Ig DIVERSITY GENERATION

- Gene rearrangement: A limited number of gene segments recombine to generate a great repertoire.

Antibody Diversity Generation - variable region

** Preview: Ab diversity is generated by four main processes.

1. Multiple copies of the different variable region gene segments recombine (VDJ gene rearrangement)
2. Additional junctional diversity during recombination process
3. Pairing of different heavy and light chain variable regions
4. Somatic hypermutation

1. V(D)J gene rearrangement

Variable regions of both light and heavy chains are in fact encoded by different gene segments that join together during DNA recombination. (Fig. 4.16) Unrearranged Ig loci are in germline configuration

* Light chain variable region is encoded by two gene segments that come together; V and J
* Heavy chain variable region is encoded by three gene segments that come together: V, D, and J

Nomenclature

V  Makes up most of the variable region sequence (95-101 aa; each variable region, which is composed of one Ig domain, is appx. 110 aa).
D  For "diversity"; only in heavy chain; adds extra degree of diversity. A few aa.
J  For "joining"; next to the C region (up to 13 aa).

Light chain variable segments: \( V_κ, λ \) and \( J_κ, λ \)
Heavy chain variable segments: \( V_H, D_H, \) and \( J_H \)
Light chain constant region gene: encoded by \( C_κ, λ \)
Heavy chain constant region gene: encoded by \( C_μ, δ, γ, α, ε \)

- There are multiple copies of the three variable region gene segments. (Figs. 4.16, 4.18)
- \( κ \) locus, \( λ \) locus and H chain locus are on different chromosomes.
- They are organized slightly differently. \( λ \) locus is different because of four paired J-C segments.

- One recombination event for light chain, two for heavy chain due to D segment. (Fig. 4.17)
- V, (D), and J gene segments join by DNA recombination.
  - Heavy chain: DJ joining first then V and DJ joining.
  - Light chain: VJ joining only (no D segment).
- DNA sequences encoding variable region and constant region are separated by great distances in genome; only in B cells are the genes rearranged so that variable and constant region sequences come close together.
- The constant region sequences are not joined to the variable region sequences by DNA recombination; instead they join at the RNA splicing level. Only after RNA splicing are all gene sequences joined as one continuous chain before translation.

Sequence of individual Ig chain production (Fig. 4.34)
- Germline (unrearranged) DNA
- DJ joining (if it is a heavy chain)
- V and (D)J joining
- Transcription of a primary transcript RNA
- Splicing out of introns and intervening sequences - generation of mRNA
- Peptide processing (leader sequence deletion) and glycosylation

Mechanism of DNA rearrangement
- Recombination signal sequence (RSS): Heptamer – 12 or 23 bases of spacer – nonamer (Fig. 4.19). Sequence of heptamer and nonamer is conserved; spacer length but not sequence cons.
- 12 or 23 base pairs = one or two helical turns in DNA. Brings heptamer and nonamer sequences to one side of the helix; can now be recognized by recombination machinery

* VDJ recombination occurs on the genes on the same chromosome (therefore not between κ and λ, or between light and heavy chains).
* VDJ recombination occurs between a gene segments with a 12mer RSS and another gene segment with a 23mer RSS (The 12/23 rule). (Fig. 4.20)
  - D_H flanked by 12mer RSS and V_H and J_H flanked by 23mer RSS: V_H and J_H therefore do not join together.

Machinery of DNA recombination
- V(D)J recombination occurs only in B and T cells (T cell receptors undergo VDJ recombination, but with different sets of V, D and J gene segments).

Definitions: endonuclease vs. exonuclease → endonuclease can cleave intact double-stranded DNA; exonuclease chews away from the ends.

V(D)J recombinase: - Complex of several enzymes that act in concert to carry out variable region gene recombination. (Fig. 4.20)
- Composed of:
  * RAG-1 and RAG-2 gene products (recombination-activating gene): RAG proteins form a heterodimeric endonuclease complex, directly recognize RSS (one 12 and one 23)
    - the two RSS are brought together and then cleavage and DNA repair proceeds
  * RAG1/2 are expressed only in developing lymphocytes (pre-B and pre-T).
  * Normal cellular DNA cleavage and repair machinery involved in DNA repair (found in many other cell types): DNA-PK, Ku70/Ku80, DNA ligase IV, XRCC4, Artemis.
Humans lacking DNA-PK have severe combined immunodeficiency (Scid) because of no mature B cells or T cells; also more sensitive to DNA damage in other cells.

- Products of reaction are **signal joint** and **coding joint**
- This joining of the coding joint is not precise – additional level of diversity

**2. Junctional diversity**
- CDR1 and 2: encoded by V segment. Note that there is some variability even in the framework regions of different V segments but that the greatest difference between V segments is in the nucleotides encoding the CDR1 and CDR2 residues
- CDR3 is encoded by the sequences at the junction between V and J in light chain/ partially by D in heavy chain.
- CDR3 diversity is aided by addition and/or deletion of nucleotides at the junction of gene segments.
- Added nucleotides are called P-nucleotides and N-nucleotides. (Fig. 4.21)
- Deletion of nucleotides by exonucleases, addition of non-templated nucleotides by enzyme called TdT

-Because of imprecise nature of joining, 2/3 of recombination events will not produce an in-frame fusion and functional protein: **non-functional rearrangement**.

- **Allelic exclusion**: once a given Ig chain is successfully rearranged and functional protein produced, no more gene rearrangement occurs on that chain (other chromosome or second light chain locus). Ensures single Ig specificity per B cell.

**3. Pairing of different heavy and light chain variable regions**
- Different pairing can generate different specificity: a given light chain can pair with any different heavy chain and generate different specificity.

* How many different specificities can the above mechanisms generate?
  1. Multiple copies of the different variable region gene segments
     \[ \kappa: 35 \text{ V}_\kappa \times 5 \text{ J}_\kappa = 175 \]
     \[ \lambda: 30 \text{ V}_\lambda \times 4 \text{ J}_\lambda = 120 \]
     295 possible light chains
     \[ 40 \text{ V}_H \times 23 \text{ D}_H \times 6 \text{ J}_H = 5,520 \text{ possible heavy chains} \]
  2. Pairing of different H and L chain variable regions
     \[ 295 \times 5,520 = 1.6 \times 10^6 \text{ different Ab specificities} \]
  1 and 2 = **Combinatorial Diversity**

Further increased diversity due to:
- 3. Additional diversity during recombination process itself (junctional diversity)
- 4. Somatic hypermutation
  An individual is thought to have \( 10^9 - 10^{11} \) Ig specificities
Co-expression of IgM and IgD on naïve mature B cells
- All Igs start out as IgMs: All B cells in late maturation stage in the bone marrow have IgM on cell surface.
- Naive mature B cells in the periphery that have not seen Ag express both IgM and IgD on cell surface. This is not from gene rearrangement; rather, a primary RNA transcript actually has both $C_\mu$ and $C_\delta$, which are encoded by exons that are adjacent in the heavy chain locus (Fig. 4.22). **Alternative splicing** allows the cells to generate two species of mRNA from the same transcriptional unit; and therefore to express both IgD and IgM. (Fig. 4.23) The function of IgD is not clear; very little is secreted.
- surface expression of IgM and IgD requires association with two proteins called Igα and Igβ (Fig. 4.25). These transmembrane proteins have longer cytoplasmic tails than Ig proteins and provide signaling function to the BCR.

Transmembrane vs. secreted immunoglobulins (Fig. 4.26)
An antibody can be either secreted by B cells or remain expressed on the cell surface.
- IgM initially on the surface of B cells.
- After an Ag encounter, some B cells differentiate into cells that secrete immunoglobulins (called plasma cells).
- Example: $C_\mu$ is actually a cluster of exons that encode different Ig domains of a heavy chain (Fig. 4.26). Each C gene has secretion-coding (SC) and membrane-coding (MC) exons. After the primary transcript is made, alternative splicing occurs and either SC or MC is lost. The remaining exon determines whether Ig is membrane-bound or secreted.

4. Somatic Hypermutation (SHM) (Figs. 4.27, 4.28)
- Occurs only after B cells respond to Ag and have had a chance to proliferate.
- One clone of B cell proliferates to become many identical B cells and they all undergo different mutations in their antibody genes and start expressing variations of the original antibody. Works on V regions (VJ or VDJ segments) not C or other genes (Fig. 4.27).
- requires an enzyme called AID (activation-induced cytidine deaminase), that is expressed only in activated B cells. The AID enzyme converts cytosine bases to uracil, which are then converted to other bases by the DNA repair machinery. Still unknown how AID activity is restricted to V region exons.
- The B cells are subsequently selected for their ability to bind the antigen. The B cells that lost its specificity to the antigen through mutation will be selected out (die by apoptosis), whereas the ones that bind Ag better will have a selective advantage.
- surviving B cells tend to have mutations affecting amino acid residues of the CDR loops rather than framework regions (Fig. 4.28)
- leads to refinement of Ab specificity (affinity maturation) during the course of B cell’s lifetime by mutation of Ig genes followed by selection.