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.

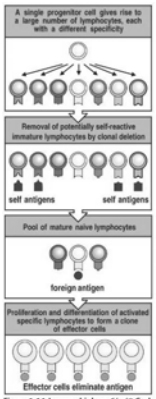
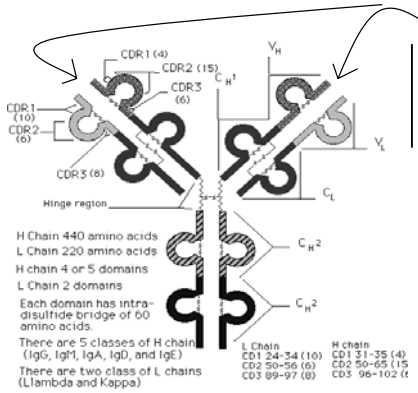


Figure 1-14 Immunobiology, 6/e. (© Garland Science 2005)

ANTIBODY: STRUCTURE AND FUNCTION



1. Antigen Recognition

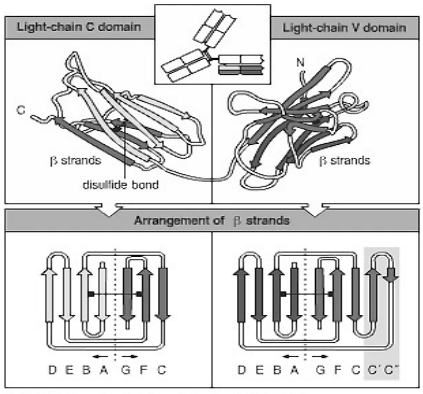
2. Antigen Elimination

CLONOTYPIC RECEPTORS

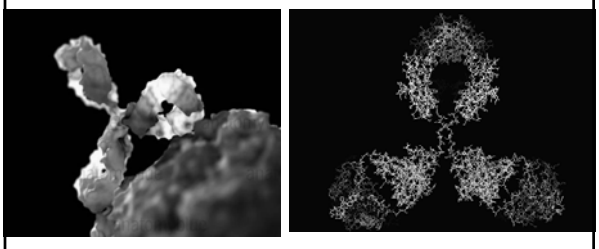
B CELLS Antibody (immunoglobulin)

T CELLS T cell receptor

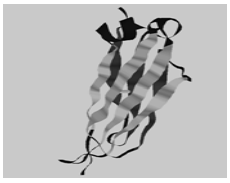
1. Protein Structure
2. Gene Organization
3. UNIQUE GENE REARRANGEMENT



ANTIBODIES

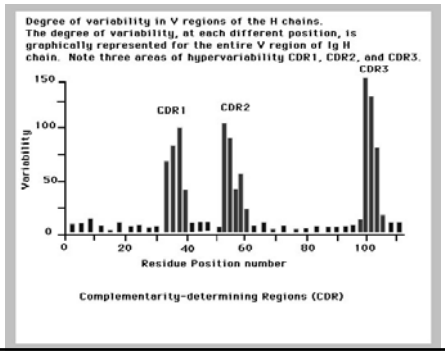


Ig CONSTANT DOMAIN

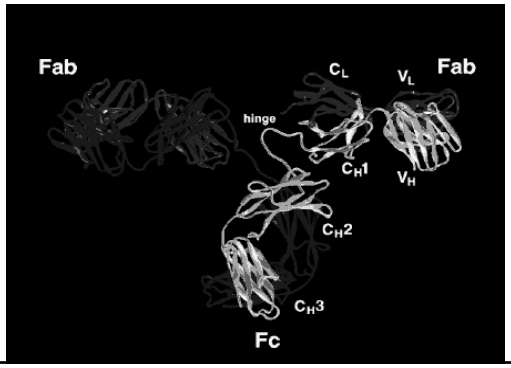


Ig VARIABLE DOMAIN

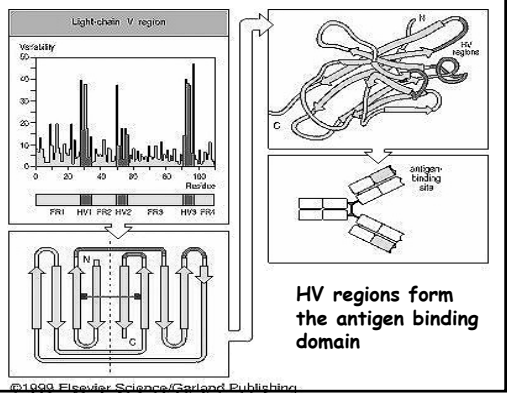
Variable Domains Have Hypervariable (HV) or Complementarity Determining Regions (CDRs)



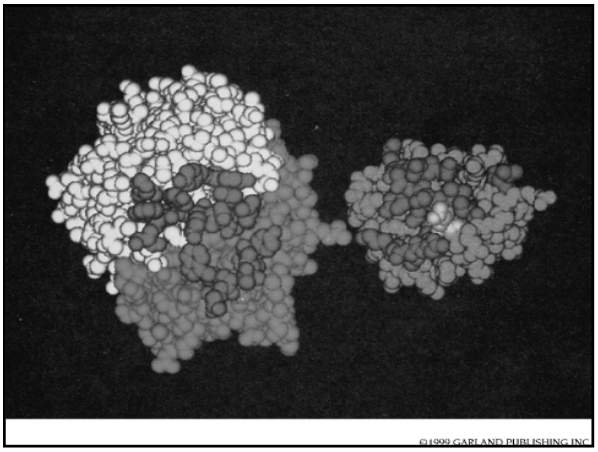
Both Heavy and Light Chains Are Required to Form The Antigen Binding Site



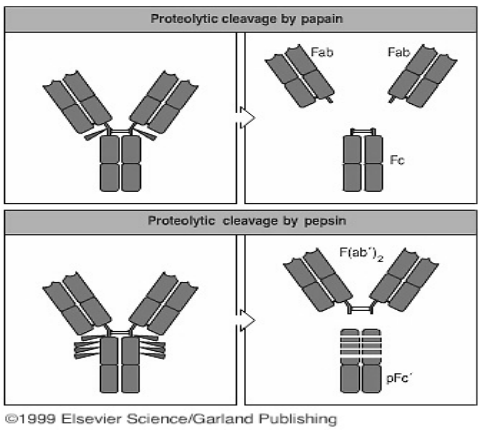
HV regions occur at loops between beta sheets



HV regions form the antigen binding domain

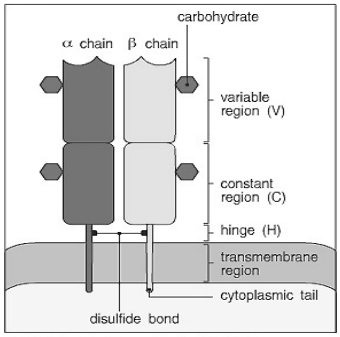


HV Regions



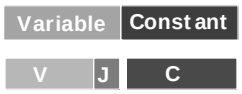
T CELL RECEPTORS ARE SIMPLER THAN ANTIBODIES

Antigen Recognition



Ig Polypeptides Are Encoded by Multiple Gene Segments

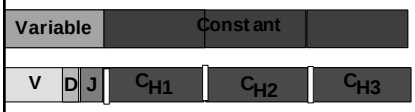
LIGHT CHAIN



Light Chain POLYPEPTIDE

Light Chain GENE SEGMENTS

HEAVY CHAIN



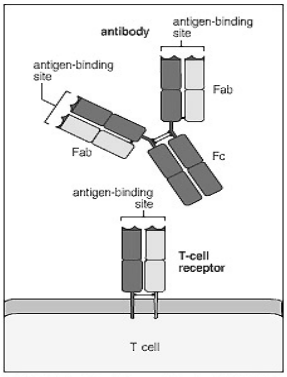
H.C. Polypeptide

Gene Segments

H.C. GENE SEGMENTS

Antibodies: Secreted or Transmembrane

TCR: Transmembrane

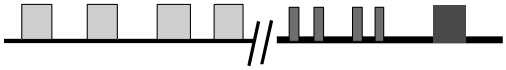


A Prototype Ig Gene: Murine Kappa

About 10 0 V_κ gene segments

4 J Gene Segment s

1 C_κ 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 10^8 clonotypic antibodies encoded?

HYPOTHESIS #1: Germline genes encode everything

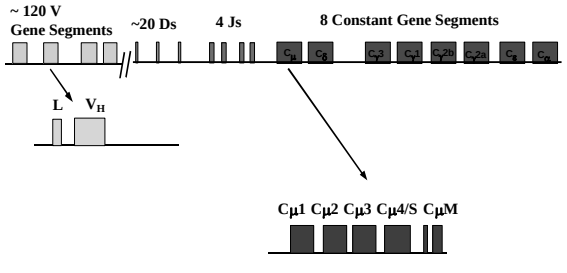
(Could there be $2x10^4$ or more Ig genes?)

HYPOTHESIS #2: Somatic mutation of single germline genes

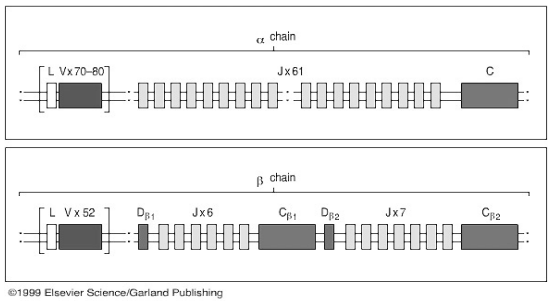
(How could the genome sustain such a high, and currently unknown, rate of somatic mutation?)

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

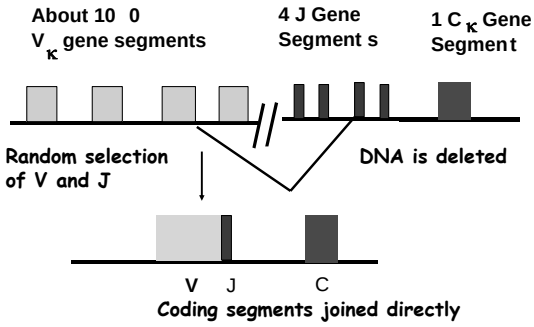
Murine Ig Heavy Chain Gene Organization



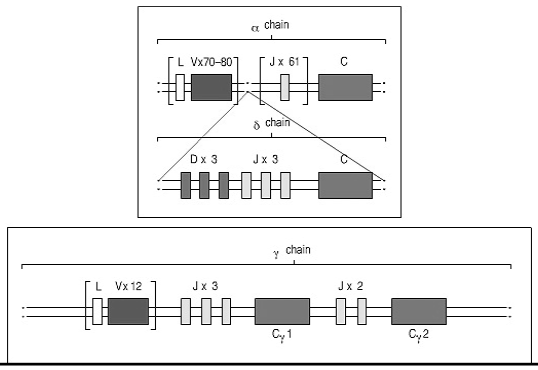
TCR Alpha and Beta Loci



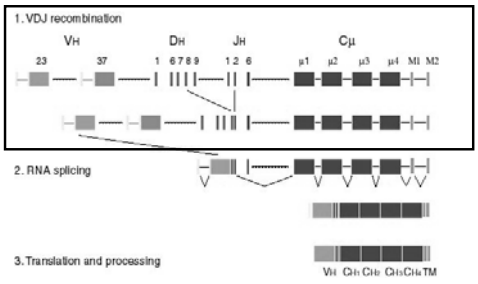
V(D)J Recombination in the Kappa Locus



TCR Delta and Gamma Loci



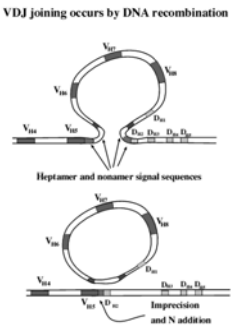
Ig Heavy Chain VDJ Recombination--2 DNA Deletions, DJ and VD



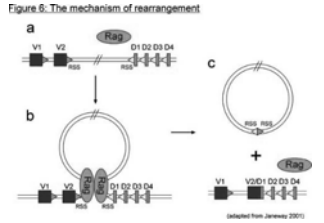
IMMUNOGLOBULIN GENES UNDERGO *TWO* DNA REARRANGEMENTS

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

VDJ Recombination Entails Deletion of Genomic DNA



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



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

- 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.

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

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.: $120x20x4=10^4$
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