Antigen Receptor Gene Rearrangement

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A progenitor lymphoid cell can become a progenitor T cell or progenitor B cell
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
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
- diversity (D)
- and joining (J) gene segments

Once it becomes a progenitor B cell, it will go through somatic recombination of the heavy chain (heavy chain rearrangement).
In developing B cells, the first antigen receptor gene to be completely rearranged is the Ig heavy chain or Ig H gene. 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.

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 and 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) which will essentially make the Ab for IgM
J and C are in close proximity. However, the V region and D region are far away from each other.
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, 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
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 somatic (VJ) recombination.

Light chain gene does not contain a D region.
What about the Light Chain gene?

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
**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.
There are many V regions, many J regions, and many D regions. This will make up a unique Ab

**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.
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.
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.
Complementarity-determining regions (CDRs)/ Hypervariable Regions

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

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

- The V(D)J recombination machinery uses consensus sequences consisting of a heptamer separated by either 12 or 23 base pairs from a nonamer (recombination signal sequence, RSS).

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.

For example V1 and V2 cannot bind together because they both have 12 bp sequences, same as V2 and V3, J1 and J2 cannot 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. Those segments can undergo recombination. They have a specific RSS motif, it can be either 23 or 12 bp spacer.
There are two ways to initiate the recombination:

First: Deletion (hairpin loop configuration)
1- the gene creates a hairpen 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 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**
18.9 V(D)J DNA Recombination Uses RSS and Occurs by Deletion or Inversion

- Recombination occurs by double-strand DNA breaks (DSBs) at the heptamers of two RSSs with different spacers: “12/23 rule.”

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 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.

Let's 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

(b) N-nucleotide addition

Hairpin

Cleavage of hairpin generates sites for the addition of P-nucleotides

TdT adds N-nucleotides
Repair enzymes add complementary nucleotides
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.