MIMG 185A/Spring 2026

Lecture 6

The Genetic Basis of Antigen Receptor Structure

4/16/2026

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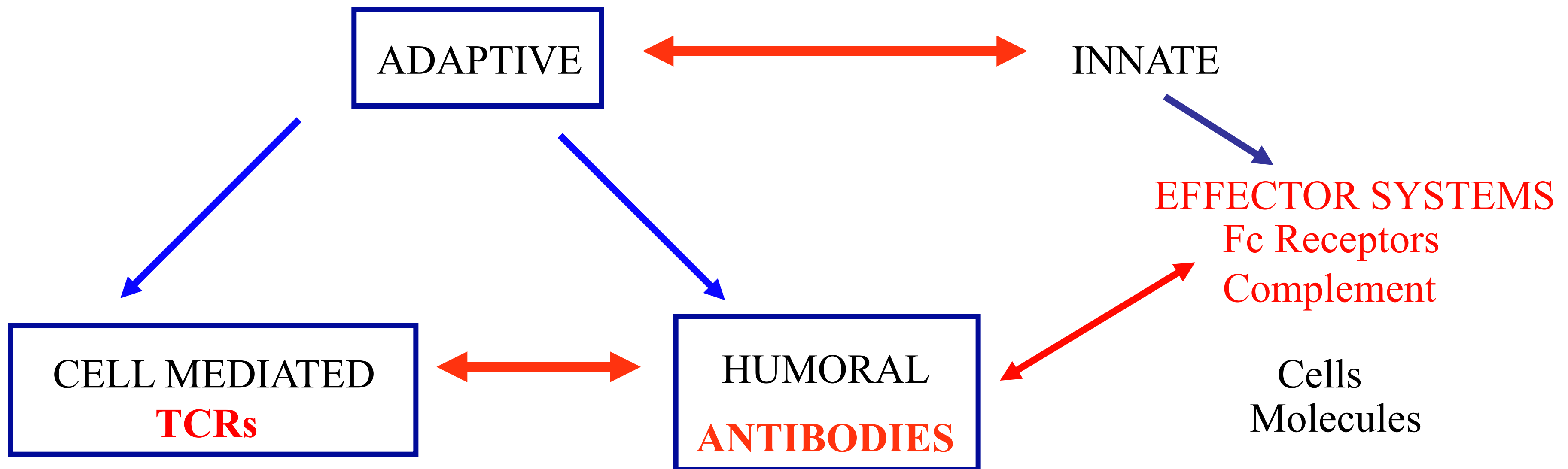


Receptors of the Adaptive Immune Response

- different than any other receptor in our bodies
- not germline-encoded, not inherited
- expressed clonally – as opposed to PRRs

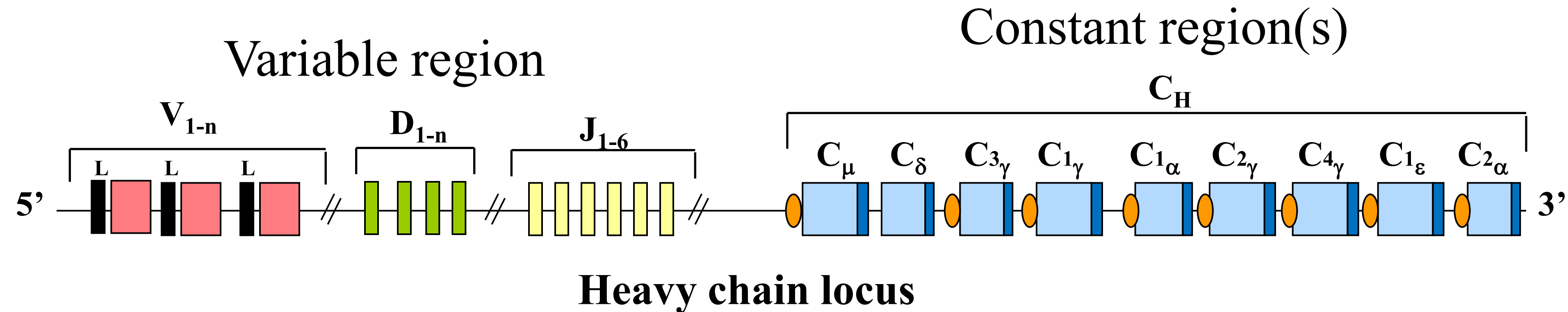
Aspects of antibody molecules that must be explained

- How can H and L chains be separated into variable and constant regions?
- How is the large number of different specificities achieved?
- How can Abs be produced in both membrane and secreted forms?
- How can different isotypes of the same specificity be produced?
- How is allelic exclusion achieved?

Genomic structure of the heavy chain locus

Antibody genes are found on several different chromosomes:

hu Heavy Chain → Chromosome 14
hu Kappa Light Chain → Chromosome 2
hu Lambda Light Chain → Chromosome 22

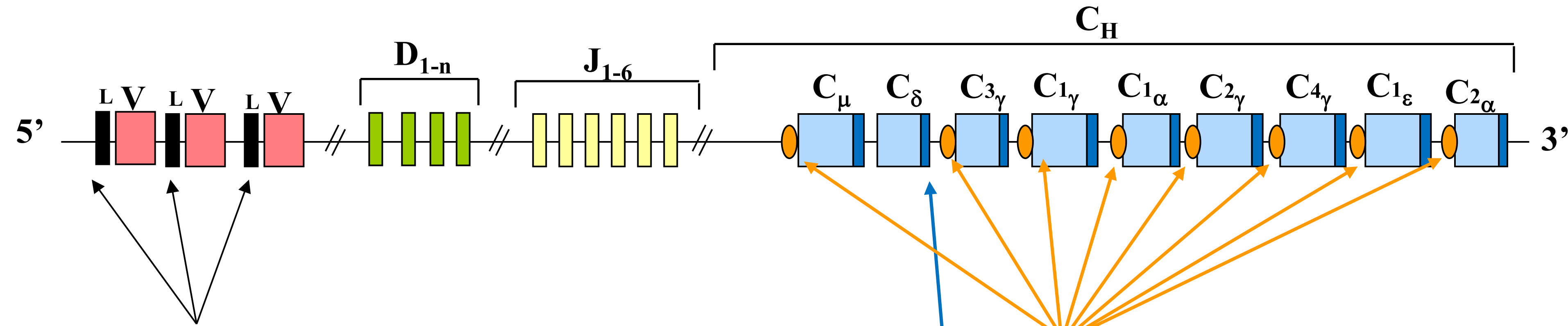


- The Ab genes are organized as discontinuous DNA segments.

- The separated DNA segments in both the heavy and light chain genes are the variable (V), joining (J), and constant (C) regions.

- Heavy chain genes also include a diversity (D) region.

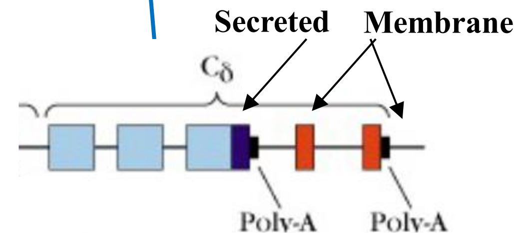
Genetic organization of the human Ab heavy chain locus



1. The small black box in front of each V-exon, is a hydrophobic leader sequence, which acts as signal sequence to direct protein to lumen of rER post-translation.

2. In front of each leader sequence is a promoter, such that once rearrangement is complete, RNA Pol can bind and transcribe the entire gene.

A "switch site" is located upstream from C_H segments for use in class switching



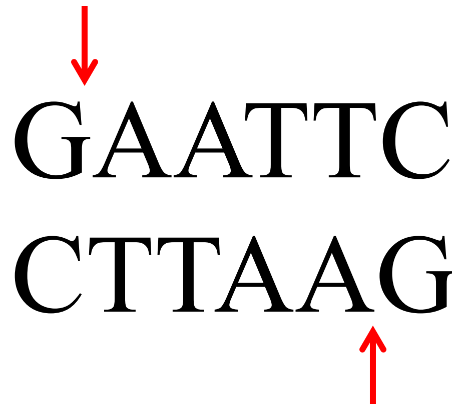
The 3' end (eventually COOH end of H-chain) of each C_H segment, contains aa sequences for both secreted & membrane forms.

Receptors of the Adaptive Immune Response

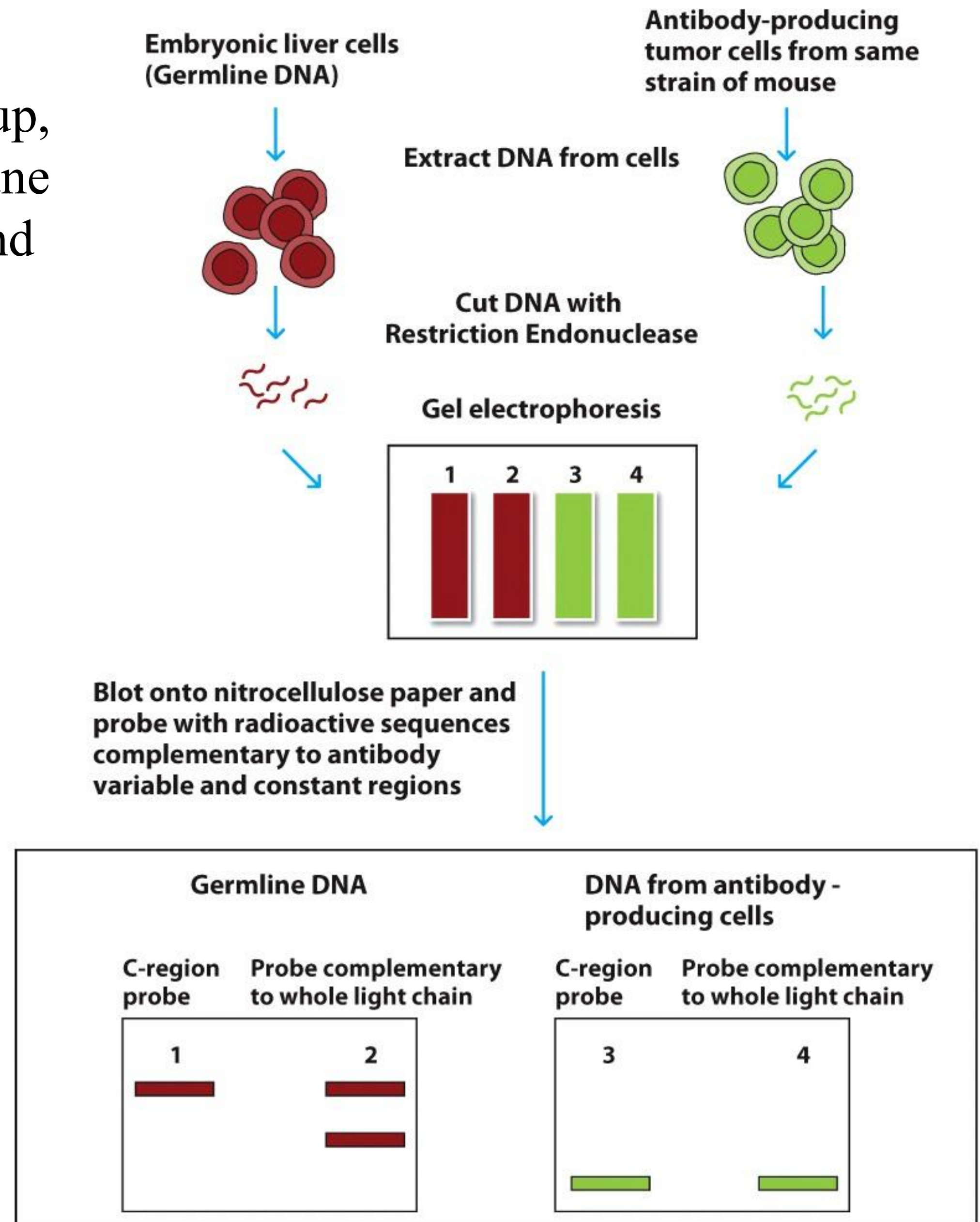
How did scientists know that Ig rearrangment occurred?
The answers came from new technology (at the time)……

Restriction endonucleases, enzymes that cut DNA at defined sequences

Example: ***EcoR I***



- Experiments by the Tonegawa group, they took two types of cells, immune and non-immune, isolated DNA and mRNA.
  - → Specifically, mRNA coding either constant region of or the whole κ light chain was isolated & labeled with ^{32}P.
- Digested the DNA of cell lines with RE, & separated by size, and then hybridized with the κ light chain/constant region ^{32}P-labeled mRNA.
- What did they see?



Box 7-1 Figure 1
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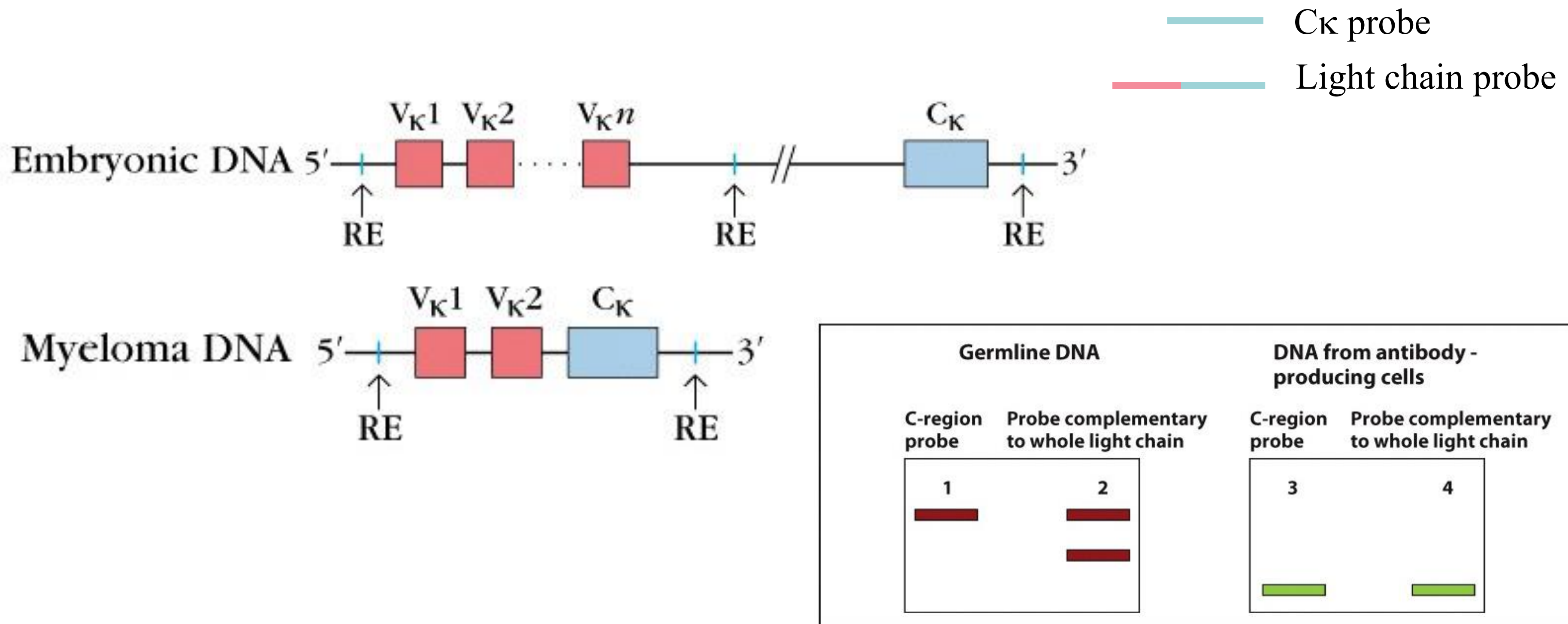

Today we know, that when you begin to make a functional heavy or light chain, there has to be physical rearrangement of the DNA!

So, in this particular case, in embryonic DNA V regions are located in a different restriction fragment than C regions.

However, in myeloma DNA, V and C have been physically rearranged & are now on same restriction fragment

Q: How does Light chain Ig rearrangement occur?

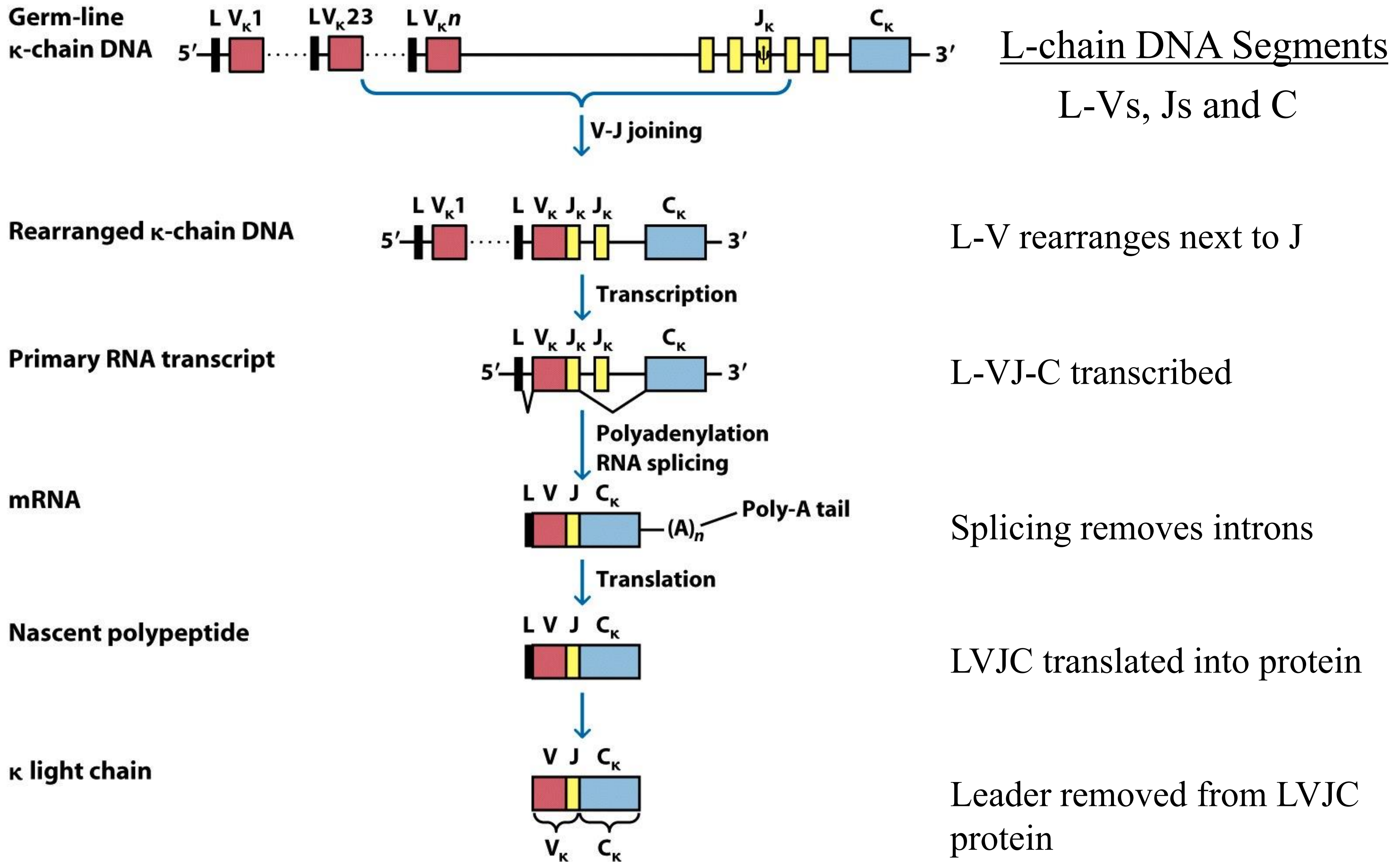

Figure 5-4
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L-chain DNA Segments

L-Vs, Js and C

L-V rearranges next to J

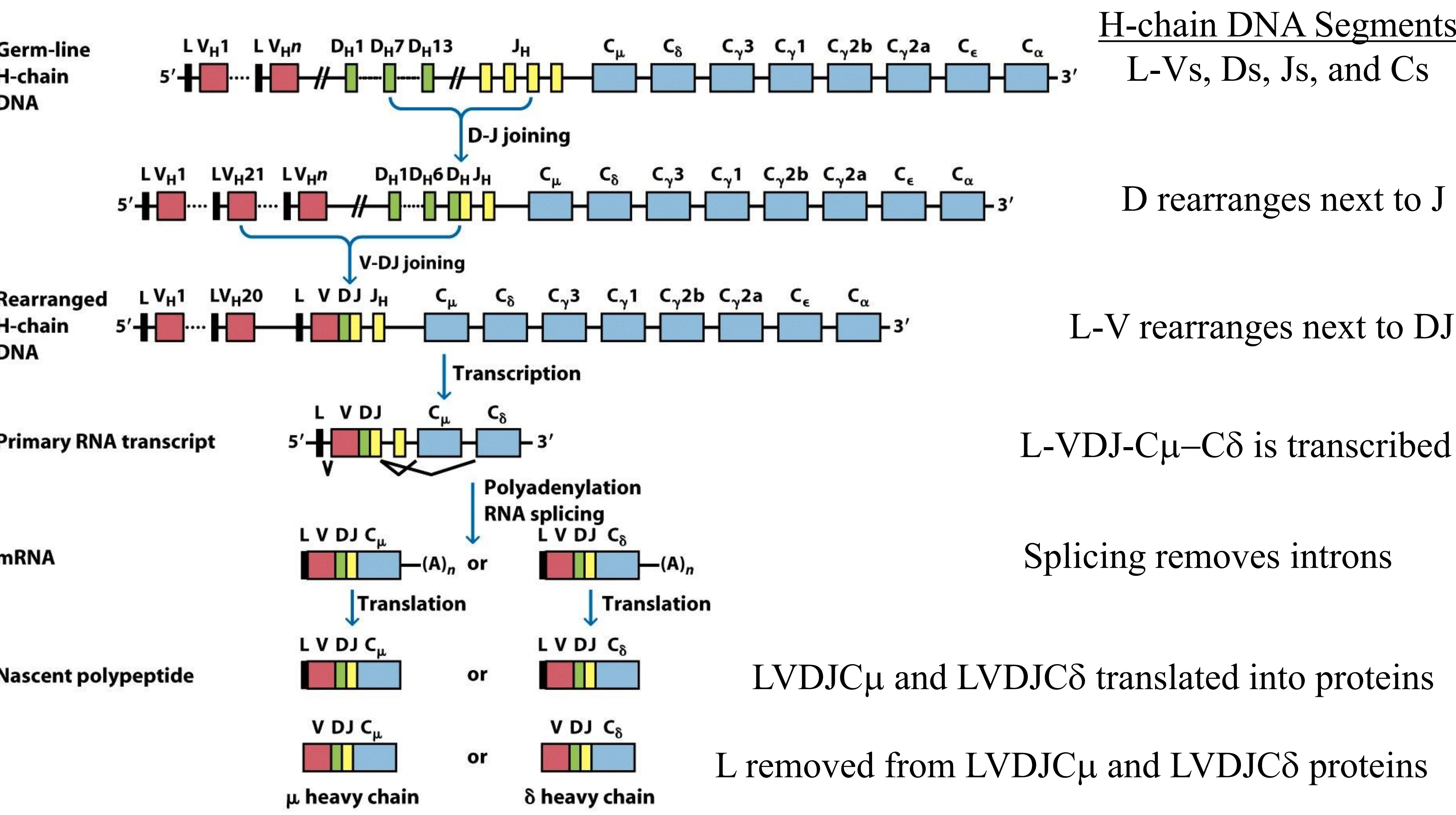
L-VJ-C transcribed

Splicing removes introns

LVJC translated into protein

Leader removed from LVJC protein

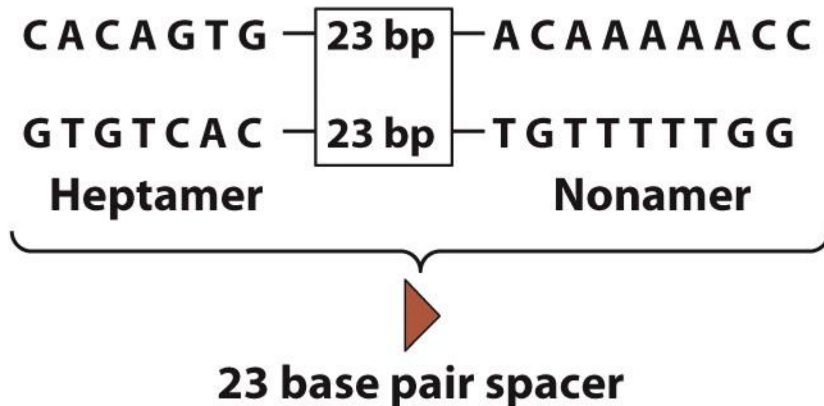
Q: How does Heavy chain Ig rearrangement occur?



There are multiple germline Vs, Ds, & Js which can be assembled into many different combinations thereby contributing to the diversity of the heavy chain.

Receptors of the Adaptive Immune Response

Recombination Signal Sequences (RSS) flank each germ-line V, D, & J gene segment. The **12/23 rule** assures proper assembly of V-regions.



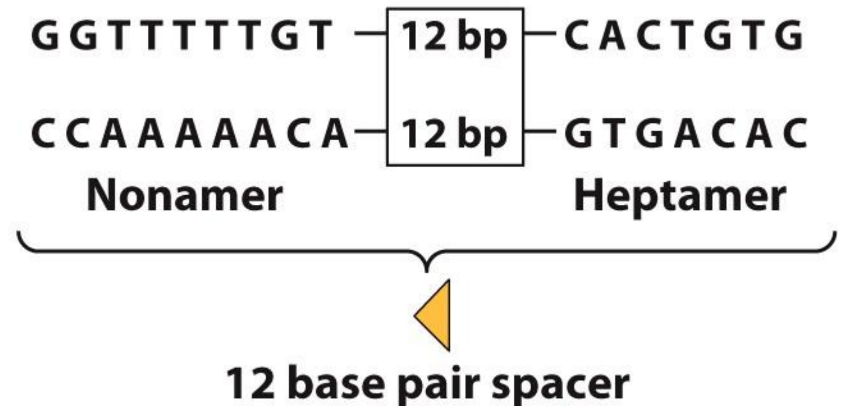


Figure 7-5a
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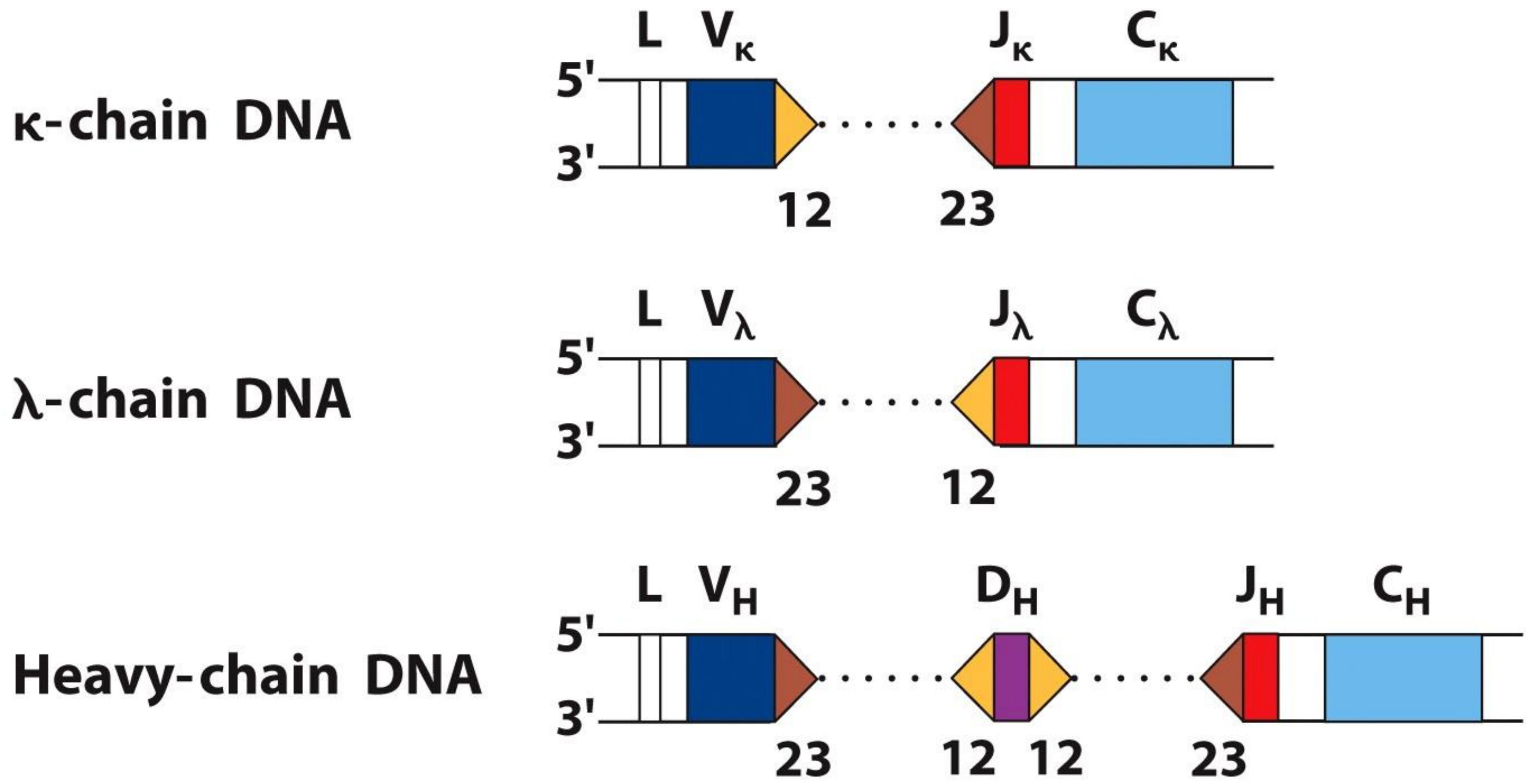
- Recombination Activating Gene-1 and -2 (RAG-1 and RAG-2) are the enzymes which recognize RSS sequences and cleave one strand of DNA at the juncture of the signal and coding sequences

Location of RSSs in germ-line immunoglobulin DNA



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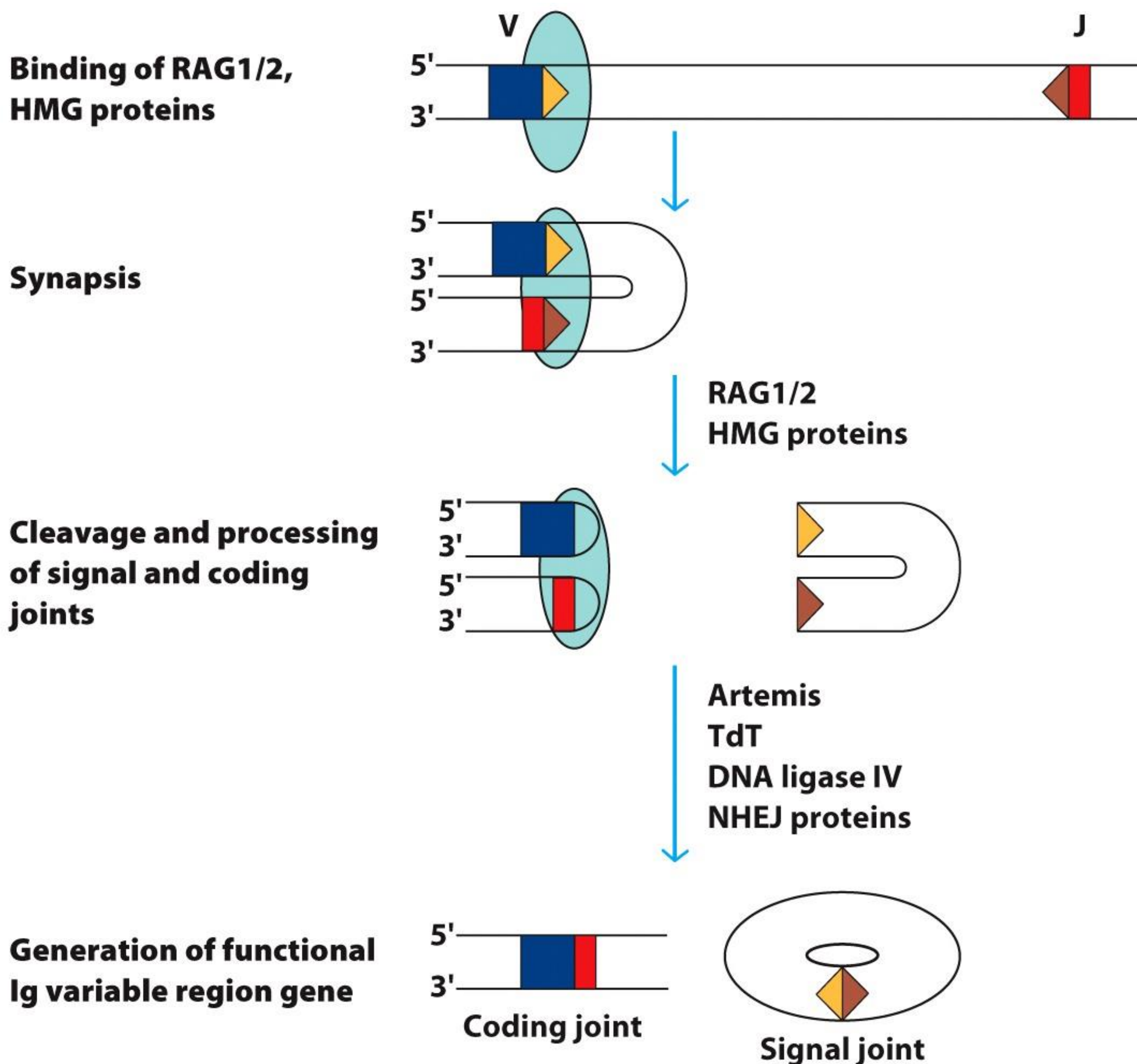
Note that V_H and J_H cannot join, assuring proper assembly.

Overview of recombination of immunoglobulin variable region genes



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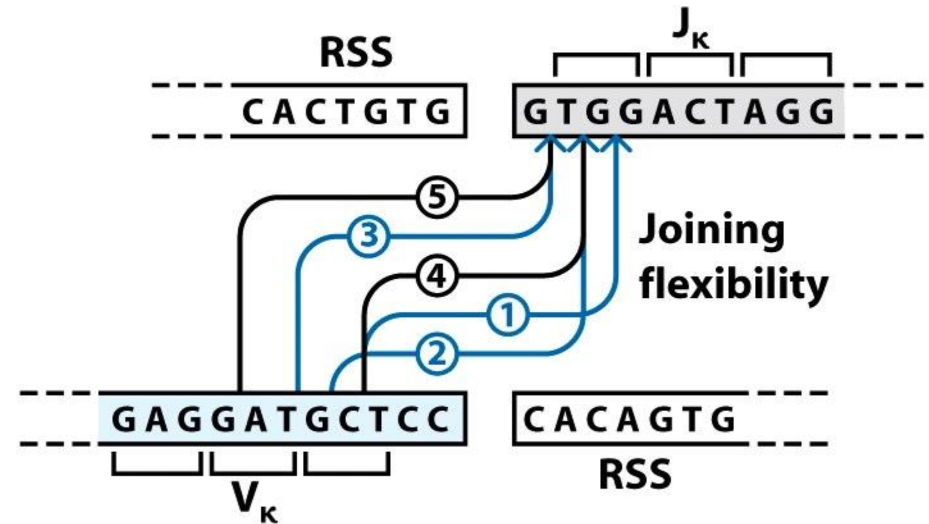
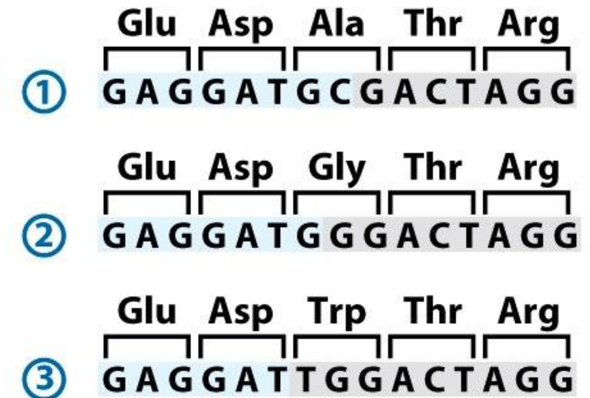
Receptors of the Adaptive Immune Response

There is variability in the joining of the coding regions, via deletion or addition of nucleotides →

The increased variability in joining generates diversity (but will lead to non-functional product as well).





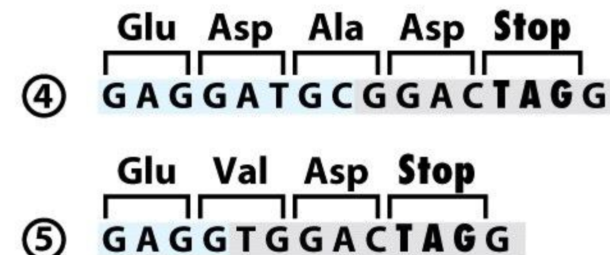


Figure 5-9
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Receptors of the Adaptive Immune Response

When RAG proteins cleave the juncture, the free 3'-OH attacks phosphodiester bond on other strand, forming a hair-pin structure.

Palindromic (**P**) nucleotide are inserted at the joining site once the hairpin is randomly cut by endonuclease Artemis (& also trimmed) –this process contributes to variability.

To join hairpins:

1. Nick
2. Fill in
3. Join ends together

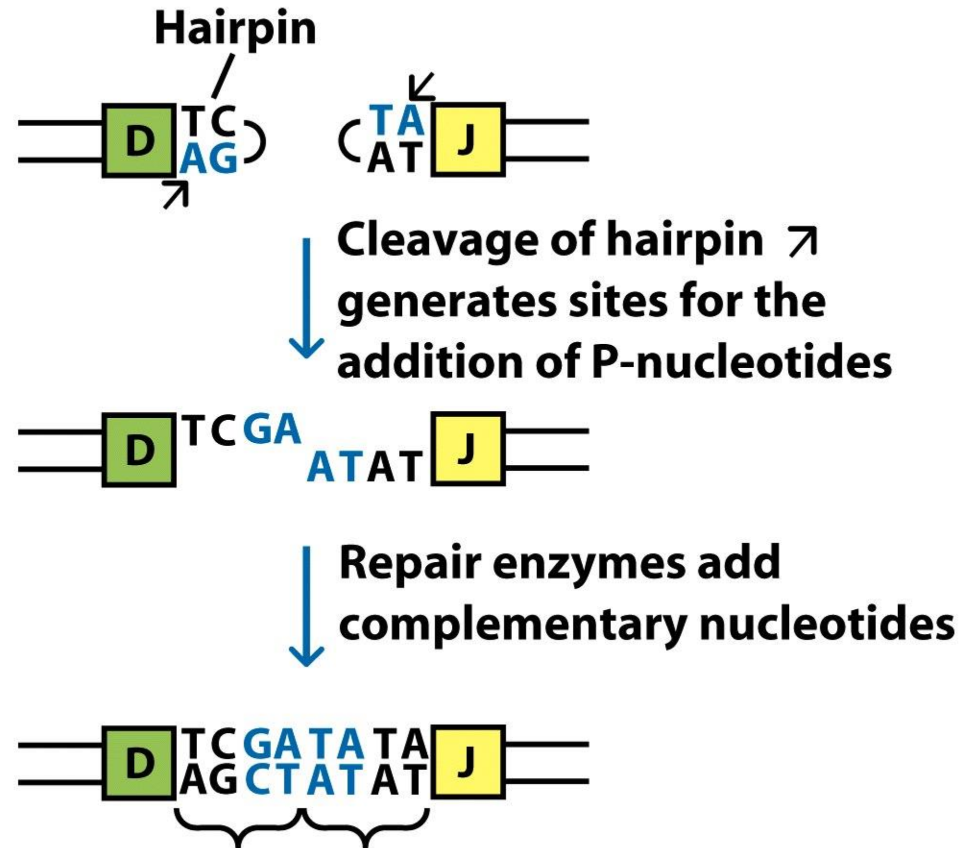

Figure 5-13a
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For **H chain** even more variability occurs because random, non-templated nucleotides (N-nucleotides, up to 15) may also be inserted between V-D and D-J by the action of the enzyme **Terminal deoxynucleotidyl transferase (TdT).**

Therefore, V_H diversity further increased with addition of P and N nucleotides, as more novel, NON-GERMLINE, nucleotides are added at joining site.

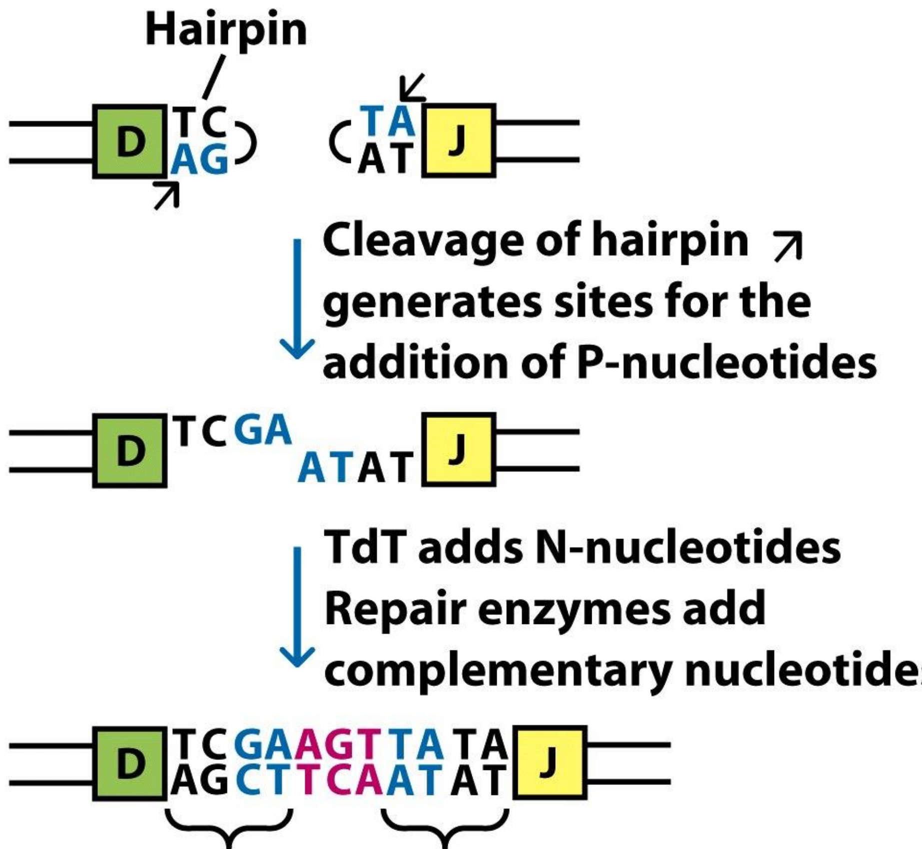

Figure 5-13b
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TABLE 7-4 Combinatorial antibody diversity in humans

Nature of segment	Number of heavy-chain segments (estimated)	Number of κ-chain segments (estimated)	Number of λ-chain segments (estimated)
V	41	41	33
D	23		
J	6	5	5
Possible number of combinations	$41 \times 23 \times 6 = 5658$	$41 \times 5 = 205$	$30 \times 5 = 165$
Possible number of heavy-light chain combinations in the human $= 5658 \times (205 + 165) = 2.09 \times 10^6$			

Table 7-4
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The number is probably closer to 10^{11} when we take into account variability introduced by exonuclease activity, P and N nucleotide additions, and somatic hypermutation!

Additional sources of diversity.... **Somatic Hypermutation**

1. Random process that introduces mutations within the rearranged VJ- or VDJ-region – approx 2Kb downstream of Ig promoter
   (mostly substitutions, not deletions or insertions)

2. Susceptible regions are called "hot spots", so not totally random.
   - ~100,000x higher rate of spontaneous mutations in this region

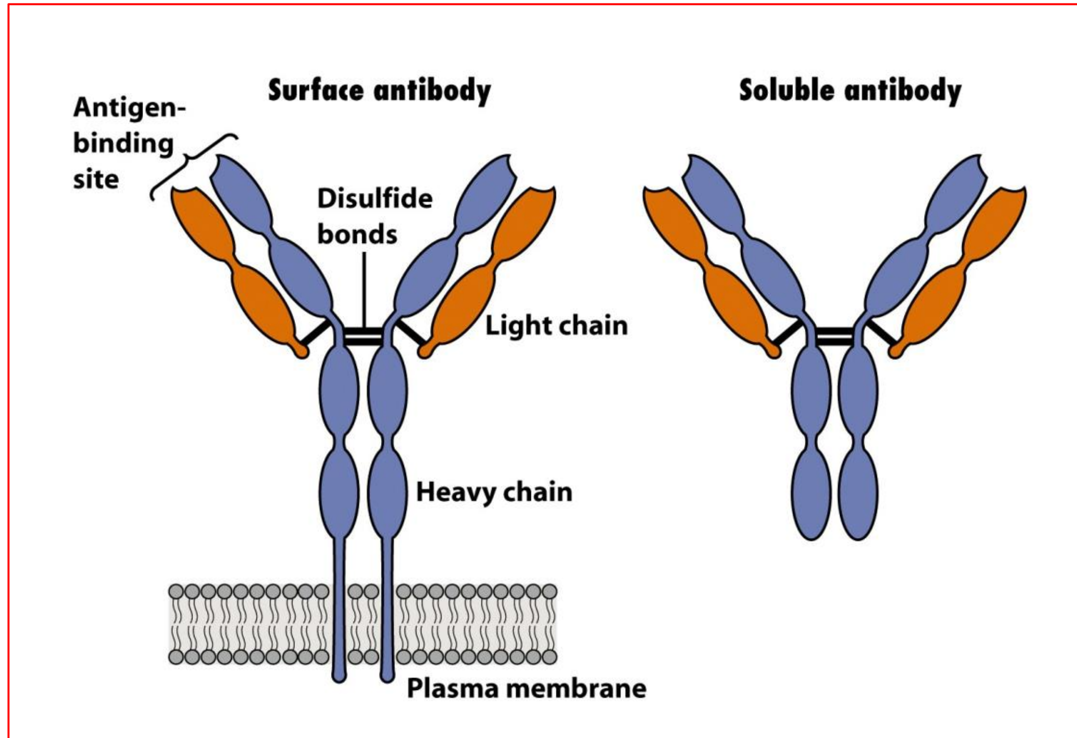
3. Takes place in germinal centers, as result of T-dependent B-cell response.
   Plays role in **affinity maturation**

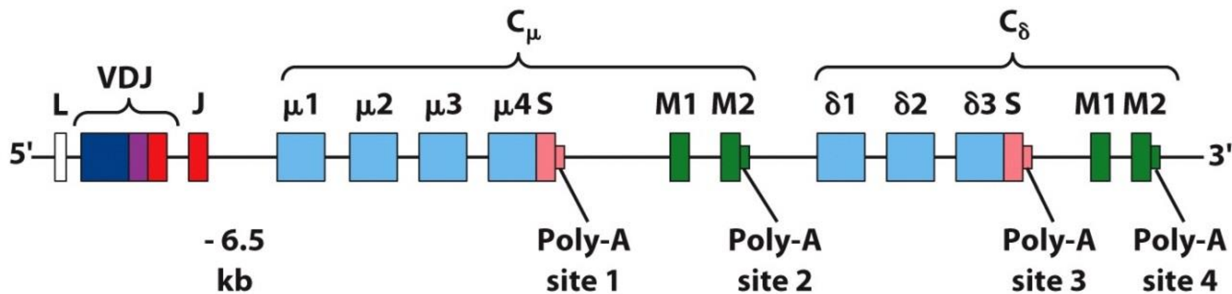
5. Requires the enzyme AID (Activation Induced Cytidine Deaminase)
   - homologous to RNA editing gene APOBEC-1
   - function still not fully explained
   also required for class switch recombination

To understand Ab structure….
Several aspects of Abs must be explained…………

- How can H and L chains be separated into variable and constant regions?
- How is the large number of different specificities achieved?
- How can Abs be produced in both membrane and secreted forms?
- How can different isotypes of the same specificity be produced?
- How is allelic exclusion achieved?

How are membrane vs. secreted Abs generated?





Splicing pattern of the primary transcript determines whether the membrane or secreted form of IgM is generated.

How are membrane vs. secreted IgM generated?

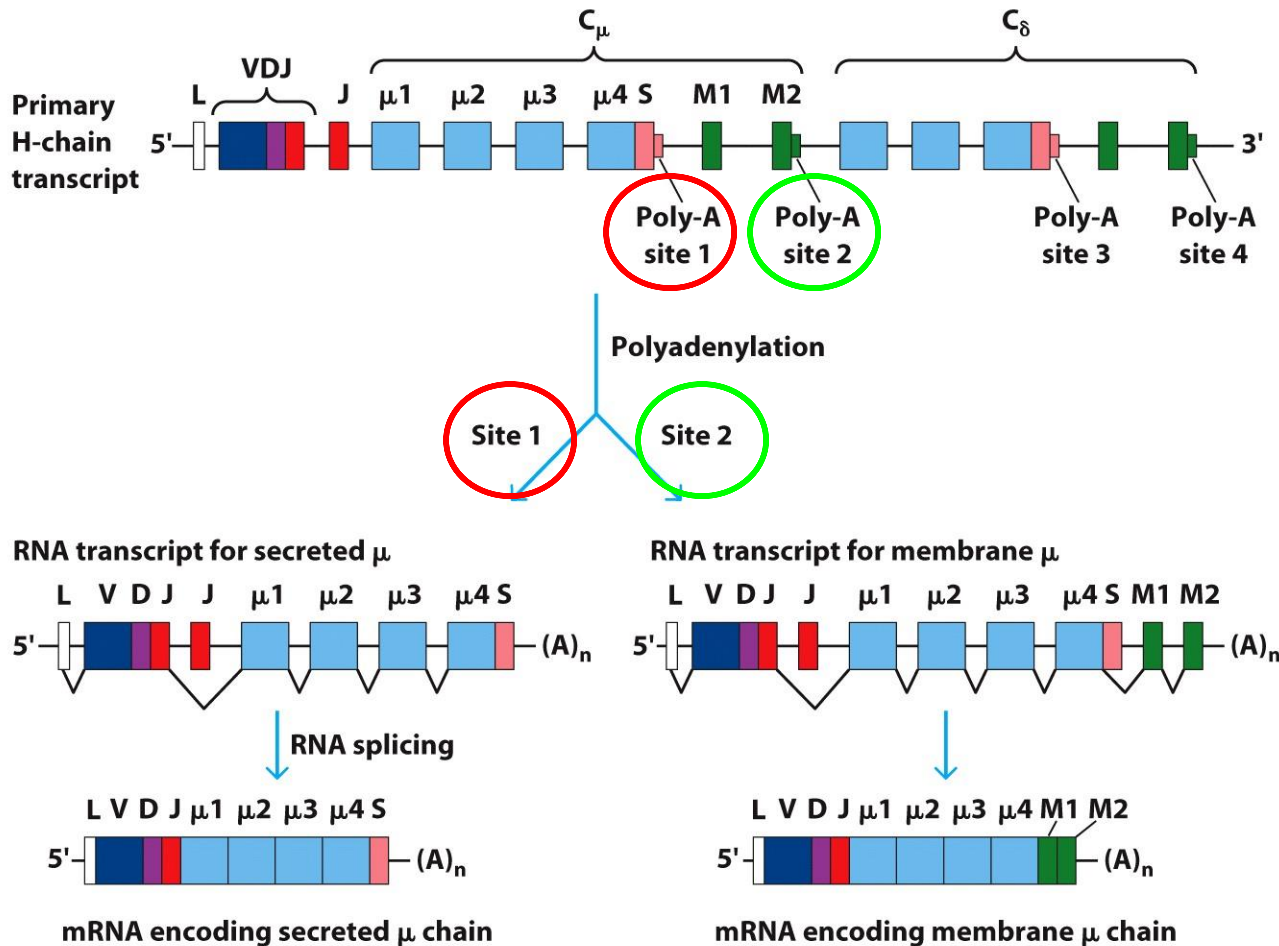


Figure 7-15a
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- Alternative processing of the 3' end of the mRNA makes it possible to produce membrane Ig and secreted Ig of the **same specificity.**
- This is true for **ALL ISOTYPES**

To understand Ab structure….
Several aspects of Abs must be explained………..

- How can H and L chains be separated into variable and constant regions?
- How is the large number of different specificities achieved?
- How can Abs be produced in both membrane and secreted forms?
- **How can different isotypes of the same specificity be produced?**
- How is allelic exclusion achieved?

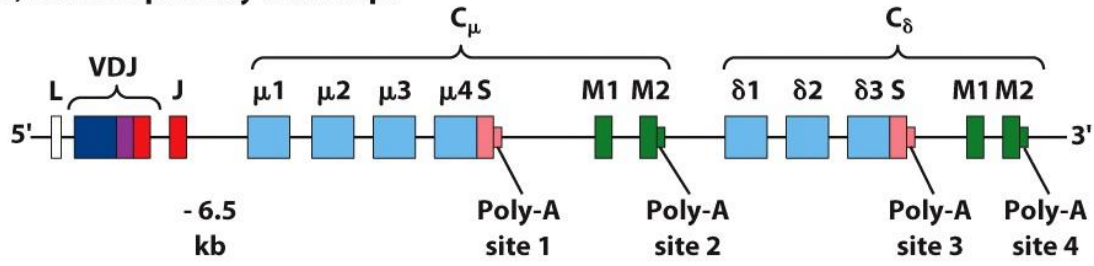
How can different isotypes of an Ab have the same specificity?

A couple different ways:

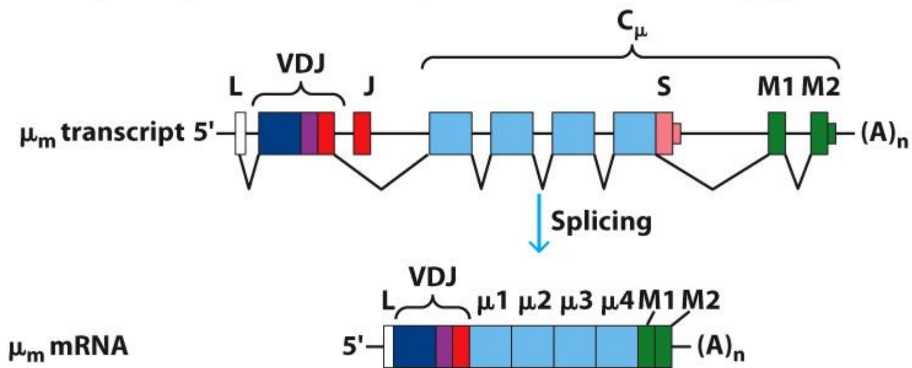
1. Through **alternative splicing** of primary transcripts
   - Alternative processing of a long primary transcript makes it possible to produce membrane IgM & IgD of the **same specificity**

2. Through **class switching** the Ab will retain the originally defined specificity, but will change isotypes.

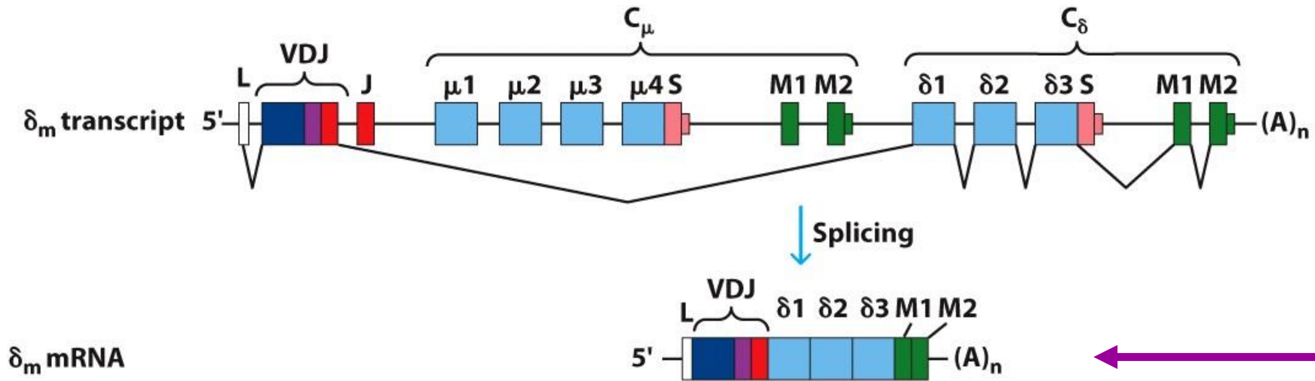
Q. How can different isotypes of an Ab have the same specificity?



1. Alternative processing of a *long primary transcript* makes it possible to produce membrane IgM & IgD of the **same specificity**



IgM expressed



IgD expressed

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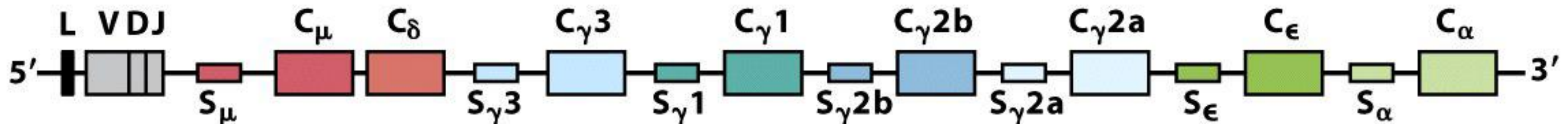
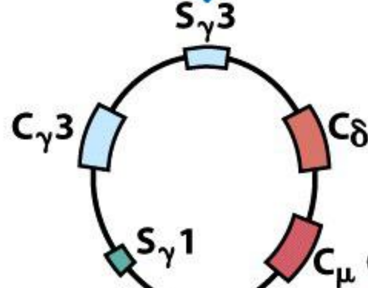
Class Switch Recombination

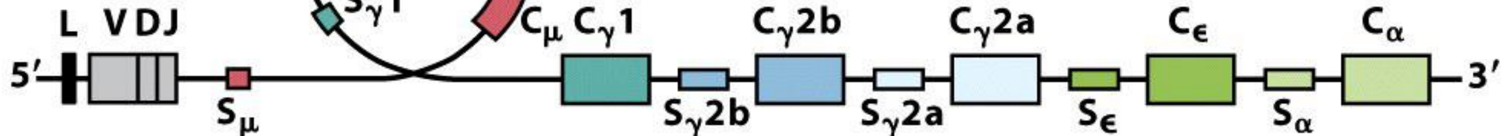


DNA looping

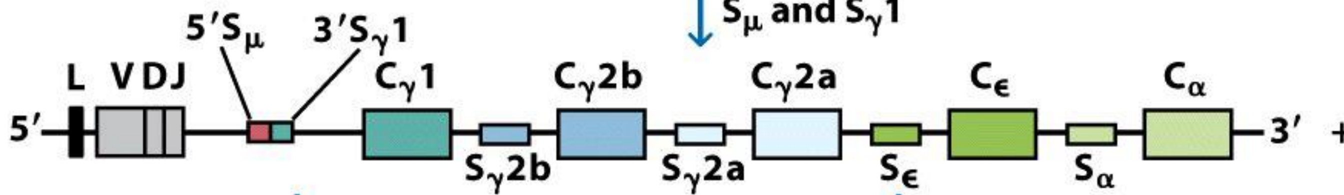
1. Switch regions are located at the 5' end of each constant region, except δ.

2. Looping of DNA, recombination at Sμ & Sγ1





Recombination at S_μ and $S_\gamma 1$



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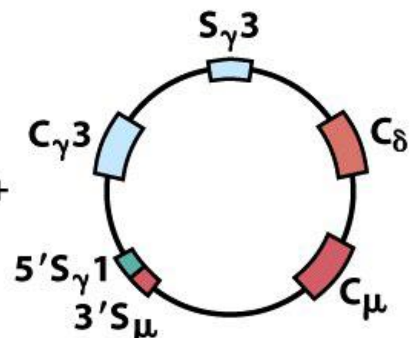
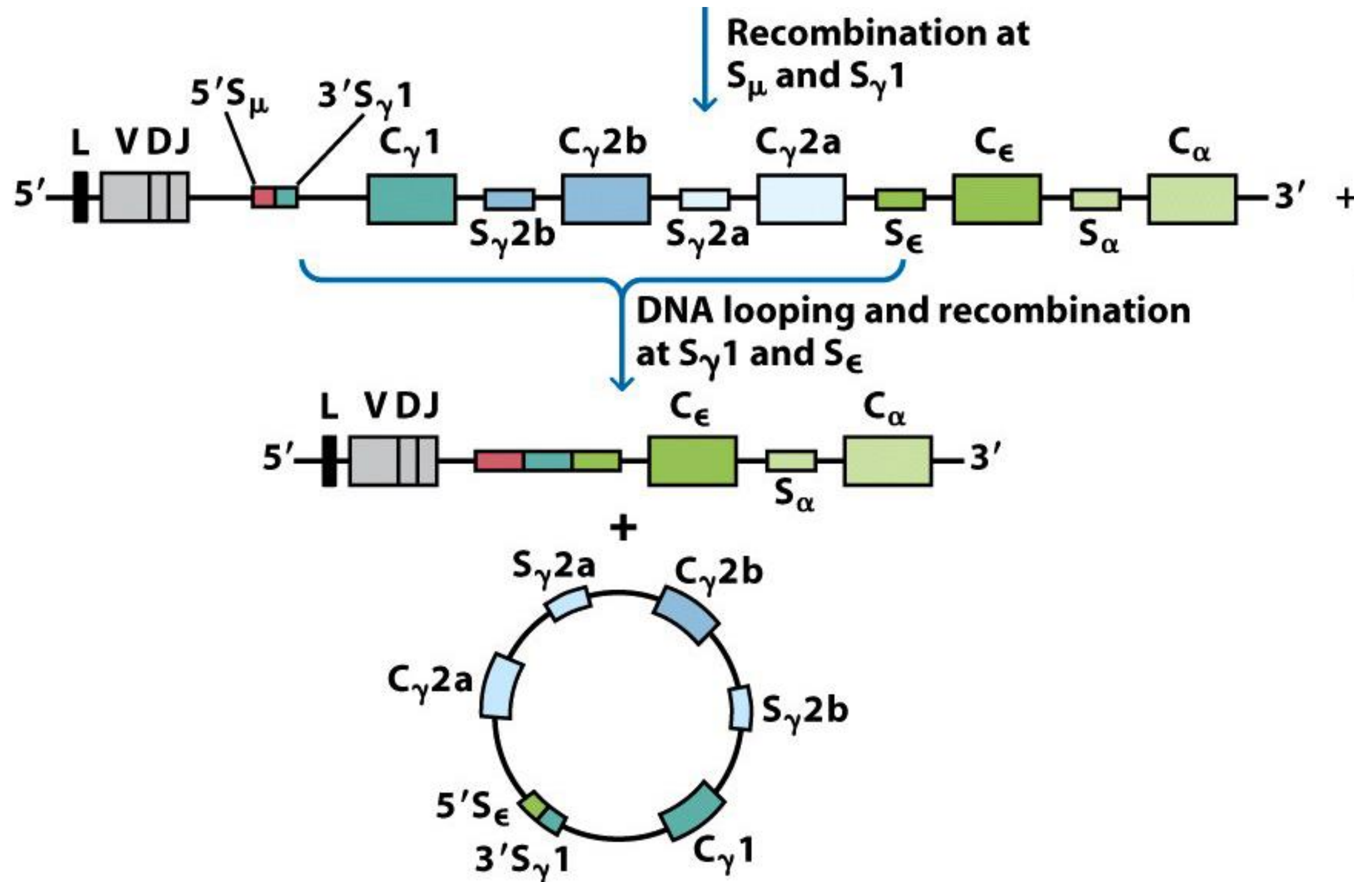


3. Rearranged L-VDJ are brought next to new constant region, small portions of the switch regions may remain, but are intronic and will be spliced out of the transcript.

4. Circular excision product, upstream constant regions are **deleted**

Class Switch Recombination



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Subsequent rearrangements to downstream Cs are possible, however upstream constant regions are deleted, so cannot go back

Class Switch Recombination

For isotype switching, a physical rearrangement of the **DNA** takes place with the functionally rearranged variable region brought next to a different constant region.

1. Induced in H chains locus following Ag stimulation + cytokines in the periphery.
   - involves DNA flanking sequences termed "**switch regions**"
   - Requires the enzyme **AID (activation induced cytidine deaminase)**.

2. For H chains, V is first adjacent to μ, thus IgM is the first isotype in immune response. All isotypes (except for δ) have a switch site.

3. A physical rearrangement of DNA takes place including the excision of portions of the DNA, which form circular products. Because DNA is excised, you can only move "forward" that is, downstream, in class switching – no switching back!

4. Isotype switching occurs with both the functional and non-functional alleles.

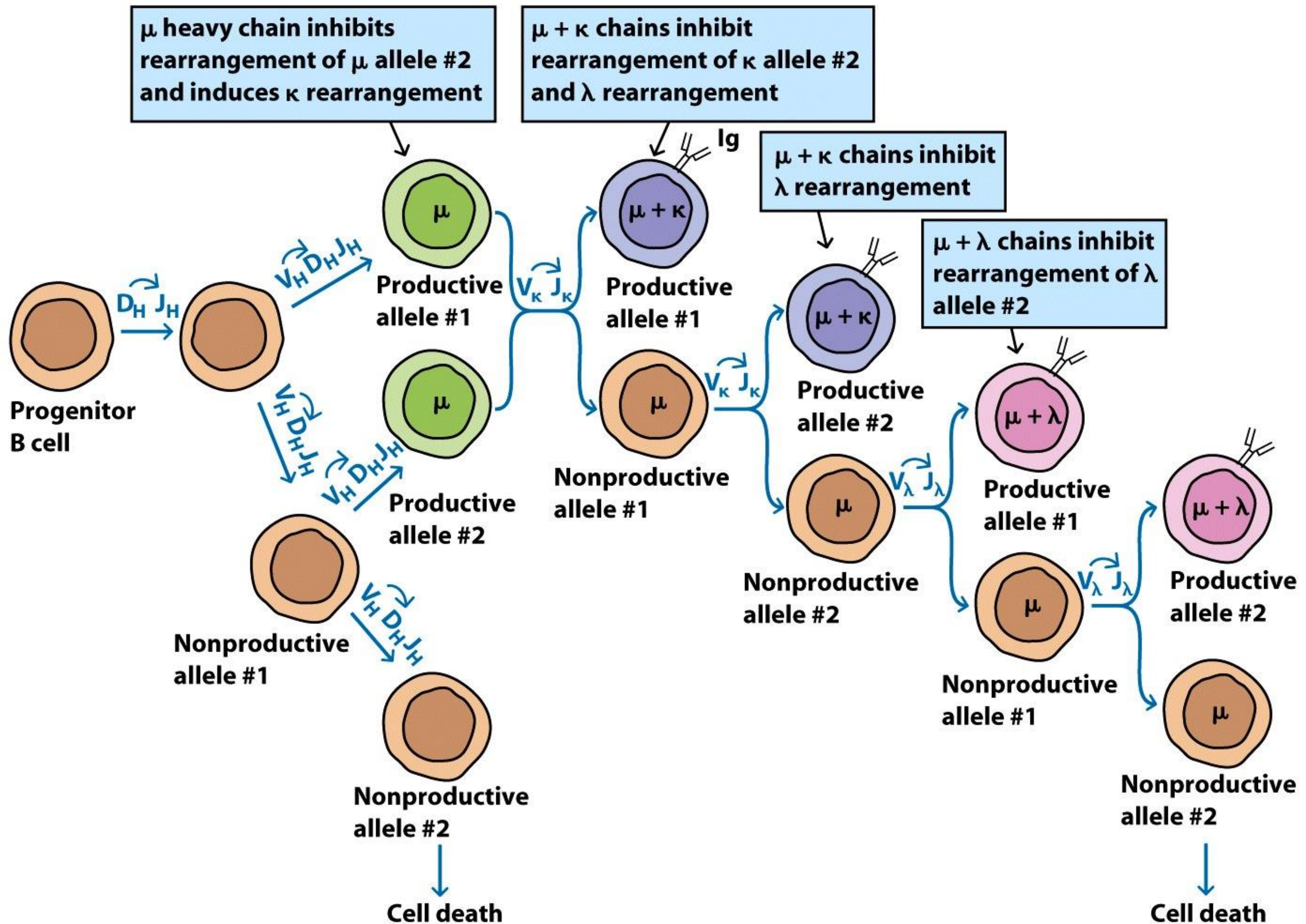
To understand Ab structure….
Several aspects of Abs must be explained………..

- How can H and L chains be separated into variable and constant regions?
- How is the large number of different specificities achieved?
- How can Abs be produced in both membrane and secreted forms?
- How can different isotypes of the same specificity be produced?
- **How is allelic exclusion achieved?**

Once productive rearrangement is attained, the presence of final protein on cell surface signals to shut down recombination machinery and prevent further gene rearrangement of the H & L chain on homologous chromosome.

Allelic Exclusion

- Allelic exclusion ensures that each B cell synthesizes only one heavy and one light chain
- Recombination is a very ordered process
- Nonproductive arrangements lead to programmed cell death (apoptosis) during development



Receptors of the Adaptive Immune Response

Q: So, *how* does Ig rearrangement occur?

-In B-cells, heavy chain rearranges first. If the first attempt at rearrangement fails, the other allele is tried.

-If H-chain rearrangement is successful, kappa light chain is rearranged.
    * approx. 60% success rate with kappa rearrangement, thus lambda is not used…

-If both kappa light chain alleles fail to successfully rearrange, then lambda is used.

-Once successful Heavy & Light chain rearrangements have occurred, the Ab is assembled in the ER & moved through vesicles for surface expression or secretion.

To understand Ab structure…. Summary

- How can H and L chains be separated into variable and constant regions?

  Different DNA segments (exons)

- How is the large number of different specificities achieved?
- How can Abs be produced in both membrane and secreted forms?
- How can different isotypes of the same specificity be produced?
- How is allelic exclusion achieved?

To understand Ab structure…. Summary

How can H and L chains be separated into variable and constant regions?
How is the large number of different specificities achieved?

a) multiple V segments
multiple D segments } **somatic assembly**
multiple J segments
b) flexibility in joining segments
c) P nucleotide insertion
d) N nucleotide insertion
e) combinatorial diversity
f) somatic hypermutation

How can Abs be produced in both membrane and secreted forms?
How can different isotypes of the same specificity be produced?
How is allelic exclusion achieved?

To understand Ab structure…. Summary

How can H and L chains be separated into variable and constant regions?
How is the large number of different specificities achieved?
How can Abs be produced in both membrane and secreted forms?

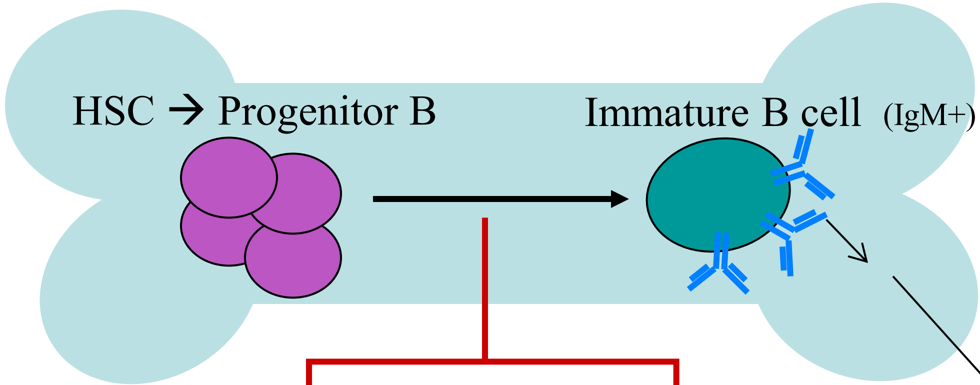
Alternative splicing

How can different isotypes of the same specificity be produced?

Alternative splicing: μ and δ
DNA rearrangement: μ to γ, α or ε

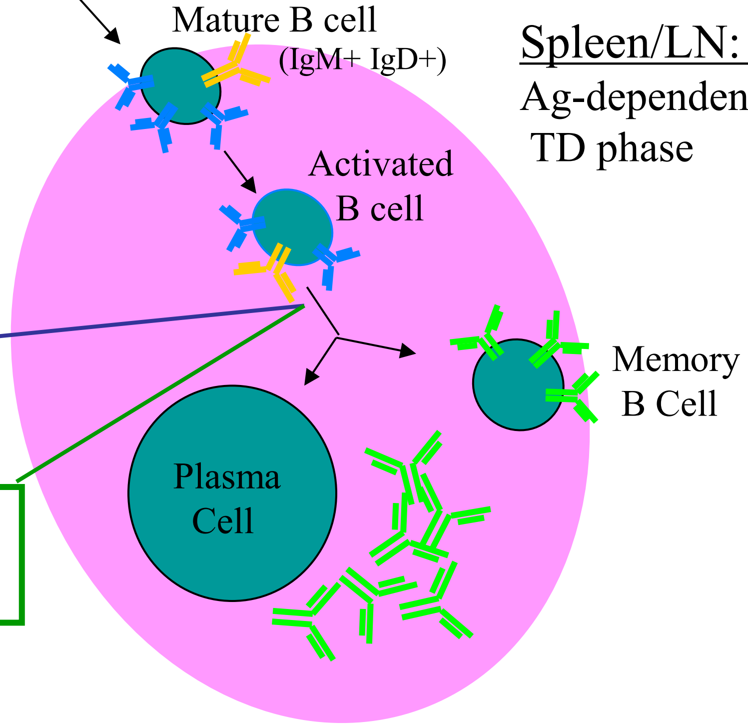
How is allelic exclusion achieved?

Bone Marrow: Ag-independent Phase



Ig Gene Rearrangement
VJ & VDJ

Spleen/LN:
Ag-dependent Phase
TD phase



Class Switching

Affinity Maturation
(Somatic Hypermutation)