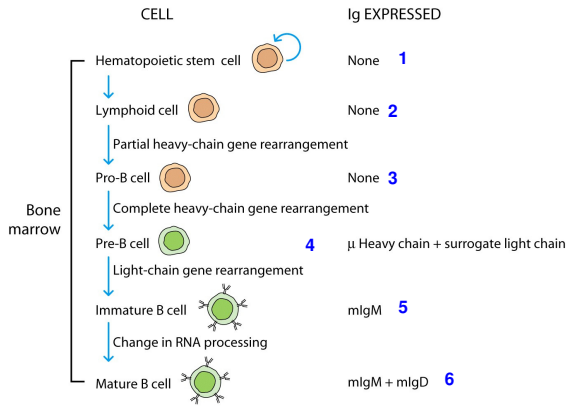
Chapter 5

Organization and Expression of Immunoglobulin Genes



Genetic Models

- **How to account for :**
  - 1) Vast diversity of antibody specificities
  - 2) Presence of Variable regions at the amino end of Heavy and Light chains, and a Constant region at the carboxyl end
  - 3) Existence of isotypes (different Heavy chains) with same antigenic specificity

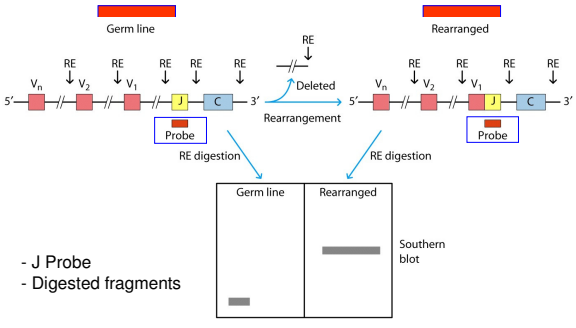
Models to Explain Antibody Diversity

1) **The Germ Line Theory**: "genome posses the large repertoire of antibody genes to account for all the antibody diversity"
2) **The Somatic Variation Theory**: "genome posses a relatively small number of antibody genes and diversity is generated by mutation and recombination of these genes during somatic development"

The two-gene model

- Developed by Dreyer and Bennet in 1965
- Two separate genes code for the Heavy and Light chains. One codes for the V region and the other for the C region
- These genes come together during at the DNA level to form a continuous message
- There are thousands of V genes in germ line but only one gene for the C region

Tonegawa (1976): Immunoglobulin gene rearrangement



Three genetic loci encode immunoglobulin molecules:

- **Two loci encoding the light chains**
  - kappa locus
  - lambda locus

- **One locus encoding the heavy chain**

These three loci are located on different chromosomes.

TABLE 5-1 Chromosomal locations of immunoglobulin genes in human and mouse

Gene	CHROMOSOME Human	Mouse
λ Light chain	22	16
κ Light chain	2	6
Heavy chain	14	12

Table 5-1
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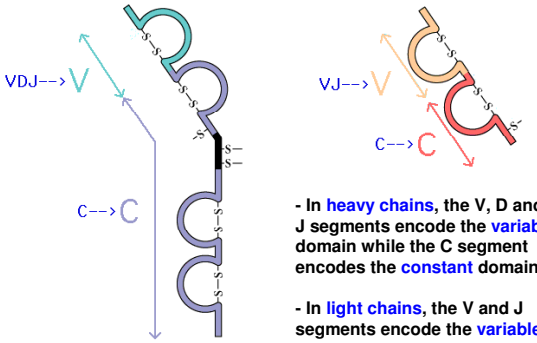
Multigene Families

- **Light Chains**: V, J and C gene segments.
- **Lambda**: Humans (30V, 4J and 7C genes)
- **Kappa**: Humans (40V, 5J and 1C genes)

- **Heavy Chains**: V, **D**, J and C gene segments
- **Heavy Chains**: Humans (50V, 25D, 6J and **8** C genes)



- In **heavy chains**, the V, D and J segments encode the **variable** domain while the C segment encodes the **constant** domain.

- In **light chains**, the V and J segments encode the **variable** domain while the C segment encodes the **constant** domain.

The loci encoding immunoglobulins have a unique structure.
- **composed of "gene segments"**

- **The heavy chain locus has multiple V (variable) segments, multiple D (diversity) segments, multiple J (joining) segments and multiple C (constant) segments.**

During maturation, one of each V, D and J segment is randomly "chosen" and used to encode the final antibody molecule.

Germline configuration of the heavy chain locus (mice)

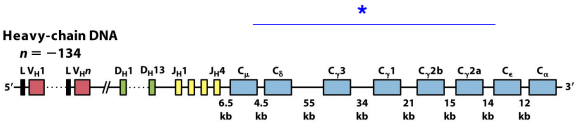


Figure 5-3c
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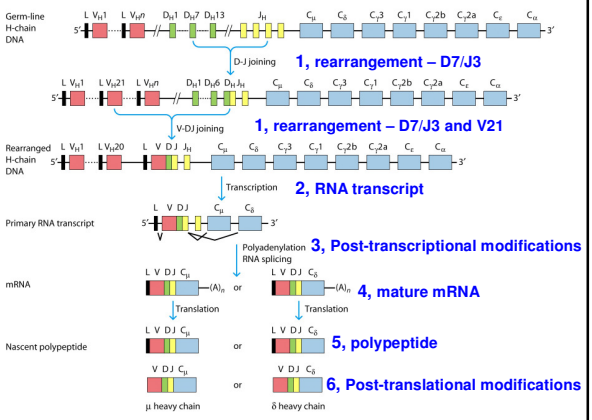

Gene rearrangement Kappa light Chain

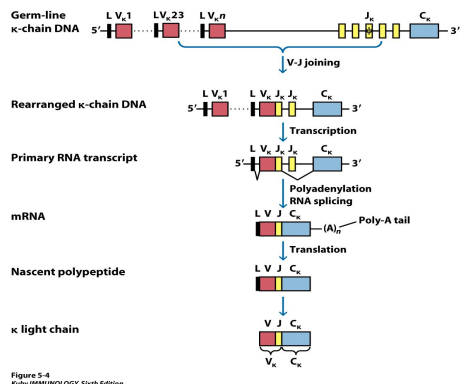

Figure 5-4
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Kuby Figure 5-4

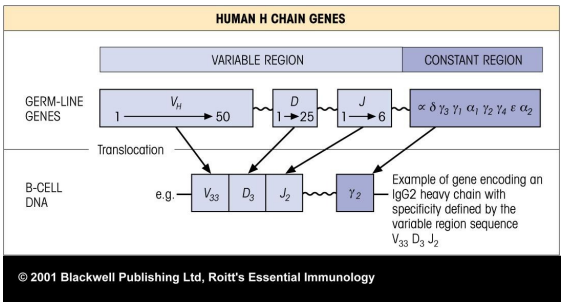
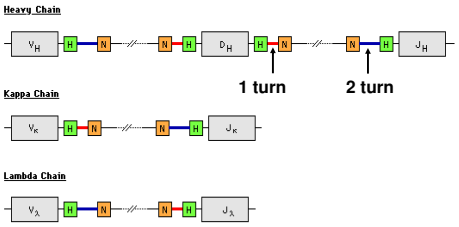


Figure 3.7

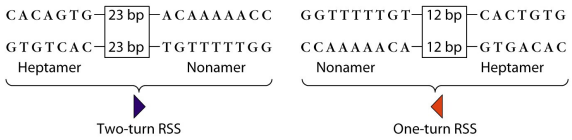
What mechanism ensures correct joining of gene segments during rearrangement of the heavy and light chain loci?

- **Recombination signal sequences** - conserved sequences in regions just upstream or downstream of gene segments.
- Consist of a conserved **heptamer** and **nonamer (orange)** with a 12 or 23 bp spacer.
- The **one-turn (red)/two-turn rule (blue) - (12/23 rule)** - recombination occurs only between a segment with a 12 bp spacer and a 23 bp spacer.



Recombinant Signal Sequences (RSS)

(a) Nucleotide sequence of RSSs

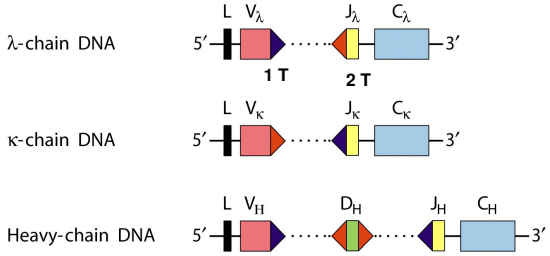


RSS – At 3' of V genes, 5' of J genes and at both sides in D genes

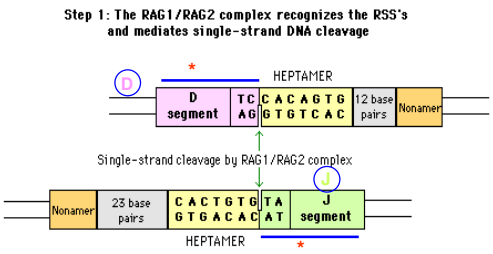
Rule: 12 (1 turn) or 23 (two turn) base pairs with conserved flanking heptamer and nonamer

*** Only 1 to 2 turn**

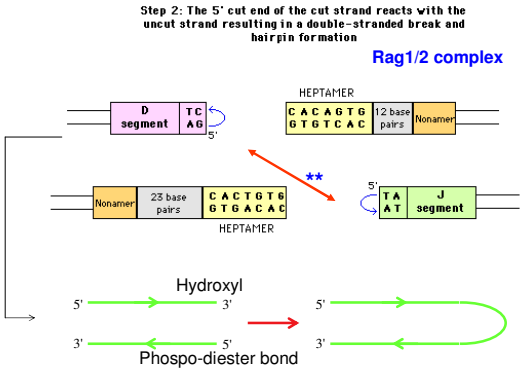
(b) Location of RSSs in germ-line immunoglobulin DNA



- **Rearrangement of gene segments is mediated by the RAG1/RAG2 enzyme complex (V(D)J recombinases).**
- **The RAG1/RAG2 complex recognizes the heptamer/nonamer sequences and cuts one strand of the DNA.**

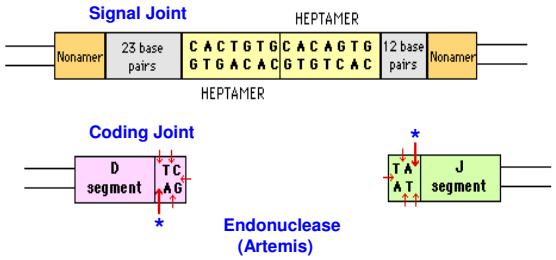


A hairpin forms…



The hairpin is cut at a random site…

Step 3: The heptamer sequences are ligated. An endonuclease cleaves the hairpin at a random site.



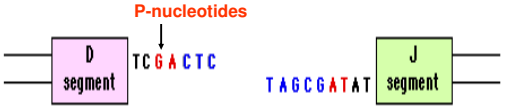
Palindromic sequences may form…

Step 4: Endonuclease cleavage may result in short palindromes - additional nucleotides resulting from this are known as P-nucleotides.

Terminal deoxynucleotidyl transferase (Tdt)

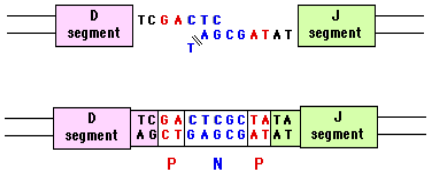
An enzyme that randomly adds in nucleotides during joining of coding gene segments.

Step 5: TdT adds N-nucleotides randomly to the single stranded ends.

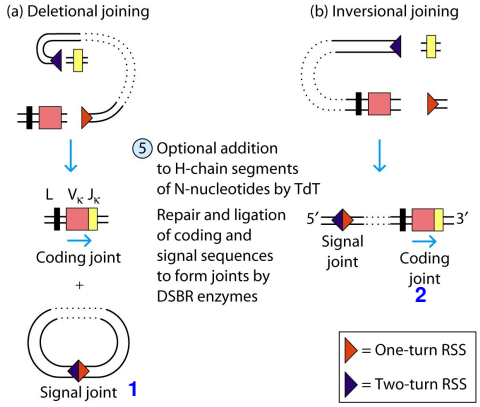


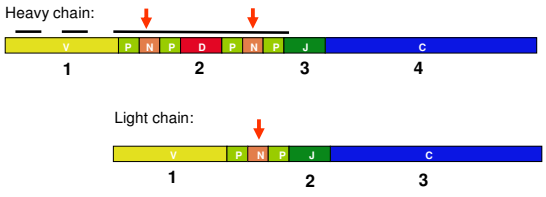
The join is repaired…

Step 6: The two single-stranded ends pair. Unpaired nucleotides are trimmed by an exonuclease and the coding joint is repaired



Note: Keep in mind that this random rearrangement can lead to PRODUCTIVE and NON-PRODUCTIVE gene rearrangements





The final "gene" encoding the antibody produced by a B cell (and T cells) consists of a number of different segments.

This process of recombination of different gene segments and addition of **P** and **N nucleotides** ensures that an enormous number of different antigen specificities are possible.

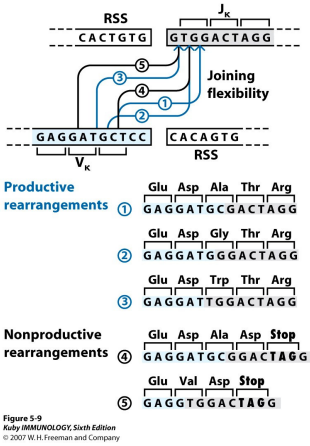


Figure 5-9
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Generation of antibody diversity

1. Multiple germline V, D and J gene segments
2. Combinatorial V-J and V-D-J joining
3. Somatic hypermutation
4. Junctional flexibility
5. P-nucleotide addition
6. N-nucleotide addition
7. Combinatorial association of heavy and light chains

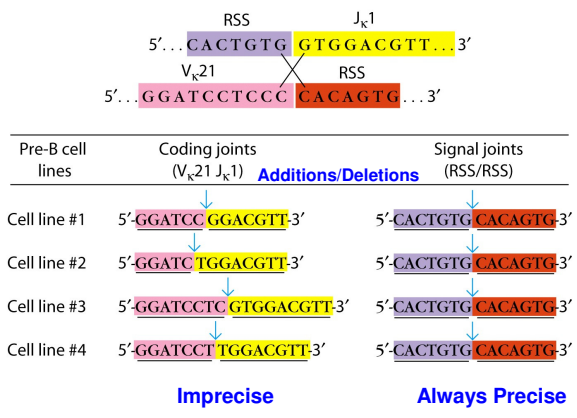
Combinatorial V-J and V-D-J joining

- Humans:
  - Heavy Chain: V (51), D (27), J (6) = 8262
  - Light Chain: Kappa – V (40), J (5) = 200
    Lambda – V(30), J (4) = 120

8262 x (200 x 120) = **2.64×10^6**

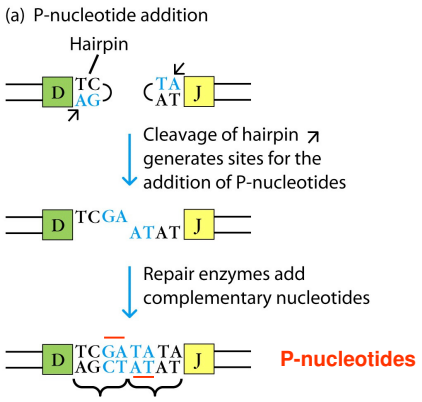
Junctional flexibility

- Generated through V, D and J combinations
- Joining of Recombination Signal Sequences = Signal Joint
- Joining of Coding Sequences = Coding Joint
- Signal Joints ALWAYS joined precisely, but joining of Coding Joints is IMPRECISE
- Good = Antibody diversity
- BAD = Non=productive rearrangements



P-nucleotide addition

- Cleavage of the Hairpin at the end of the coding sequence by endonuclease (Artemis) is random
- Generates a short single strand of nucleotides at the end of the Coding sequence
- Addition of complementary nucleotides to this strand forms a palindrome sequence (P nucleotides)



N-nucleotide addition

- Once complementary nucleotides to this strand have been added to form a palindrome sequence (P nucleotides)
- The enzyme TdT (terminal deoxynucleotidyl transferase) fills the gap with N nucleotides.
- This enzyme can add randomly up to 15 N nucleotides (non-genomic)
- N nuclotides can be added to the **D-J** and **V-DJ** in the H chain (and to the **V-J** in the L chain)

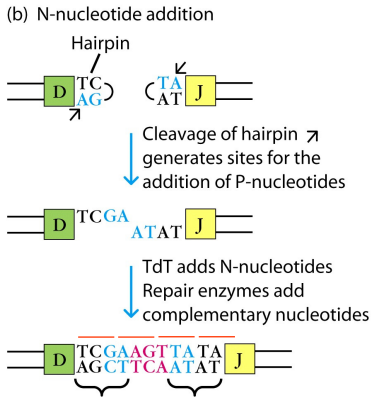
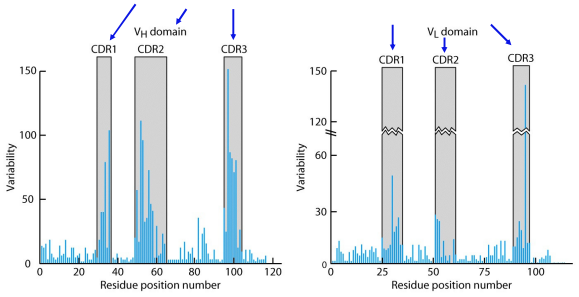


TABLE 5-3 Sources of sequence variation in complementarity-determining regions of immunoglobulin heavy- and light-chain genes

Source of variation	CDR1	CDR2	CDR3 ****
Sequence encoded by:	V segment	V segment	V_L-J_L junction; V_H-D_H-J_H junctions
Junctional flexibility	–	–	+
P-nucleotide addition	–	–	+
N-nucleotide addition*	–	–	+
Somatic hypermutation	+	+	+

*N-nucleotide addition occurs only in heavy-chain DNA.

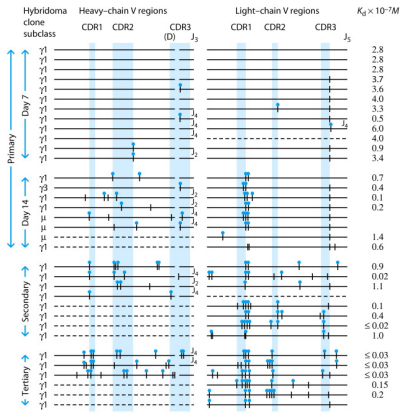
Complementarity-Determining Regions, or CDRs.



Heavy Chain **Light Chain**

Somatic Hypermutation

- Generated **point mutations** in gene segments for variable regions (VDJ and VJ segments)
- Takes place in secondary lymphoid organs (~ 1 week after contact with antigen)
- In mature B cells mutations are clustered in CDRs regions
- **Somatic hypermutation leads to Affinity maturation-** selection process leading to survival of those B cells with high affinity for the antigen



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2.64 x 10^6 to 7.2 x x 10^7 variabilities!!!!

ALLELIC EXCLUSION:

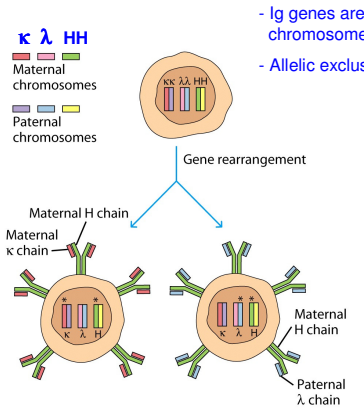
-We have two copies (alleles) of each Ig gene - one inherited from our father and one from our mother.

- In most cases, both genes are expressed.

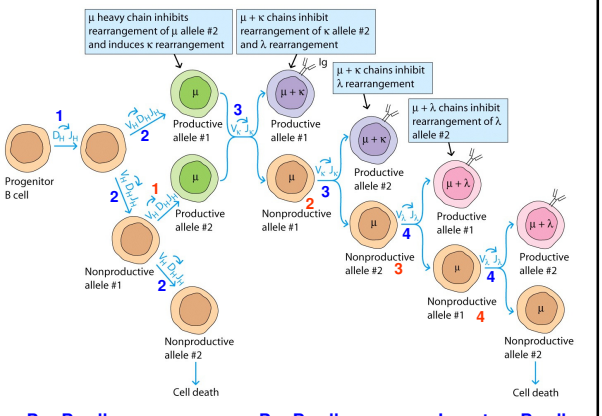
- But Antibody genes are different! …. Only one heavy chain allele and one light chain allele is expressed!!!

- This is termed allelic exclusion (one allele is excluded). Once a productive arrangement is made, the other allele is suppressed

- Why? To ensure that each B cell makes antibody of a single specificity.



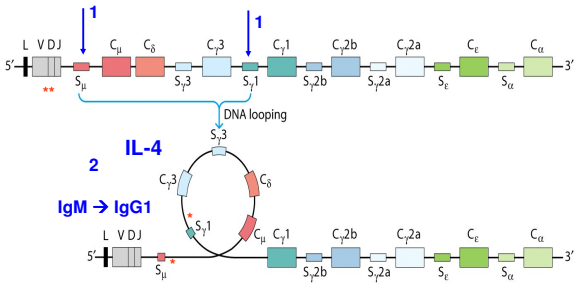
- Ig genes are located in 3 chromosomes
- Allelic exclusion

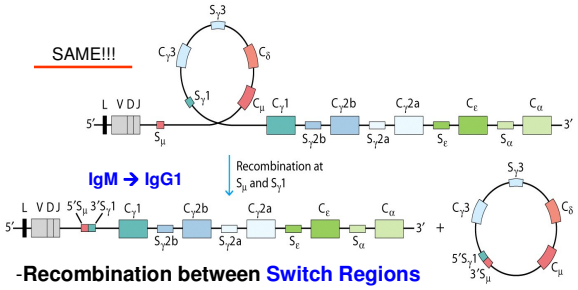


Class Switching

- Antigen stimulation of a B cells → Antibodies with same variable Heavy (VDJ) with any C_H gene segment
- Process dependent on Switch Regions
- Switch Regions (2-3 kb) are located upstream from each C_H segment, except IgD (Cδ)
- Process driven by cytokines:
  - IL-4 → IgM to IgG1 or IgE
  - IFN-γ → IgM to IgG2a
- Players in regulation: 1) switch regions, 2) switch recombinases, 3) cytokine signals

Class Switching





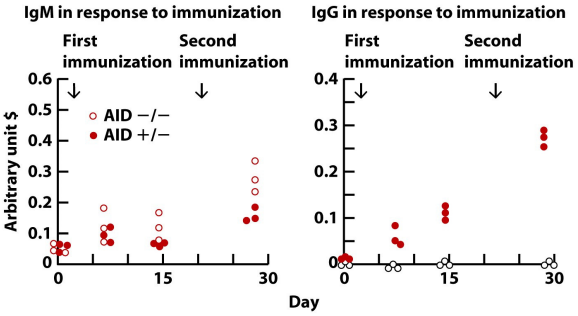
-Recombination between Switch Regions

-Switching only proceeds downstream

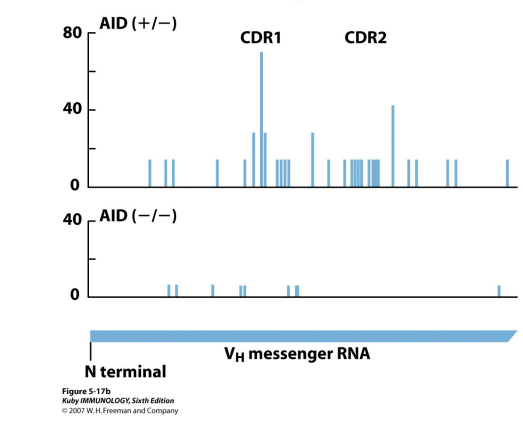
Note: Same specificity but different H chain

AID Enzyme

- Activation induced cytidine deaminase
- RNA editing enzyme
- Deamination of cytosine → uracyl → repair induces base modifications!!!
- Mediates SOMATIC HYPERMUTATION, GENE CONVERSION, and CLASS switching recombination

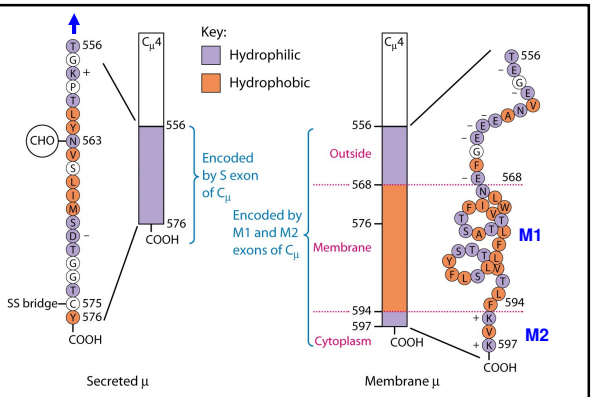


1) No class switching
2) Little or no point mutations

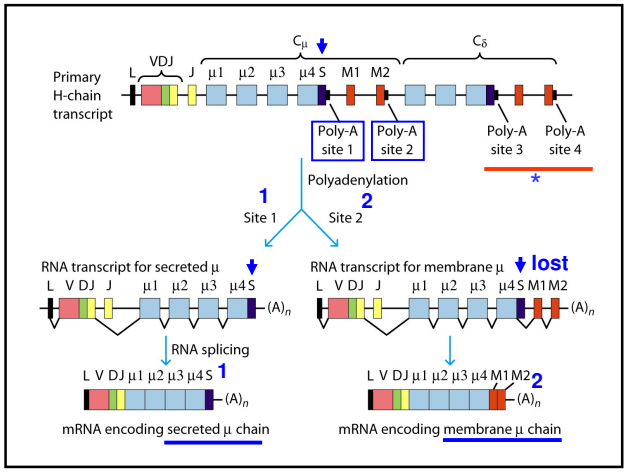


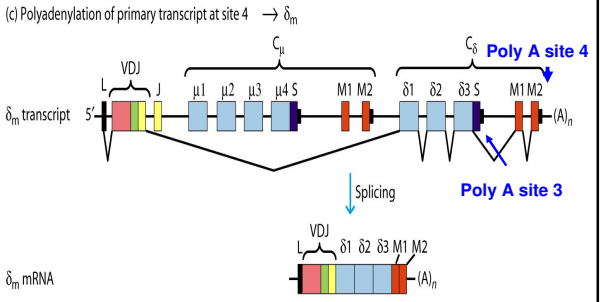
Expression of membrane or secreted Immunoglobulin

- In mature B cells → membrane forms; in Plasma cells→ secreted forms
- Process depends on **differential processing** of primary transcript
- Remember: IgG, IgD, IgA (3 C_H domains), IgM and IgA (4 C_H domains).
- Domain 3/4 contains the Secretory (hydrophilic) nucleotide sequence (S) at its 3'.
- Two Exons at 3' encode the M1 (trans-membrane) and M2 (cytoplasmic) segments.
- Primary transcript contains two PolyA sites: If cleavage at Poly A site I = Secreted Form. If cleavage at PolyA site 2 = Membrane Form



S component **M1 and M2 components (exons)**





In mature B cells: both IgM and IgD, then cleavage must occurs at SITE 4 and both processing pathways MUST occur simultaneously

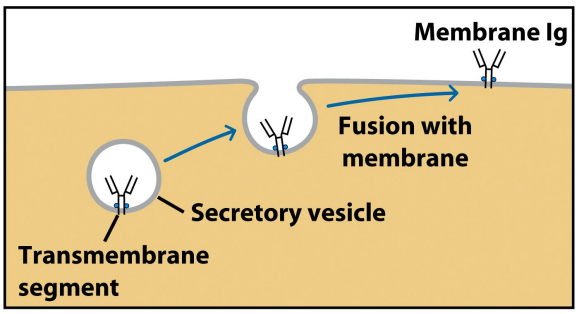


Figure 5-20 part 1
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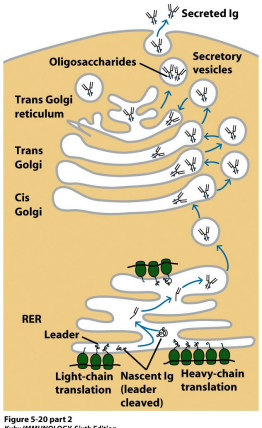

Figure 5-20 part 2
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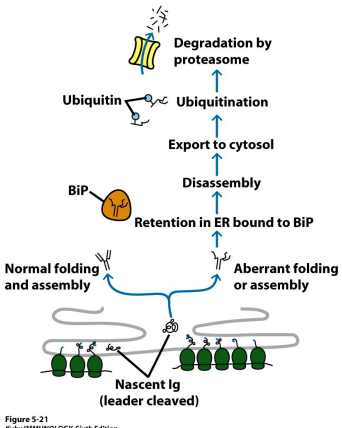

Figure 5-21
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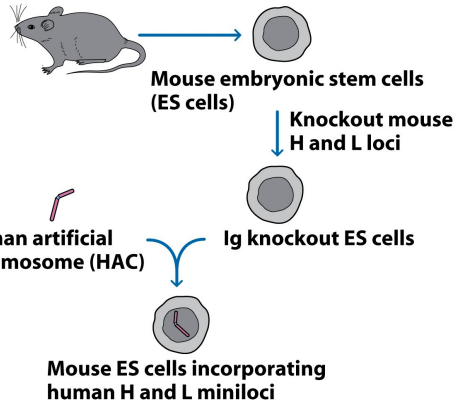

Figure 5-24 part 1
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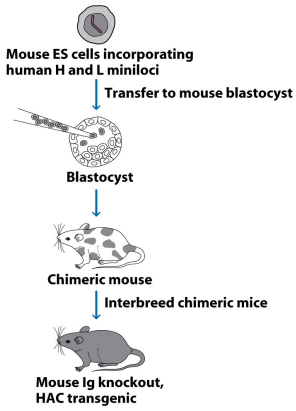

Figure 5-24 part 2
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Some monoclonal antibodies in clinical use

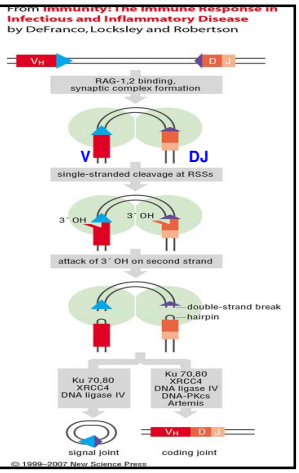
Monoclonal antibody [mAB] (product name)	Nature of antibody	Target (antibody specificity)	Treatment for
Muromonab-CD3 (Orthoclone OKT3)	Mouse mAB	T cells (CD3, a T-cell antigen)	Acute rejection of liver, heart, and kidney transplants
Abciximab (ReoPro)	Human-mouse chimeric	Clotting receptor of platelets (GP IIb/IIIa)	Blood clotting during angioplasty and other cardiac procedures
Daclizumab (Zenapax)	Humanized mAB	Activated T cells (IL-2 receptor alpha subunit)	Acute rejection of kidney transplants
Infliximbab (Remicade)	Human-mouse chimeric	Tumor necrosis factor (TNF), a mediator of inflammation (TNF)	Rheumatoid arthritis and Crohn's disease
Palivizumab (Synagis)	Humanized mAB	Respiratory syncytial virus (RSV) (F protein, a component of RSV)	RSV infection in children, particularly infants
Gemtuzumab (Mylotarg)	Humanized mAB	Many cells of the myeloid lineage (CD33, an adhesion molecule)	Acute myeloid leukemia (AML)
Alemtuzumab (Campath)	Humanized mAB	Many types of leukocytes (CD52 a cell surface antigen)	B-cell chronic lymphocytic leukemia
Trastuzumab (Herceptin)	Humanized mAB	An epidermal growth factor receptor (HER2 receptor)	HER2-receptor-positive advanced breast cancers
Rituximab (Rituxan)	Human-mouse chimeric	B cells (CD20, a B-cell surface antigen)	Relapsed or refractory non-Hodgkins lymphoma
Ibritumomab (Zevalin)	Mouse mAB	B cells (CD20, a B-cell surface antigen)	Relapsed or refractory non-Hodgkins lymphoma

SOURCE: Adapted from P. Carter. 2001, Improving the efficacy of antibody-based cancer therapies, *Nature Reviews/Cancer* 1:118.

Unnumbered table pg 141
Kuby IMMUNOLOGY, Sixth Edition

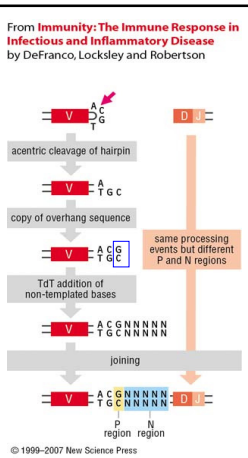
The End

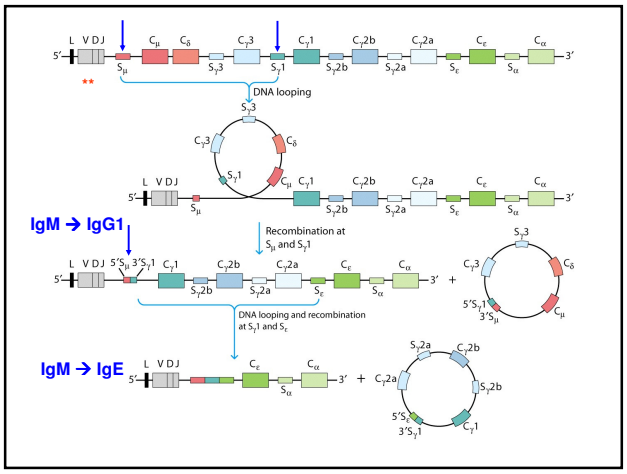
- **RAG-1/RAG-2 cleave ONE strand of DNA**
- **This occurs at the border of the RSS heptamer and the coding gene**
- **The 3' OH group attacks a phosphodiester bond on the other DNA strand**
- **This results in hairpin DNA strand on the coding region.**
- **Other enzymes get involved and remove the "junk" and bring together the coding regions**



Junctional Diversity:

- Terminal deoxynucleotidyl transferase **(TdT)** is important for creating junctional diversity
- What are **SIGNAL JOINTS** and **CODING JOINTS**?
- Hairpin must be opened → the enzyme **Artemis**
- Cleavage is random and can happened at any site in the hairpin
- Replication results in a short inverted repeat or palindrome = **P nucleotides**
- TdT now can introduce random nucleotides into the coding joints = **N nucleotides**
- Keep in mind that all this introduced variability may results in functional and non-functional Ig (or TCR) genes.




L V D J
DNA looping
IgM → IgG1
Recombination at
DNA looping and recombination
IgM → IgE