

CLONAL SELECTION

1. Each clone expresses one unique receptor.
2. Receptors form independent of antigen encounter.
3. Self-reactive clones are eliminated (tolerance).
4. Antigen encounter selects specific clones for proliferation and differentiation.

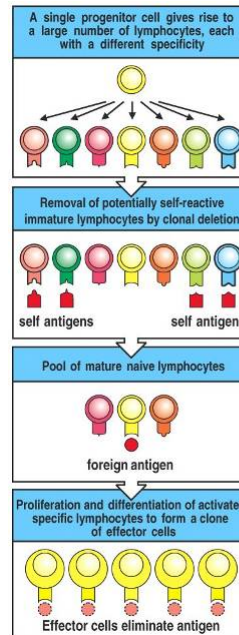


Figure 1-14 Immunobiology, 6/e. (© Garland Science 2005)

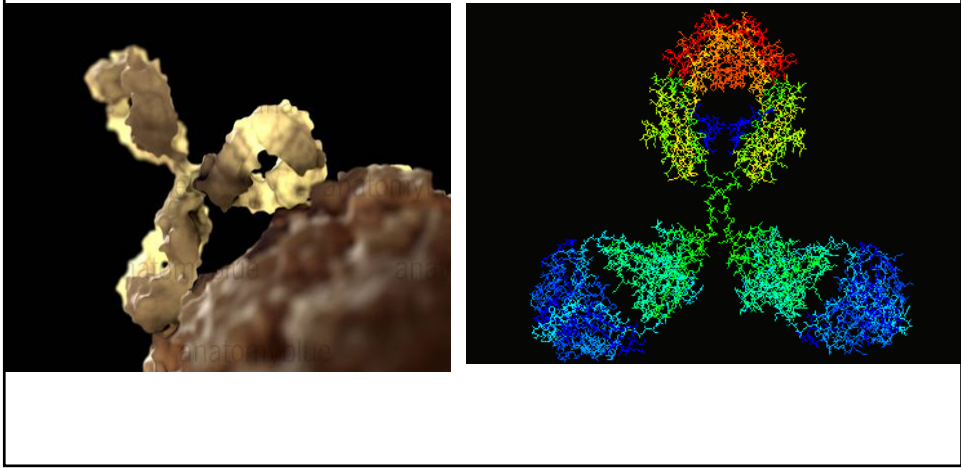
CLONOTYPIC RECEPTORS

B CELLS Antibody (immunoglobulin)

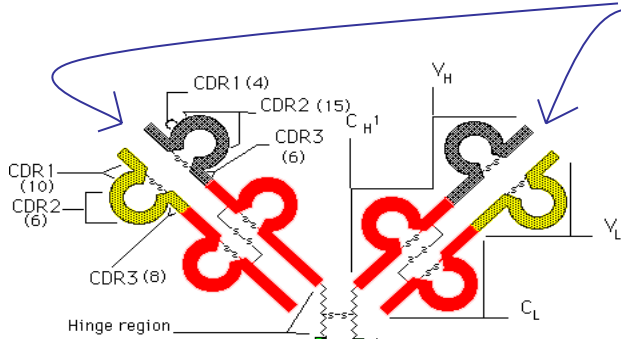
T CELLS T cell receptor

1. Protein Structure
2. Gene Organization
3. **UNIQUE GENE REARRANGEMENT**

ANTIBODIES



ANTIBODY: STRUCTURE AND FUNCTION



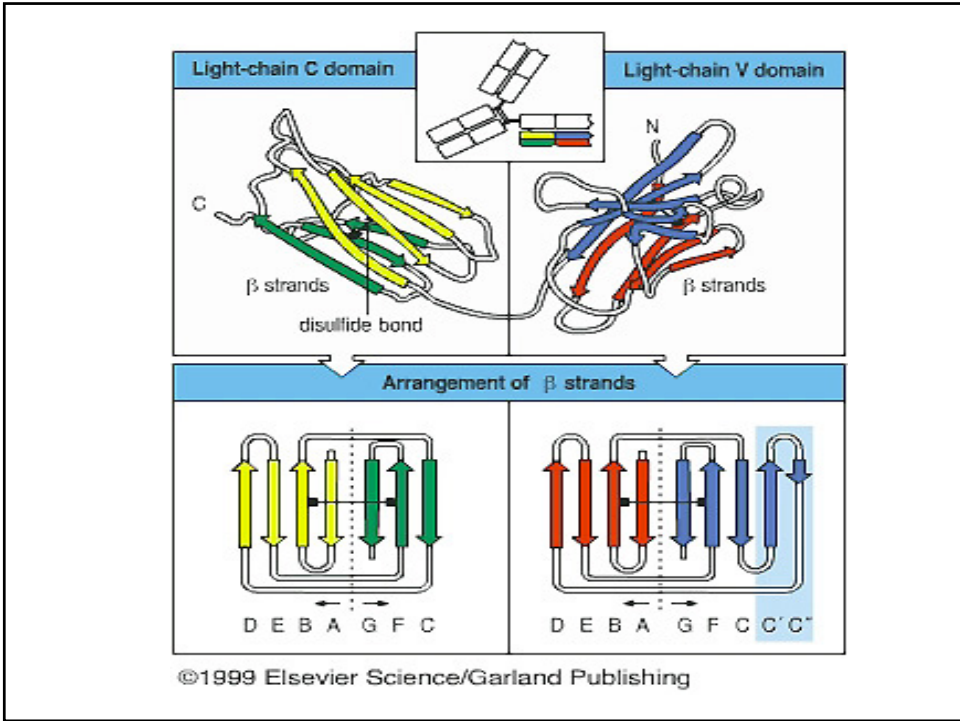
1. Antigen Recognition

2. Antigen Elimination

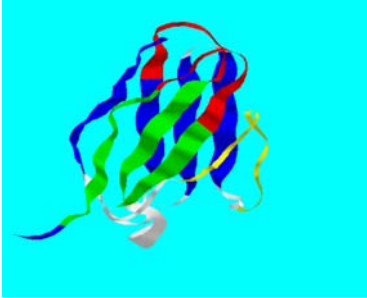
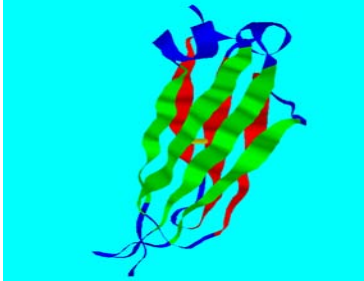
H Chain 440 amino acids
 L Chain 220 amino acids
 H chain 4 or 5 domains
 L Chain 2 domains
 Each domain has intra-disulfide bridge of 60 amino acids.

There are 5 classes of H chain (IgG, IgM, IgA, IgD, and IgE)
 There are two class of L chains (Lambda and Kappa)

L Chain	H chain
CD1 24-34 (10)	CD1 31-35 (4)
CD2 50-56 (6)	CD2 50-65 (15)
CD3 89-97 (8)	CD3 96-102 (6)

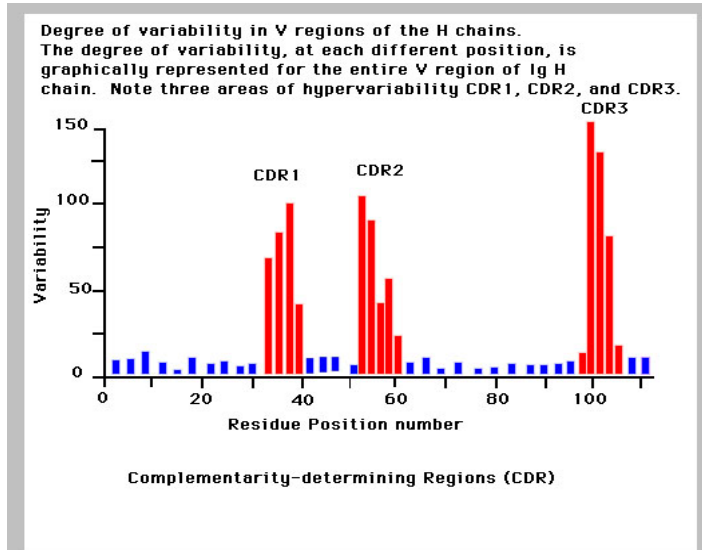


Ig CONSTANT DOMAIN

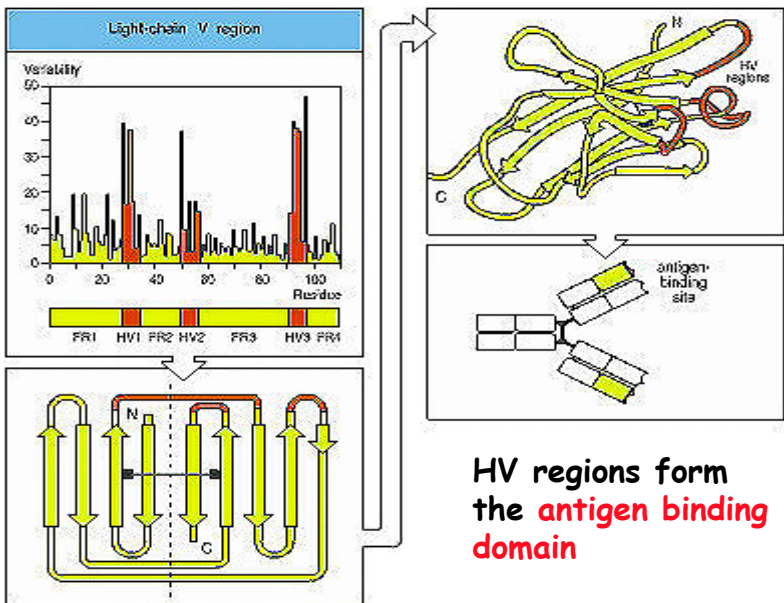


Ig VARIABLE DOMAIN

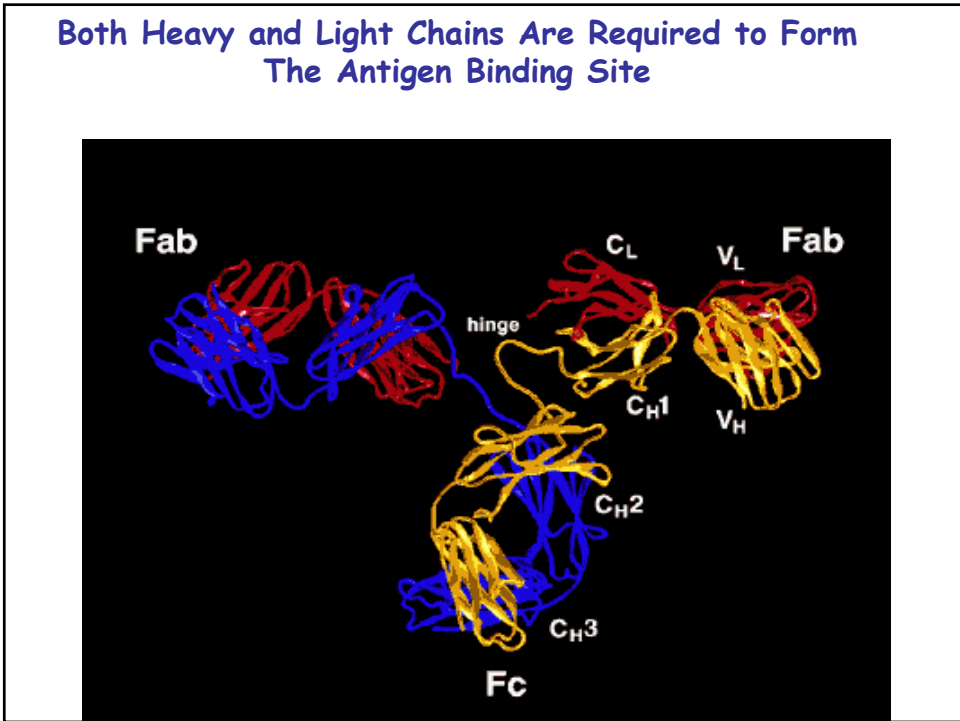
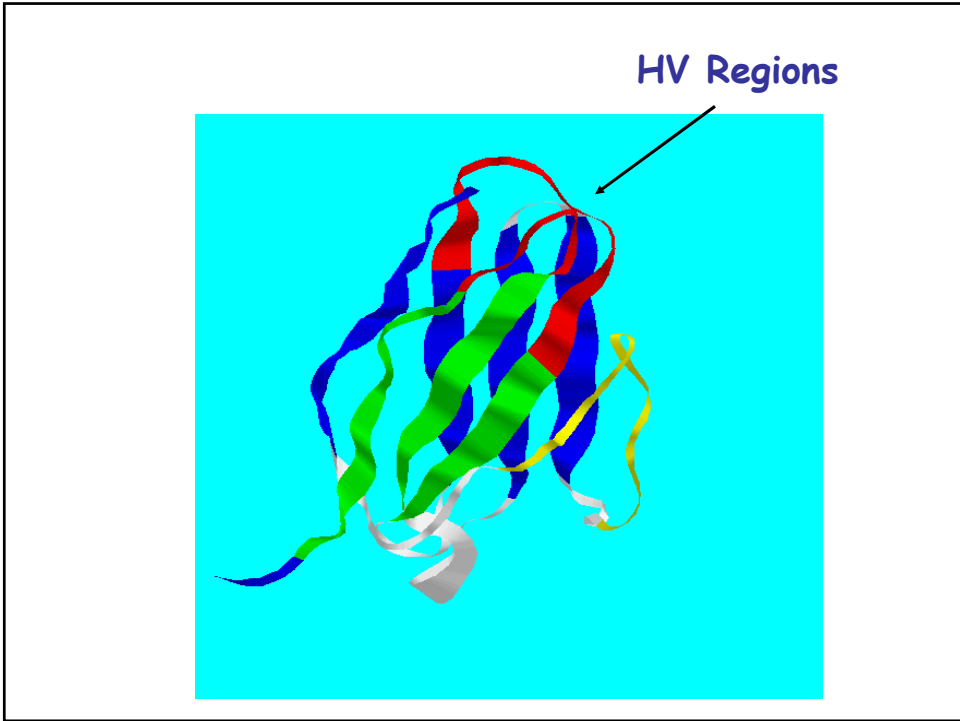
Variable Domains Have Hypervariable (HV) or Complementarity Determining Regions (CDRs)

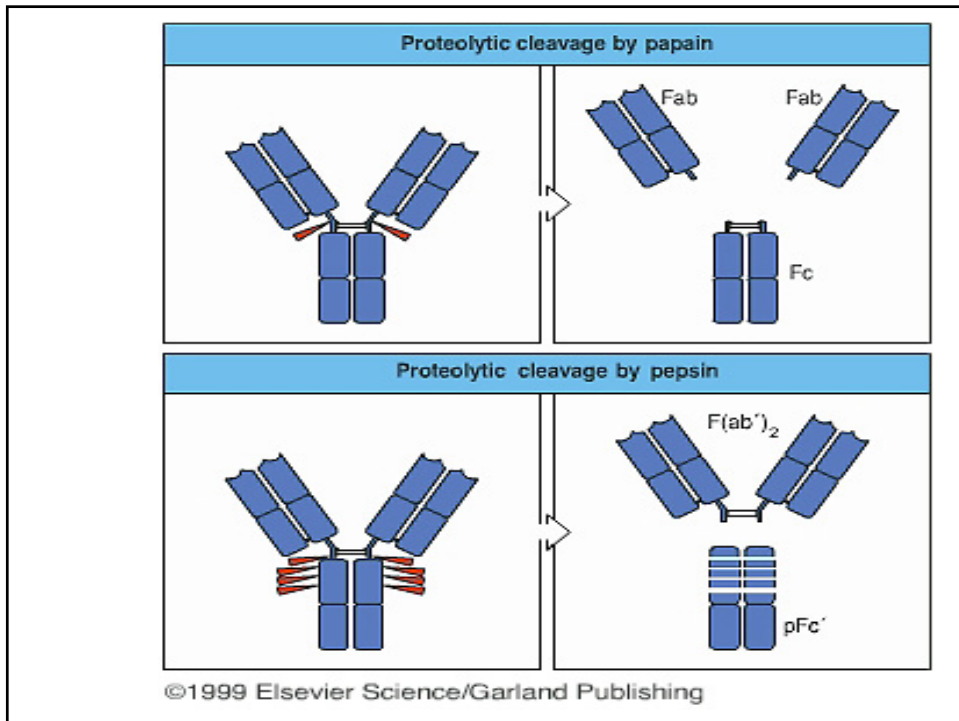
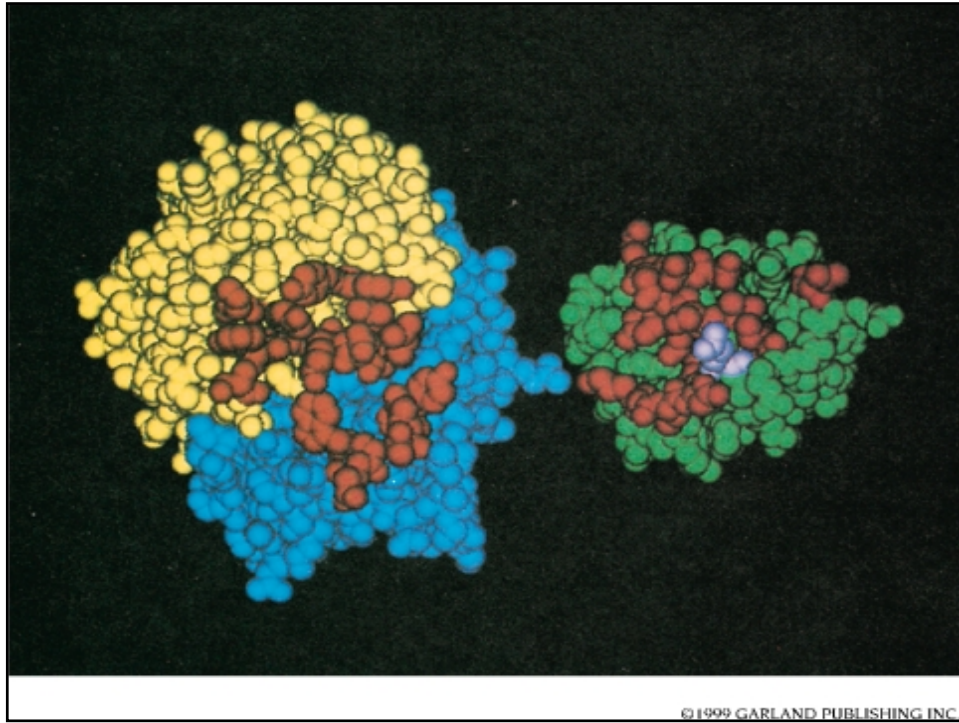


HV regions occur at loops between beta sheets



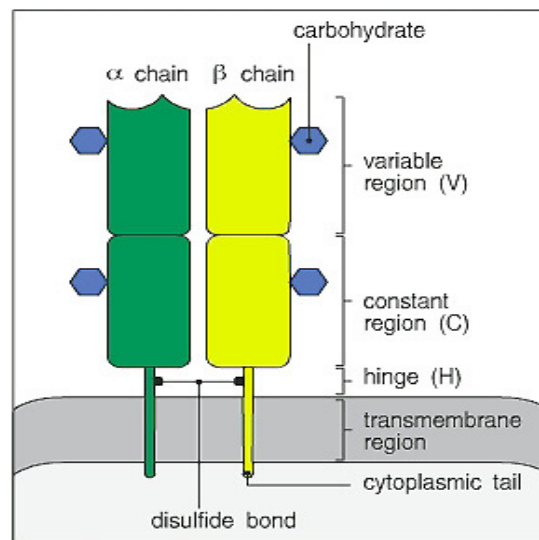
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T CELL RECEPTORS ARE SIMPLER THAN ANTIBODIES

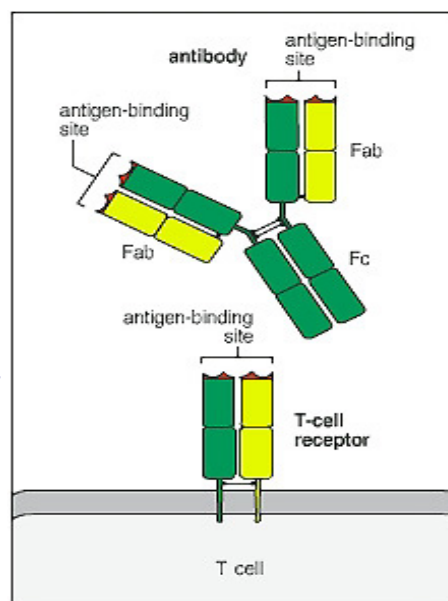
Antigen Recognition



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Antibodies:
Secreted or
Transmembrane

TCR: Transmembrane



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The Diversity Problem:

How are 10^8 clonotypic antibodies encoded?

HYPOTHESIS #1: Germline genes encode everything

(Could there be 2×10^4 or more Ig genes?)

HYPOTHESIS #2: Somatic mutation of single germline genes

(How could the genome sustain such a high, and currently unknown, rate of somatic mutation?)

ANSWER LIES IN ORGANIZATION AND UNIQUE REARRANGEMENT OF Antibody and TCR GENES

Ig Polypeptides Are Encoded by Multiple Gene Segments

Variable Constant Light Chain POLYPEPTIDE

V J C Light Chain GENE SEGMENTS

Variable Constant H.C. POLYPEPTIDE

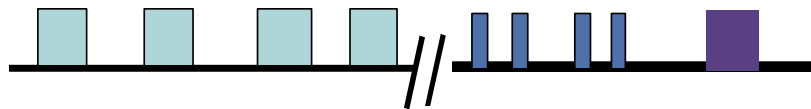
V D J C_{H1} C_{H2} C_{H3} H.C. GENE SEGMENTS

A Prototype Ig Gene: Murine Kappa

About 10-20
V_κ gene segments

4 J Gene
Segments

1 C_κ Gene
Segment

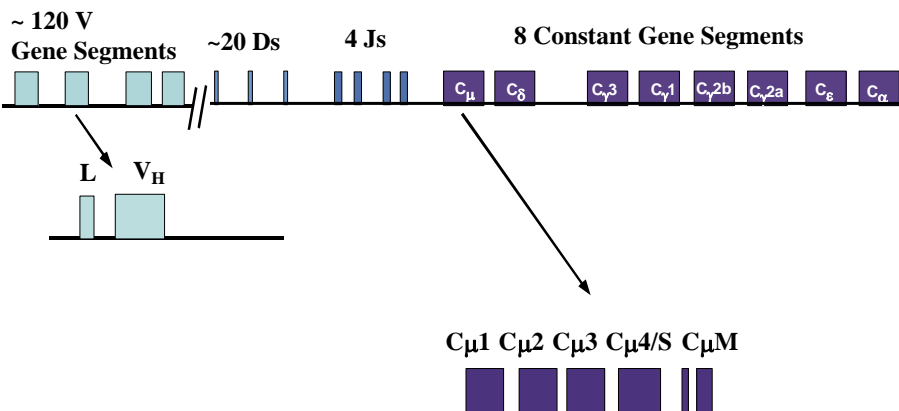


Multiple V gene segments, distant from J and C

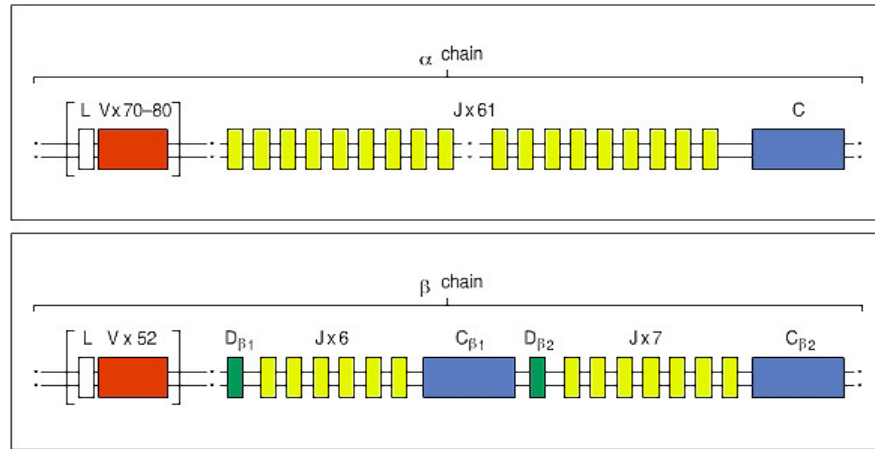
A few J gene segments

One C gene segment

Murine Ig Heavy Chain Gene Organization

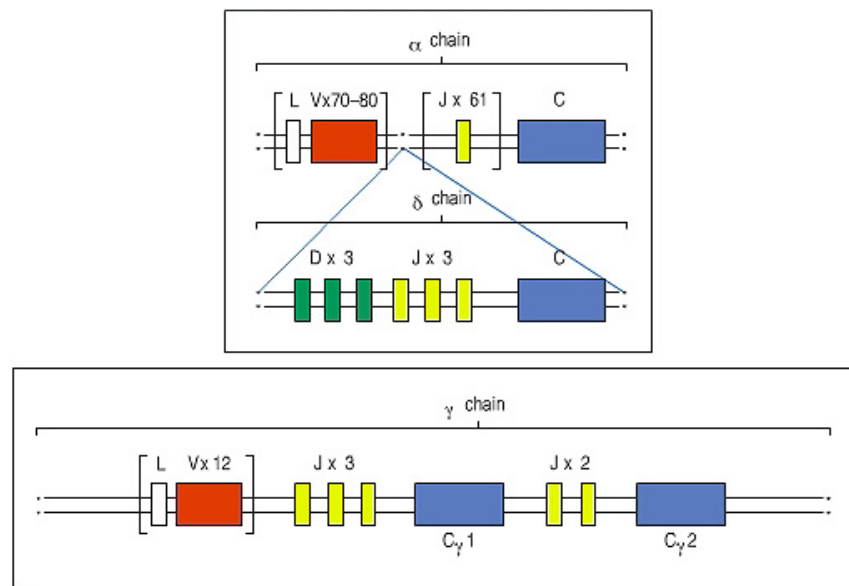


TCR Alpha and Beta Loci



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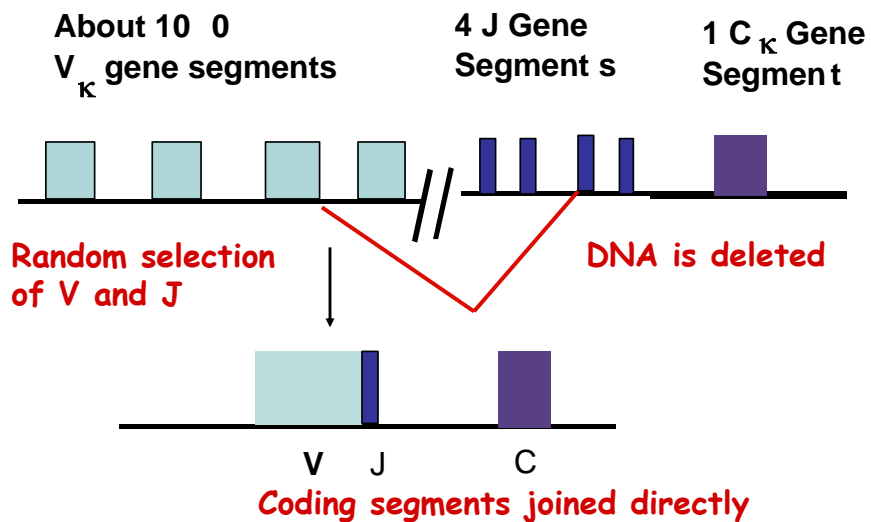
TCR Delta and Gamma Loci



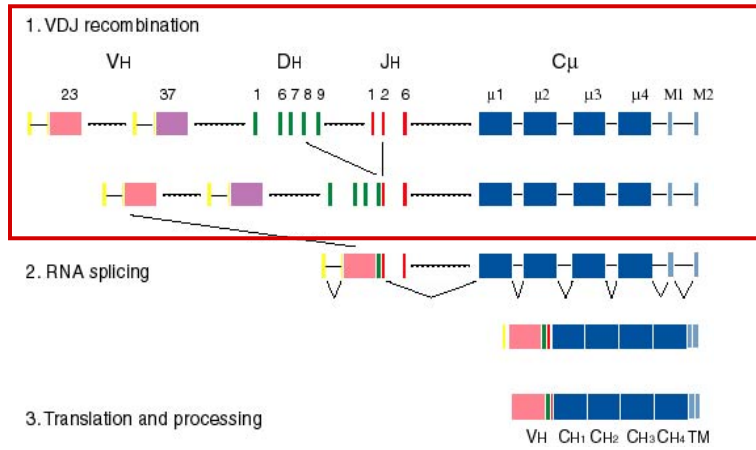
IMMUNOGLOBULIN GENES UNDERGO TWO DNA REARRANGEMENTS

1. **V(D)J Recombination:** both light and heavy chains
2. **Class switch recombination:** heavy chains only

V(D)J Recombination in the Kappa Locus

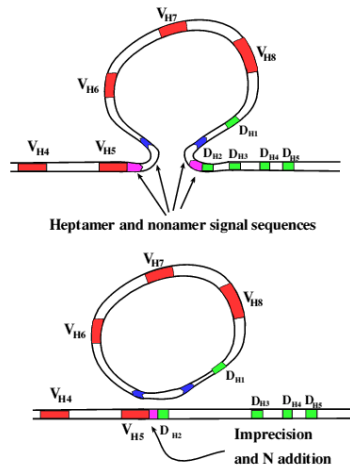


Ig Heavy Chain VDJ Recombination--2 DNA Deletions, DJ and VD



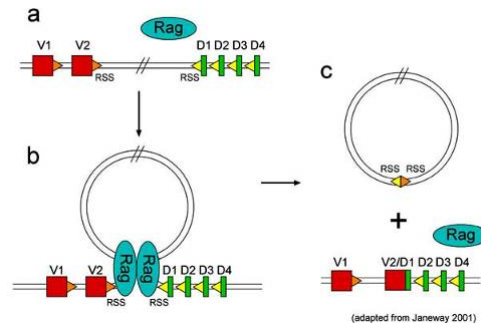
VDJ Recombination Entails Deletion of Genomic DNA

VDJ joining occurs by DNA recombination



RAG PROTEINS are lymphocyte-specific and mediate precise DNA recognition and cutting.

Figure 6: The mechanism of rearrangement



DNA repair enzymes, that are NOT lymphoid specific, rejoin the cut ends of DNA.

Omenn Syndrome: Mutation in RAG-1 Gene

An infant with a skin rash and recurrent bacterial and fungal infections

- Presented at two weeks with severe generalized dermatitis and diarrhea.

- Developed a life-threatening disseminated infection with *Staphylococcus aureus*.

- Diagnosis was suspected after noting absence of thymic shadow on X-ray, markedly reduced serum immunoglobulins, absent B cells and reduced numbers of T cells from peripheral blood.

- In vitro V(D)J recombination assay was 10% of normal. Sequencing of the *RAG-1* gene revealed a missense mutation.

- Bone marrow transplantation is only therapeutic option.

QuickTime™ and a TIFF (Uncompressed) decoder are needed to see this pict

CONSEQUENCES OF V(D)J RECOMBINATION (in addition to formation of a functional gene)

- 1. Combinatorial diversity:** # of possible combinations is the product of the # of recombining segments
i.e. for mouse h.c.: $120 \times 20 \times 4 = 10^4$
- 2. Junctional diversity at CDR3**
 - Deletion of bases at junctions
 - N region additions at junctions
 - P region additions at junctions
- 3. Activates transcription of the rearranged gene**
Juxtaposition of intronic enhancers with V region promoters.
- 4. Allows receptor editing** to alter potentially self-reactive antibodies

SUMMARY-PROTEIN STRUCTURE

1. Antibodies are the clonotypic receptors for B cells. T cell receptors are clonotypic receptors for T cells.
2. Antibodies are tetramers of 2 identical light chains and 2 identical heavy chains. Each chain has variable constant regions.
3. Antibody variable regions recognize antigen; antibody heavy chain constant regions eliminate antigen.
4. Hypervariable regions within the variable domains are antigen-contact sites.
5. HV regions from both light and heavy chains are necessary to form an antigen binding site.
6. TCRs resemble two Ig light chains; their sole function is to recognize antigen.

SUMMARY-Ig and TCR GENE REARRANGEMENT

- 1. Ig and TCR genes are encoded by 30-150 V gene segments, several J and D gene segments and few C gene segments.**
- 2. Unrearranged Ig and TCR genes are inactive.**
- 3. VDJ recombination forms functional Ig and TCR genes.**
- 4. VDJ recombination involves deletion of DNA.**
- 5. RAG1 and RAG2 genes are lymphoid specific components of VDJ recombination and are required for formation of Ig and TCR genes.**
- 6. VDJ recombination provides a mechanism to generate huge diversity, primarily via combinatorial mechanisms.**