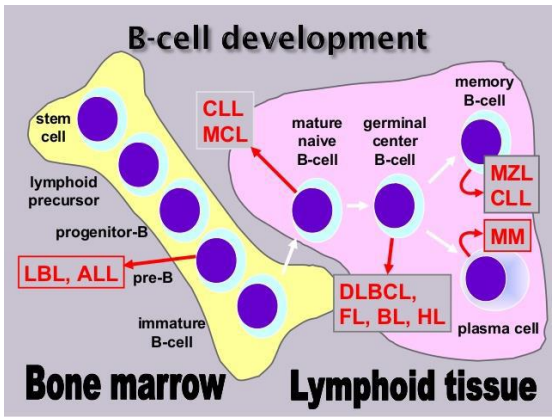
Antigen Receptor Gene Rearrangement

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 very 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 human body cells ***(except eggs and sperms)*** are genetically identical. This means if you sequence human cells from liver, skin, hematopoietic stem cells or any other cell, the DNA will be identical. However, not all of 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.

- The bone marrow is a heterogenous 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 for B or T cells.

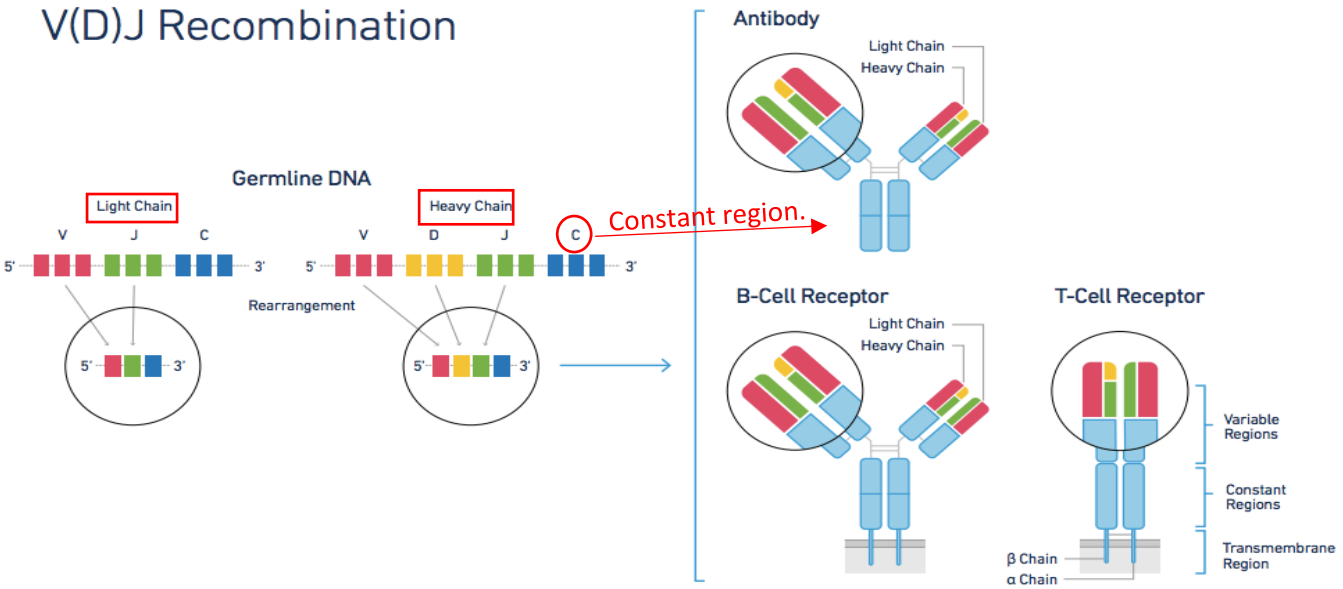


- B cell development begins in Progenitor Lymphoid Cell through somatic recombination will make an immature B cell with a unique Ab that binds to a specific antigen.

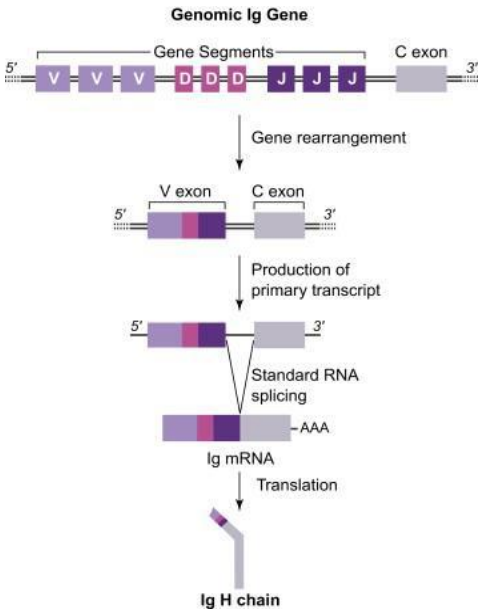
Rearrangement of antigen receptor genes lymphocytes:

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, diversity (D) and joining (J) gene segments.***

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

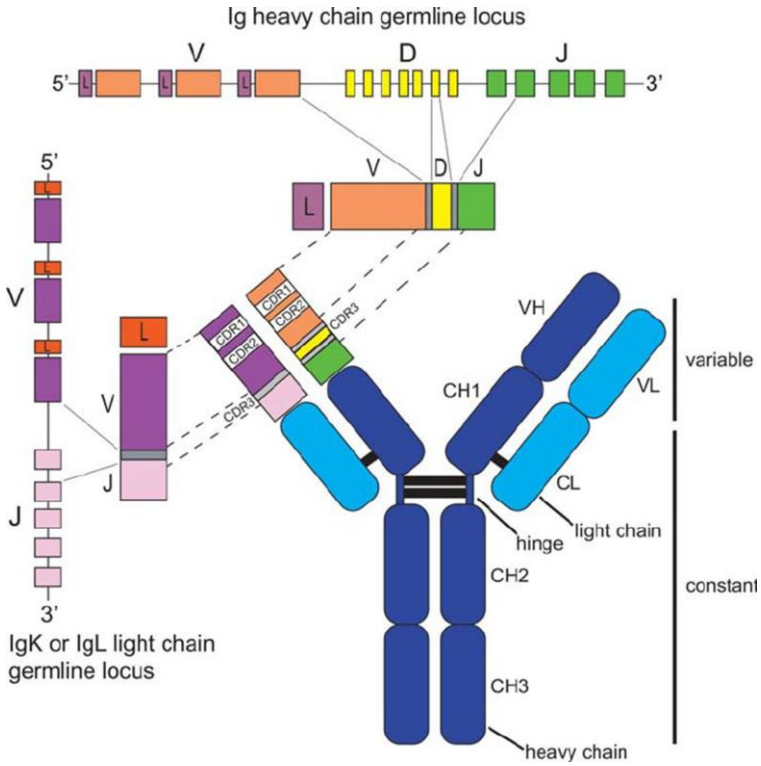


- There are millions of antibodies and T cell receptors, if each one of them has it is own gene, this mean we have millions of genes, but this is not the case as we only have 22000 genes. Through alternative splicing (skipping exons) on the RNA level, you can make 100000 proteins out of these 22000 genes. 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.
- The lymphocyte precursor DNA will be changed randomly.
- In developing B cells, the first antigen receptor gene to be completely rearranged is ***the Ig heavy chain or Ig H gene***.
- Only on 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.***



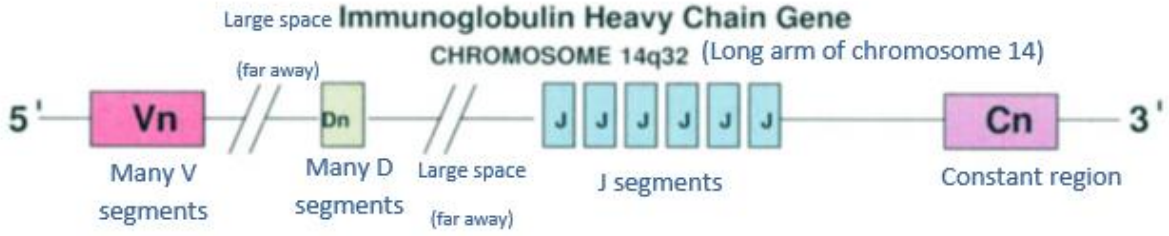
- When it becomes a precursor B cell, this precursor B cell will undergo another somatic recombination but this time of the light chain, this will give rise to ***immature B cell.***

- 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 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 are the same except the mature T and B cells where the process of somatic rearrangement of heavy and light chains occurred.
- VDJ recombination involves the VDJ segments. There are many V segments, many D segments, and many J segments.

- 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 the mitotic division as the daughter cell misses DNA segment from the original cell. This is an exception which only happens in the bone marrow hematopoietic stem cells.

- ***Further explanation:***

The first process that occurs is **J and D** recombination

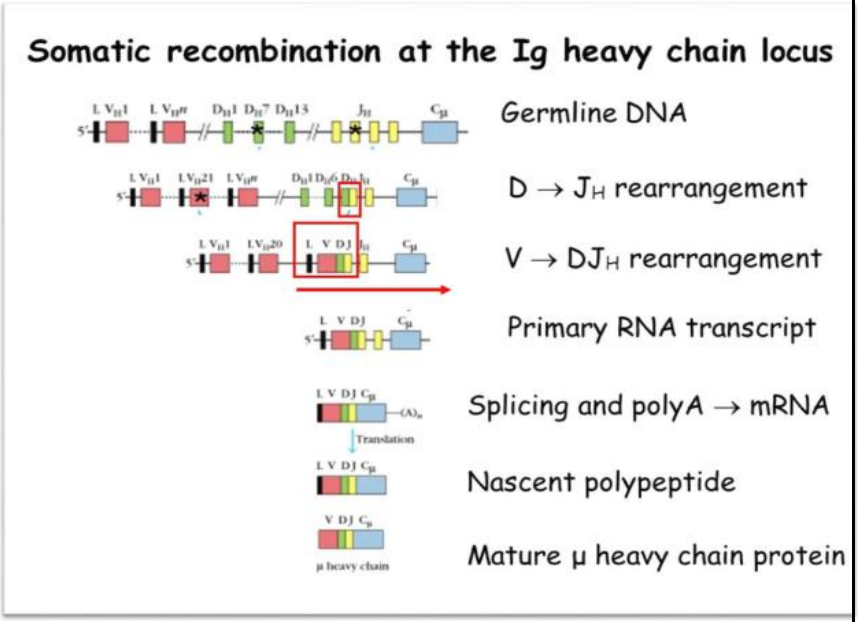
This will cause **J and D** regions to bind.
This will bring the C in close proximity.

Next we have VDJ recombination with the **D and J segment will bind** to the variable region brining 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

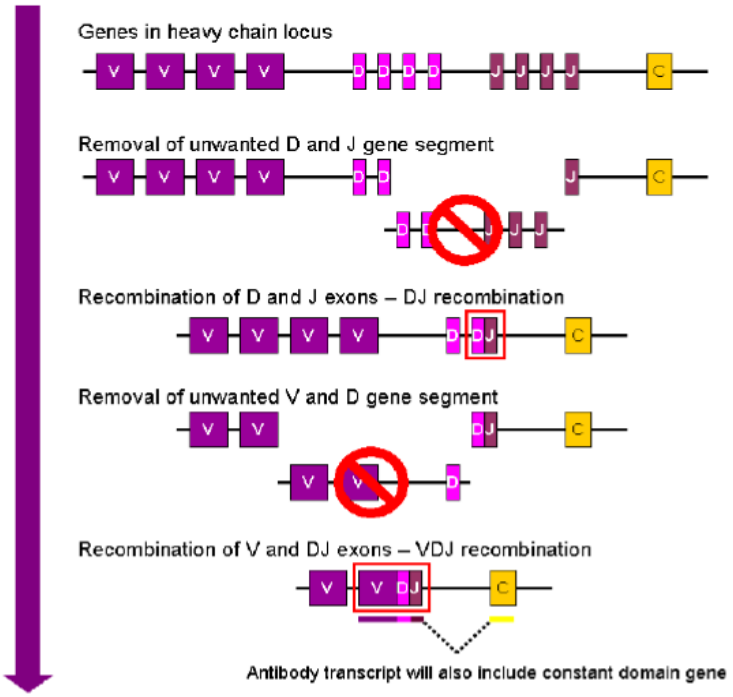
The heavy chain gene has:
1-40 V segments
1-23 D segments, and
1-6 joining segments

The first thing, the heavy chain gene will undergo DJ recombination (D and J segments will join together)

Next, VDJ recombination, where essentially previously bound D and J will bind to one of the variable segments, and then you transcribe.

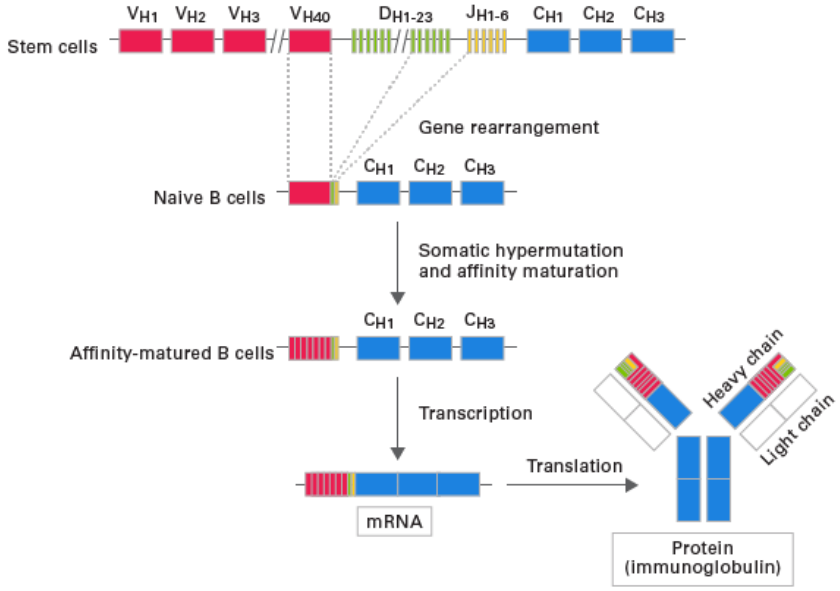
Eventually, you will have one V, one D and one J segment and four Constant regions (Cu)

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 heavy chain, light chain gene segment undergoes somatic recombination.
- There are two types of light chains: ***kappa and lambda***.

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



Kappa (K) light chain rearrangement:

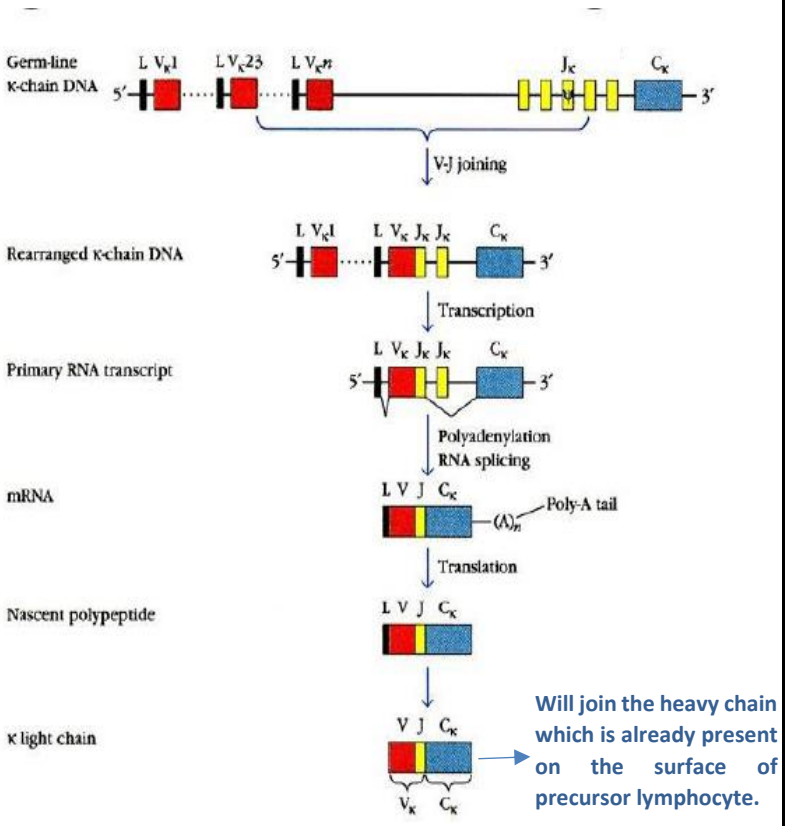
Both K and L don't have a Diversity segment

K light chain region consisting of
1-38 V regions
1-5 J segments
1 Ck (kabba constant)

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

First thing that happens is the J and V recombination
with the J and V regions bind together to bring the constant region to close proximity.

This is transcribed to RNA which will be spliced



Will join the heavy chain which is already present on the surface of precursor lymphocyte.

- 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).

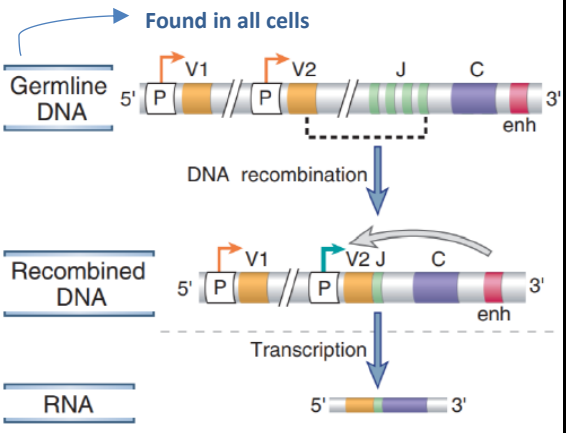


FIGURE 8–11 Transcriptional regulation of Ig genes. V-D-J recombination brings promoter sequences (shown as P) close to the enhancer (enh). The enhancer promotes transcription of the rearranged V gene (V2, whose active promoter is indicated by a bold green arrow). Many receptor genes have an enhancer in the J-C intron and another 3′ of the C region. Only the 3′ enhancer is depicted here.

Look at the different probabilities which make different unique antibodies:

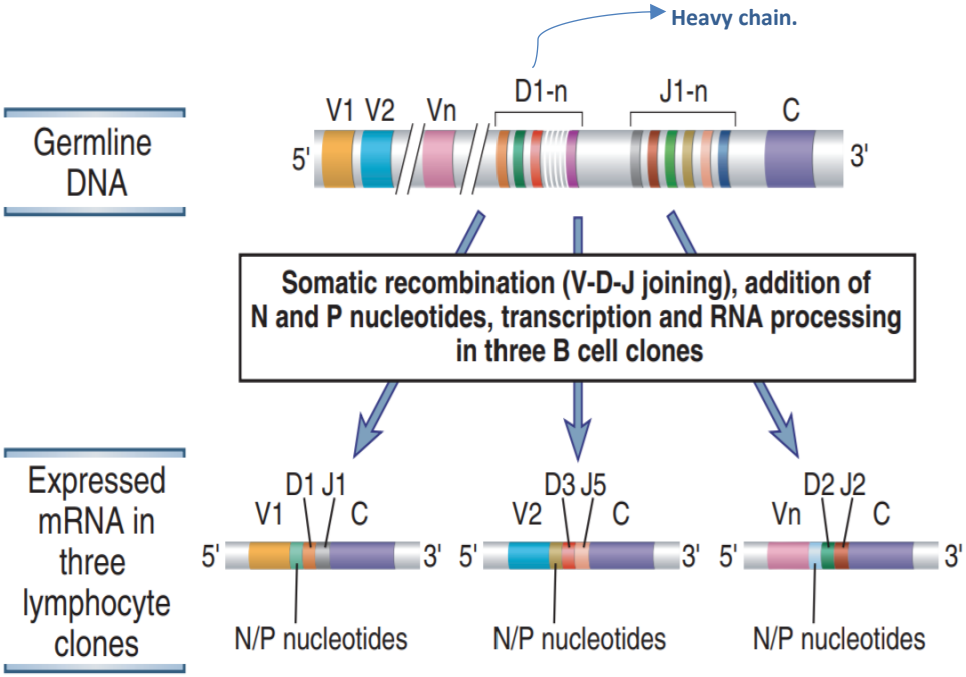
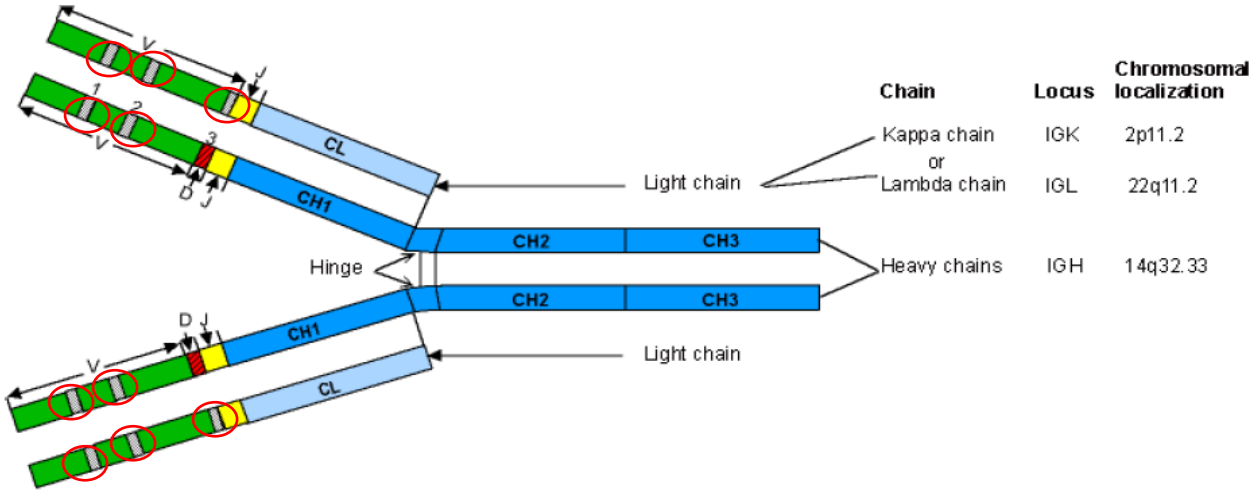


FIGURE 8–9 Diversity of antigen receptor genes. From the same germline DNA, it is possible to generate recombined DNA sequences and mRNAs that differ in their V-D-J junctions. In the example shown, three distinct antigen receptor mRNAs are produced from the same germline DNA by the use of different gene segments and the addition of nucleotides to the junctions.

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



Chain	Locus	Chromosomal localization
Kappa chain or Lambda chain	IGK / IGL	2p11.2 / 22q11.2
Heavy chains	IGH	14q32.33

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

- **VH**: V-DOMAIN of the immunoglobulin heavy chain
- **VL**: V-DOMAIN of the immunoglobulin light chain
- **CH1, CH2, CH3**: C-DOMAIN of the immunoglobulin heavy chain
- **CL**: C-DOMAIN of the immunoglobulin light chain

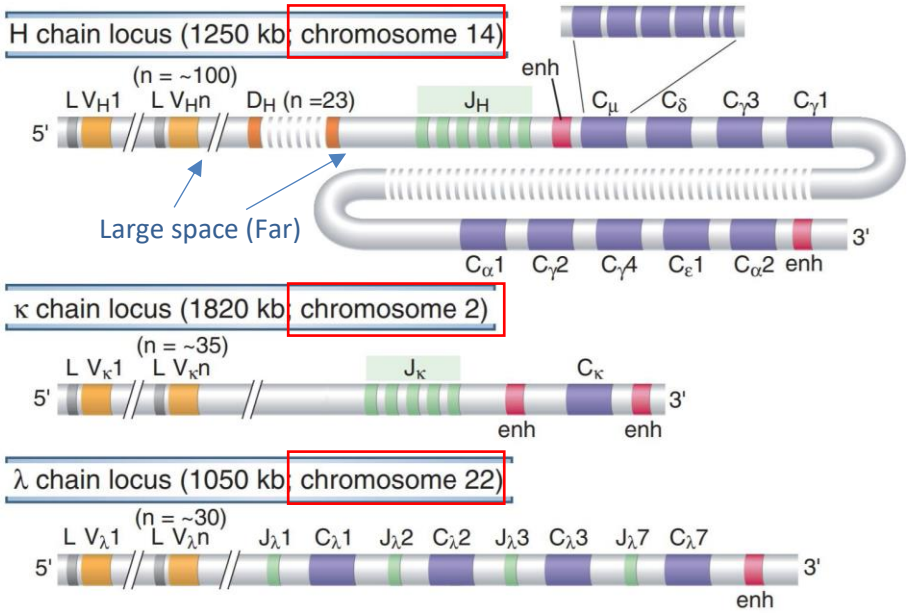
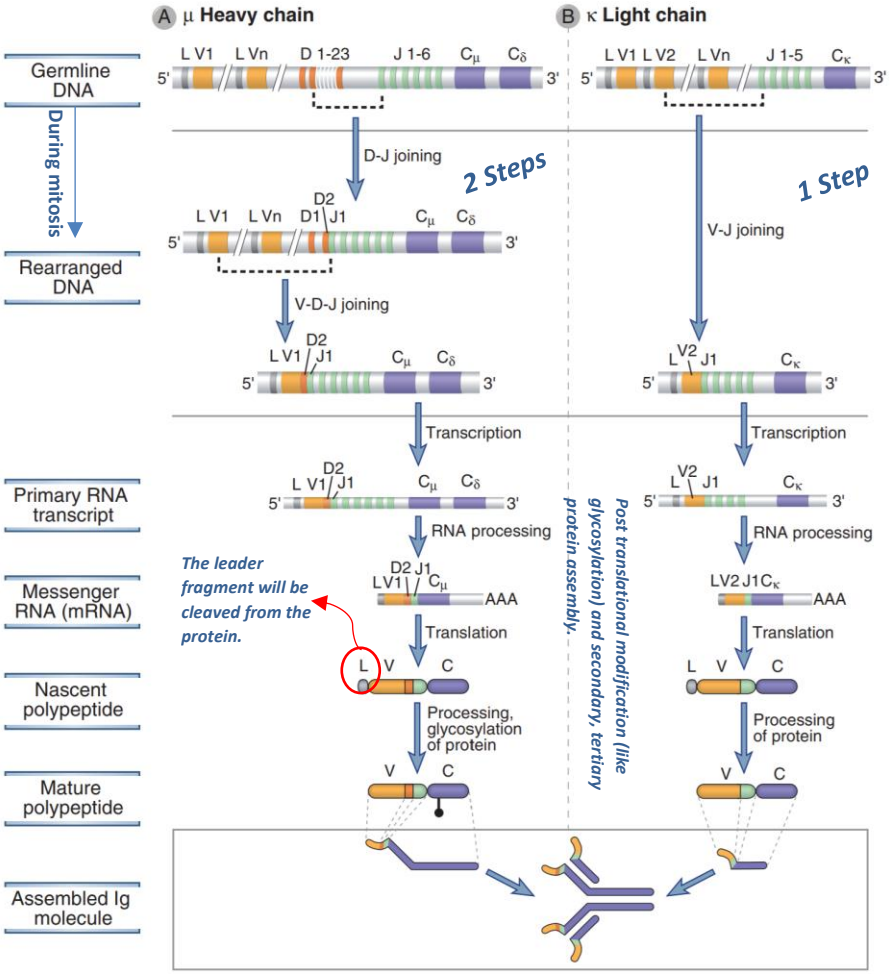


FIGURE 8–5 Germline organization of human Ig loci. The human heavy chain, κ light chain, and λ light chain loci are shown. Only functional genes are shown; pseudogenes have been omitted for simplicity. Exons and introns are not drawn to scale. Each C_H gene is shown as a single box but is composed of several exons, as illustrated for C_μ. Gene segments are indicated as follows: L, leader (often called signal sequence); V, variable; D, diversity; J, joining; C, constant; enh, enhancer.

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

Comparison between heavy chain and light chain:



- 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

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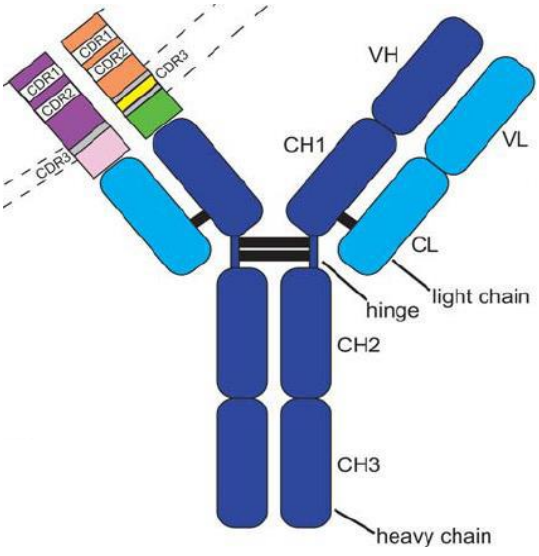
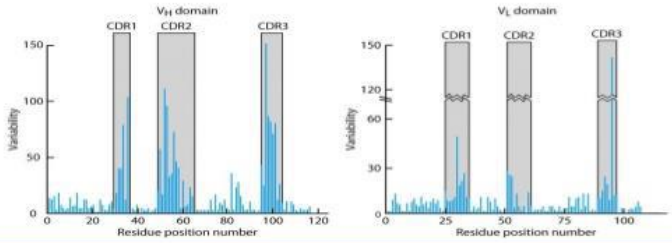
Complementarity-determining regions (CDRs) (Hypervariable Regions):

- 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.



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



To understand hypervariability, we need to go back to the germline DNA of the variable region:

- 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 nonamer (9 nucleotides).

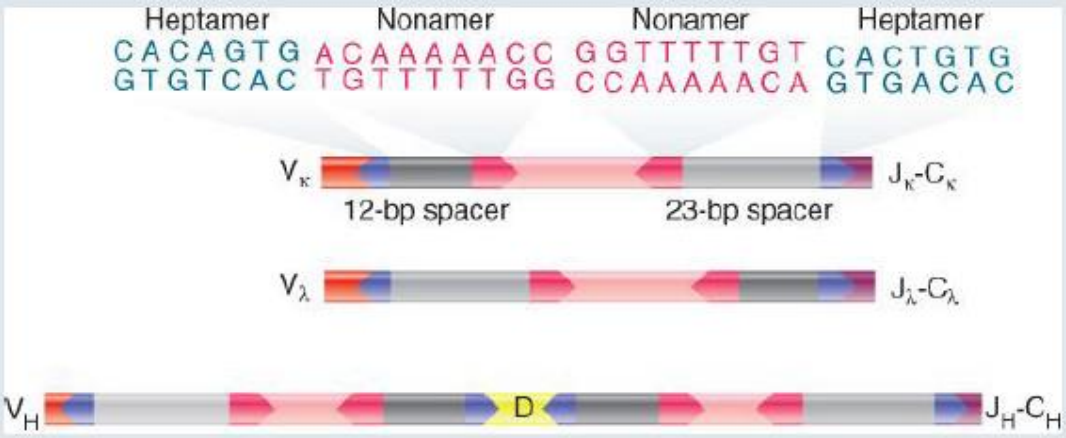
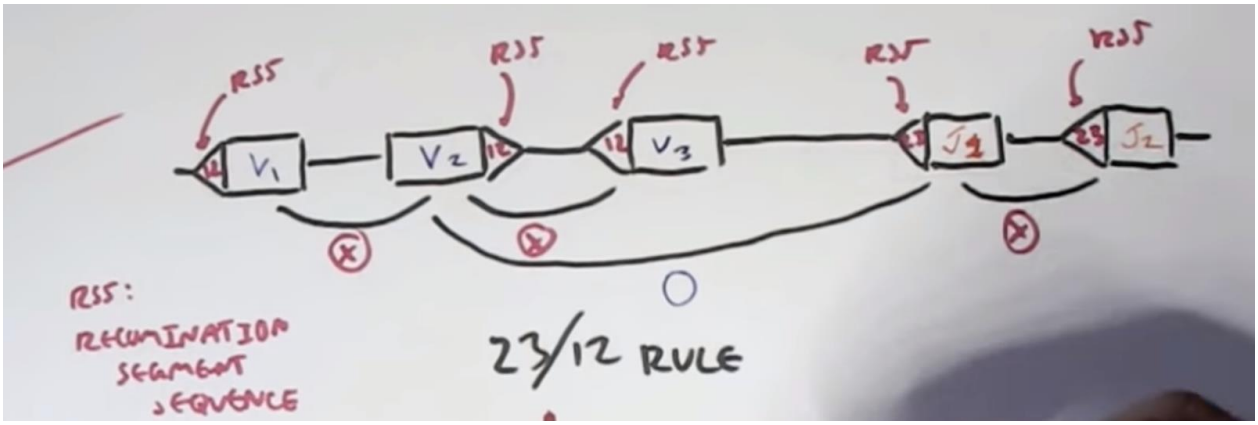


Figure 18.14: RSS sequences are present in inverted orientation at each pair of recombining sites.

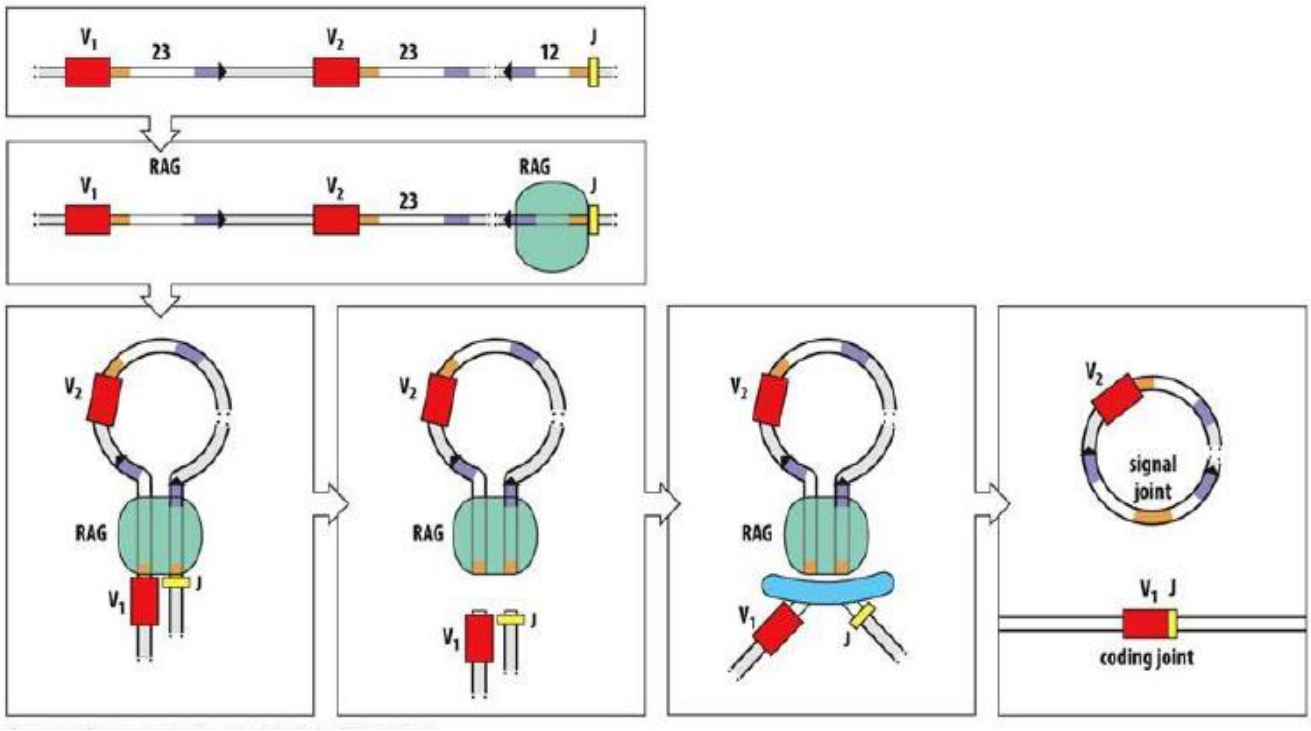
- The RSS motifs have a special rule called 23/12 rule where essentially a 23 and 12 can bind together. This means 23 can't recombine with 23 and 12 can't recombine with 12.

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



If you take part of the K light chain gene, we have 2 Variable segments (V1, V2) and 1 Joining (J) segment (recombination will occur between either V1 or V2 with J):

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



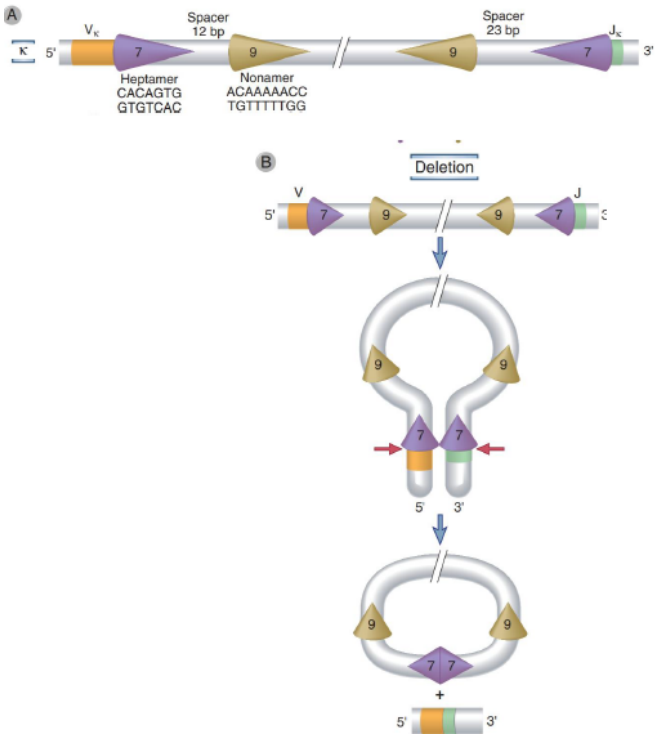
The 12 and the 23 spacers will lie parallel to each other forming a hairpin loop.

A special enzyme called RAG will break the double stranded DNA on both the V and the J.

The loop will be deleted.

V and J will combine together.

Further explanation:



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 sequence 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 and J

which will then get transcribed into RNA, which will ther go through splicing to remove intors 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**

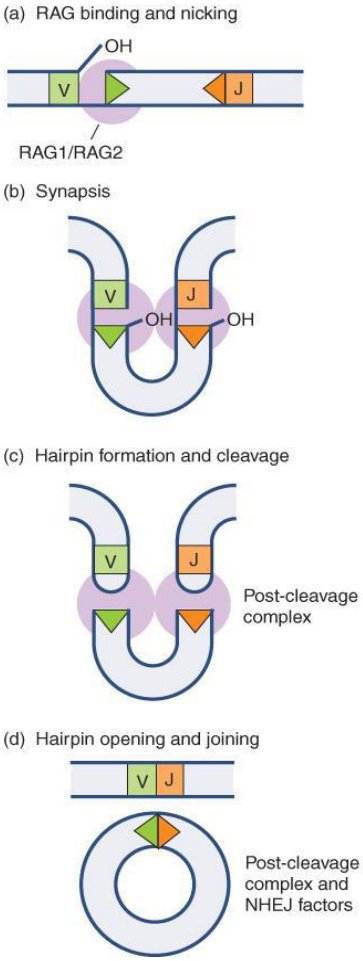
RAG1 and RAG 2 will identify the RSS.

Nicking and breaking of double stranded DNA.

Loop deletion.

V and J joining.

Nucleotides will be inserted randomly.



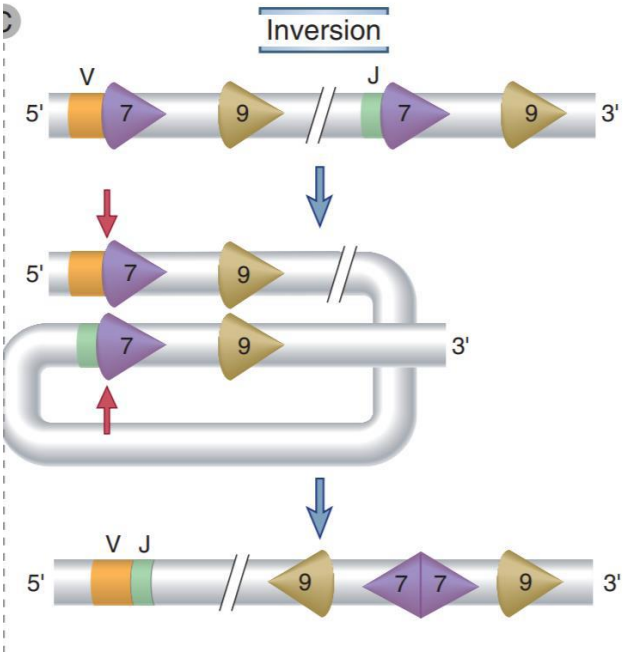
Second: Inversion (Tangled configuration):

V and J are far from each other.

DNA tangling so that V and J get closer to each other.

RAG proteins will cut at the RSS (red arrows).

D and J recombine and the rest of the sequence does not get deleted, it is rather inverted.



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doctor didn't add anything for the following slides:

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

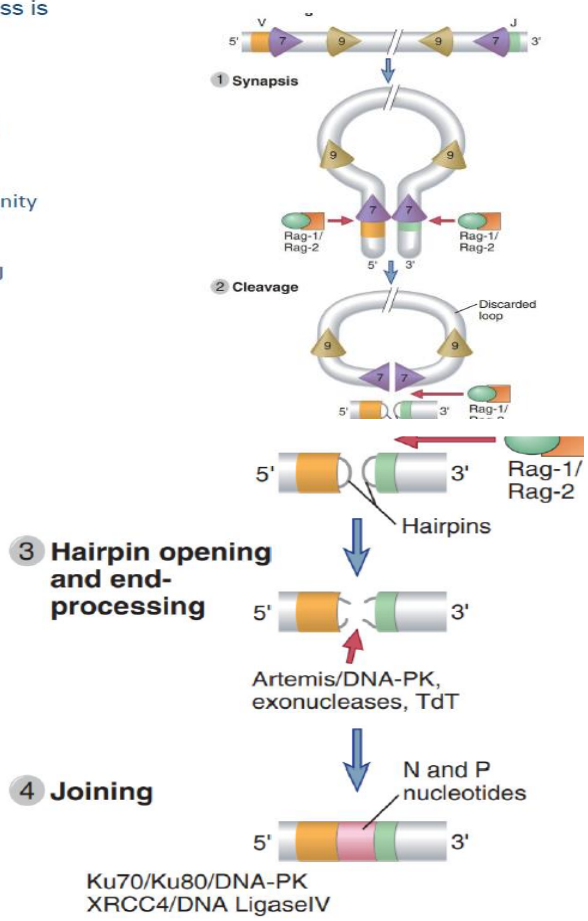
Ku proteins initiate repair by forming hairpin loop where RAG1/2 has broken the RSS

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

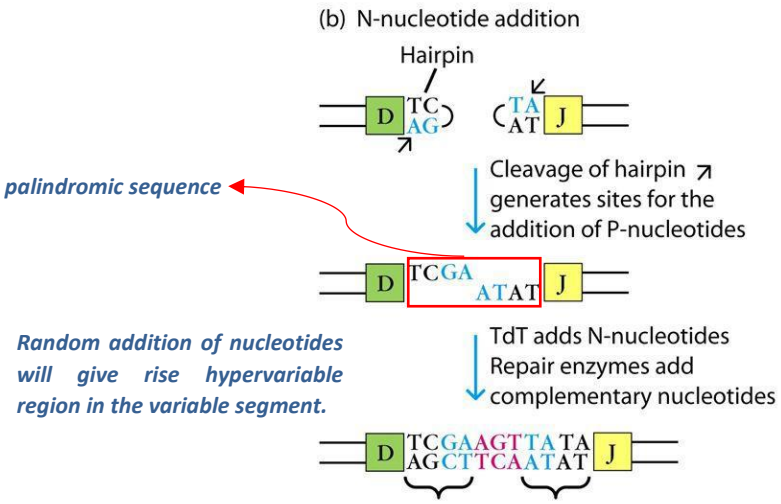
Following this another protein will come in called terminal deoxynucleotidyl transferase (TDT) which will add in nucleotides into the separated variable and joining segments

The TDT adds nucleotides randomly

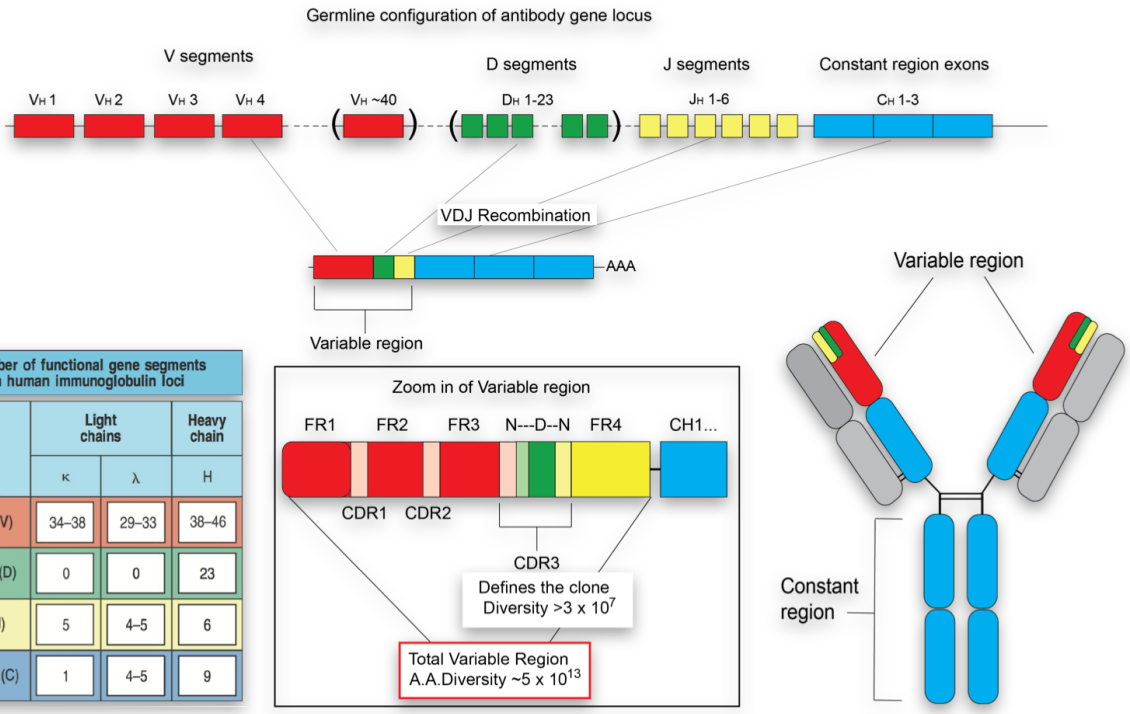
Because they are separated, DNA ligase and XRCC4 will ligate the ends together. Which will essentially form a repaired and unique V and J recombinant segment



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		Heavy chain
	κ	λ	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

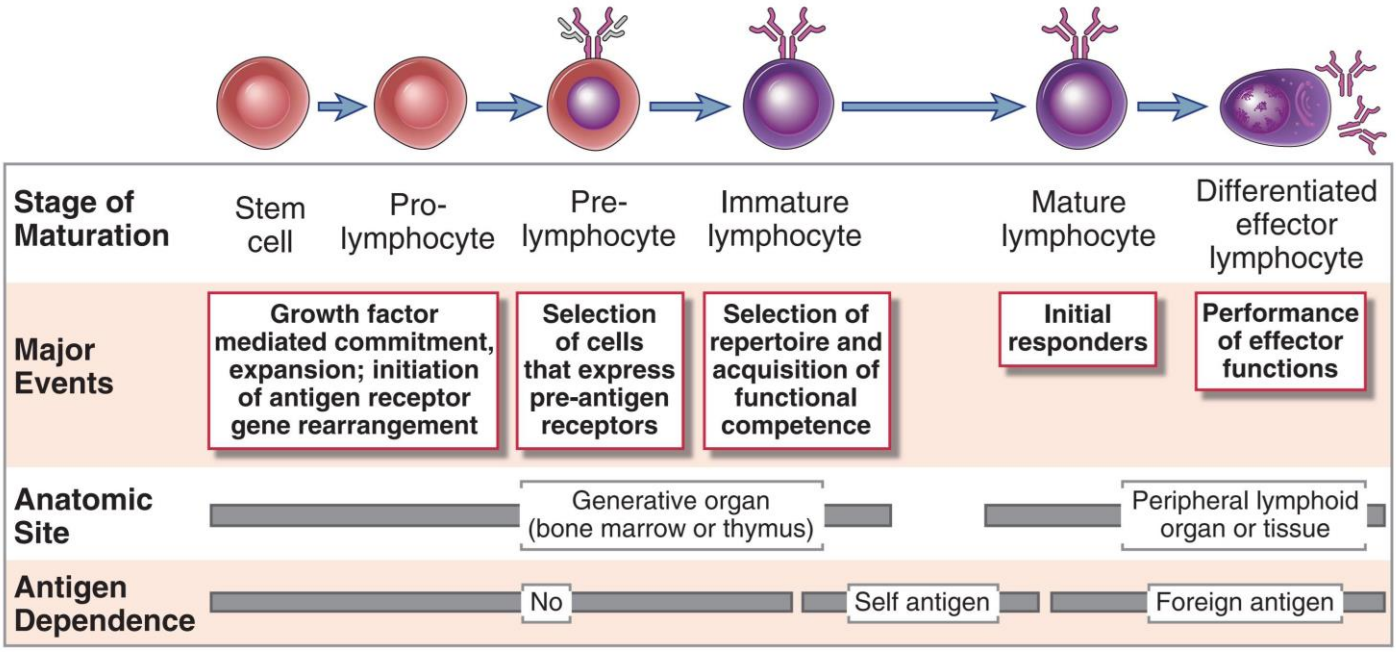


FIGURE 8–1 Stages of lymphocyte maturation. Development of both B and T lymphocytes involves the sequence of maturational stages shown. B cell maturation is illustrated, but the basic stages of T cell maturation are similar.

The End