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Joining Variable Constant Region Genes

M1 – Immunology Sequence

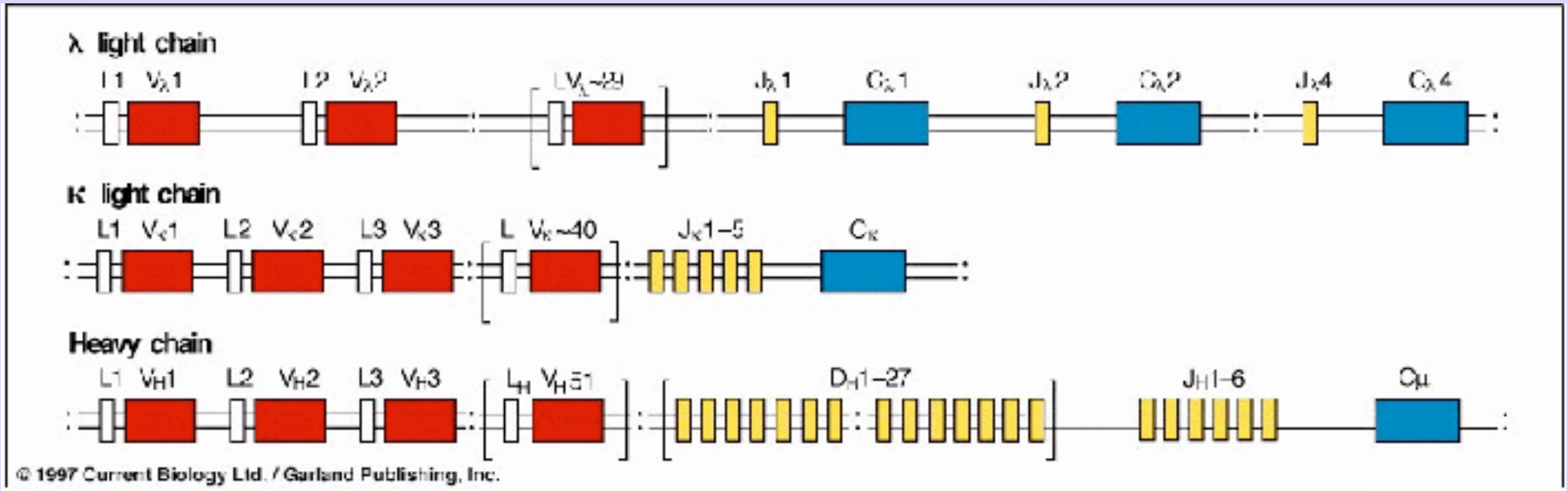
Winter 2009

University of Michigan
Medical School

1. How is diversity in the germline further expanded by DNA rearrangement in B cells?

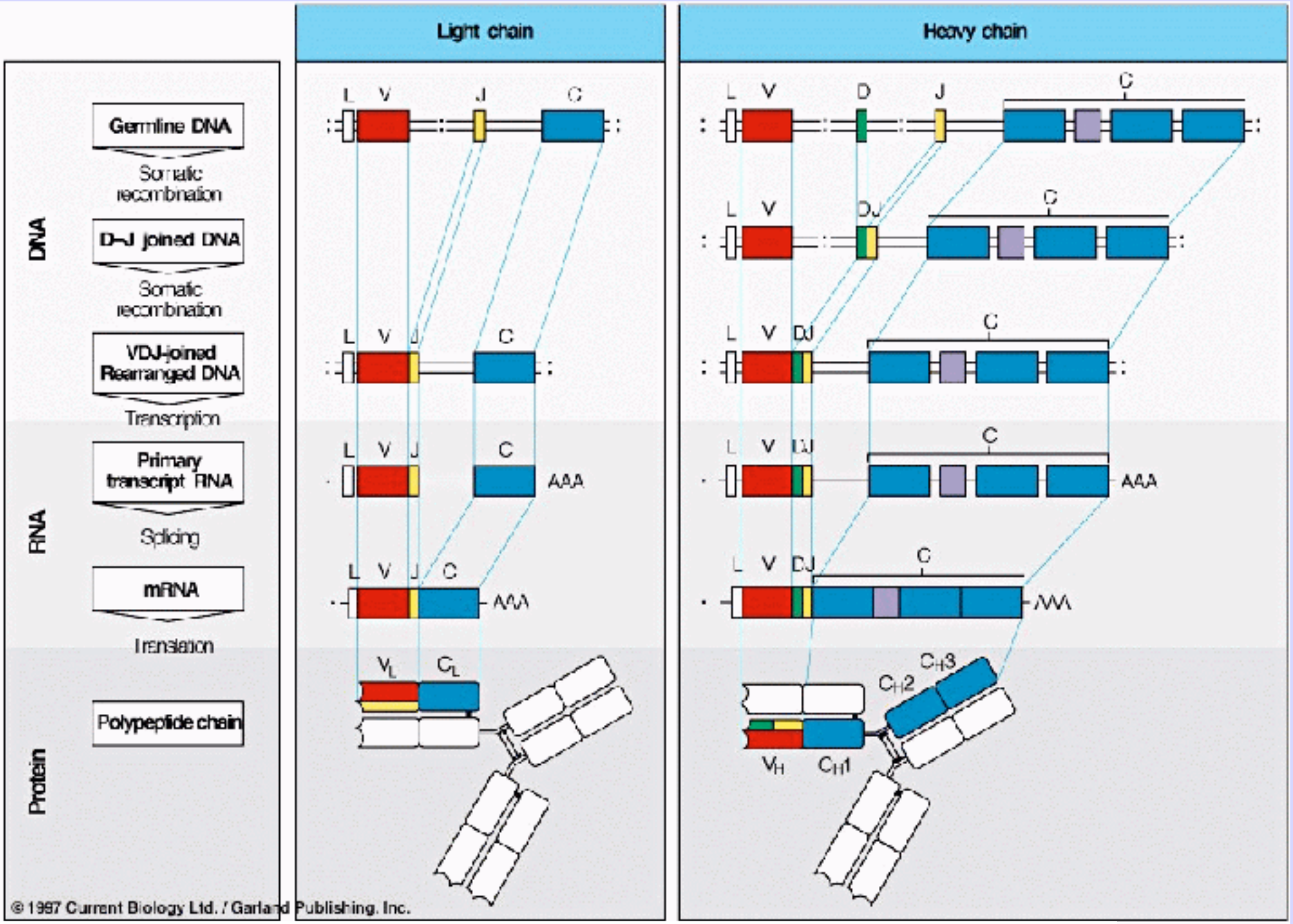
2. What are the changes in immunoglobulin gene rearrangement associated with various stages of antigen-independent B cell differentiation?

3. How can IgM and IgD be expressed simultaneously on the surface of a mature, but naive, B cell?



PD-INEL Janeway. Immunobiology: The Immune System in Health and Disease. Current Biology Ltd./Garland Publishing, Inc. 1997

Note multiple Cλ genes, each with one J region. Heavy chain: D segments encode 2-8 amino acids and are both preceded and followed by recombination signal sequences. The heavy chain variable region encodes amino acids 1-99 and JH encodes an additional 14-20 amino acids.



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Methods to generate diversity.

1. Germline diversity is generated by the use of any one of the one hundred variable region genes, several Ds, and four to ten Js encoded in DNA.

2. Combinatorial diversity is generated by joining of any variable region gene to any D to any J, and by combination of any heavy chain variable region with any light chain variable region.

50 V x 30 D x 6 JH = 9000 different VH

40 V x 5 Jκ = 200 different Vκ

9000 x 200 = $2x10^6$ binding sites

9000 x 300 = $3x10^6$ binding site (VHVλ)

3. Junctional diversity is generated during V(D)J joining by variation in the exact point of recombination between a heavy chain variable region gene and a D segment, or a D segment and a heavy chain J segment, or a light chain variable region gene and J segment.

Germline sequences:

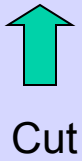
CCC GGA CGA AGC TTC GTG A CACAGTG VH

DJ CACTGTG GAT TAC TAC GGT AGT : TGG GAC

Cutting next to CACAGTG

CCC GGA CGA AGC TTC GTG A CACAGTG

CACTGTG GAT TAC TAC GGT AGT : TGG



Ligation of CACAGTG to CACAGTG

CACTGTG:CACAGTG

Exonuclease digestion of coding sequences from ends

CCC GGA CGA AGC TTC GTG A GAT TAC TAC GGT AGT

CCC GGA CGA AGC T AT TAC TAC GGT AGT

Ligation of coding ends

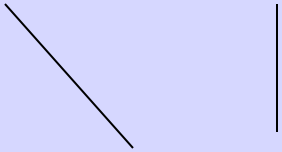
CCC GGA CGA AGC T : AT TAC TAC GGT AGT

Digestion to a different extent and ligation

CCC GGA CGA AGC TTC GTG A GAT TAC TAC GGT AGT

CCC GGA CGA AGC TTC GT T TAC TAC GGT AGT

CCC GGA CGA AGC TTC GT : T TAC TAC GGT AGT



CCC GGA CGA AGC T : AT TAC TAC GGT AGT

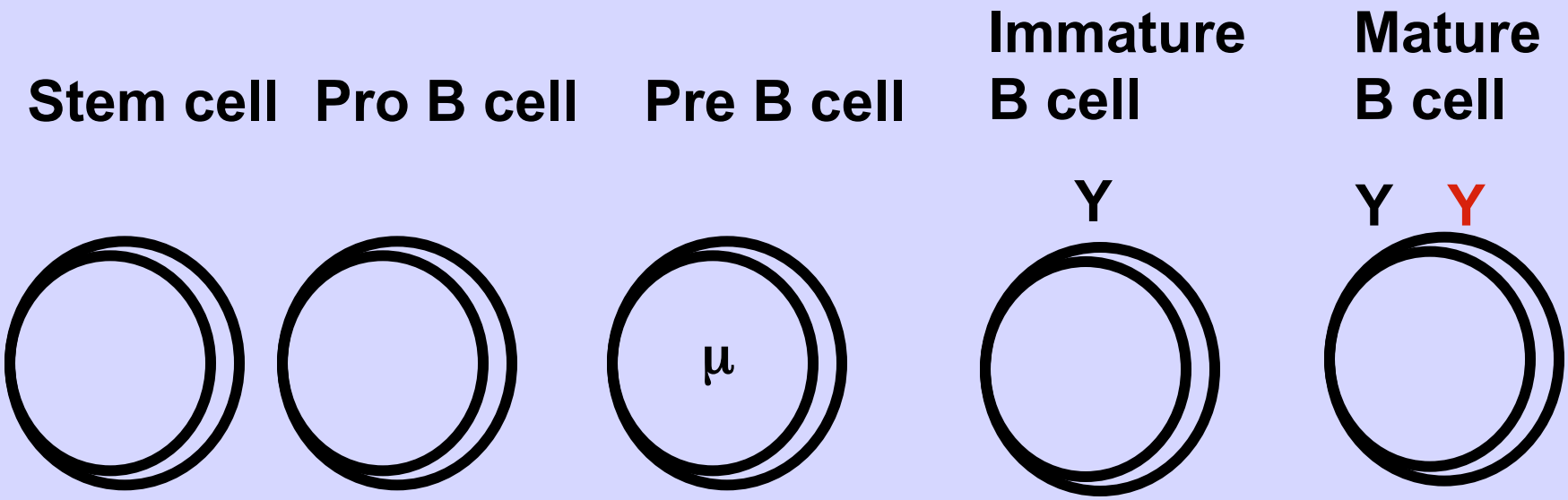
(for comparison; this is the sequence from the previous slide)

Junctional diversity often results in exactly the same number of codons at the V(D)J junction (particularly in the light chain variable region), but a different sequence, and hence, a different amino acid.

4. N region addition occurs by addition of nucleotides by terminal deoxynucleotide transferase to variable region, D segment, or J segment ends. These nucleotides are not encoded by a template.

CCC GGA CGA AGC TT : GCC : T TAC TAC GGT AGT

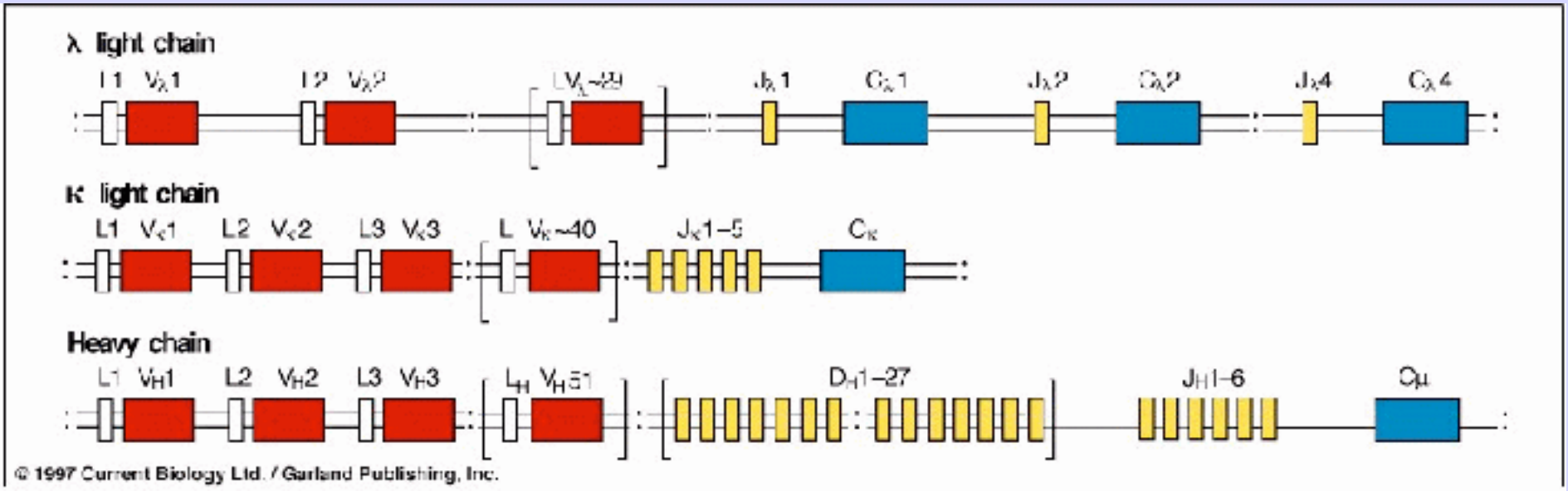
When in B cell differentiation do immunoglobulin gene rearrangements take place?



In pro B cells in the bone marrow, during antigen-independent B cell differentiation, first a D segment is rearranged to a heavy chain J segment on both chromosomes.

Then, a heavy chain variable region is rearranged to the DJ on one chromosome.

If this V(D)J rearrangement does not result in mu heavy chain expression (because a heavy chain variable region pseudogene is used, or the VDJ exon is out of frame), the pro B cell attempts to rearrange a heavy chain variable region to DJ on the other chromosome.



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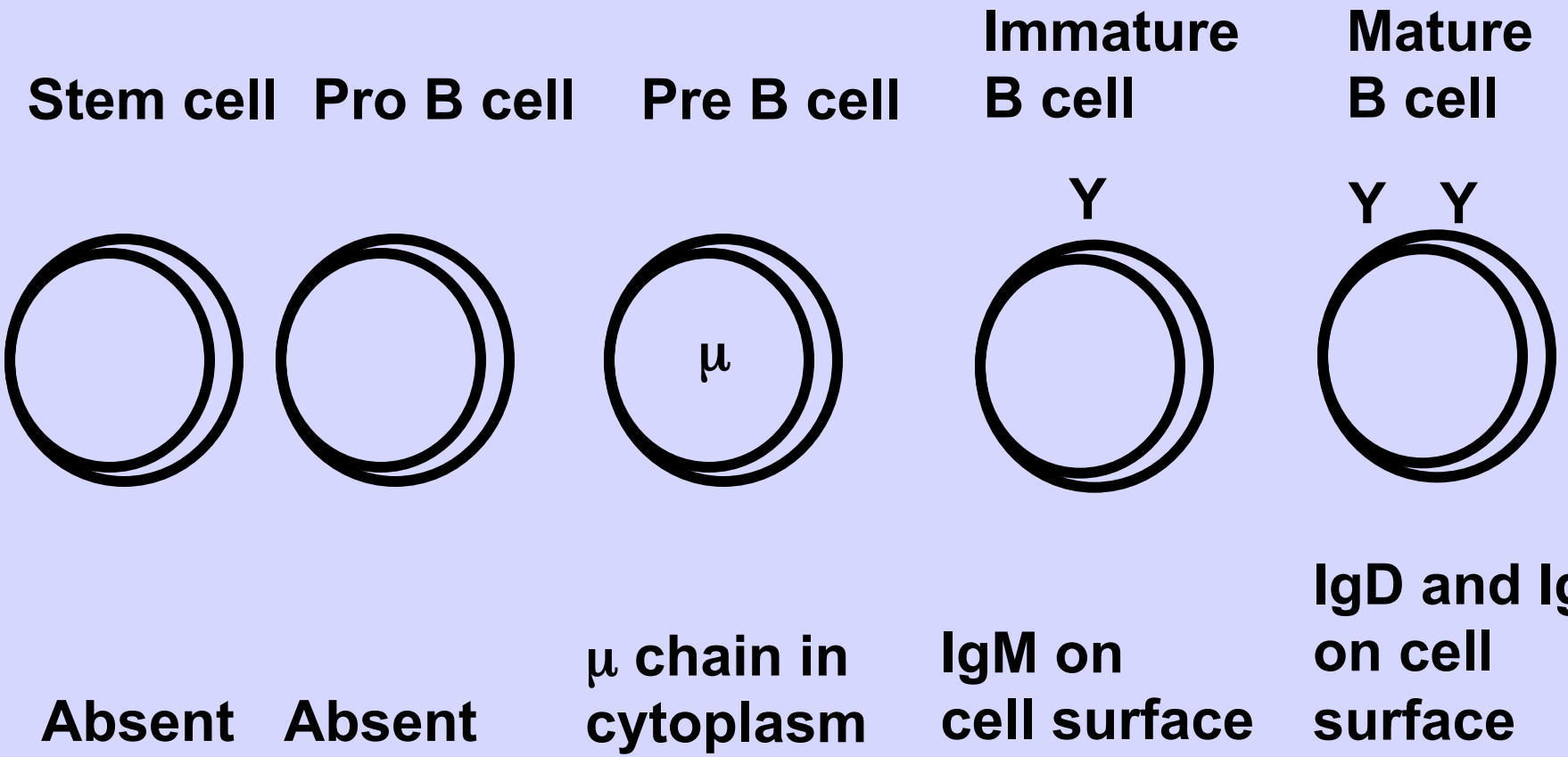
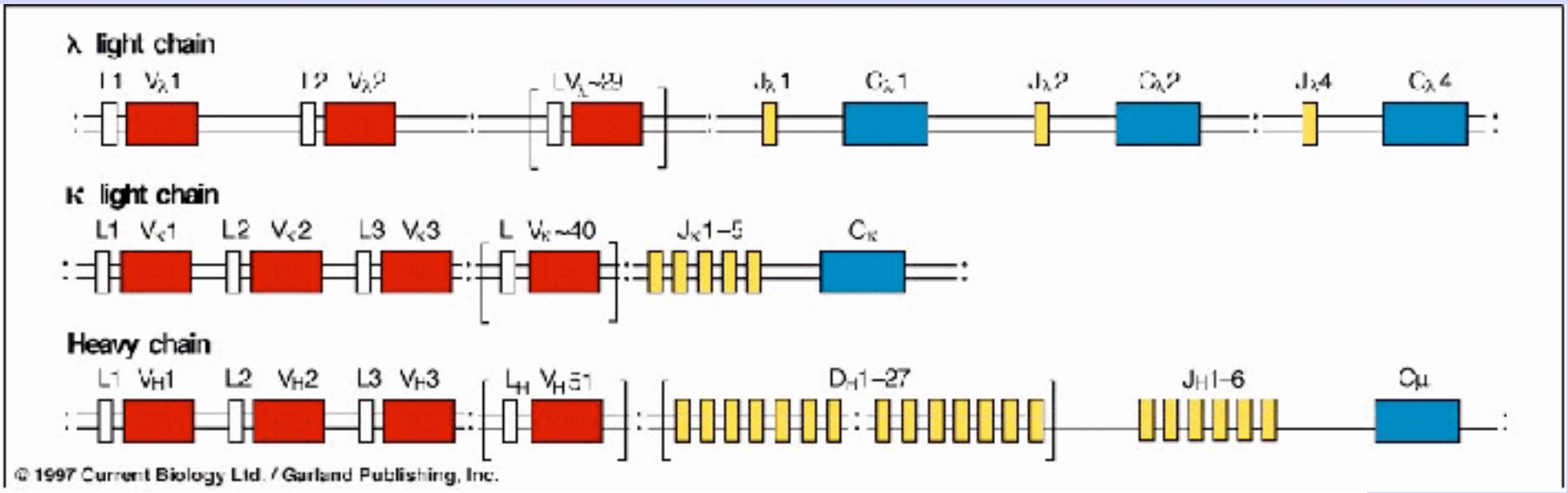
If heavy chain variable region to DJ joining results in expression of a μ heavy chain, the pro B cell becomes a pre B cell and also attempts light chain variable region to J segment rearrangement on one chromosome


Stem cell
Pro B cell
Pre B cell
Immature B cell
Mature B cell
Y
Y Y
μ
Absent
Absent
μ chain in cytoplasm
IgM on cell surface
IgD and IgM on cell surface



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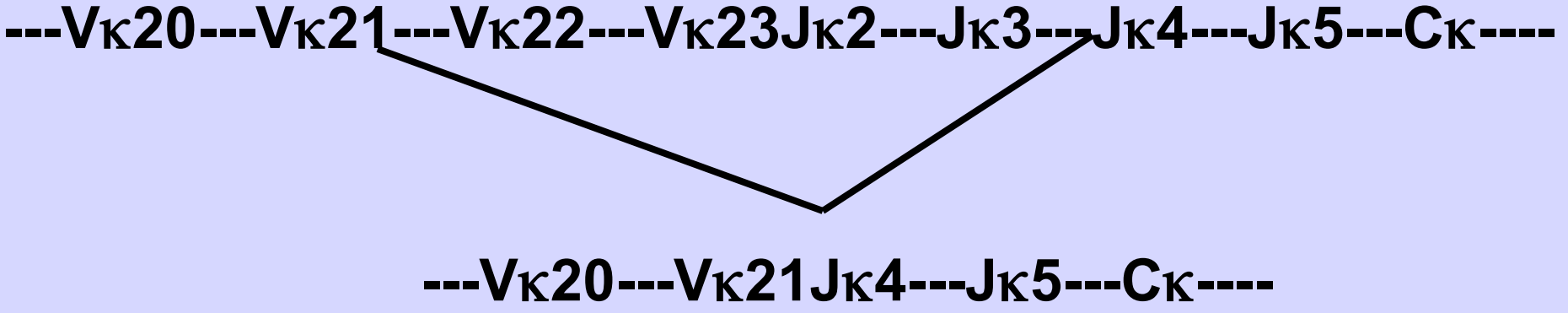
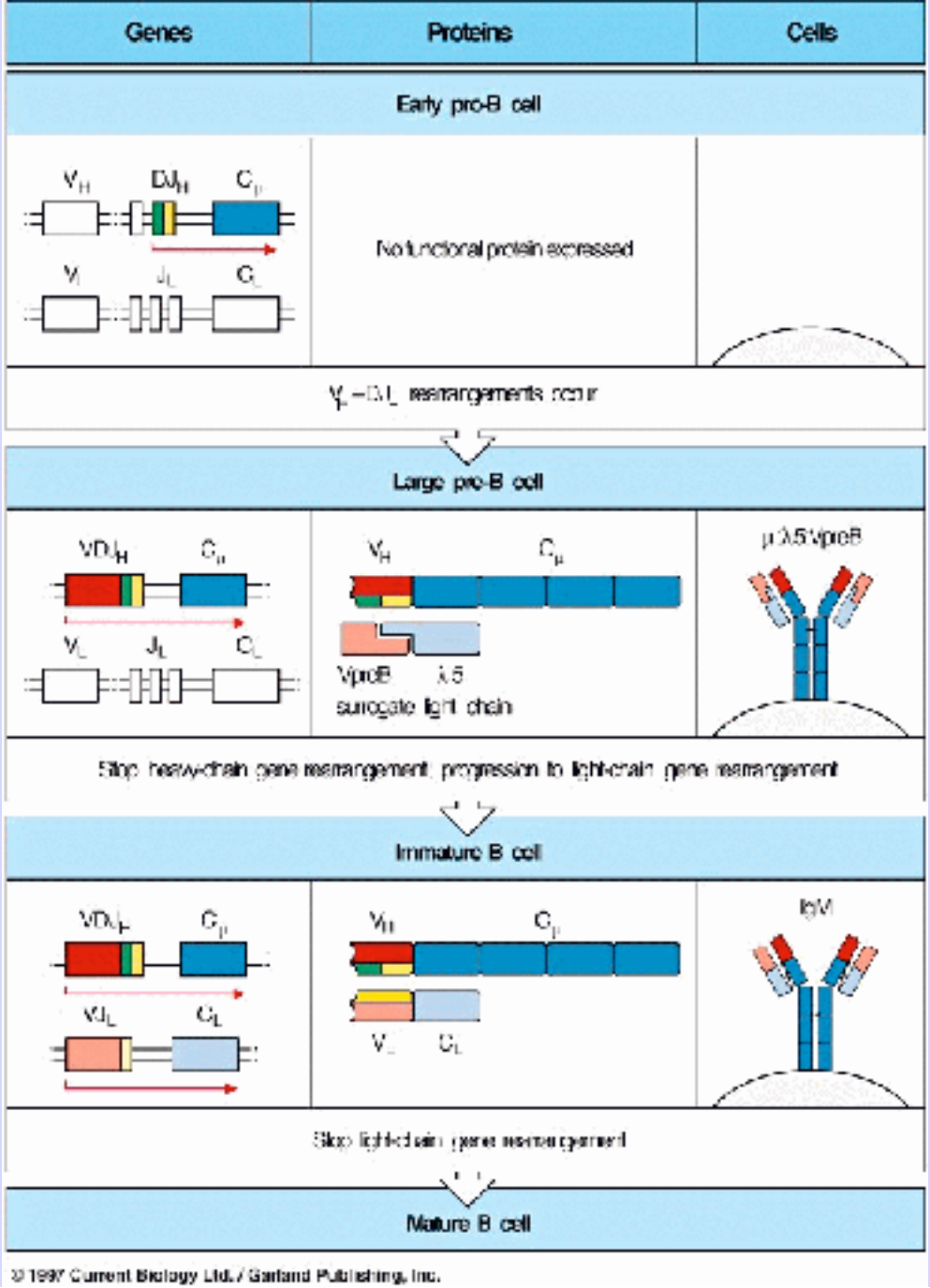
In general Vκ to Jκ precedes Vλ to Jλ, because κ rearrangement is favored 20 to 1 over λ rearrangement.

Each cell has four light chain loci that could undergo variable region to J segment rearrangement. There are several VJ rearrangements possible in each light chain locus.



If light chain variable region to J segment joining results in light chain production and an assembled IgM molecule on the cell surface, the pre B cell goes on to be an immature B cell.



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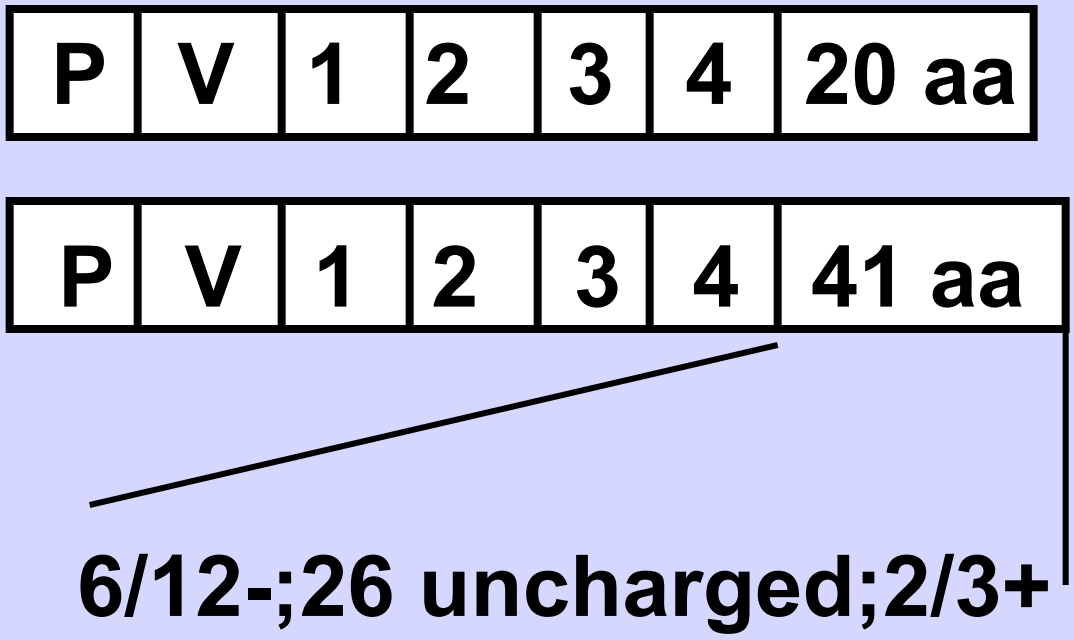
If V(D)J joining results in expression of a mu heavy chain (the joining is in-frame, and does not use a pseudo VH), then further VH to DJ joining is shut off.

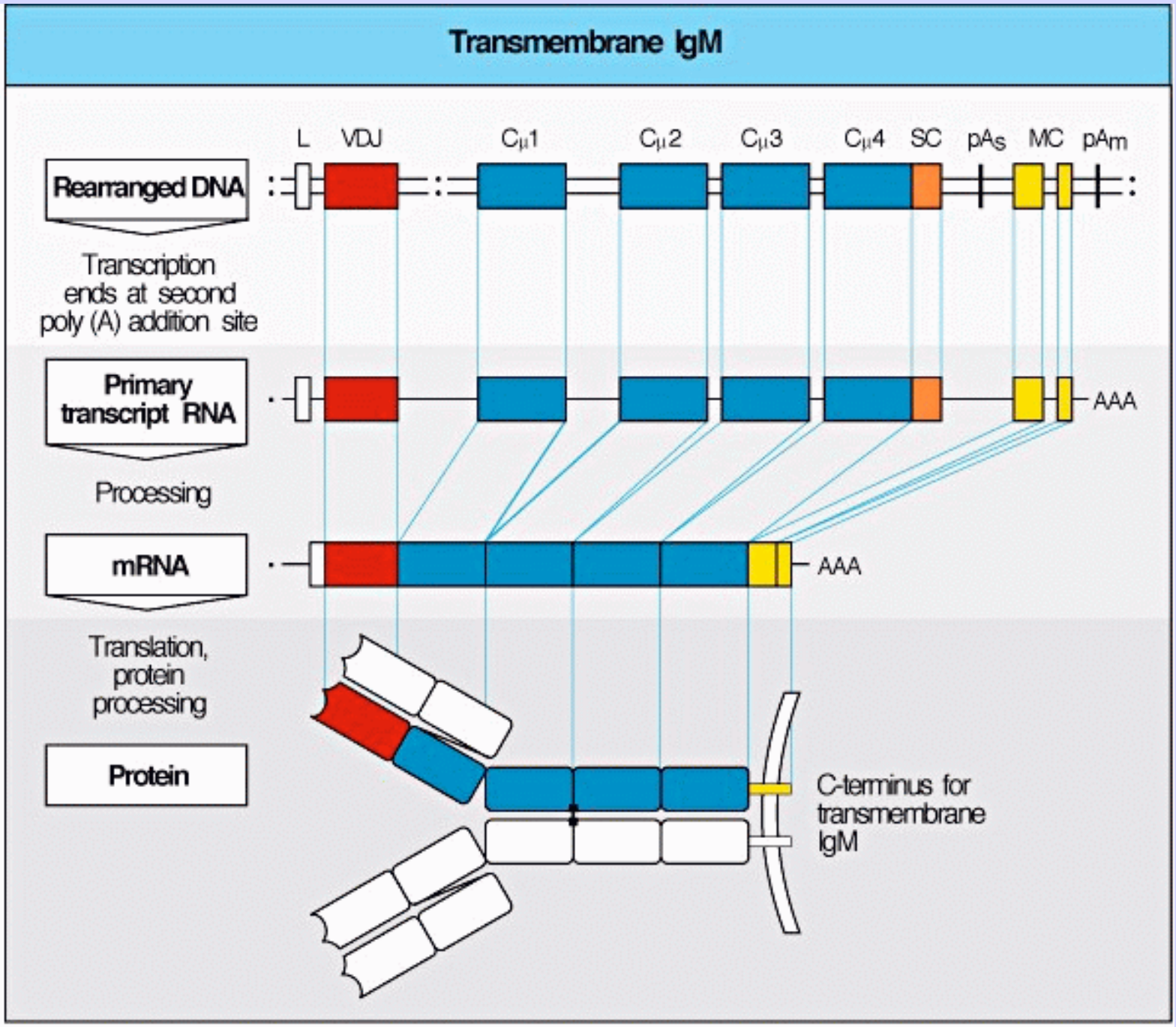
If VL to JL joining results in expression of a light chain, then further VL to JL joining is shut off for both the kappa and lambda genes.

This feed-back regulation of V(D)J joining is the basis of allelic exclusion. The shut-off of further V(D)J joining prevents a B cell from expressing two heavy chains or two light chains.

How does a mature B cell express first the membrane form of IgM, and later, the secreted form of IgM, with the same idiotype (same heavy chain variable region)?



P = the hydrophobic, 26 amino acid, region that is cleaved off as the translated protein crosses the endoplasmic reticulum.



SC=20 aa

MC=41 aa

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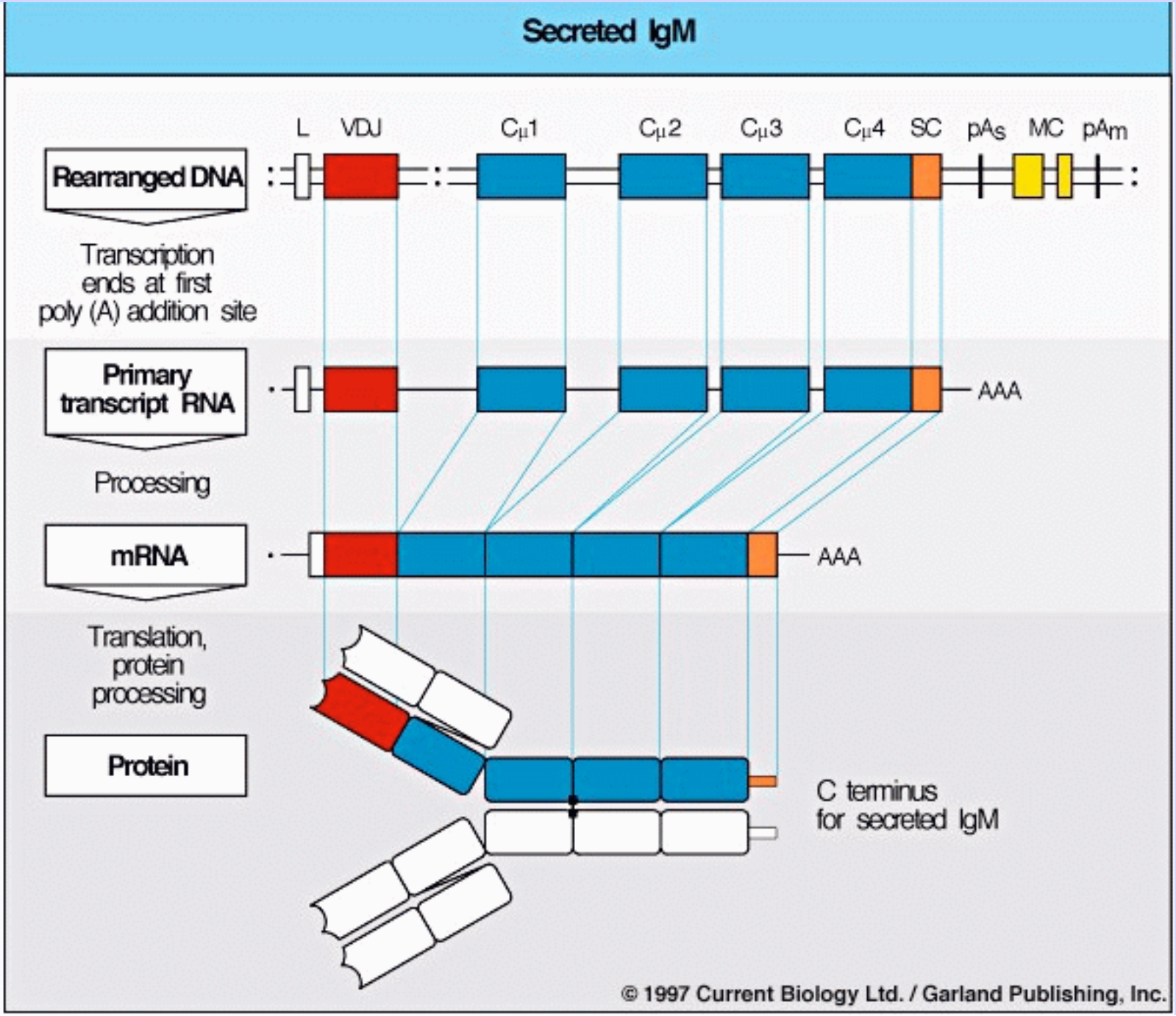

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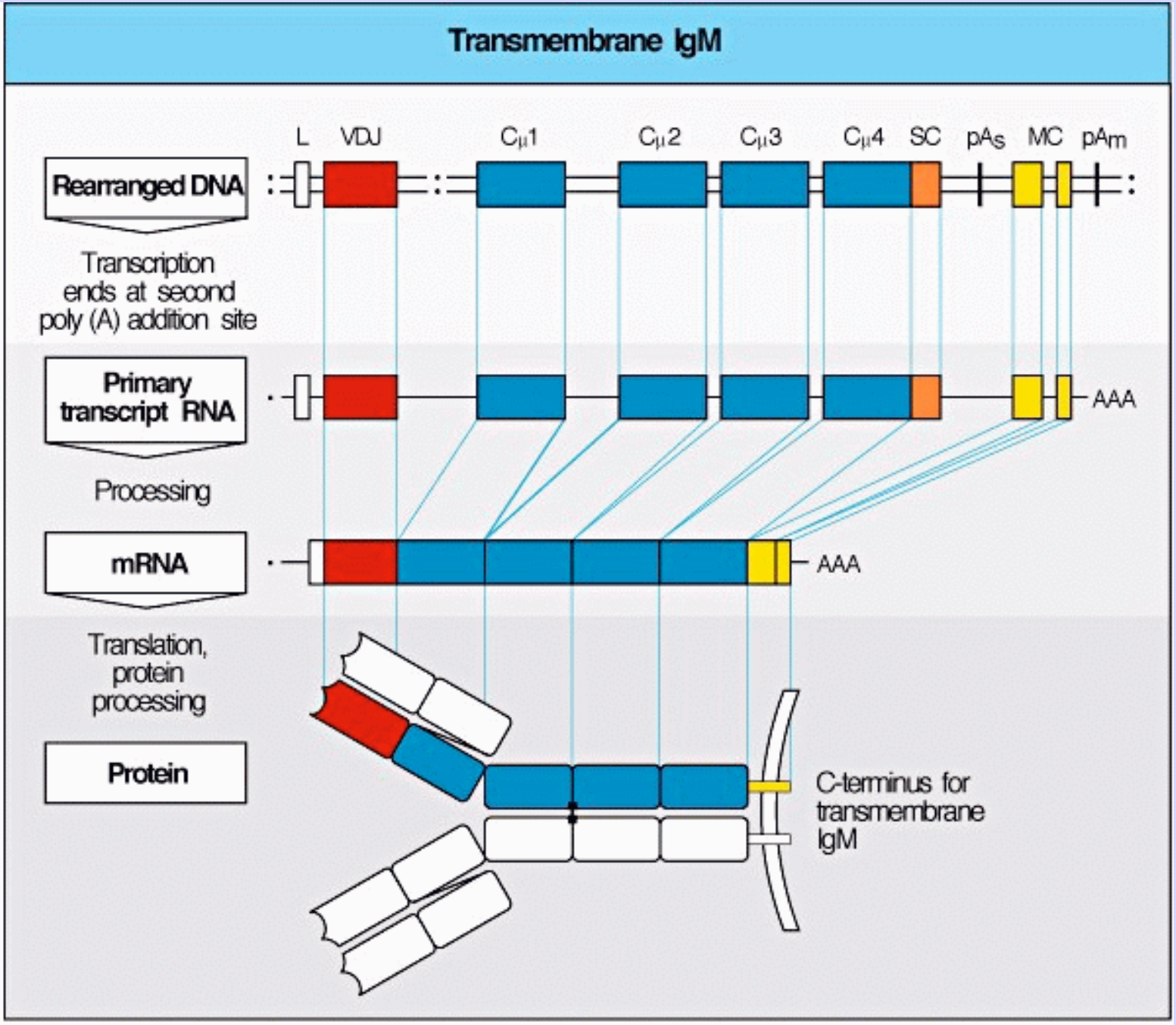

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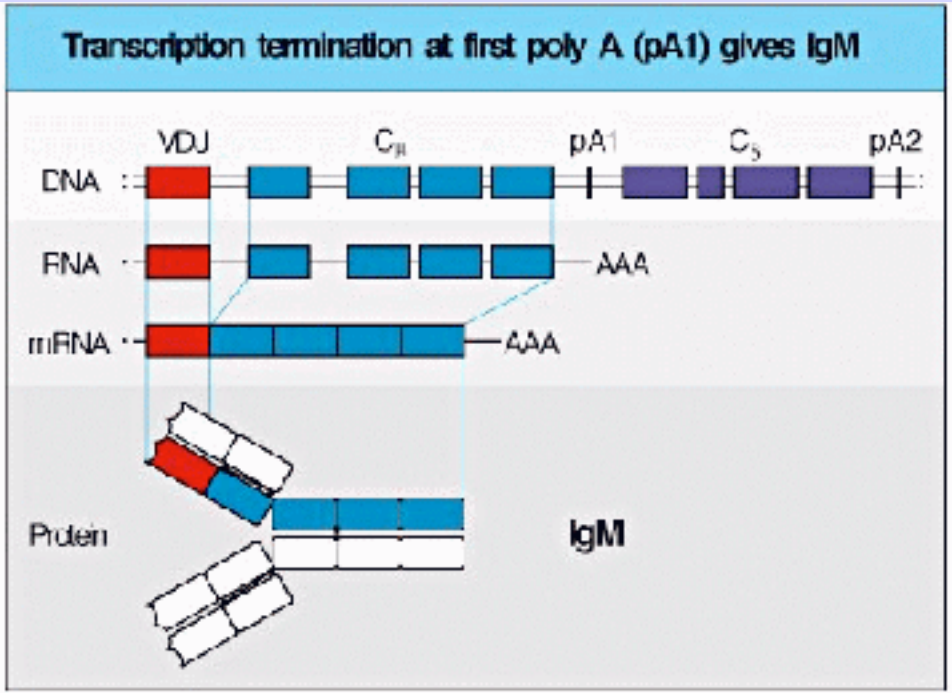

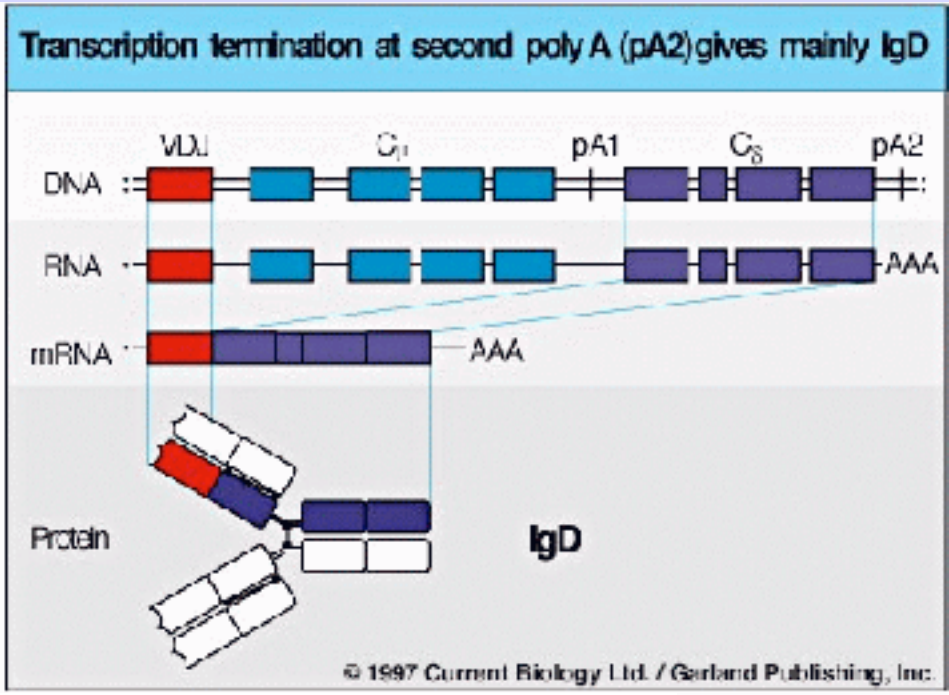

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Changes in membrane μ, secreted μ, and membrane δ expression are mediated by RNA splicing; the genes themselves do not change. Hence, these changes in expression are reversible.

Summary

1. Combinatorial diversity is generated by joining of one of many variable regions to one of a few D segments and J segments, independently of antigenic stimulation.

2. Junctional and N region diversity are generated at V-D, D-J, and VL-JL junctions.

3. V(D)J joining is regulated in that it occurs only in the B cell lineage, the gene segments are recombined in specific order, and V(D)J joining is stopped by productive expression of heavy and light chain protein.

4. Membrane μ, secreted μ, and δ expression are mediated by RNA splicing.

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