

## Chapter 5: Organization and Expression of Immunoglobulin Genes

- I. Genetic Model Compatible with Ig Structure
  - A. Two models for Ab structure diversity
    1. Germ-line theory: maintained that the genome contributed by the germ cells, egg and sperm, contains a large repertoire of immunoglobulin genes
    2. Somatic-variation theory: maintained that the genome contains a small number of immunoglobulin genes, from which a large number of Ab specificities are generated in the somatic cells by mutation or recombination

## Chapter 5 Cont'd

- B. The Two-Gene Model of Dreyer and Bennett
  - said that two separate genes encode a single immunoglobulin heavy or light chain, one gene for the V region (variable region) and the other for the C region (constant region)
  - these two genes must come together at the DNA level to form a continuous message that can be transcribed and translated into a single Ig heavy or light chain
  - their theory was essentially proven correct in time

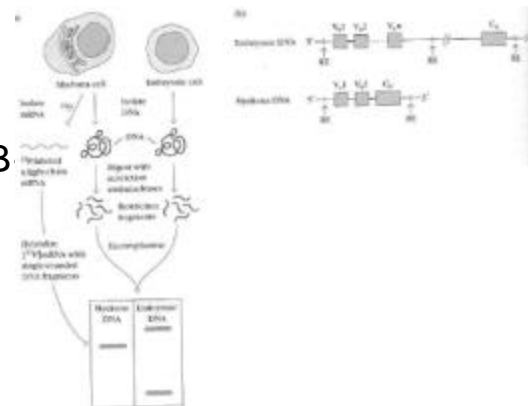
## Chapter 5 Cont'd

### C. Verification of the Dreyer and Bennett Hypothesis

- S. Tonegawa and N. Hozumi found that separate genes encode the V and C regions of Ig's and that the genes are rearranged in the course of B-cell differentiation
- experimental procedure and results are shown on the next slide
- results prove the two-gene model is correct: one gene encodes the V region and one encodes the C region

## Chapter 5 Cont'd

Experimental demonstration that genes encoding K light chains are rearranged during B cell development



## Chapter 5 Cont'd

Chromosomal locations  
of Ig genes in mice  
and humans

TABLE 5-1 CHROMOSOMAL  
LOCATIONS OF IMMUNOGLOBULIN  
GENES IN HUMAN AND MOUSE

| Gene                  | Chromosome |       |
|-----------------------|------------|-------|
|                       | Human      | Mouse |
| $\lambda$ Light chain | 22         | 16    |
| $\kappa$ Light chain  | 2          | 6     |
| Heavy chain           | 14         | 12    |

## Chapter 5 Cont'd

### II. Multigene Organization of Ig Genes

- germ-line DNA contains several coding sequences, called gene segments, separated by noncoding regions
- gene segments are rearranged during B cell maturation to form functional Ig genes
- K and lambda light-chain families contain V, J, and C segments; the rearranged VJ segments encode the variable region of the light chains

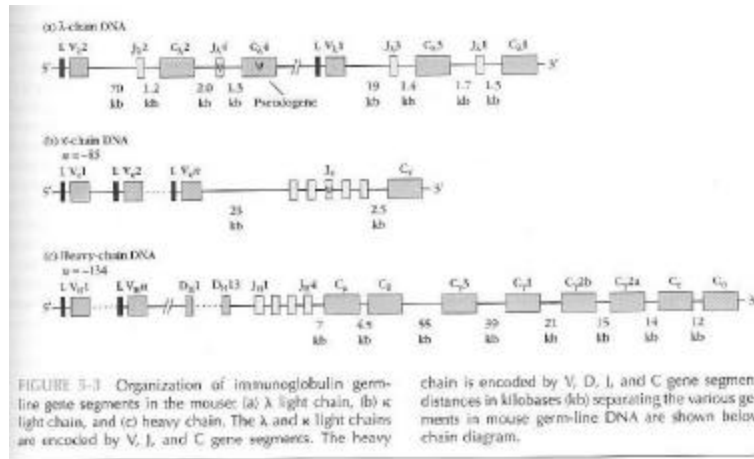
## Chapter 5 Cont'd

- the heavy-chain family contains V, D, J, and C gene segments; the rearranged VDJ gene segments encode the variable region of the heavy chain
- each V gene segment is preceded at its 5' end by a signal or leader (L) peptide that guides the heavy or light chain through the endoplasmic reticulum
- signal peptide is cleaved from the nascent light and heavy chains before assembly of the final Ig molecule

## Chapter 5 Cont'd

- A. lambda-chain multigene family
- functional lambda variable-region gene contains two coding segments – a 5' V segment and a 3' J segment – which are separated by a noncoding DNA sequence in unrearranged germ-line DNA
  - Mouse:
    - has two V-lambda gene segments, four J-lambda gene segments, and four C-lambda gene segments
    - J-lambda4 and C-lambda4 are pseudogenes, which are defective genes that are incapable of encoding protein

## Chapter 5 Cont'd



## Chapter 5 Cont'd

### B. K-chain Multigene Family

- in the mouse, the V-K and J-K gene segments encode the variable region of the K light chain, and the C-K gene segment encodes the constant region

### C. Heavy-chain Multigene Family

- see previous slide for heavy-chain structure

## Chapter 5 Cont'd

### III. Variable-Region Gene Rearrangements

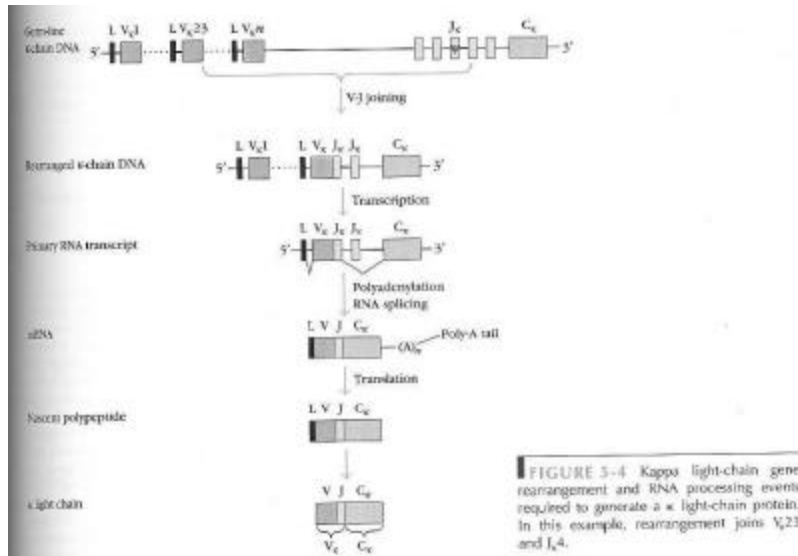
- this mechanism produces mature, immunocompetent B cells; each such cell is committed to produce antibody with a binding site encoded by the particular sequence of its rearranged V genes

## Chapter 5 Cont'd

### A. V-J Rearrangements in Light-Chain DNA

- in humans, any of the functional V-lambda genes can combine with any of the four functional J-lambda-C-lambda combinations
- in human and mouse K light-chain DNA, any one of the V-K gene segments can be joined with any one of the functional J-K gene segments
- see next slide for Kappa light-chain gene rearrangement and RNA processing events required to generate a K light-chain protein

## Chapter 5 Cont'd

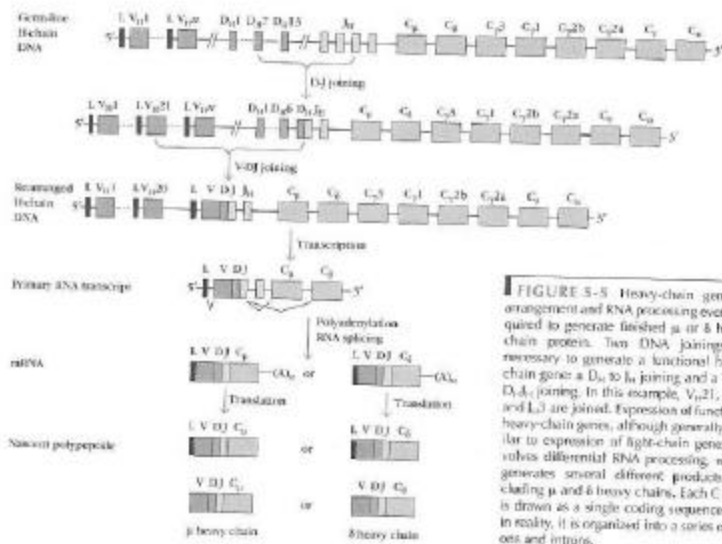


## Chapter 5 Cont'd

### B. V-D-J Rearrangements in Heavy-Chain DNA

- look at next slide for heavy-chain gene rearrangement and RNA processing events required to generate finished  $\mu$  or  $\delta$  heavy chain protein

## Chapter 5 Cont'd



## Chapter 5 Cont'd

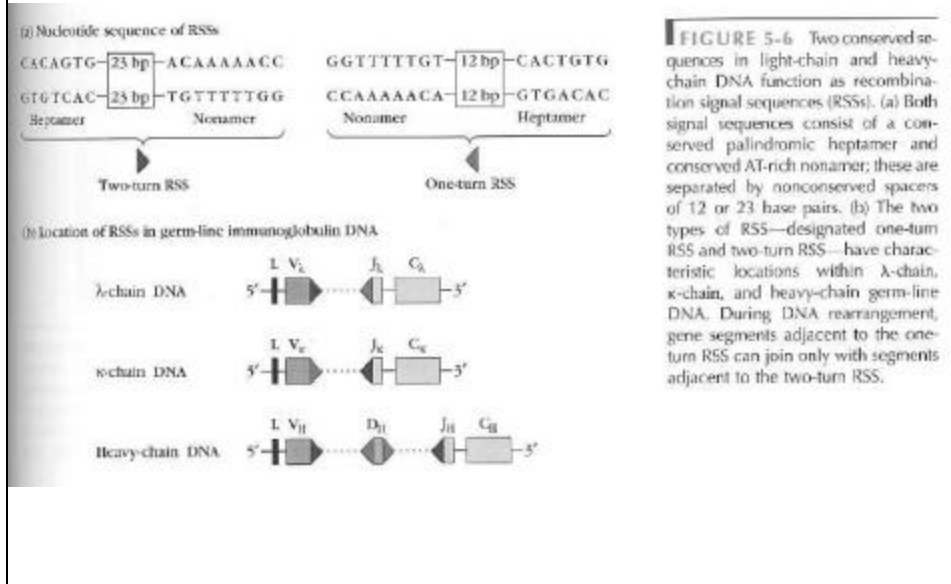
### IV. Mechanism of Variable-Region DNA Rearrangements

#### A. Recombination Signal Sequences

- flank each germ-line V, D, and J gene segment
- function as signals for the recombination process that rearranges the genes
- see next slide



## Chapter 5 Cont'd

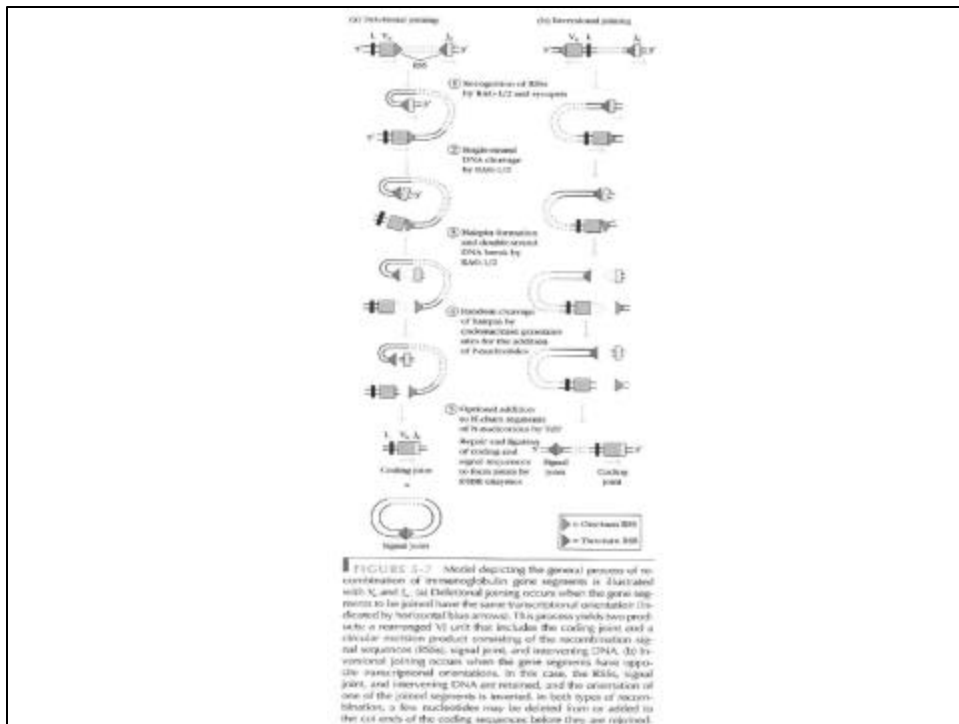


**FIGURE 5-6** Two conserved sequences in light-chain and heavy-chain DNA function as recombination signal sequences (RSSs). (a) Both signal sequences consist of a conserved palindromic heptamer and conserved AT-rich nonamer; these are separated by nonconserved spacers of 12 or 23 base pairs. (b) The two types of RSS—designated one-turn RSS and two-turn RSS—have characteristic locations within  $\lambda$ -chain,  $\kappa$ -chain, and heavy-chain germ-line DNA. During DNA rearrangement, gene segments adjacent to the one-turn RSS can join only with segments adjacent to the two-turn RSS.

## Chapter 5 Cont'd

### B. Enzymatic Joining of Gene Segments

- V(D)J recombinase: catalyzes V-(D)-J recombination, which takes place at the junctions between RSSs and coding sequences
- recombination-activating genes (RAG-1 and RAG-2) encode for proteins that act synergistically to mediate V-(D)-J joining
- next slide shows that recombination of variable-region gene segments is a multistep process catalyzed by a system of recombinase enzymes



## Chapter 5 Cont'd

### C. Defects in Ig-Gene Rearrangements

- knockout mice lacking either RAG-1 or RAG-2 are unable to initiate the recombination process because they cannot introduce double-strand DNA breaks between the RSSs and coding sequences in germ-line immunoglobulin DNA

- result: V, D, and J gene segments remain unrearranged

## Chapter 5 Cont'd

- since both B and T cells utilize the same recombination machinery, the RAG-1- and RAG-2- knockout mice lack mature T and B cells and consequently exhibit a severe combined immunodeficiency (SCID)

- next slide demonstrates these two recombination defects

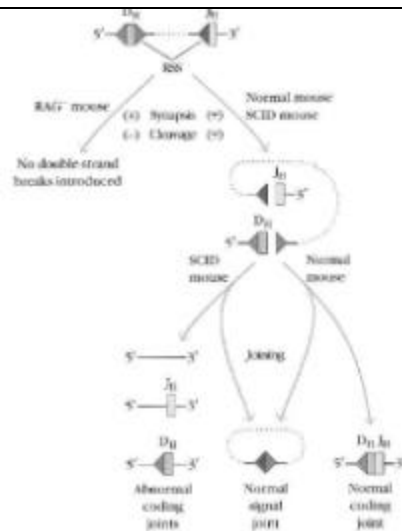


FIGURE 5-10 Recombination defects have been identified in RAG-deficient mice and SCID mice. Mice that lack a functional RAG-1 or RAG-2 cannot even start the recombination process. In contrast, SCID mice can carry out synapsis between  $D_H$  and  $J_H$  gene segments, introduce double-strand breaks to produce normal recombination intermediates, and form a normal signal joint. However, SCID mice cannot properly join the coding sequences. Both types of defective mice lack mature B and T cells and thus exhibit a severe combined immunodeficiency. Adapted from FW Alt et al. 1992. [https://doi.org/10.1016/0092-8674\(92\)90001-0](https://doi.org/10.1016/0092-8674(92)90001-0)

## Chapter 5 Cont'd

### D. Productive and Nonproductive Rearrangements

- nonproductive rearrangement: imprecise joining that results in gene segments that are joined out of phase, so that the triplet reading frame for translation is not preserved.

- resulting VJ or VDJ unit will contain numerous stop codons, which interrupt translation

## Chapter 5 Cont'd

- Productive rearrangement: the resulting VJ or VDJ unit can be translated in its entirety, which will yield a complete antibody

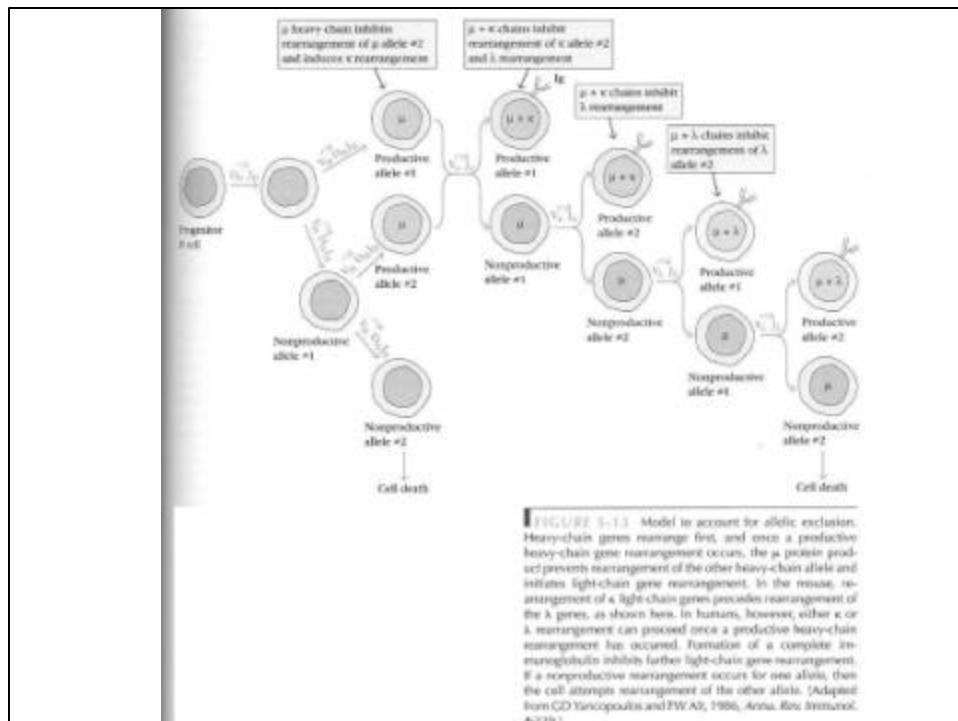
- these two type of rearrangements that occur produce only about 8% of pre-B cells in the bone marrow that undergo maturation and leave as mature, immunocompetent B cells

## Chapter 5 Cont'd

### E. Allelic Exclusion

- even though a B cell is diploid, it expresses the rearranged heavy-chain genes from only one chromosome and the rearranged light-chain genes from only one chromosome. This is known as allelic exclusion

- this ensures that functional B cells never contain more than one VDJ from the heavy chain and one VJ unit from the light chain



## Chapter 5 Cont'd

### V. Generation of Antibody Diversity

#### A. Multiple Germ-Line V, D, and J Gene Segments

- contribute to the diversity of the antigen-binding sites in antibodies

#### B. Combinatorial V-J and V-D-J Joining

- in humans, the ability of any of the 51 V<sub>H</sub> gene segments to combine with any of the 27 D<sub>H</sub> segments and any of the 6 J<sub>H</sub> segments allows a considerable amount of heavy-chain gene diversity to be generated

## Chapter 5 Cont'd

### C. Junctional Flexibility

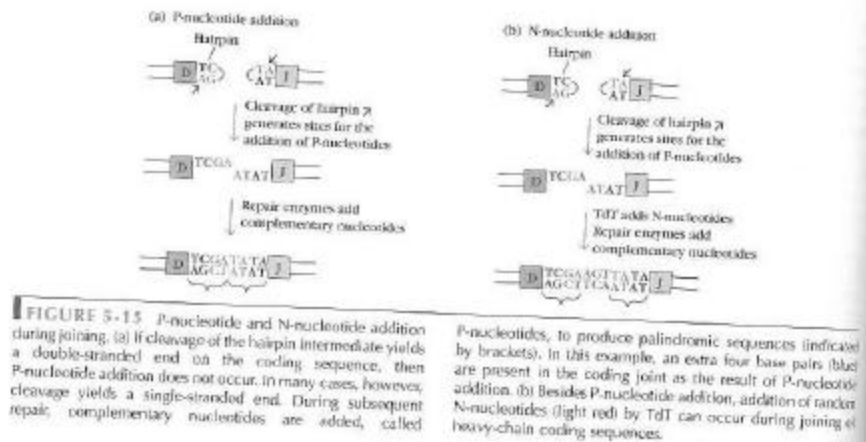
- increases the enormous diversity generated by means of V, D, and J combinations

- can lead to many nonproductive rearrangements, but it also generates several productive combinations that encode alternative amino acids at each coding joint, thereby increasing antibody diversity

## Chapter 5 Cont'd

### D. P-Addition

### E. N-Addition



## Chapter 5 Cont'd

### F. Somatic Hypermutation

- results in additional antibody diversity that is generated in rearranged variable-region gene units

- also causes individual nucleotides in VJ or VDJ units to be replaced with alternatives, thus possibly altering the specificity of the encoded immunoglobulins

## Chapter 5 Cont'd

- usually occurs within germinal centers
- targeted to rearranged V-regions located within a DNA sequence containing about 1500 nucleotides, which includes the whole of the VJ or VDJ segment
- largely random
- affinity maturation: following exposure to antigen, those B cells with higher affinity receptors will be selected for survival because of their greater ability to bind to the antigen
  - this takes place in germinal centers

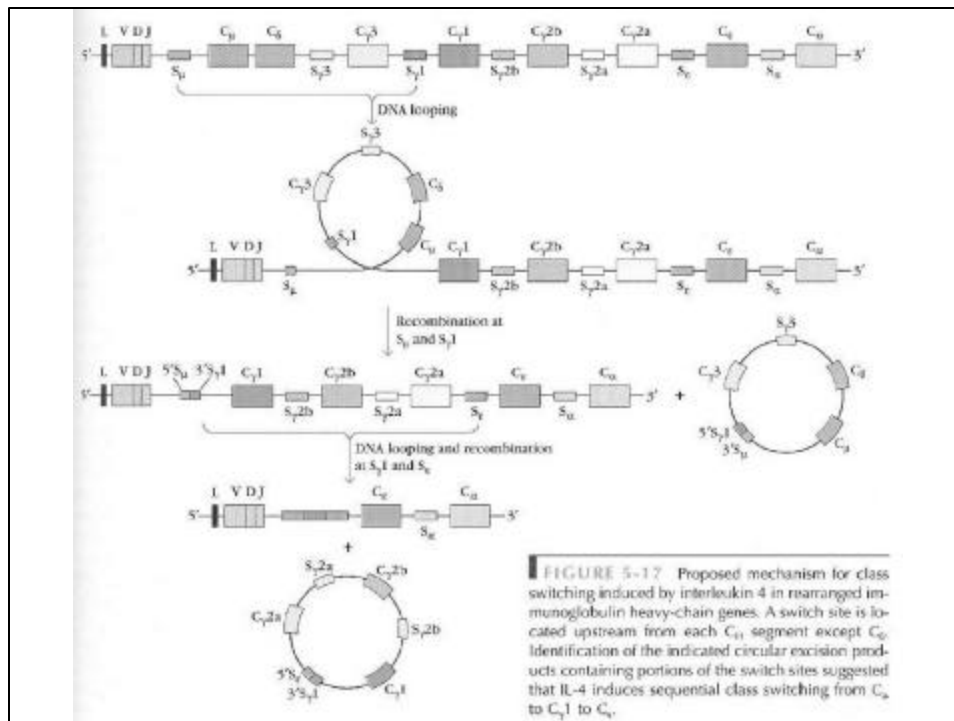
## Chapter 5 Cont'd

- G. Association of Heavy and Light Chains
  - combinational association of H and L chains also can generate antibody diversity
- VI. Class Switching Among Constant-Region Genes
  - class switching: after antigenic stimulation of a B cell the heavy chain DNA can undergo a further rearrangement in which the VhDhJh unit can combine with any Ch gene segment



## Chapter 5 Cont'd

- switch region: DNA flanking sequences located 2-3 kb upstream from each Ch segment that are involved in class switching
- example of class switching on next slide



## Chapter 5 Cont'd

### VII. Expression of Ig Genes

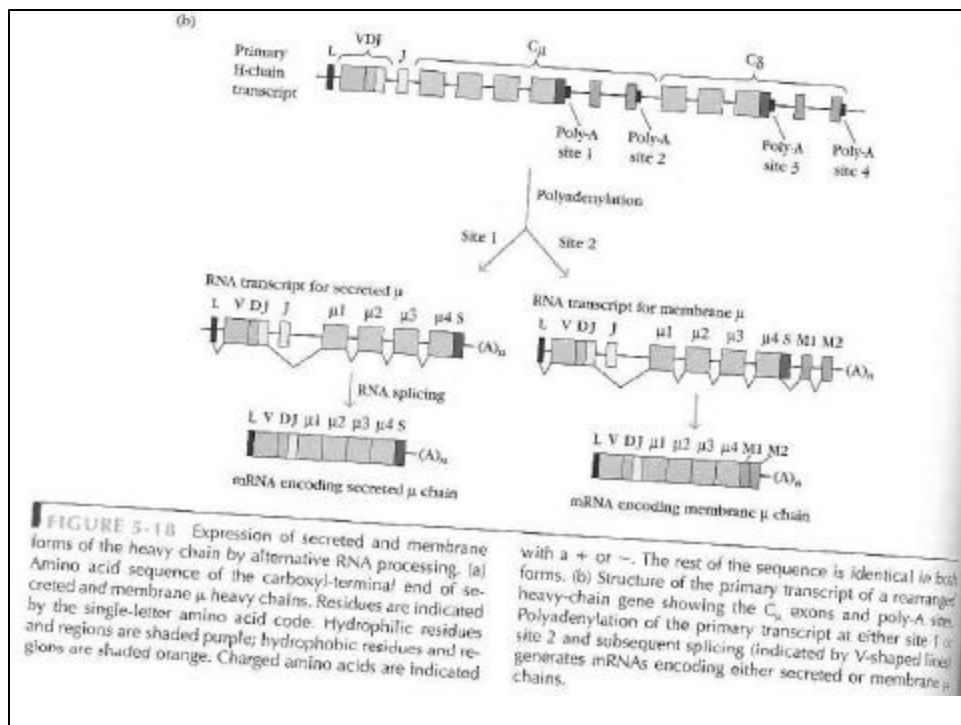
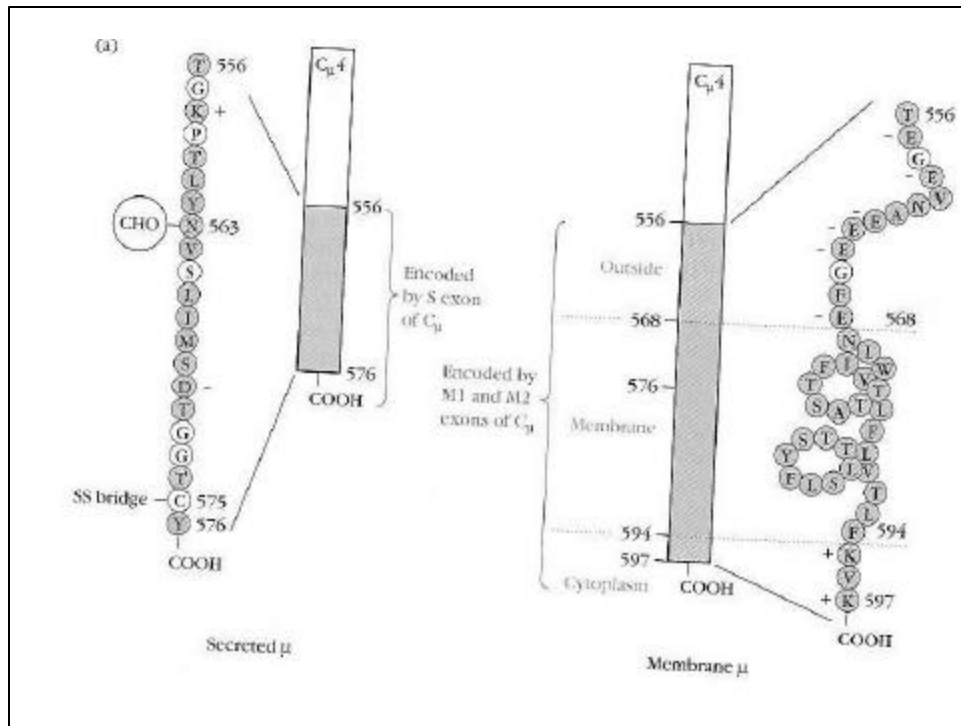
#### A. Differential RNA Processing of Heavy-Chain Primary Transcripts

- this can yield different mRNA's
- this processing explains the production of secreted or membrane-bound forms of a particular immunoglobulin and the simultaneous expression of IgM and IgD by a single B cell

## Chapter 5 Cont'd

### 1. Expression of Membrane or Secreted Immunoglobulin

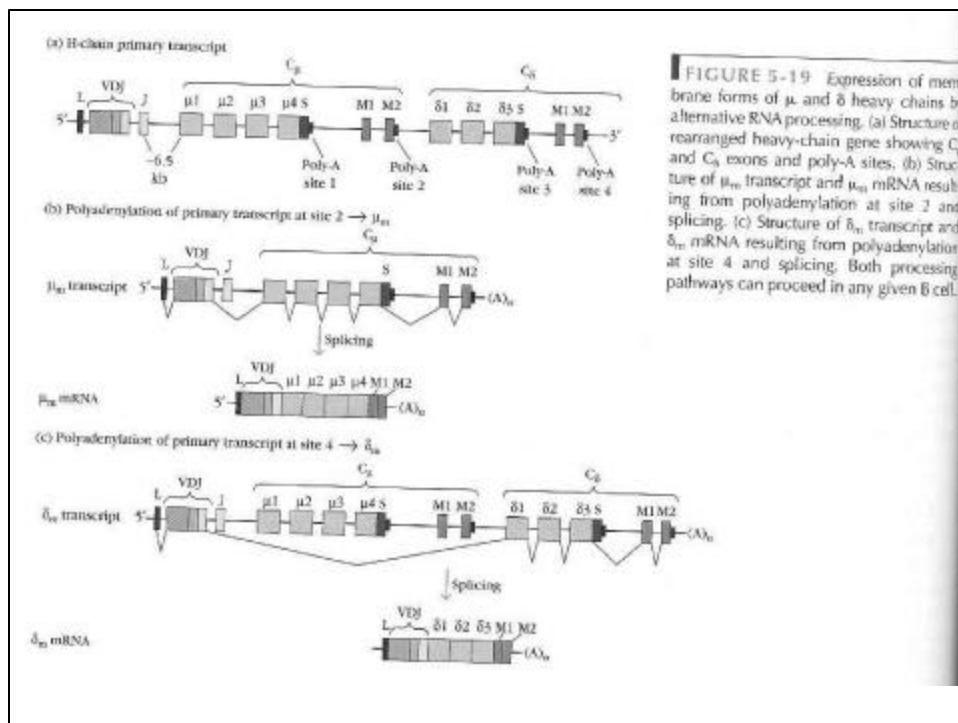
- difference depends on differential processing of a common primary transcript
- mature naïve B cells produce only membrane-bound antibody
- differentiated plasma cells produce secreted antibodies



## Chapter 5 Cont'd

### 2. Simultaneous Expression of IgM and IgD

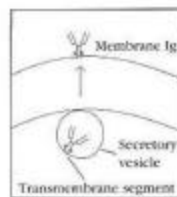
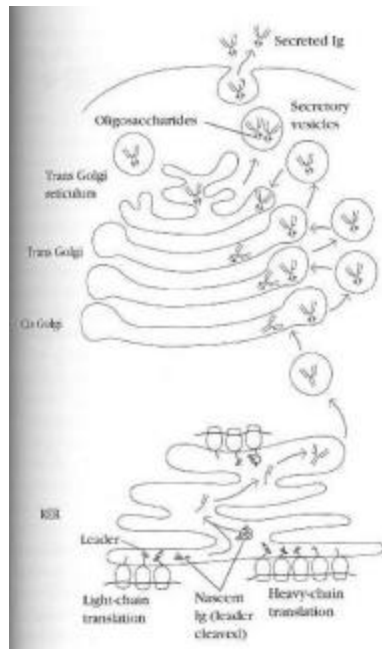
- controlled by differential RNA processing
- if the heavy chain transcript is cleaved and polyadenylated at site 2 after the C $\mu$  exons, then the mRNA will encode the membrane form of the  $\mu$  heavy chain
- if polyadenylation is instead further downstream at site 4, after the C $\delta$  exons, then RNA splicing will remove the intervening C $\mu$  exons and produce mRNA encoding the membrane form of the delta heavy chain



## Chapter 5 Cont'd

### B. Synthesis, Assembly, and Secretion of Immunoglobulin

- heavy and light chains are synthesized on separate polyribosomes of the rough ER
- next slide shows this process



**FIGURE 5-20** Synthesis, assembly, and secretion of the immunoglobulin molecule. The heavy and light chains are synthesized on separate polyribosomes (polysomes). The assembly of the chains to form the disulfide-linked and glycosylated immunoglobulin molecule occurs as the chains pass through the cisternae of the rough endoplasmic reticulum (RER) into the Golgi apparatus and then into secretory vesicles. The main figure depicts assembly of a secreted antibody. The inset depicts a membrane-bound antibody, which contains the carboxyl-terminal transmembrane segment. This form becomes anchored in the membrane of secretory vesicles and then is inserted into the cell membrane when the vesicles fuse with the membrane.

## Chapter 5 Cont'd

### VIII. Regulation of Ig-Gene Transcription

- three regulatory sequences in DNA regulate transcription of immunoglobulin genes:

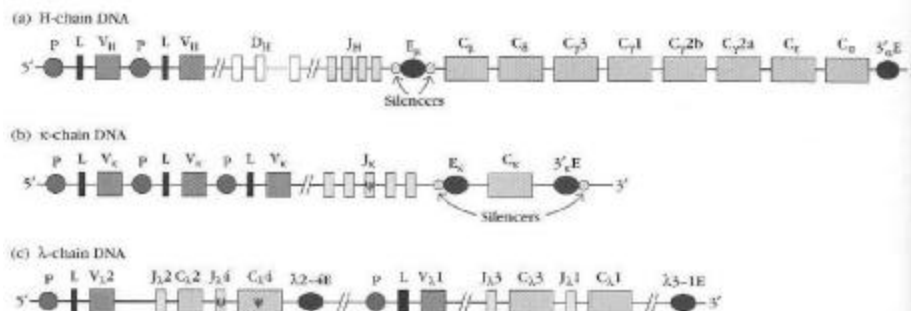
1. promoters: promote initiation of RNA transcription in a specific direction
  - located about 200 bp upstream from transcription initiation site

## Chapter 5 Cont'd

2. Enhancers: nucleotide sequences located some distance upstream or downstream from a gene that activate transcription from the promoter sequence in an orientation-independent manner
3. Silencers: nucleotide sequences that down-regulate transcription, operating in both directions over a distance

## Chapter 5 Cont'd

- each Vh and Vl gene segment has a promoter located just upstream from the leader sequence
- these promoters contain a TATA Box
- location of these regulatory sequences are shown on next slide



**FIGURE 5-21** Location of promoters (dark red), enhancers (green), and silencers (yellow) in mouse heavy-chain,  $\kappa$  light-chain, and  $\lambda$  light-chain germ-line DNA. Variable-region DNA rearrangement moves an enhancer close enough to a promoter that the enhancer can activate transcription from the promoter.

## Chapter 5 Cont'd

### A. Effect of DNA Rearrangement on Transcription

- rate of transcription of a rearranged VJ<sub>H</sub>I or V<sub>H</sub>D<sub>H</sub>J<sub>H</sub> unit is as much as 10,000X the rate of transcription of unrearranged V<sub>I</sub> or V<sub>H</sub> segments
- oncogenes: genes that promote cellular proliferation or prohibit cell death
  - these can often translocate to the immunoglobulin heavy or light chain loci

## Chapter 5 Cont'd

### B. Inhibition of Ig-Gene Expression in T cells

- complete Ig-gene rearrangement of H and L chains occurs only in B cells
- complete TCR-gene rearrangement is limited to T cells



## Chapter 5 Cont'd

### IX. Antibody Genes and Antibody Engineering

- possible to design and construct genes that encode immunoglobulin molecules in which the variable regions come from one species and the constant regions come from another

## Chapter 5 Cont'd

### A. Chimeric and Hybrid Monoclonal Antibodies

- to make an antibody one needs to clone recombinant DNA containing the promoter, leader, and variable-region sequences from a mouse antibody gene and the constant-region exons from a human antibody gene
- the Ab encoded by such a recombinant gene is a mouse-human chimera, also known as humanized antibody

## Chapter 5 Cont'd

- its antigenic specificity is determined by the variable region and is derived from the mouse DNA
- its isotype is determined by the constant region and is derived from the human DNA
- the process is shown on the next slide

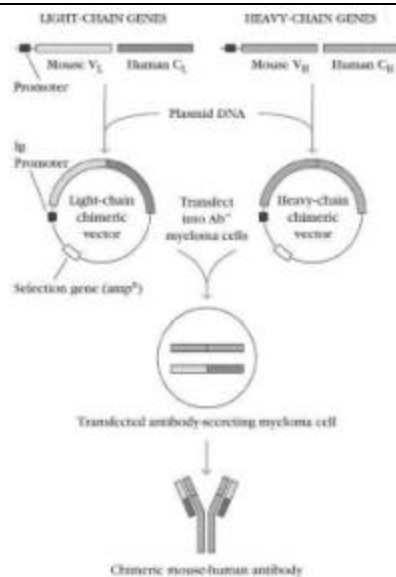
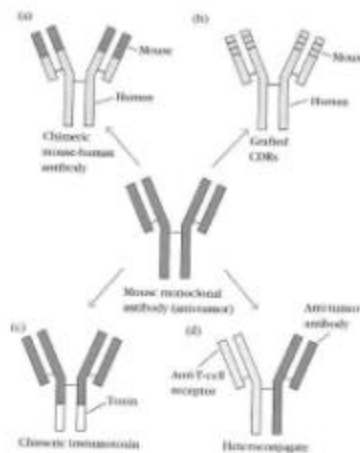


FIGURE 5-22 Production of chimeric mouse-human monoclonal antibodies. Chimeric mouse-human heavy- and light-chain expression vectors are produced. These vectors are transfected into Ab<sup>+</sup> myeloma cells. Culture in ampicillin medium selects for transfected myeloma cells which secrete the chimeric antibody. [Adapted from M Verhoeyen and I. Reichmann, 1988. *BioEssays* 10:74.]

## Chapter 5 Cont'd

- heteroconjugates (or bispecific antibodies): hybrids of two different Ab molecules

- constructed by chemically crosslinking two different Ab's or synthesized in hybridomas consisting of two different monoclonal-Ab-producing cell lines that have been fused



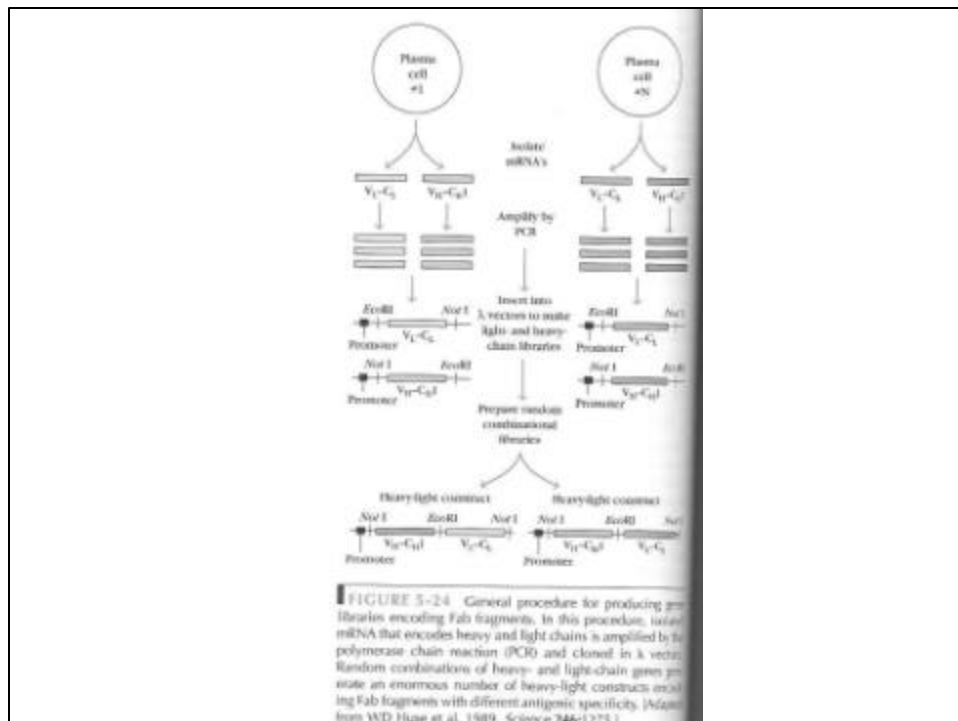
**FIGURE 5-23** Chimeric and hybrid monoclonal antibodies engineered by recombinant DNA technology. (a) Chimeric mouse-human monoclonal antibody containing the V<sub>H</sub> and V<sub>L</sub> domains of a mouse monoclonal antibody (blue) and C<sub>H</sub> and C<sub>L</sub> domains of a human monoclonal antibody (gray). (b) A chimeric monoclonal antibody containing only the CDRs of a mouse monoclonal antibody (blue bands) grafted within the framework regions of a human monoclonal antibody. (c) A chimeric monoclonal antibody in which the terminal Fc domain is replaced by toxin chains (white). (d) A heteroconjugate in which one-half of the mouse antibody molecule is specific for a tumor antigen and the other half is specific for the CD3/7-cel6 receptor complex.

## Chapter 5 Cont'd

### B. Monoclonal Antibodies Constructed from Ig-Gene Libraries

- use of PCR to amplify the DNA that encodes Ab heavy chain and light chain Fab fragments from hybridoma cells or plasma cells

- process shown on next slide

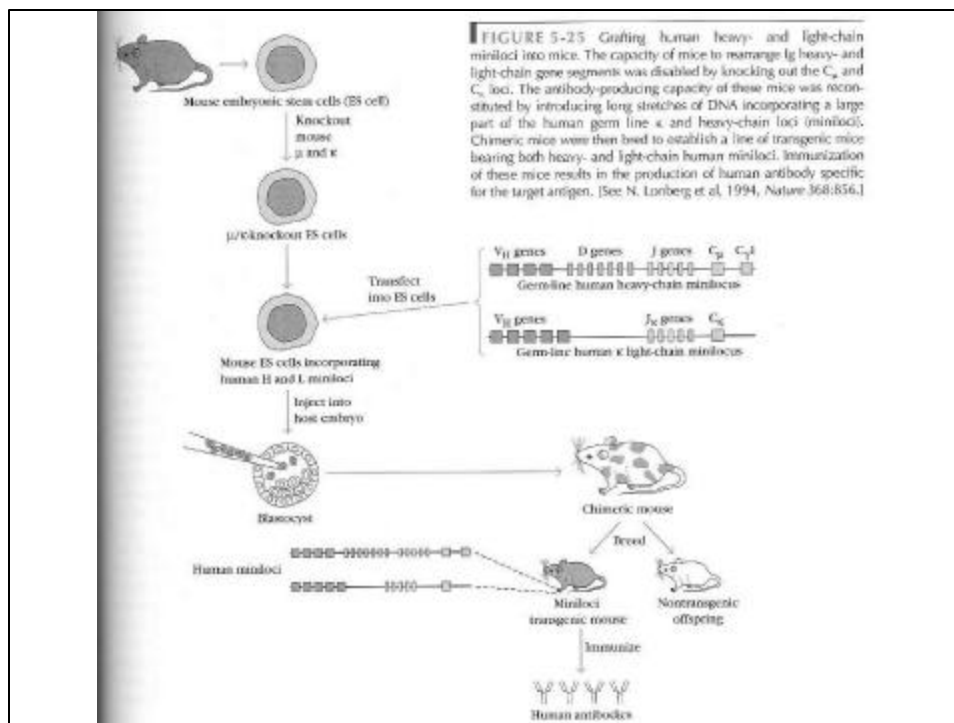


## Chapter 5 Cont'd

- this procedure generates a diversity of Ab specificities; clones containing these random combinations of H + L chains can be rapidly screened for those secreting Ab to a particular Ag

### C. Mouse with Human Instead of Mouse Immunoglobulin Loci

- one can knockout the heavy and light chain immunoglobulin loci in mouse embryonic stem cells and introduce very large DNA sequences containing human heavy and light chain gene segments



## Chapter 5 Cont'd

- advantage of this process: completely human antibodies are made in cells of the mouse B-cell lineage, from which antibody-secreting hybridomas are readily derived by cell fusion
- therefore, this process offers a solution to the problem of producing human monoclonal Ab's of any specificity desired