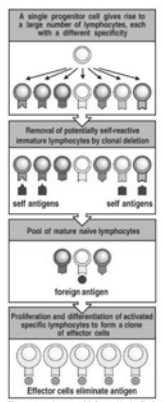


## CLONAL SELECTION

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.

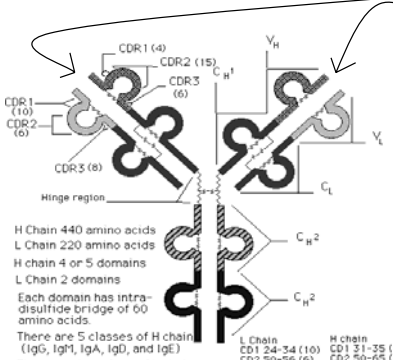


The diagram illustrates the clonal selection process in four stages:
 

- A single progenitor cell gives rise to a large number of lymphocytes, each with a different specificity.
- Removal of potentially self-reactive immature lymphocytes by clonal deletion. Self-antigens are shown binding to receptors, leading to the elimination of those cells.
- Pool of mature naive lymphocytes. A foreign antigen is introduced.
- Proliferation and differentiation of activated specific lymphocytes to form a clone of effector cells. Effector cells eliminate the antigen.

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

## ANTIBODY: STRUCTURE AND FUNCTION



The diagram shows the Y-shaped structure of an antibody, composed of two heavy chain (H) and two light chain (L) domains. Key regions labeled include:
 

- CDR1 (4), CDR2 (15), CDR3 (6), CDR4 (6), CDR5 (6)
- H<sub>H</sub><sup>1</sup>, H<sub>L</sub><sup>1</sup>, H<sub>H</sub><sup>2</sup>, H<sub>L</sub><sup>2</sup>
- Hinge region

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)

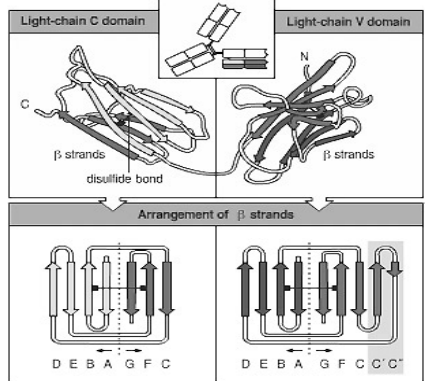
L Chain: CD1 24-34 (10), CD2 50-56 (6), CD3 89-97 (8)  
 H chain: CD1 31-35 (4), CD2 50-56 (6), CD3 96-102 (6)

1. Antigen Recognition
2. Antigen Elimination

## CLONOTYPIC RECEPTORS

**B CELLS**    Antibody (immunoglobulin)  
**T CELLS**    T cell receptor

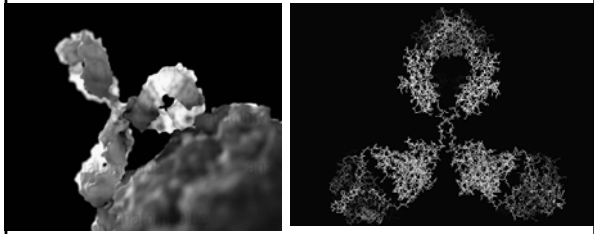
1. Protein Structure
2. Gene Organization
3. UNIQUE GENE REARRANGEMENT



The diagram compares the arrangement of beta strands in the Light-chain C domain and Light-chain V domain. Both domains feature a beta-barrel structure with beta strands labeled D, E, B, A, G, F, C. The V domain has an additional C' strand. Disulfide bonds are shown connecting the strands.

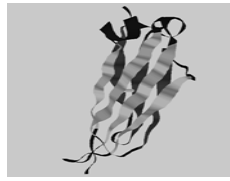
©1999 Elsevier Science/Garland Publishing

## ANTIBODIES




Two electron micrographs showing the structure of antibodies. The left image shows a single antibody molecule with its characteristic Y-shape. The right image shows a cluster of multiple antibody molecules.

## Ig CONSTANT DOMAIN

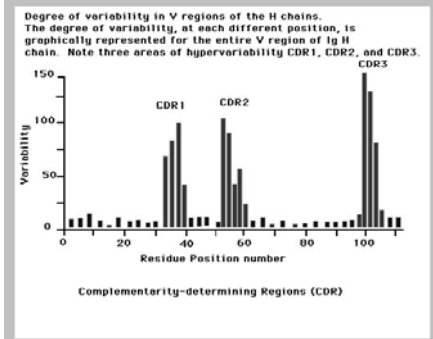


## Ig VARIABLE DOMAIN

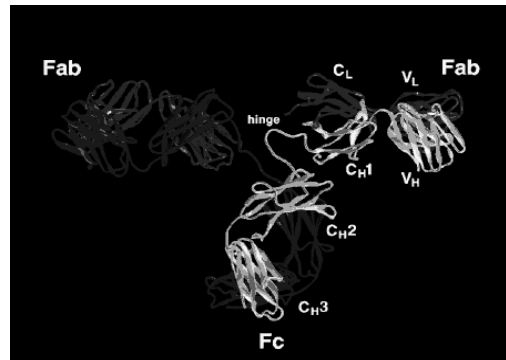


The diagram shows two 3D ribbon models of antibody domains. The left model is the Ig CONSTANT DOMAIN, which is more rigid and has a characteristic shape. The right model is the Ig VARIABLE DOMAIN, which is more flexible and contains the antigen-binding sites.

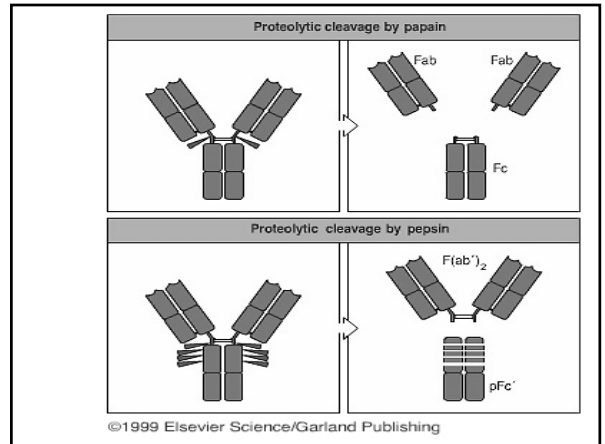
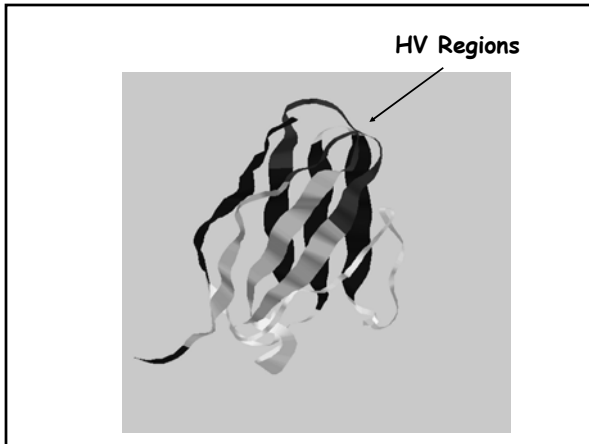
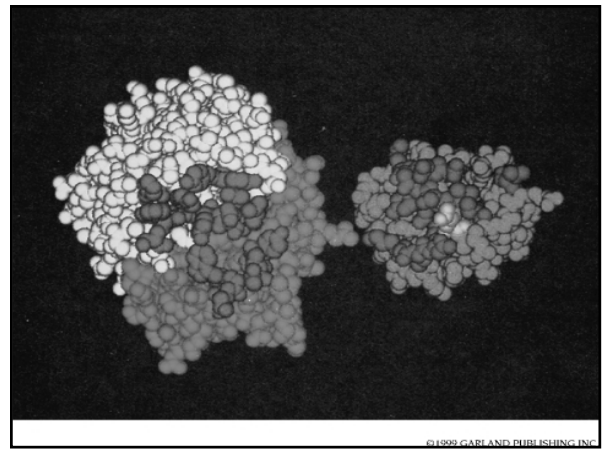
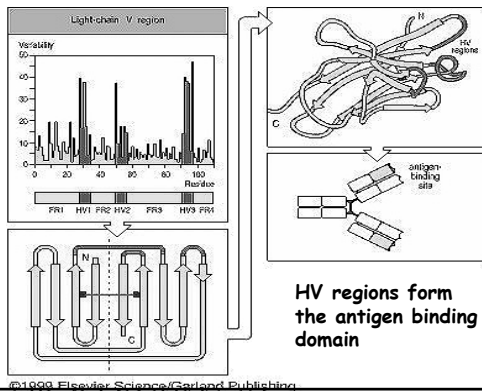
**Variable Domains Have Hypervariable (HV) or Complementarity Determining Regions (CDRs)**



**Both Heavy and Light Chains Are Required to Form The Antigen Binding Site**

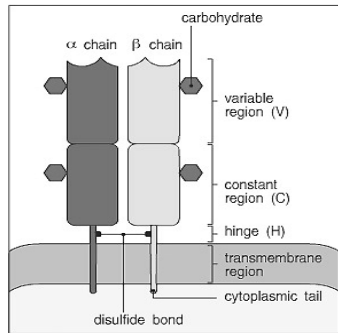


**HV regions occur at loops between beta sheets**



**T CELL RECEPTORS ARE SIMPLER THAN ANTIBODIES**

**Antigen Recognition**

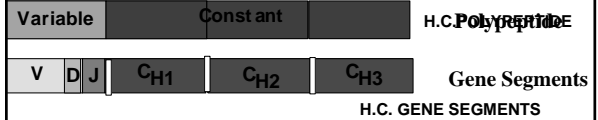


©1999 Elsevier Science/Garland Publishing

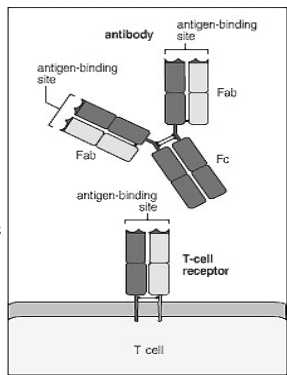
**Ig Polypeptides Are Encoded by LIGHT CHAIN Multiple Gene Segments**



**HEAVY CHAIN**



**Antibodies: Secreted or Transmembrane**

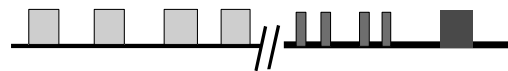


©1999 Elsevier Science/Garland Publishing

**TCR: Transmembrane**

**A Prototype Ig Gene: Murine Kappa**

About 100 V<sub>κ</sub> gene segments      4 J Gene Segments      1 C<sub>κ</sub> Gene Segment



Multiple V gene segments, distant from J and C

A few J gene segments

One C gene segment

**The Diversity Problem:**

How are 10<sup>8</sup> clonotypic antibodies encoded?

**HYPOTHESIS #1: Germline genes encode everything**

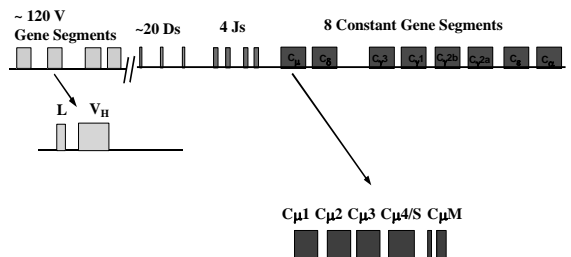
(Could there be 2x10<sup>4</sup> or more Ig genes?)

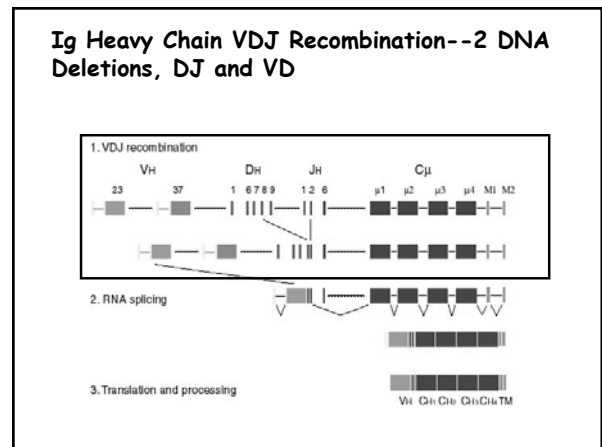
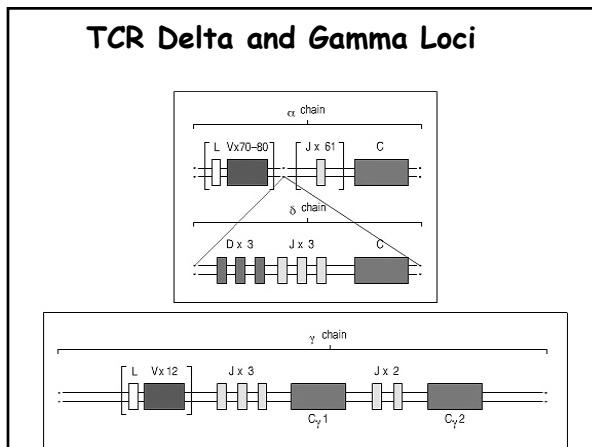
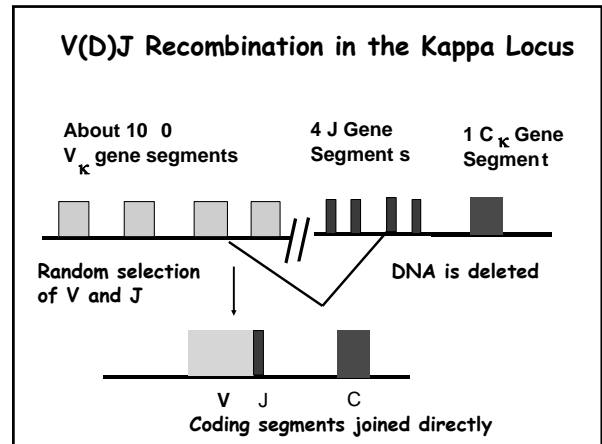
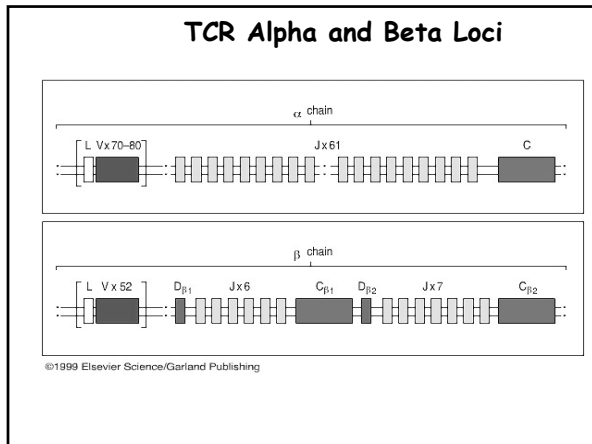
**HYPOTHESIS #2: Somatic mutation of single germline genes**

(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**

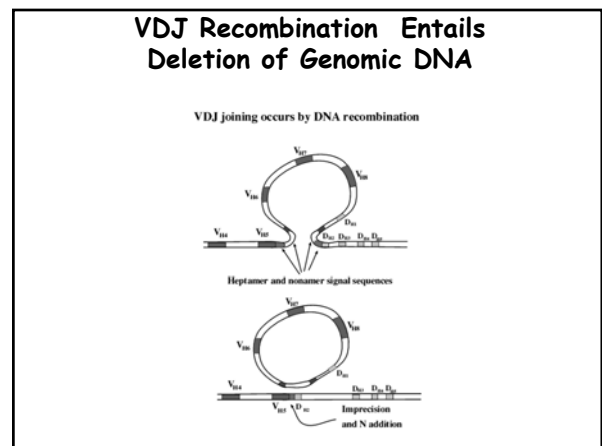
**Murine Ig Heavy Chain Gene Organization**





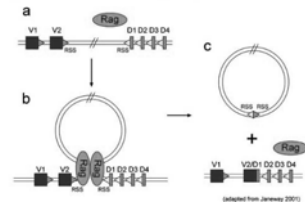
## IMMUNOGLOBULIN GENES UNDERGO TWO DNA REARRANGEMENTS

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



**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.

### 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.

### Omenn Syndrome: Mutation in RAG-1 Gene

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

QuickTime™ and a TIFF (Uncompressed) library are needed to see this picture.

- 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.

### 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.

### 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