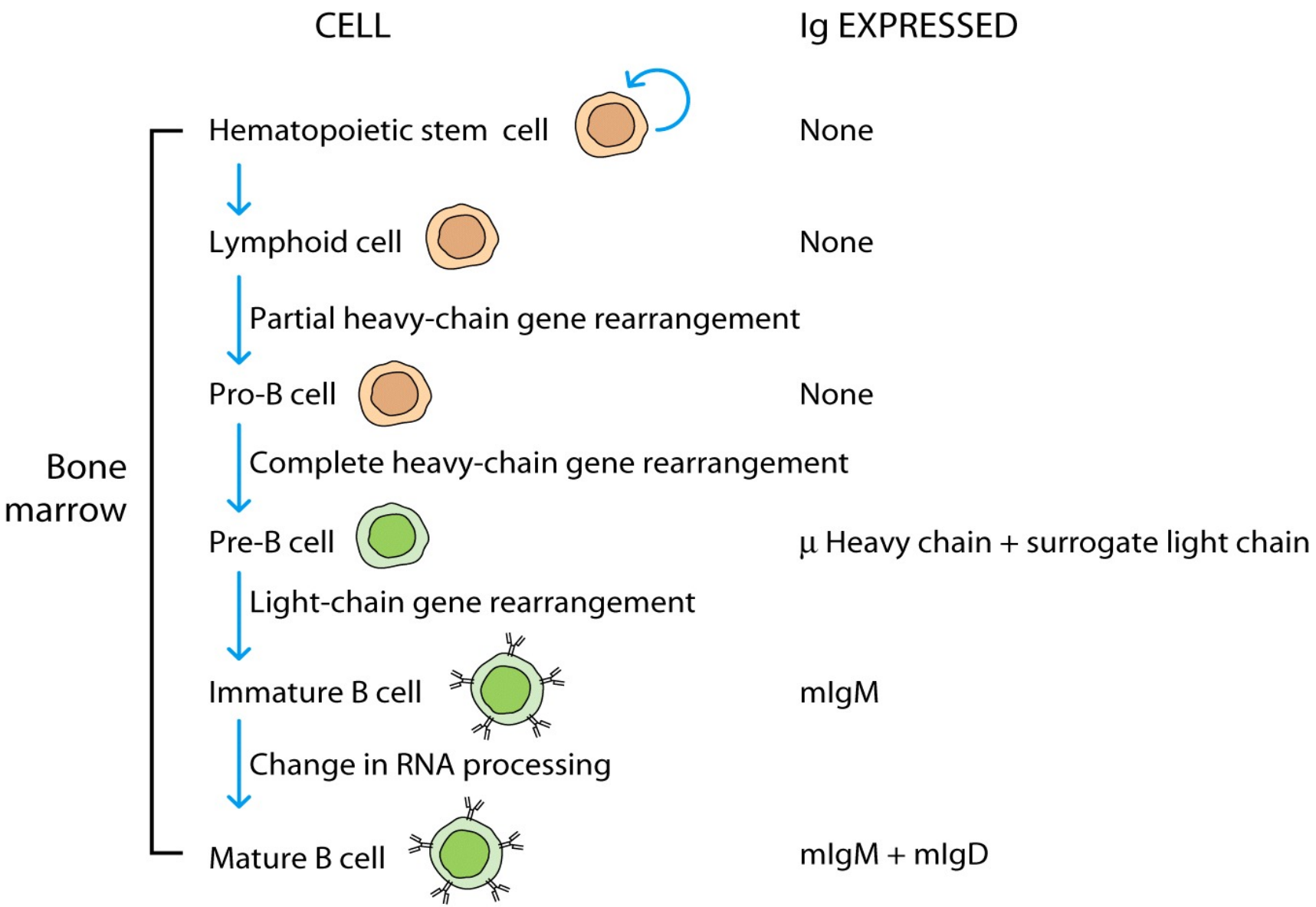
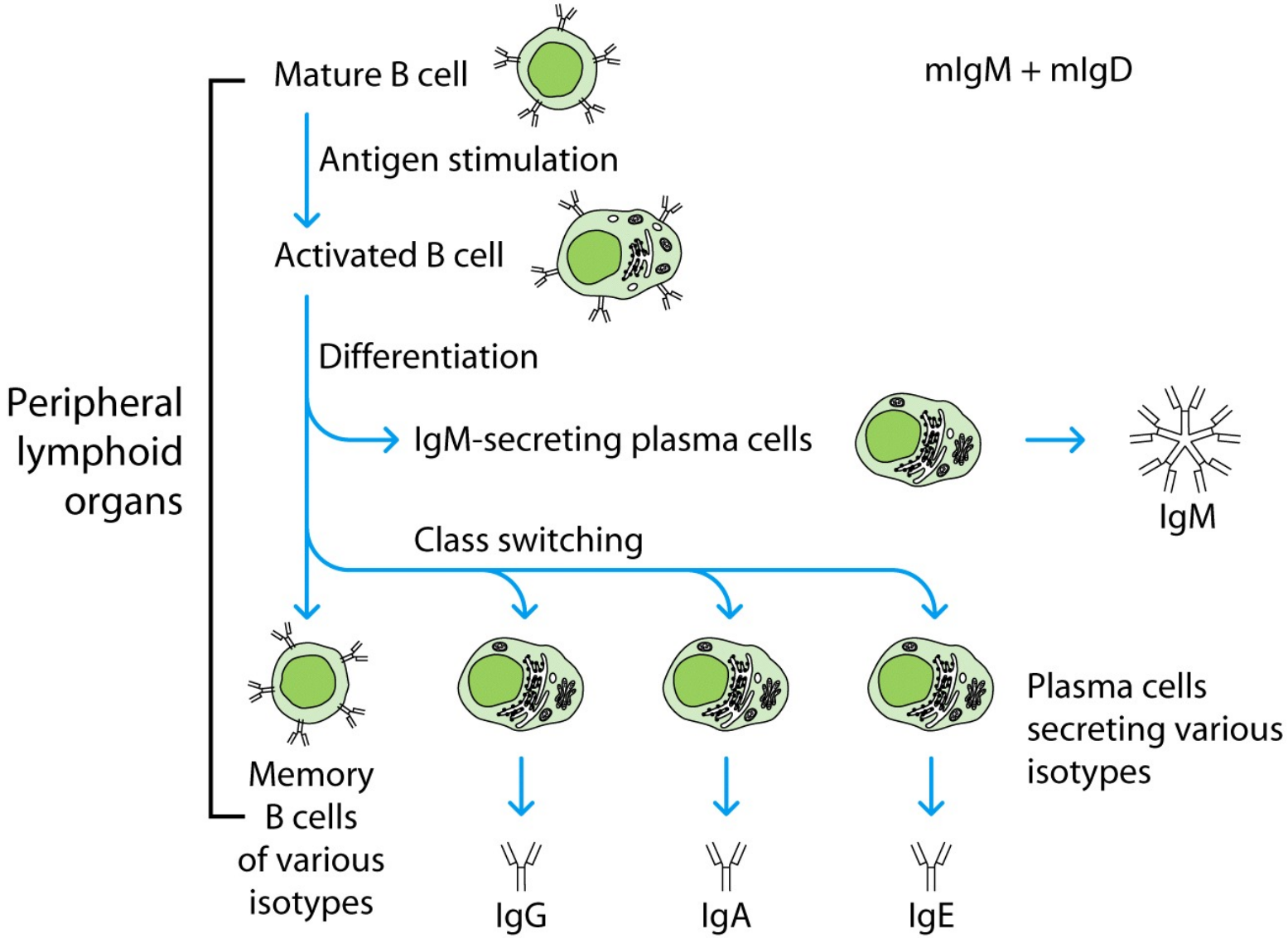
Organization and Expression of Immunoglobulin Genes And Antibody Diversity

B lymphocyte development




Mature B cell
mIgM + mIgD
Antigen stimulation
Activated B cell
Differentiation
Peripheral lymphoid organs
IgM-secreting plasma cells
IgM
Class switching
Memory B cells of various isotypes
Plasma cells secreting various isotypes
IgG
IgA
IgE

Problem…the immune system makes over one billion different antibody proteins

In 1950's: central dogma stated DNA→ RNA→ protein

One gene for each protein

Required millions of genes just for the immune system

Does not seem possible, but most scientists thought it might be

Today we know the human genome is less than 30,000 genes

So, what is really going on???

Dryer and Bennett, 1965

They proposed radical theory to account for diversity of antibodies

►Each antibody was coded for by two separate genes

- **One for the variable region**
- **One for the constant region**

►These genes combined at the DNA level and expressed single mRNA

►Suggested 1000's of variable region genes and only one constant region gene

Most biomedical scientists did not like this idea and rejected it!!!

Genetic models of the 1960's were also unable to explain:

- **How B cells shut down the Ig genes on just one of their chromosomes.**

  All other genes known at the time were expressed co-dominantly. B cells expressed a light chain from one parent only and a heavy chain from one parent only (evidence from allotypes).

- **A genetic mechanism to account for increased antibody affinity in an immune response**

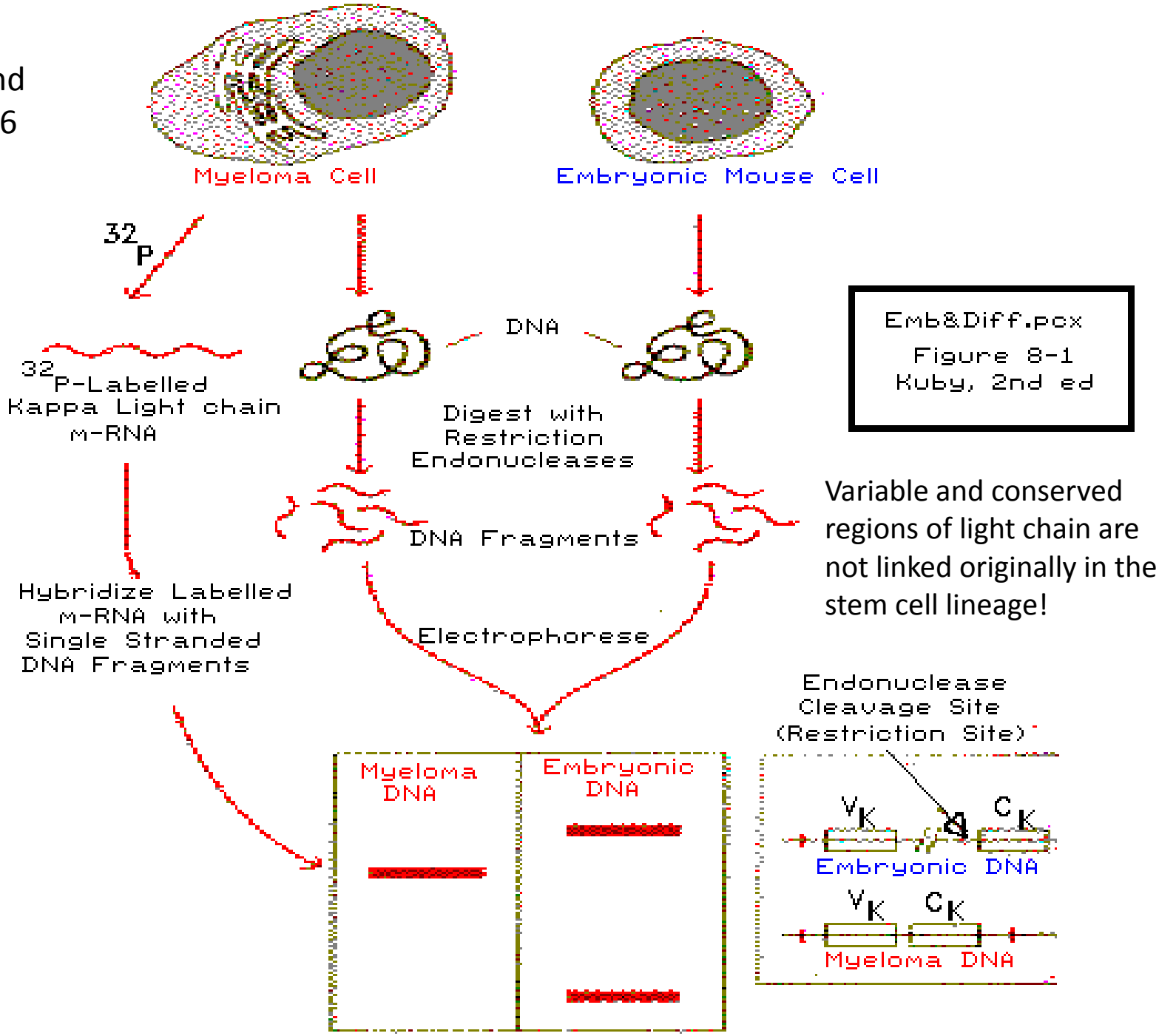
- **How a single specificity of antibody sequentially switched isotype.**

- **How the same specificity of antibody was secreted and simultaneously expressed on the cell surface of a B cell.**

Current theory must account for the following known properties of antibodies

- The vast diversity of antibody specificities
- The presence in Ig heavy and light chains of a variable region at the amino-terminal end and a constant region at the carboxyl-terminal end
- The existence of isotypes with the same antigenic specificity, which result from the association of a given variable region with different heavy-chain constant regions

Tonegawa and Hozumi, 1976



Emb&Diff.pcx
Figure 8-1
Kuby, 2nd ed

Variable and conserved regions of light chain are not linked originally in the stem cell lineage!

Tonegawa's demonstration

1976—used restriction enzymes and DNA probes to show that germ cell DNA contained several smaller DNA segments compared to DNA taken from developed lymphocytes (myeloma cells)

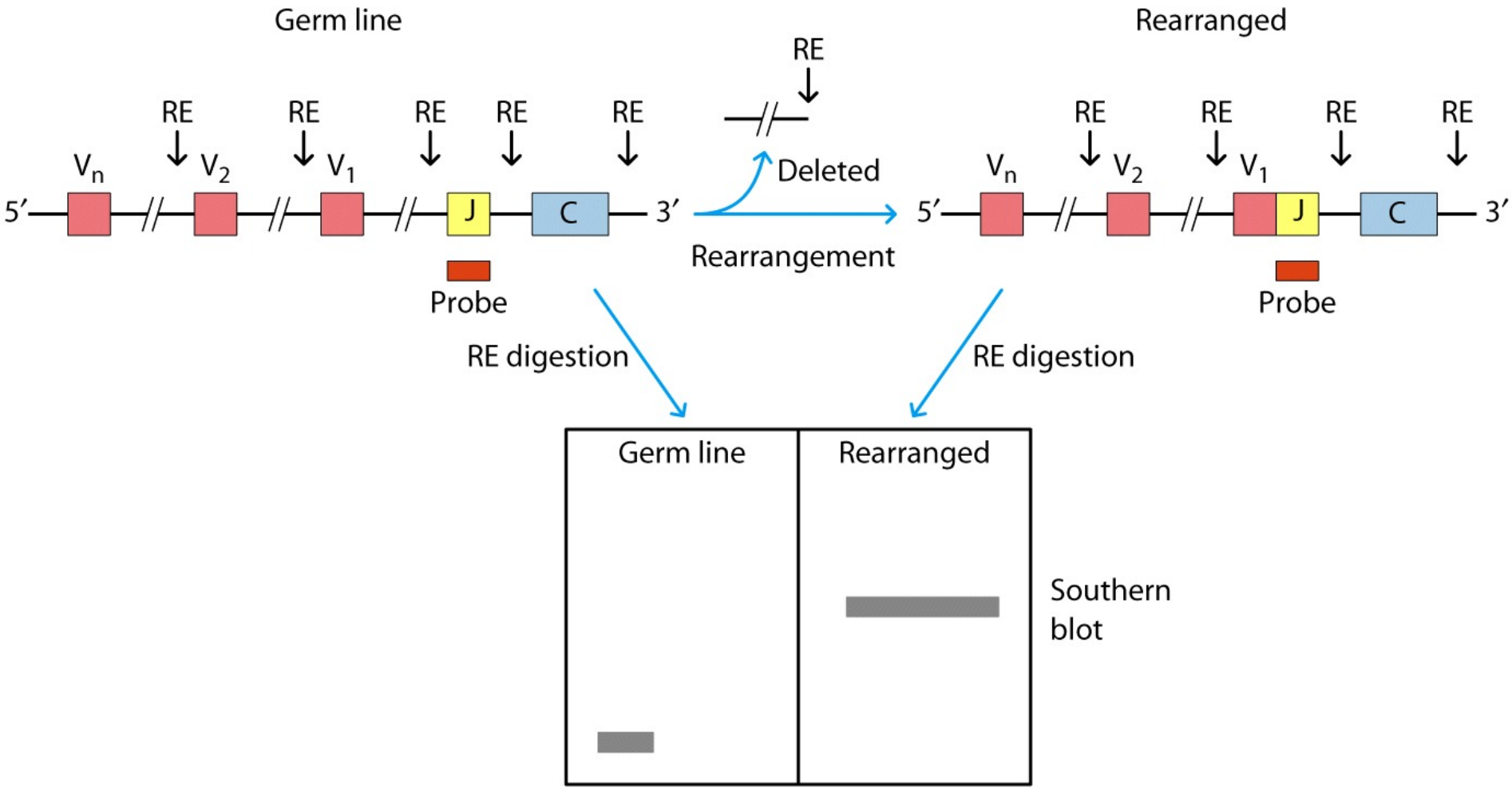
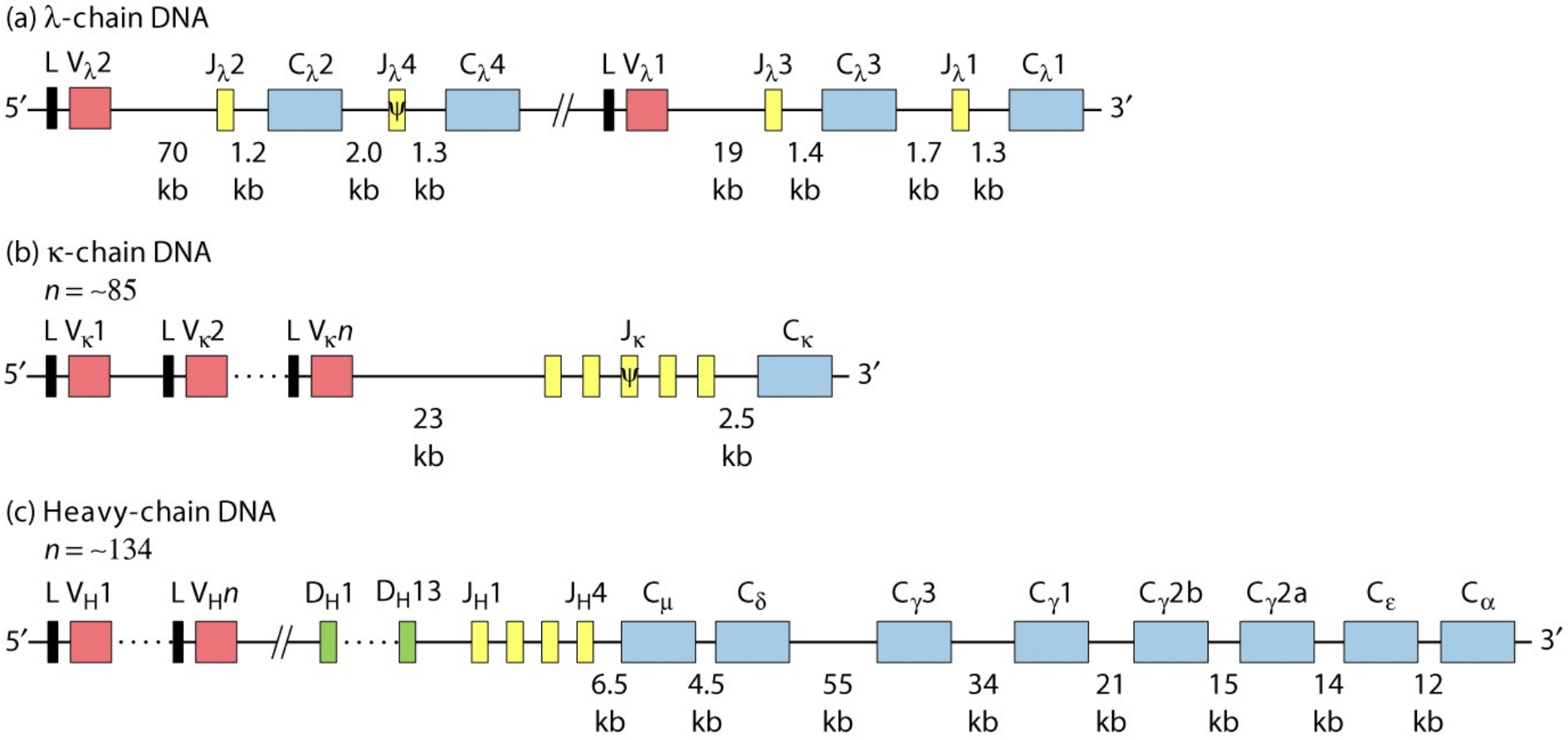


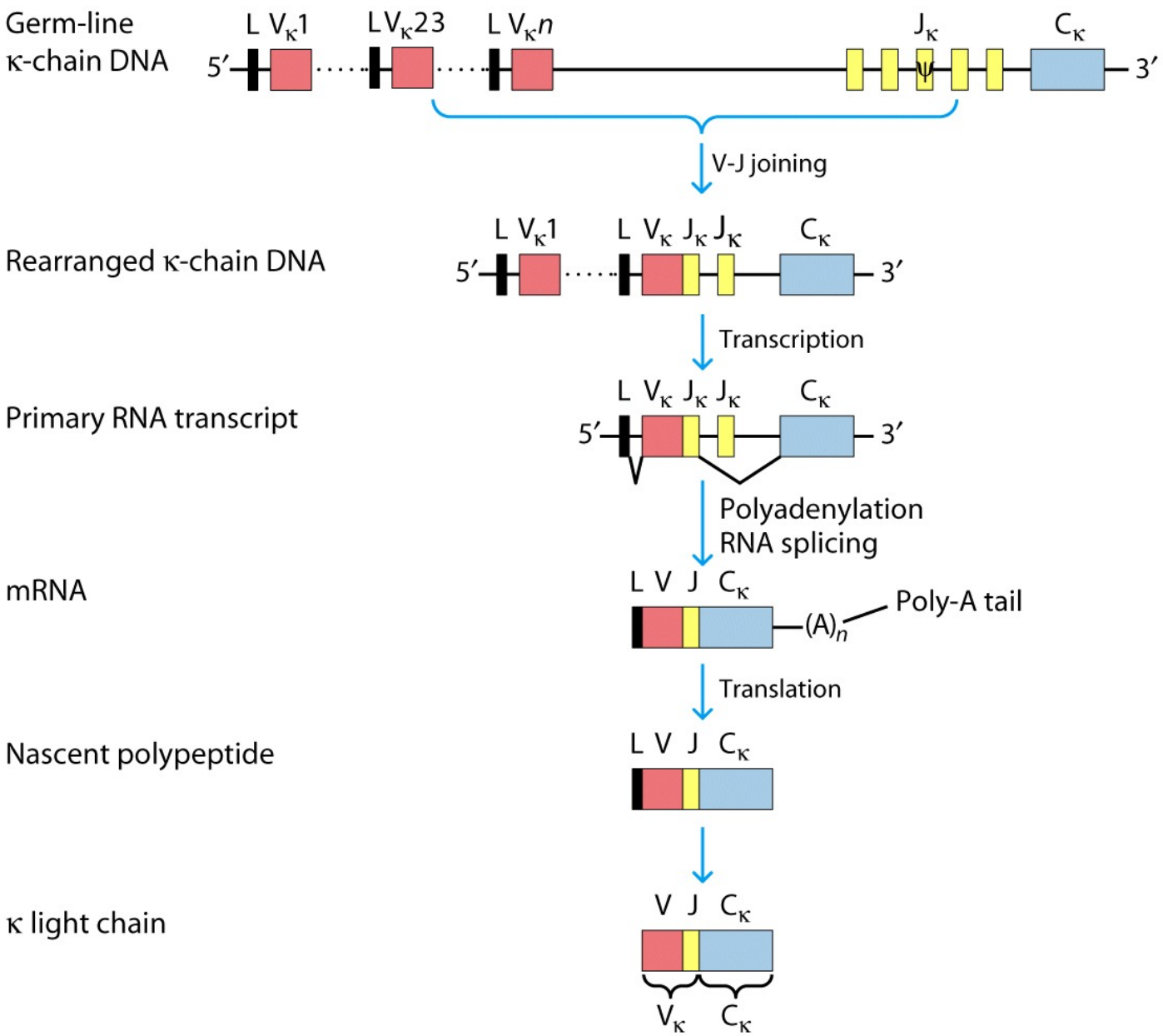
TABLE 5-1 Chromosomal locations of immunoglobulin genes in human and mouse

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

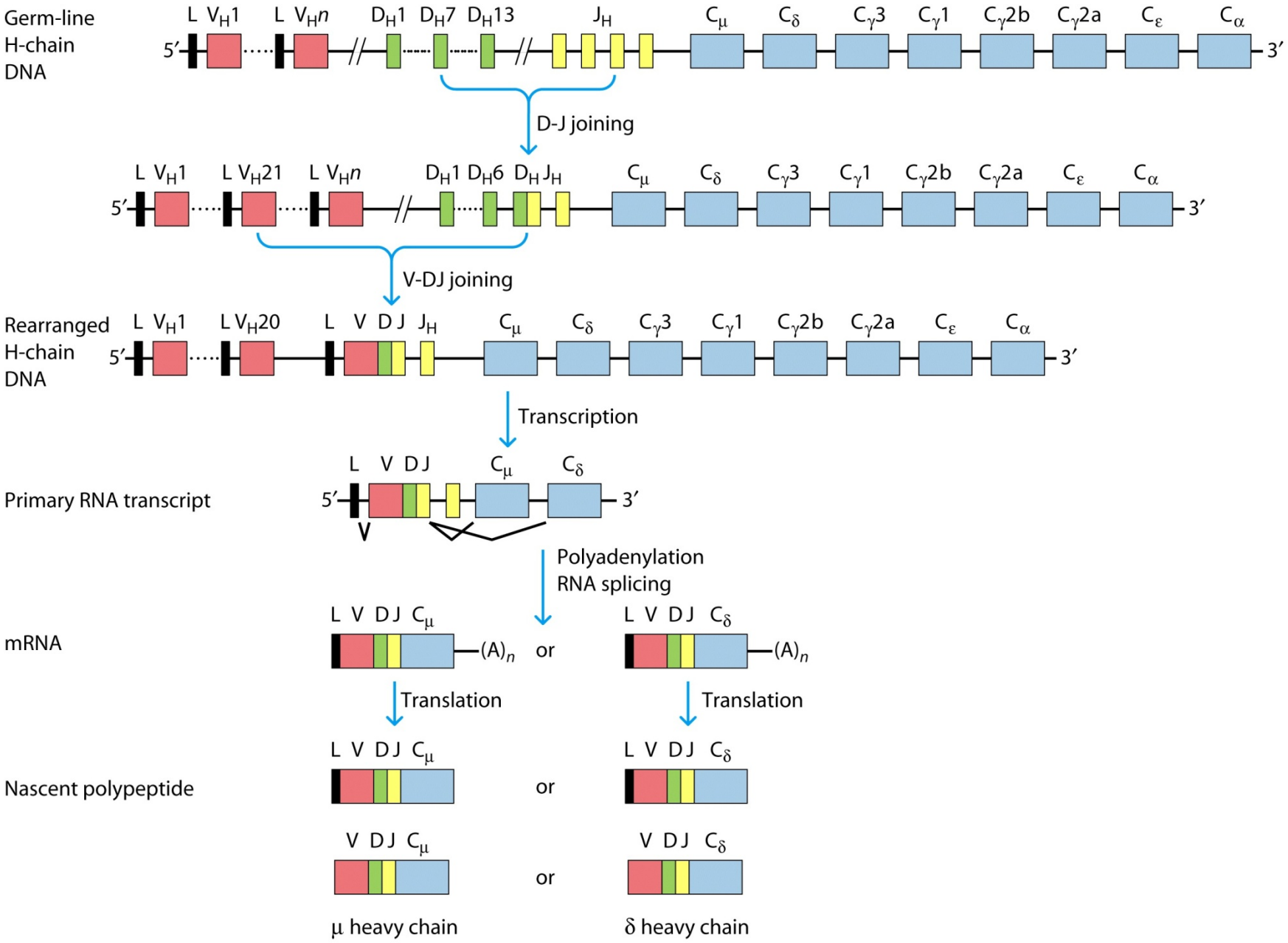
Multigene organization of Ig genes



Kappa light chain rearrangement



Heavy chain rearrangement



Mechanism of Variable-Region DNA rearrangements

- Recombination signal sequences (RSSs)
  - Between V, D, and J segments
  - Signal for recombination
  - 2 kinds
    - » 12 base pairs (bp) – 1 turn of DNA
    - » 23 bp – 2 turns of DNA
    - » 12 can only join to 23 and vice versa

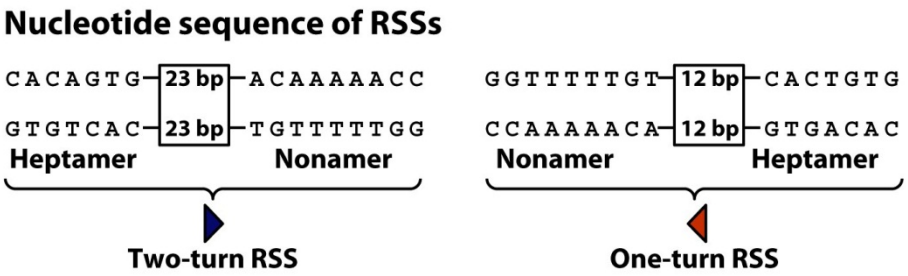


Figure 5-6a
Kuby IMMUNOLOGY, Sixth Edition

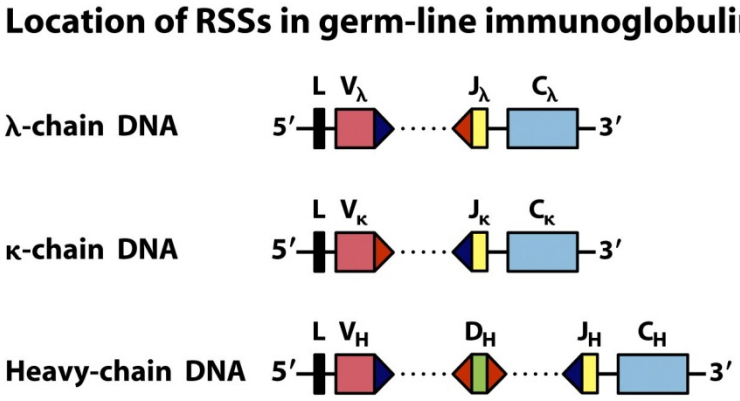
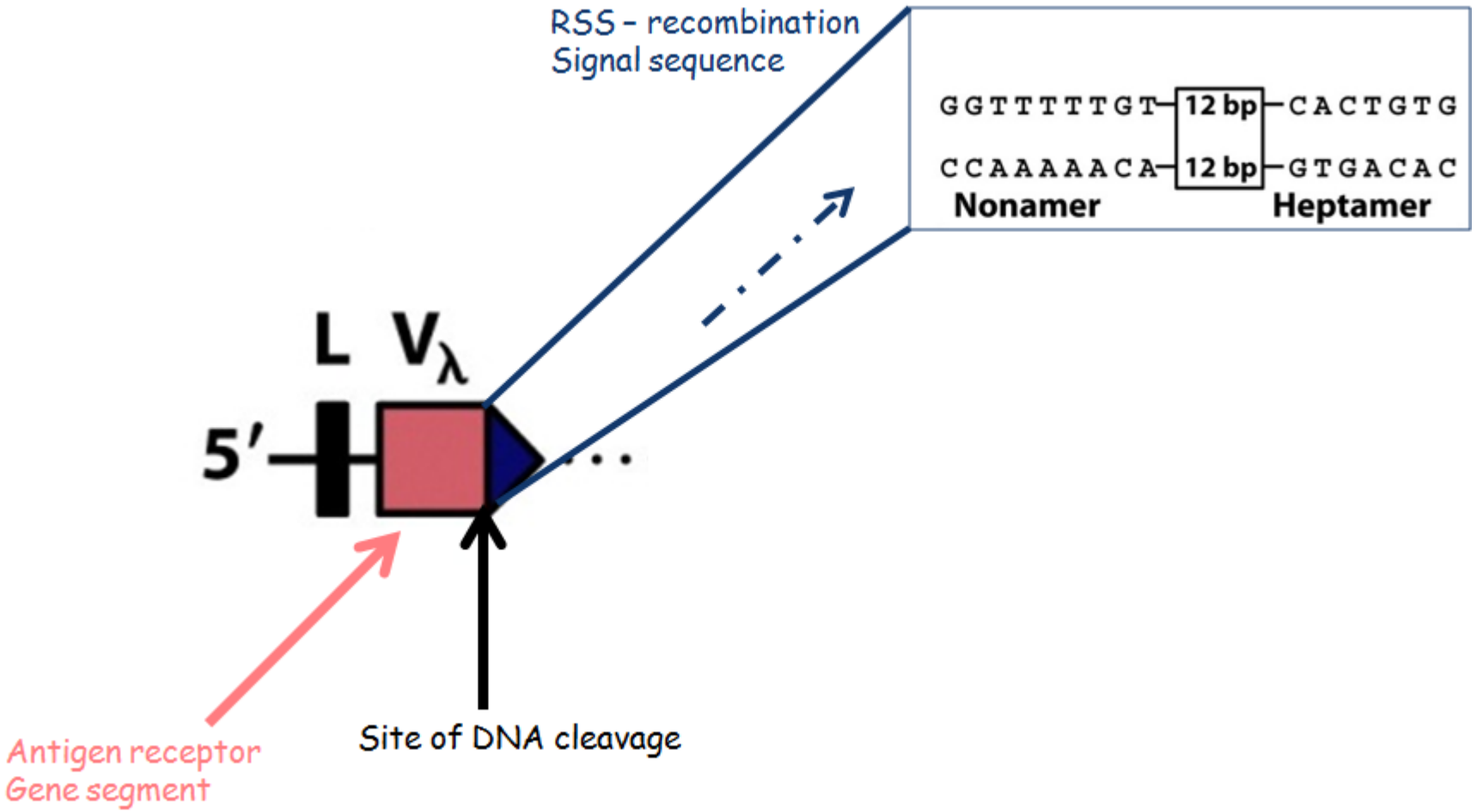
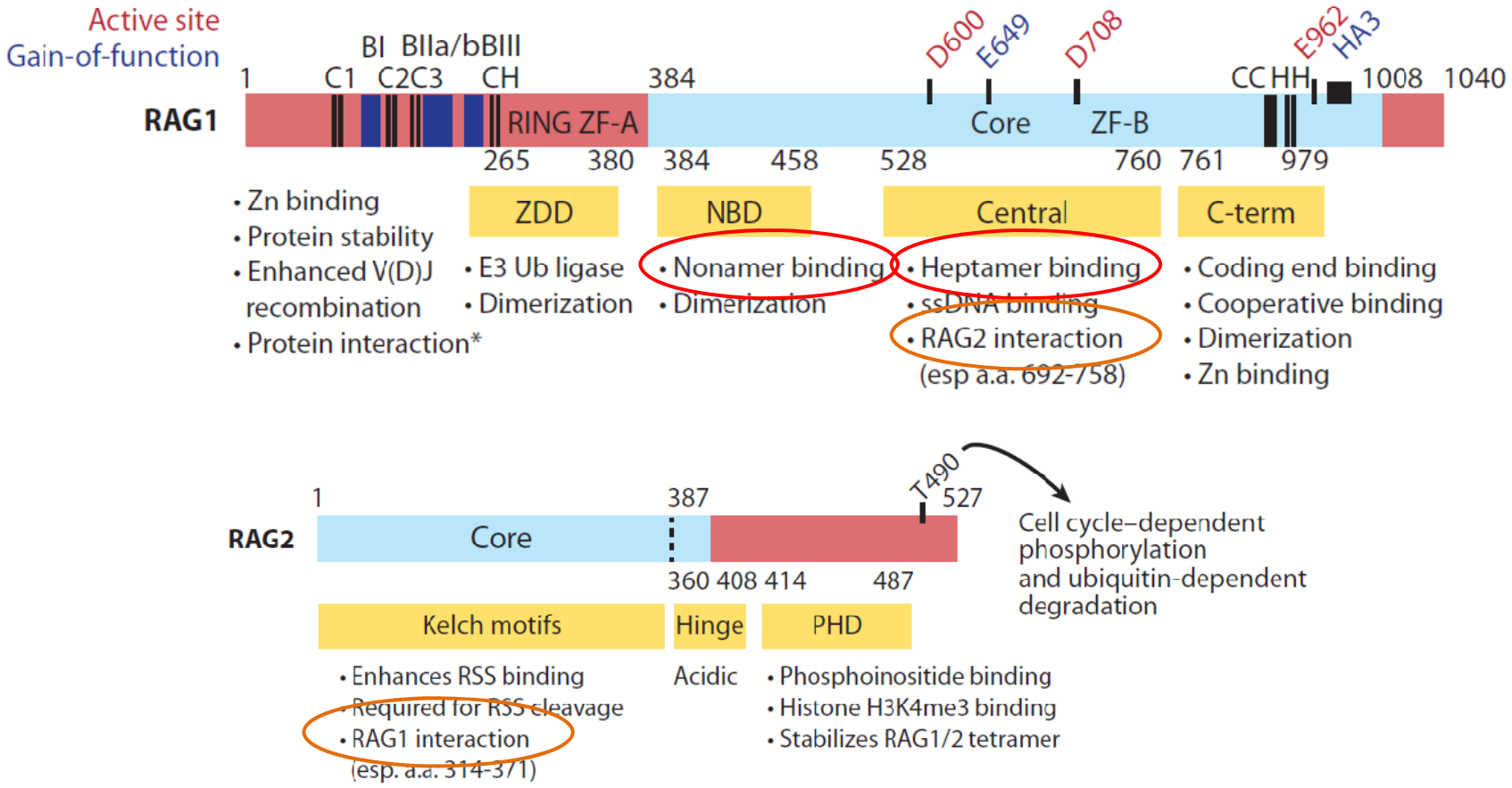


Figure 5-6b
Kuby IMMUNOLOGY, Sixth Edition


RSS – recombination
Signal sequence
GGTTTTTGT
12 bp
CACTGTG
CCAAAAACA
12 bp
GTGACAC
Nonamer
Heptamer
L
Vλ
5′
Antigen receptor
Gene segment
Site of DNA cleavage

Recombination Activating Gene (RAG):



Schatz, David G., and Patrick C. Swanson. "V (D) J recombination: mechanisms of initiation." *Annual review of genetics* 45 (2011): 167-202.

V(D)J recombination:

Nicking: first step of DNA cleavage by RAG in which one DNA strand is broken 5' of the heptamer

Hairpin formation: second step of DNA cleavage by RAG in which the 3'-hydroxyl of the nicked strand attacks the other strand

HMGB: high mobility group box

Paired (synaptic) complex (PC): protein-DNA complex in which the two RSSs are held in close juxtaposition by the RAG proteins

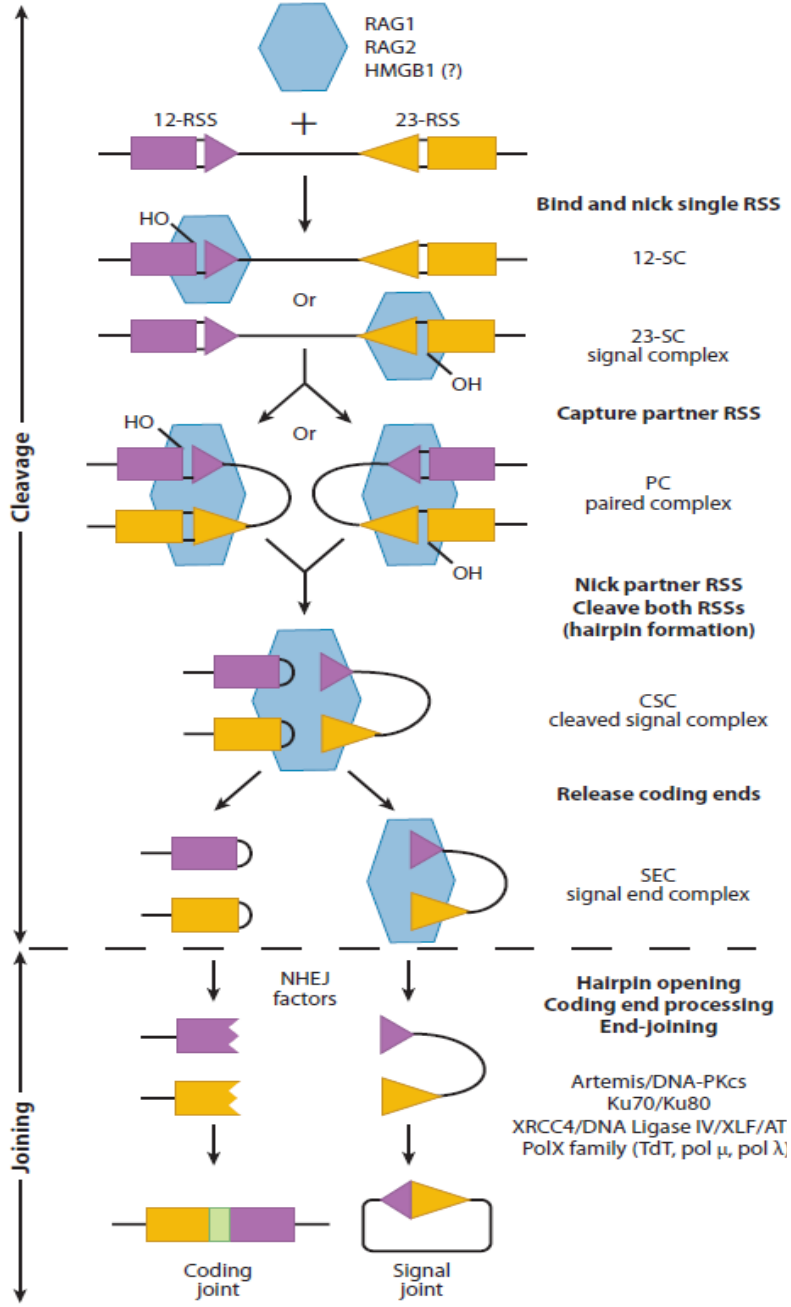
Signal end: after DNA cleavage by the RAG proteins, the DNA end that terminates in the RSS

Coding end: after DNA cleavage by the RAG proteins, the DNA end that terminates in the coding segment

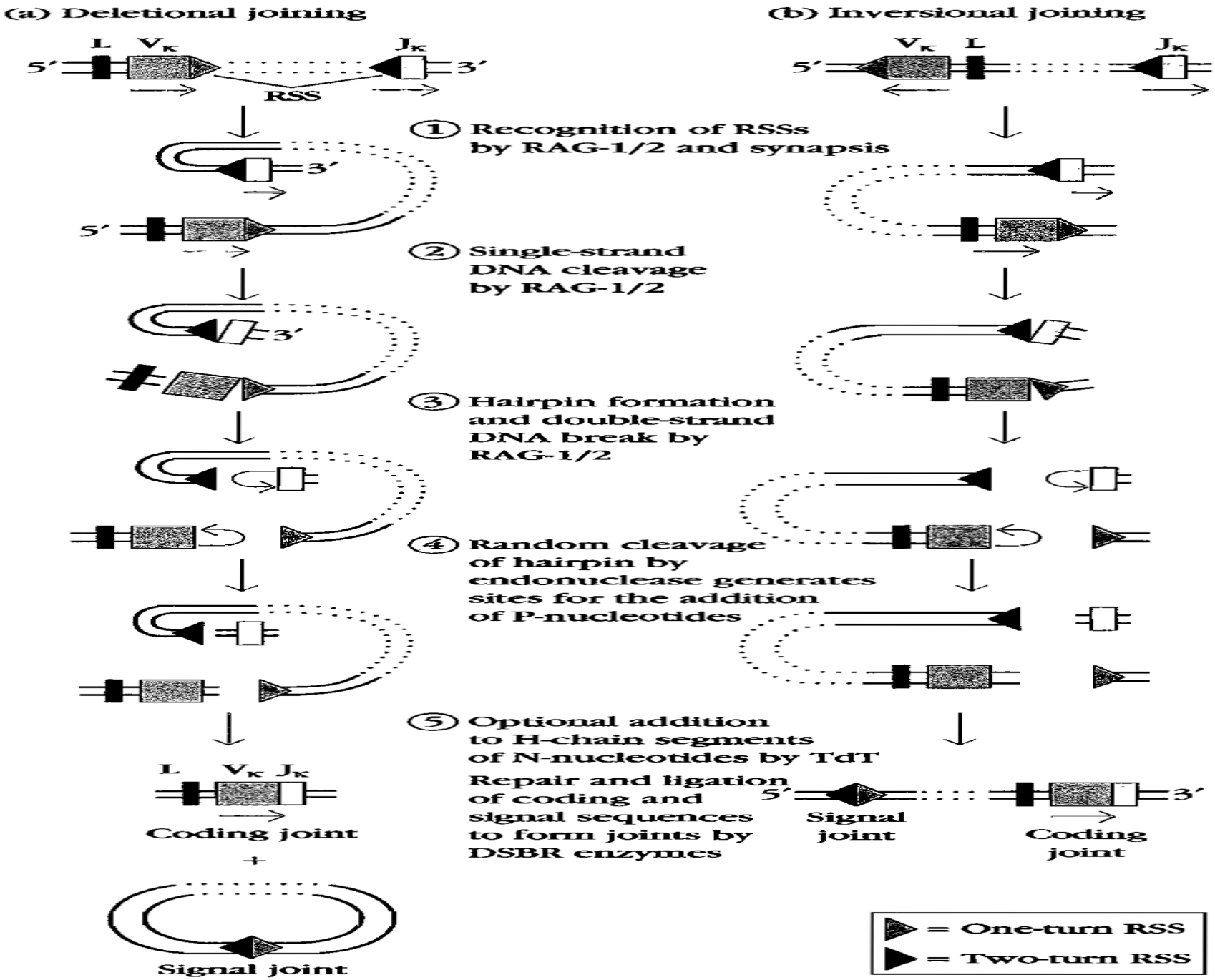
CSC: cleaved signal complex

SEC: signal end complex

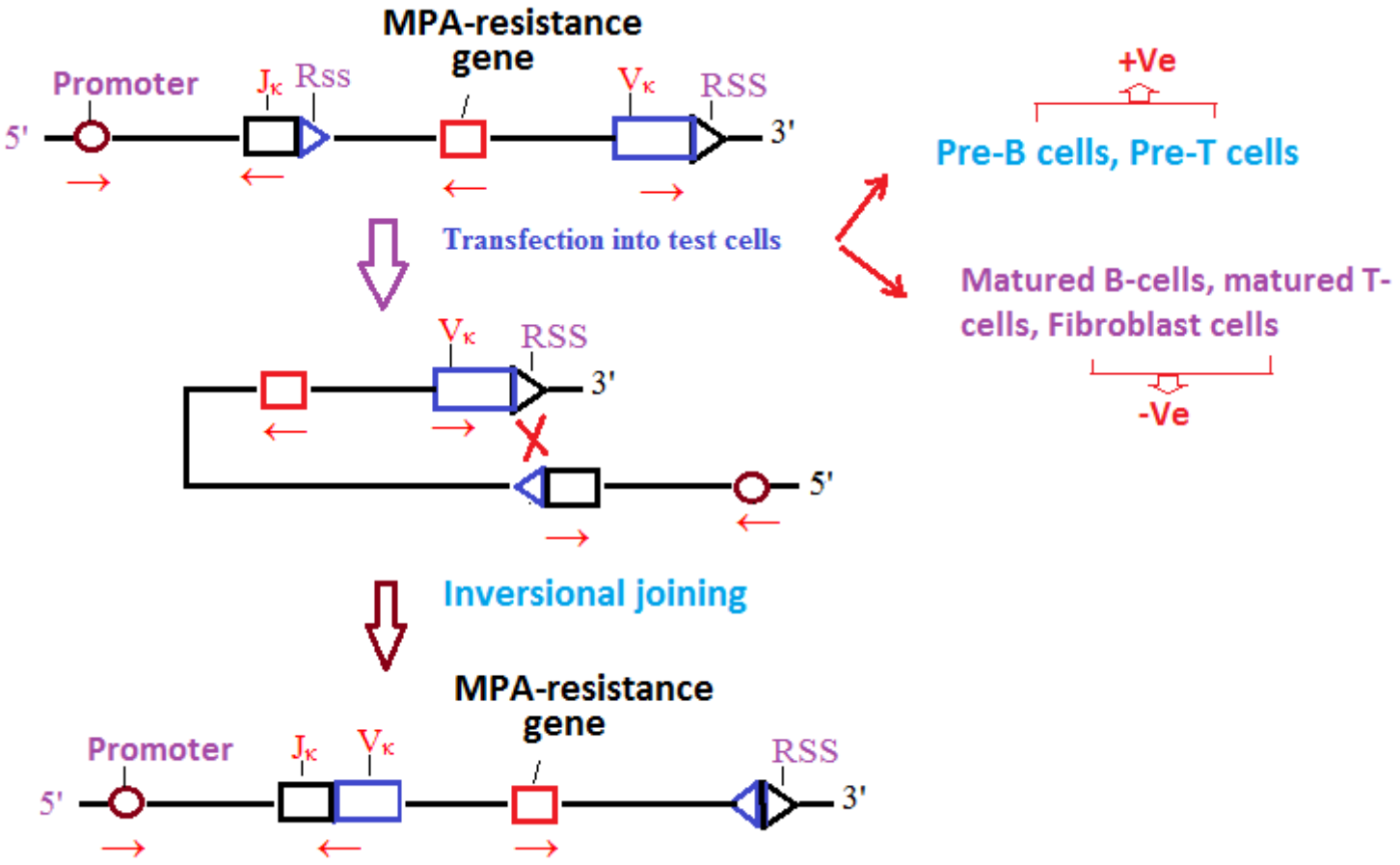
Nonhomologous end joining (NHEJ): a DNA repair process that joins broken DNA ends (double-strand breaks) without using homologous DNA as a template



Schatz, David G., and Patrick C. Swanson. "V (D) J recombination: mechanisms of initiation." *Annual review of genetics* 45 (2011): 167-202.

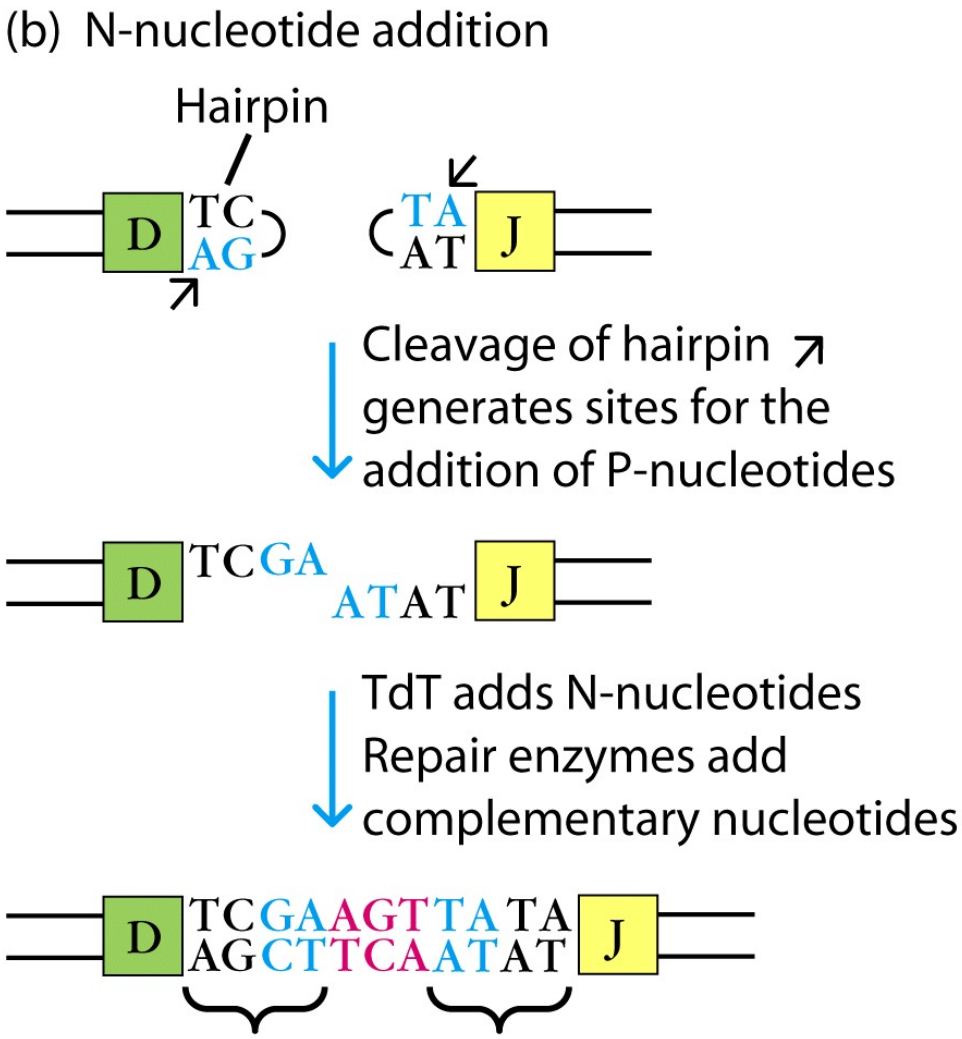

(a) Deletional joining
(b) Inversional joining
L
V_K
J_K
5′
3′
RSS
① Recognition of RSSs by RAG-1/2 and synapsis
② Single-strand DNA cleavage by RAG-1/2
③ Hairpin formation and double-strand DNA break by RAG-1/2
④ Random cleavage of hairpin by endonuclease generates sites for the addition of P-nucleotides
⑤ Optional addition to H-chain segments of N-nucleotides by TdT
Repair and ligation of coding and signal sequences to form joints by DSBR enzymes
Coding joint
+
Signal joint
Signal joint
Coding joint
= One-turn RSS
= Two-turn RSS

Retroviral construct

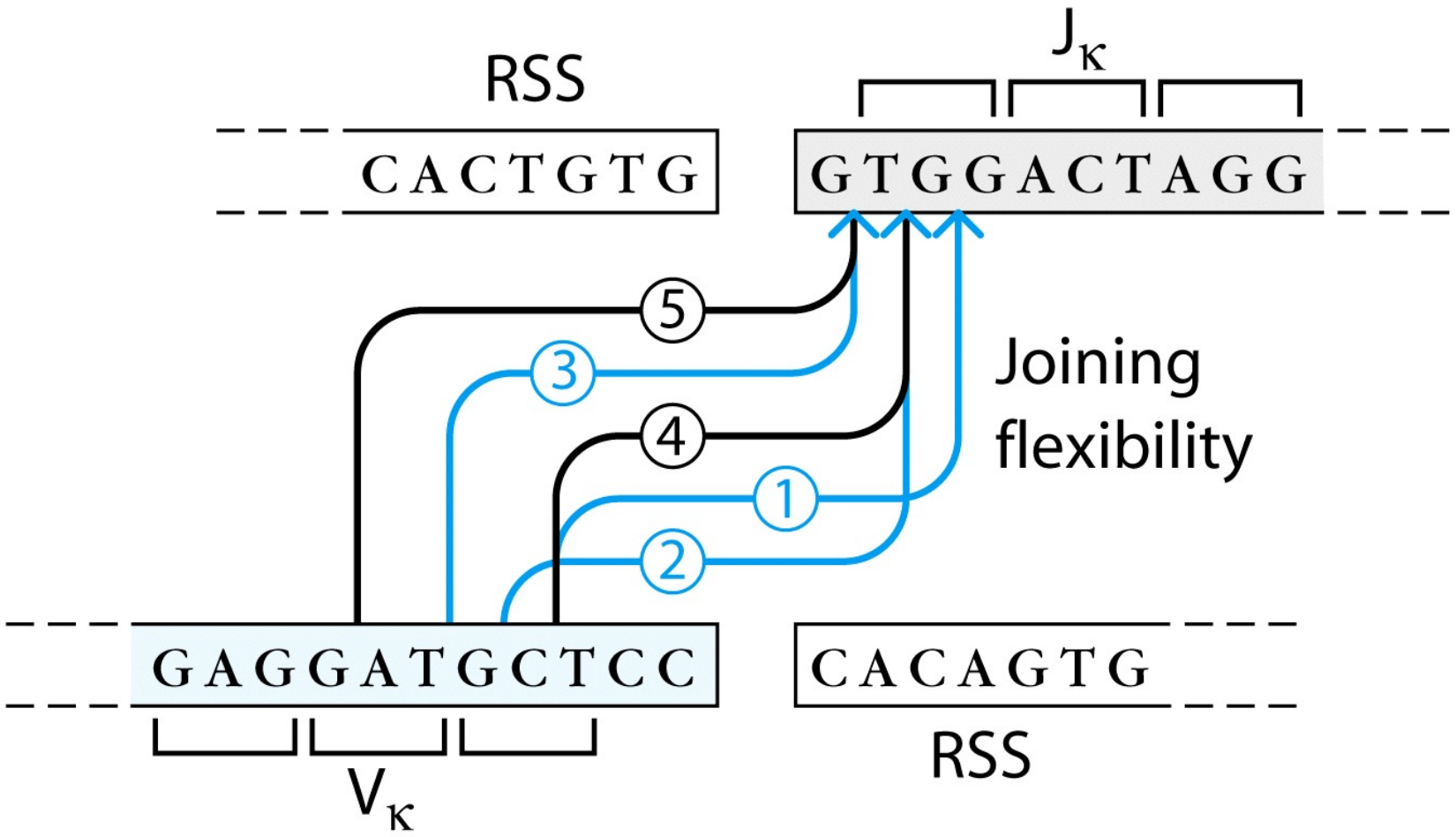


Assay for demonstrating recombination of Ig gene segments

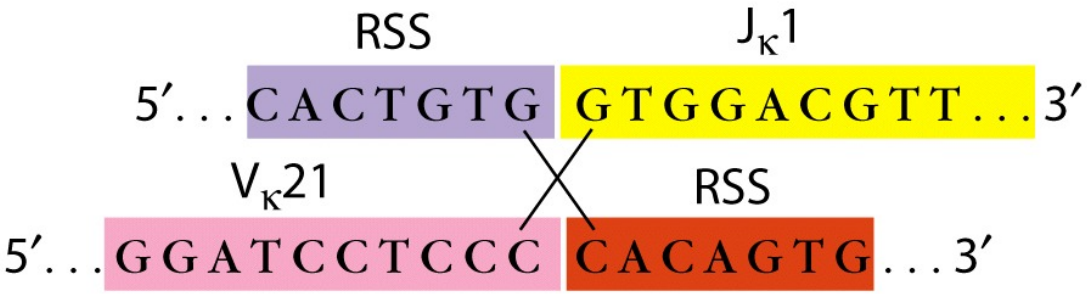
N nucleotide addition at joining segments: the addition of random



Randomness in joining process helps generate diversity by creating hypervariable of antigen binding site

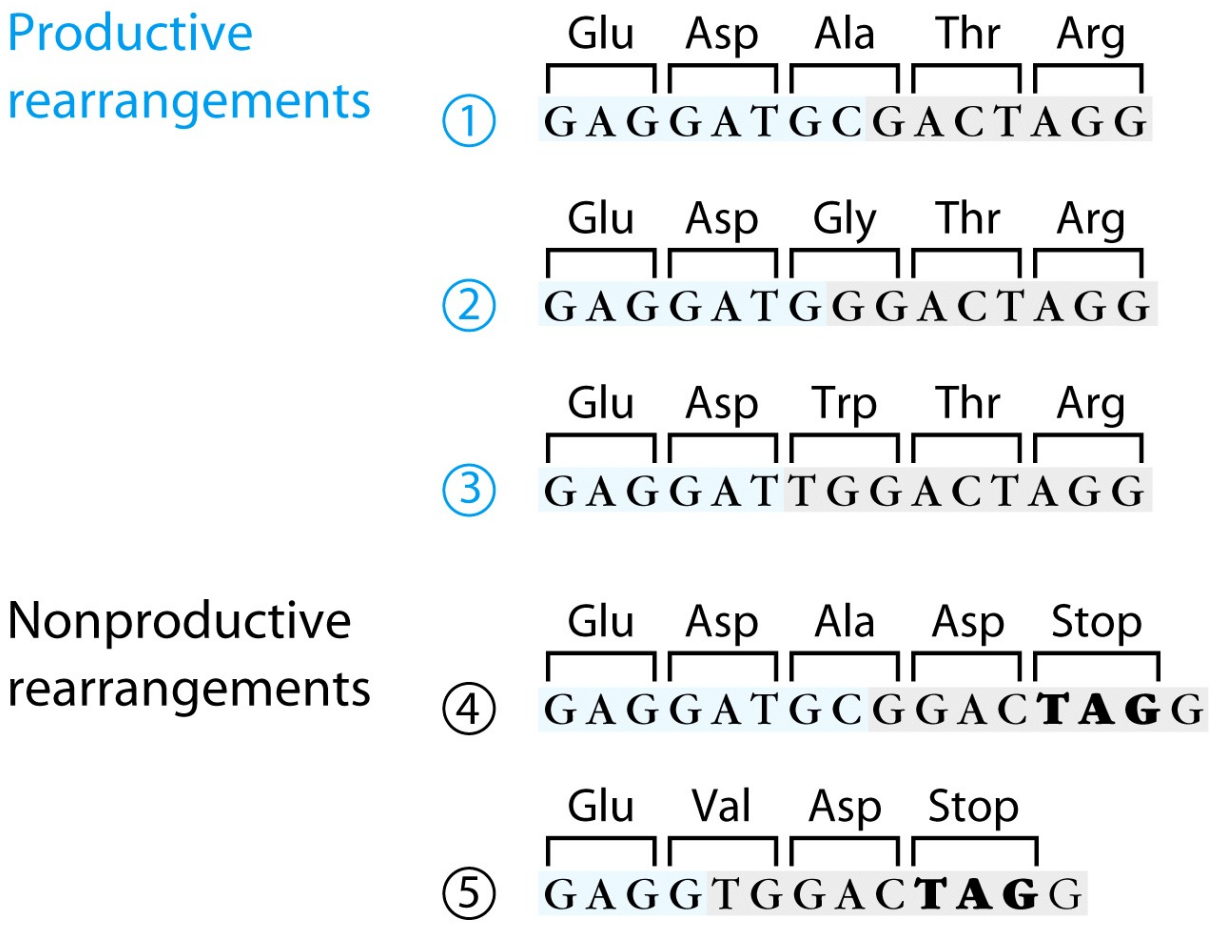


Imprecise joining generates diversity

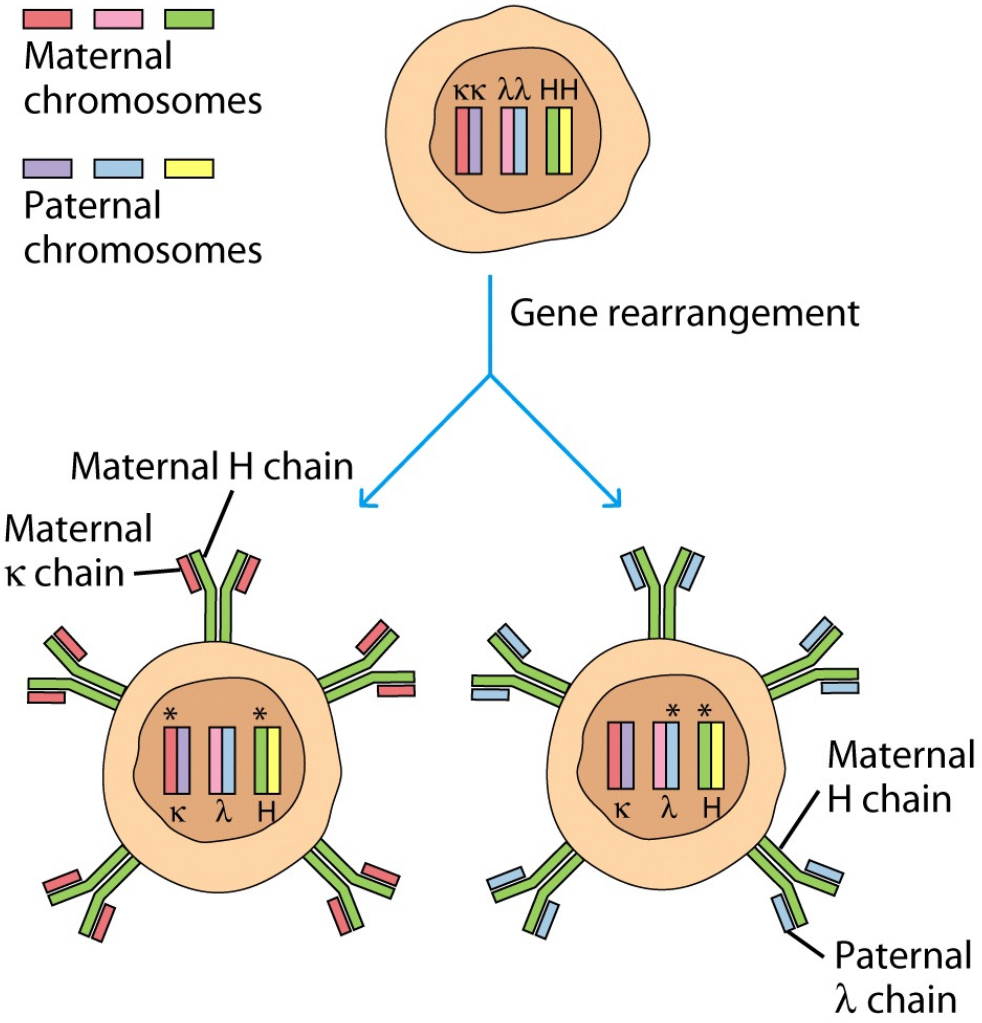


Pre-B cell lines	Coding joints ($V_\kappa 21$ $J_\kappa 1$)	Signal joints (RSS/RSS)
Cell line #1	5′-GGATCC GGACGTT-3′	5′-CACTGTG CACAGTG-3′
Cell line #2	5′-GGATC TGGACGTT-3′	5′-CACTGTG CACAGTG-3′
Cell line #3	5′-GGATCCTC GTGGACGTT-3′	5′-CACTGTG CACAGTG-3′
Cell line #4	5′-GGATCCT TGGACGTT-3′	5′-CACTGTG CACAGTG-3′

Some rearrangements are productive, others are non-productive: frame shift alterations are non-productive



Allelic exclusion: only one chromosome is active in any one lymphocyte



7 means of generating antibody diversity

- Multiple germ-line gene segments
- Combinatorial V-(D)-J joining
- Junctional flexibility
- P-region nucleotide addition (P-addition)
- N-region nucleotide addition (N-addition)
- Somatic hypermutation
- Combinatorial association of light and heavy chains

Although the exact contribution of each of these avenues of diversification to total antibody diversity is not known, they each contribute significantly to the immense number of distinct antibodies that the mammalian immune system is capable of generating.

TABLE 5-2 Combinatorial antibody diversity in humans and mice

Multiple germ-line segments	Heavy chain	LIGHT CHAINS κ	LIGHT CHAINS λ
	ESTIMATED NUMBER OF SEGMENTS IN HUMANS*		
V	51	40	30
D	27	0	0
J	6	5	4
Combinatorial V-D-J and V-J joining (possible number of combinations)	$51 \times 27 \times 6 = 8262$	$40 \times 5 = 200$	$30 \times 4 = 120$
Possible combinatorial associations of heavy and light chains†	$8262 \times (200 + 120) = 2.64 \times 10^6$		
	ESTIMATED NUMBER OF SEGMENTS IN MICE*		
V	134	85	2
D	13	0	0
J	4	4	3
Combinatorial V-D-J and V-J joining (possible number of combinations)	$134 \times 13 \times 4 = 6968$	$85 \times 4 = 340$	$2 \times 3 = 6$
Possible combinatorial associations of heavy and light chains†	$6968 \times (340 + 6) = 2.41 \times 10^6$		

Location of variability occurs within CDR regions of V domains (antigen binding sites)

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.

Somatic hyper mutation:

(1) At a frequency ~ 10^{-3} /bp/generation > 10^{5} fold than spontaneous mutation rate (10^{-8} /bp/generation)

(2) It occurs within germinal centers in secondary lymphoid organs within a week or so of immunization with an antigen at active T-cells dependent B-cells resonse.

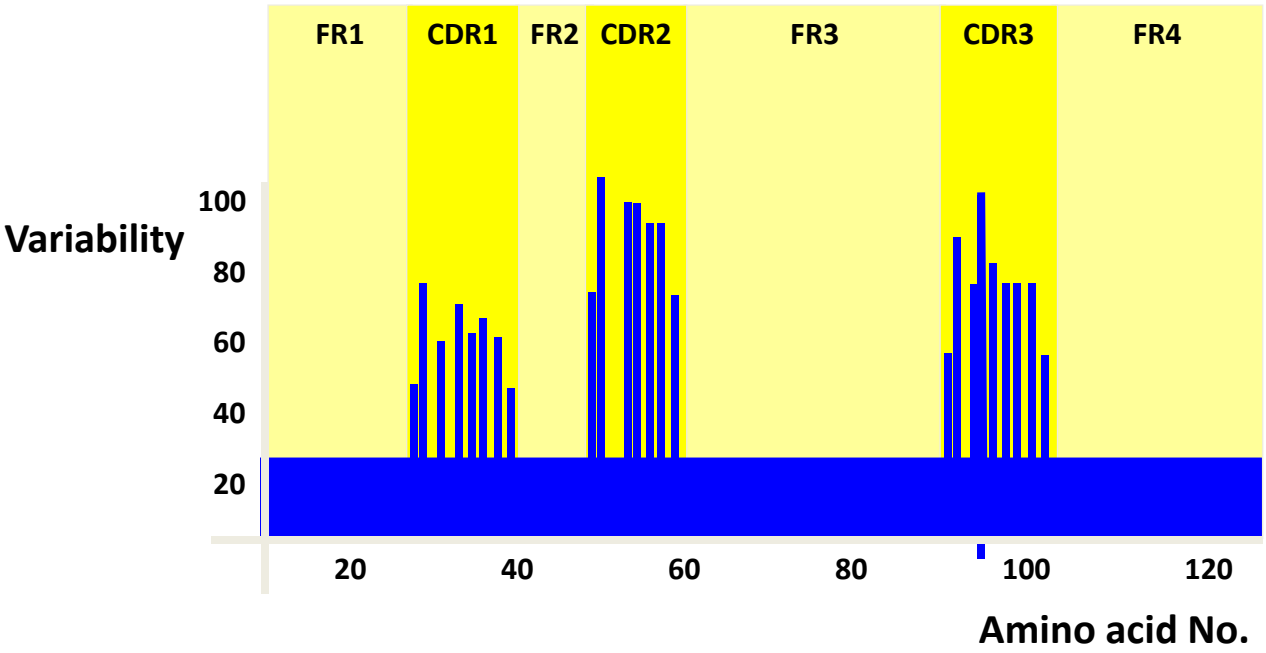
(3) Target sites → 1500 nucleotides (VJ or VDJ segments)

AID (Activation induced cytidine deaminase) :

Somatic hyper mutation
Class switiching

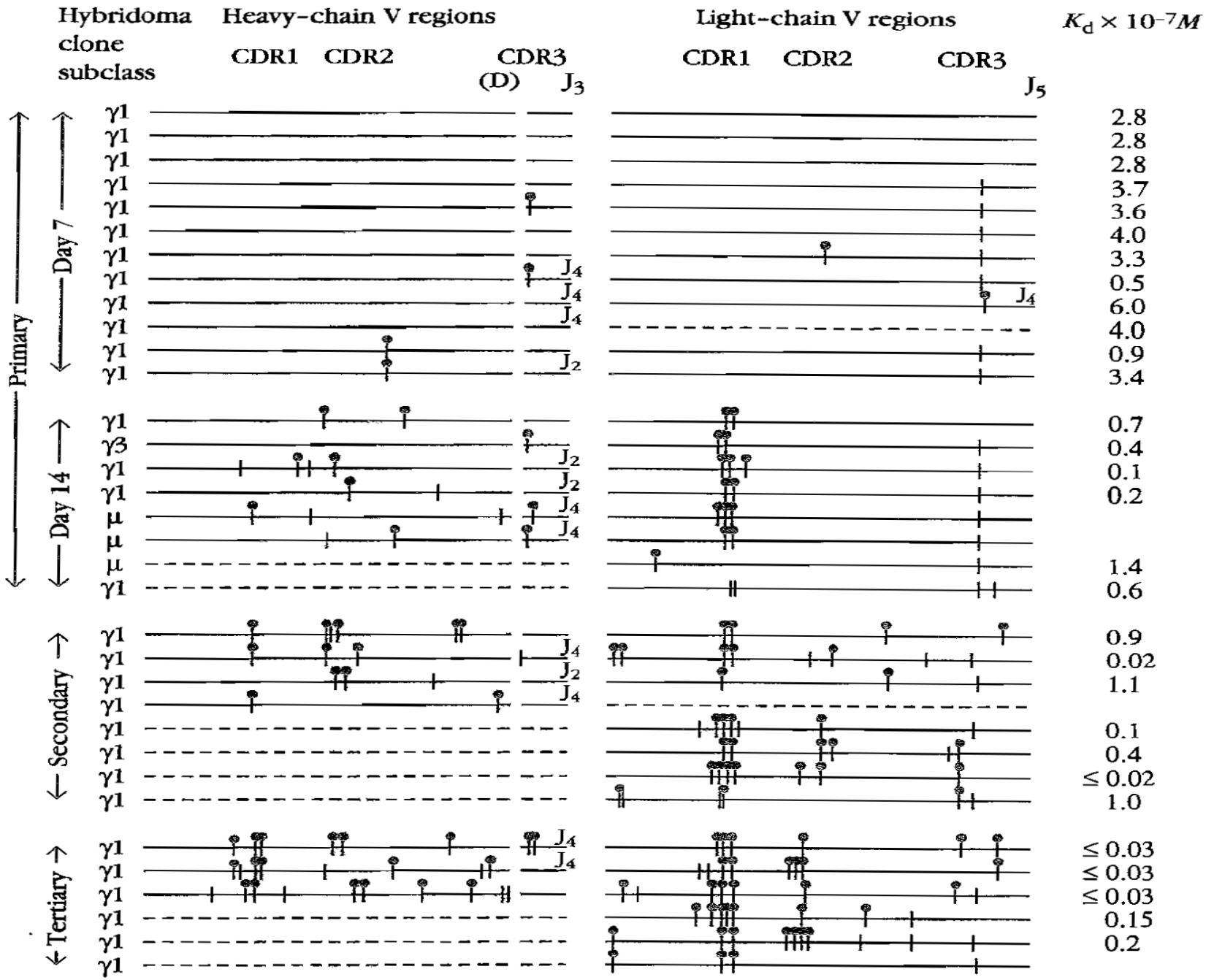
Affinity maturation

Somatic hypermutation

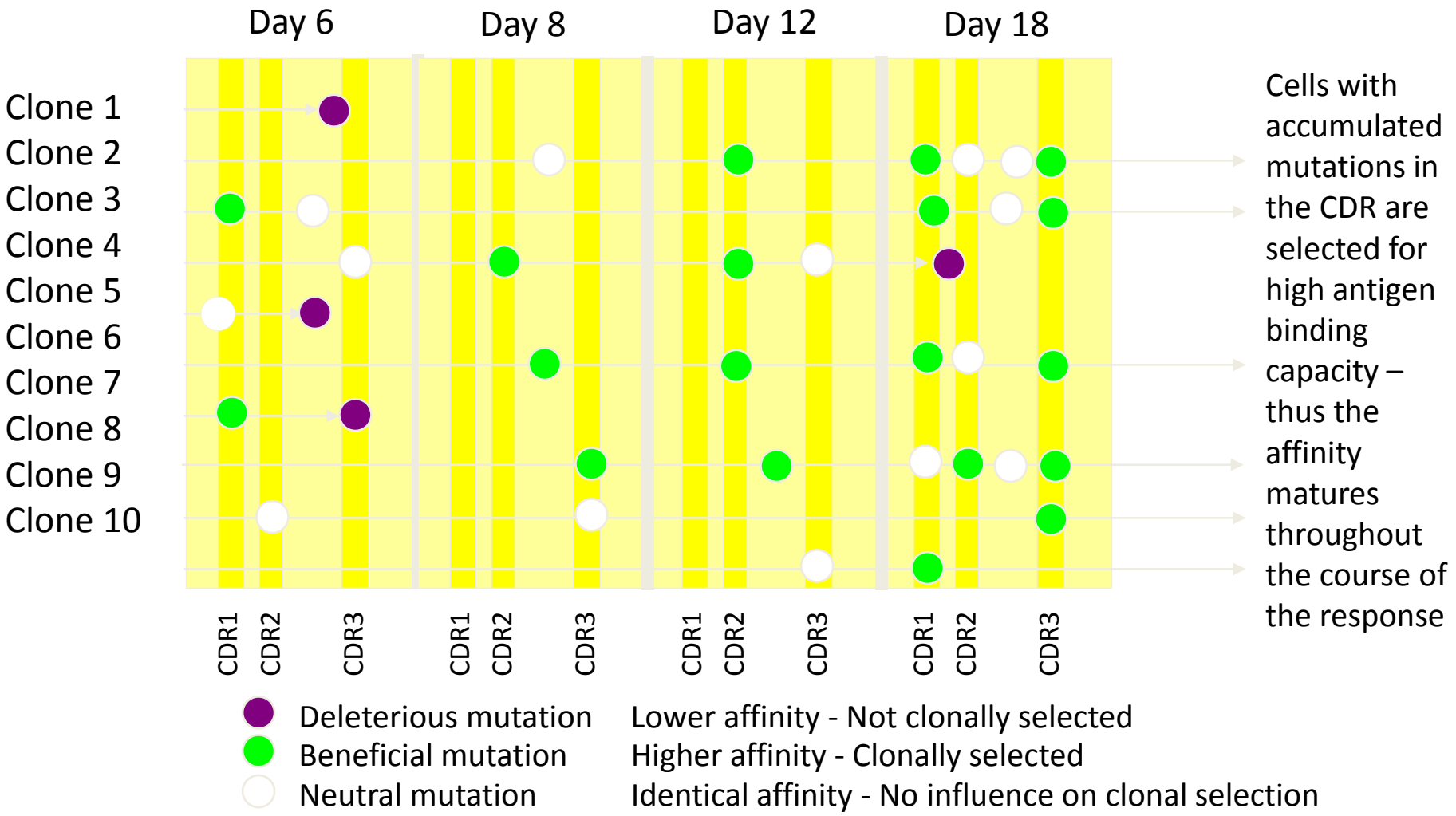


Wu - Kabat analysis compares point mutations in Ig of different specificity.

What about mutation *throughout* an immune response to a single epitope?
How does this affect the specificity and affinity of the antibody?


Hybridoma clone subclass
Heavy-chain V regions
CDR1
CDR2
CDR3
(D) J3
Light-chain V regions
CDR1
CDR2
CDR3
J5
Kd × 10−7M
Primary
Day 7
Day 14
Secondary
Tertiary
γ1
γ3
μ
J4
J2
2.8
2.8
2.8
3.7
3.6
4.0
3.3
0.5
6.0
4.0
0.9
3.4
0.7
0.4
0.1
0.2
1.4
0.6
0.9
0.02
1.1
0.1
0.4
≤ 0.02
1.0
≤ 0.03
≤ 0.03
≤ 0.03
0.15
0.2

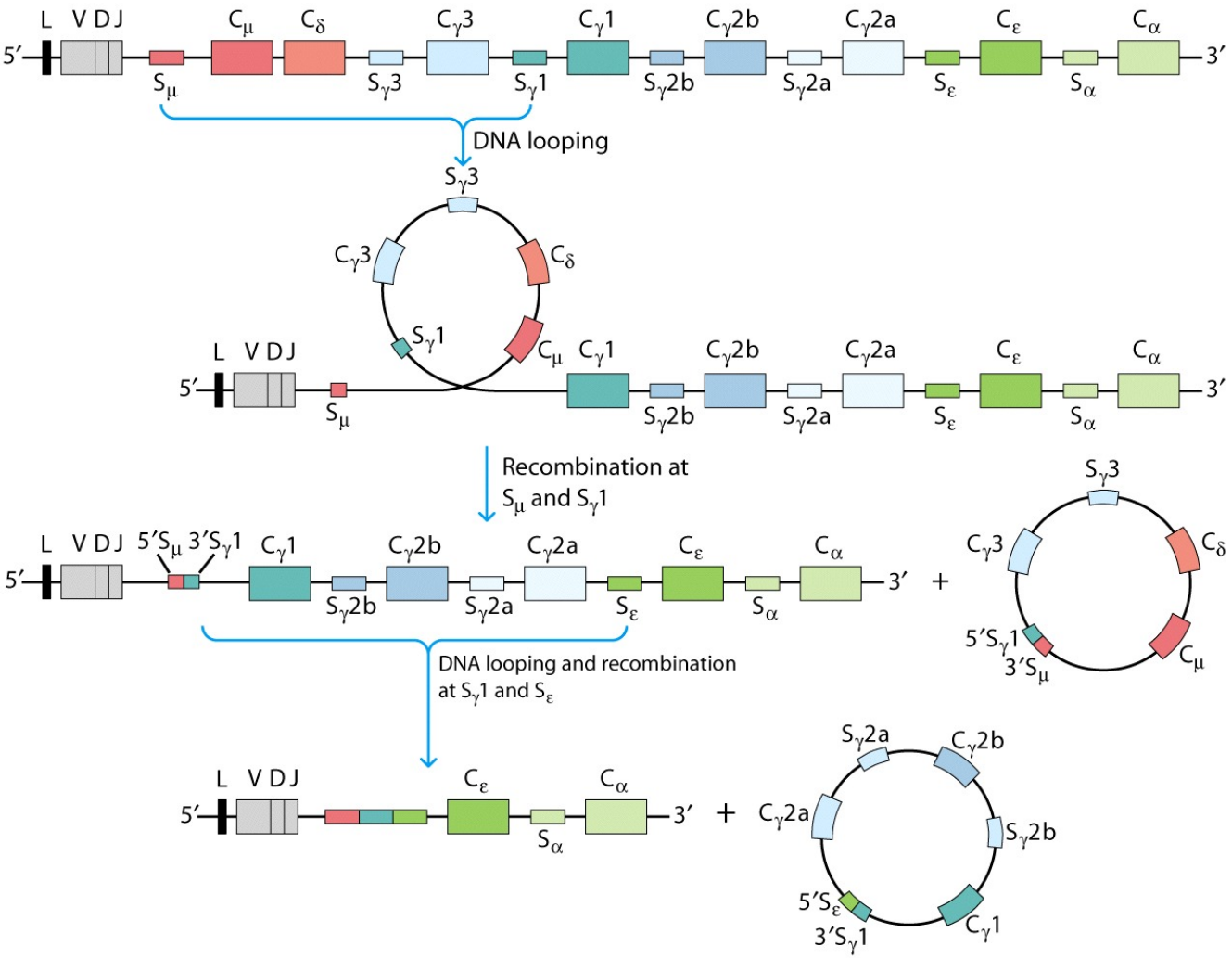
Somatic hypermutation leads to affinity maturation



Hypermutation is T cell dependent

Mutations focussed on 'hot spots' (i.e. the CDRs) due to double stranded breaks repaired by an error prone DNA repair enzyme.

Class switching among constant regions: generation of IgG, IgA and IgE with same antigenic determinants—idiotypes



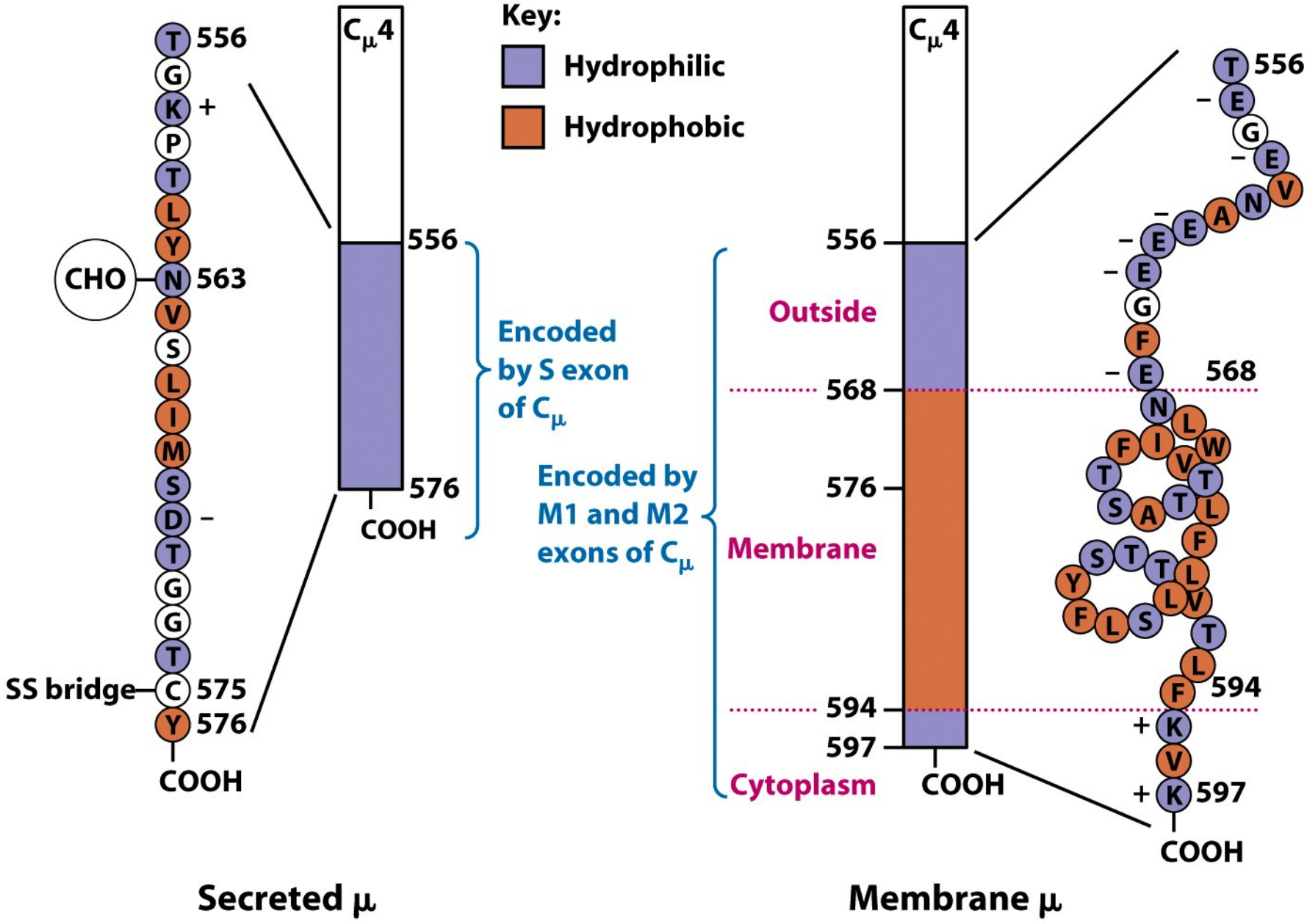


Figure 5-18a
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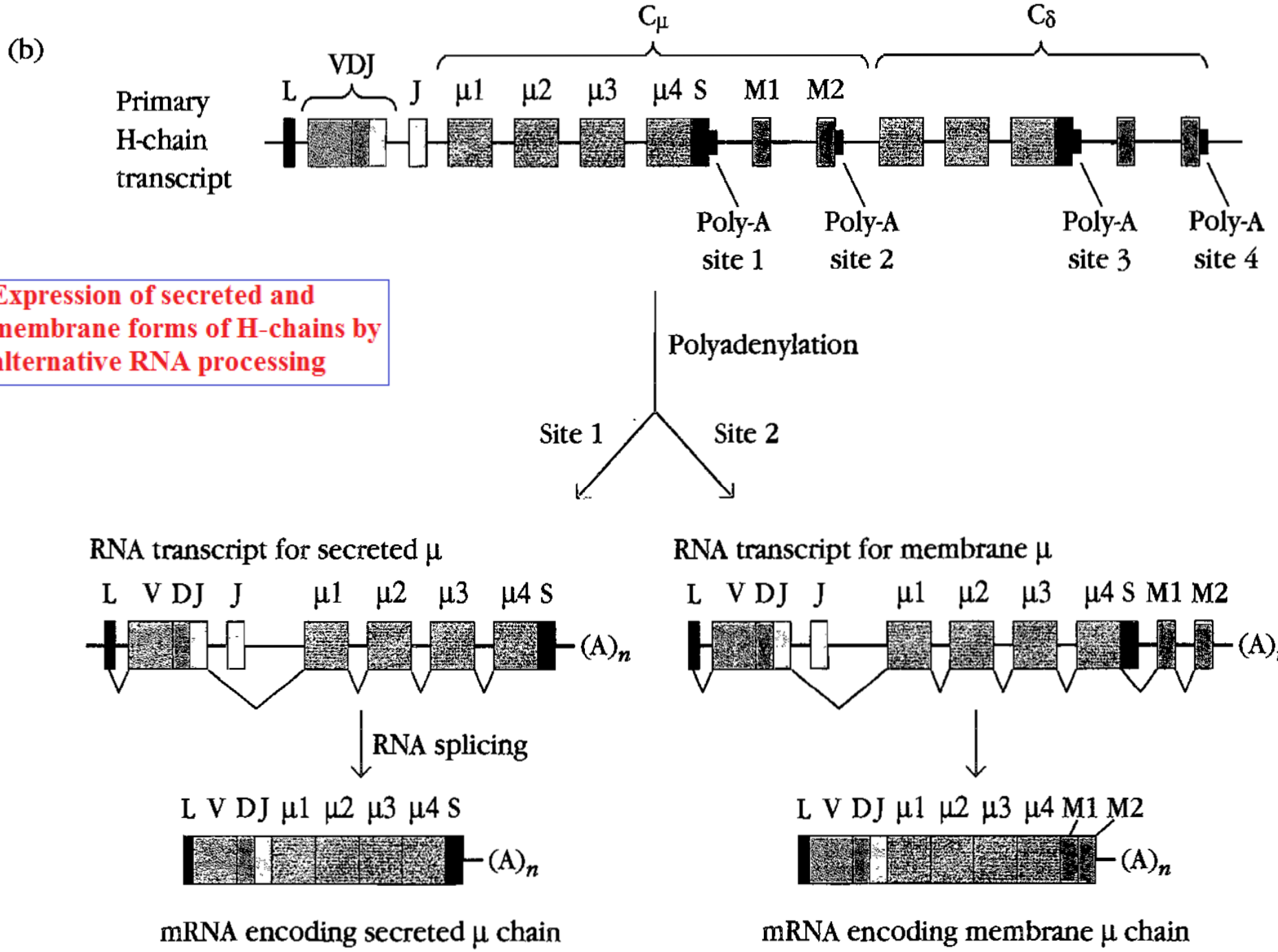
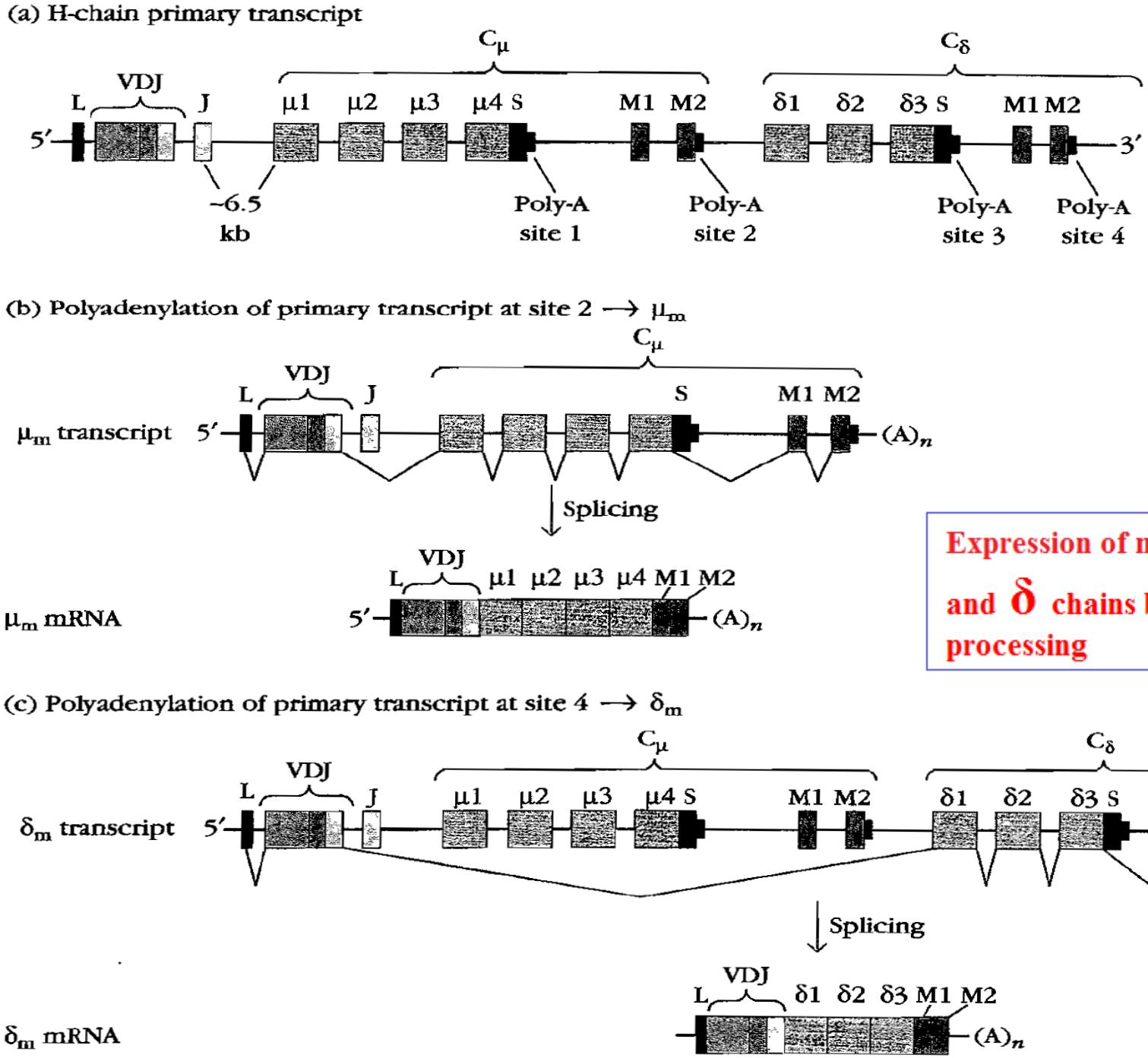
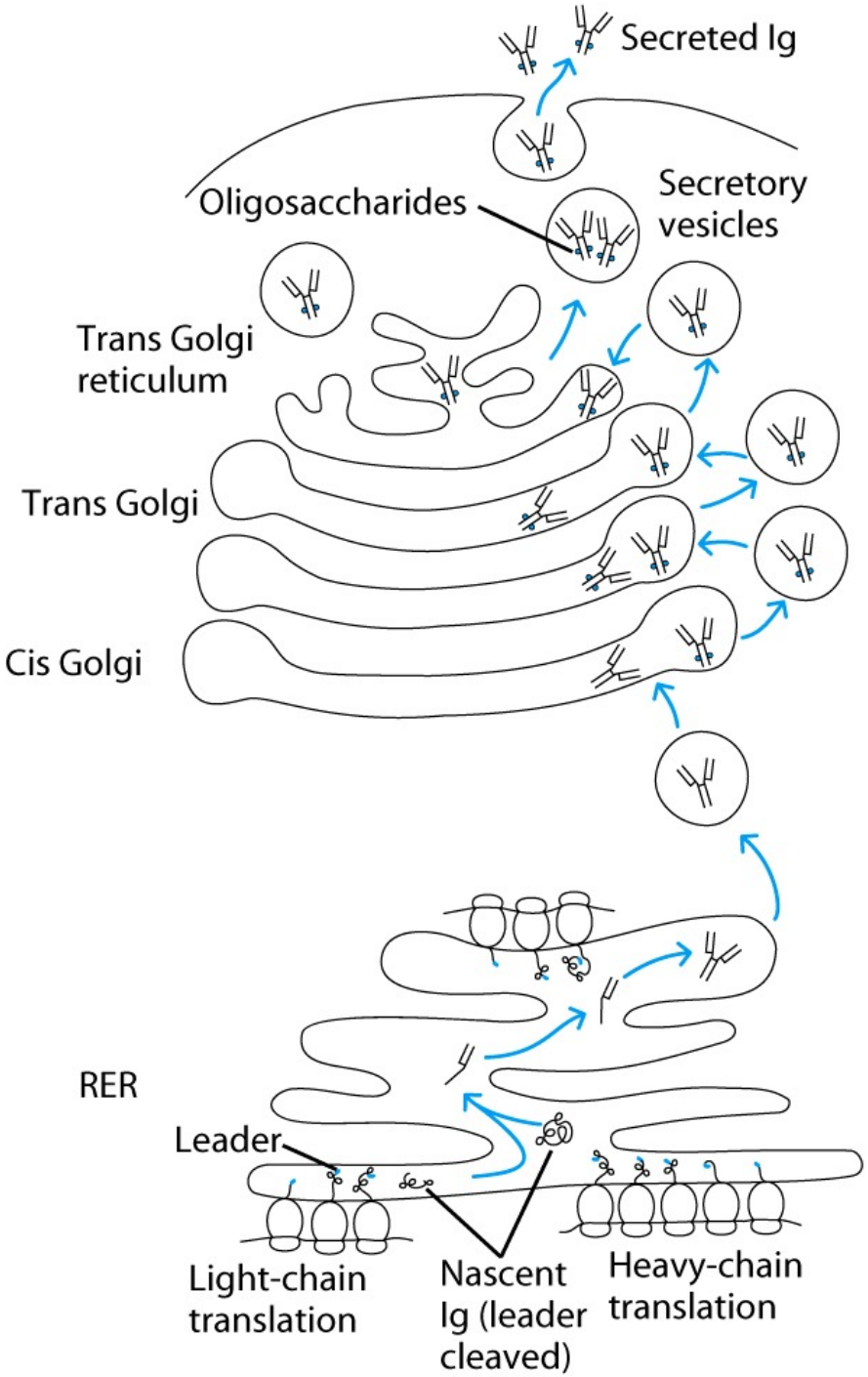

(b)
Cμ
Cδ
VDJ
L
J
μ1
μ2
μ3
μ4
S
M1
M2
Primary
H-chain
transcript
Poly-A
site 1
Poly-A
site 2
Poly-A
site 3
Poly-A
site 4
Expression of secreted and membrane forms of H-chains by alternative RNA processing
Polyadenylation
Site 1
Site 2
RNA transcript for secreted μ
L V DJ J μ1 μ2 μ3 μ4 S
(A)n
RNA splicing
L V DJ μ1 μ2 μ3 μ4 S
(A)n
mRNA encoding secreted μ chain
RNA transcript for membrane μ
L V DJ J μ1 μ2 μ3 μ4 S M1 M2
(A)n
L V DJ μ1 μ2 μ3 μ4 M1 M2
(A)n
mRNA encoding membrane μ chain



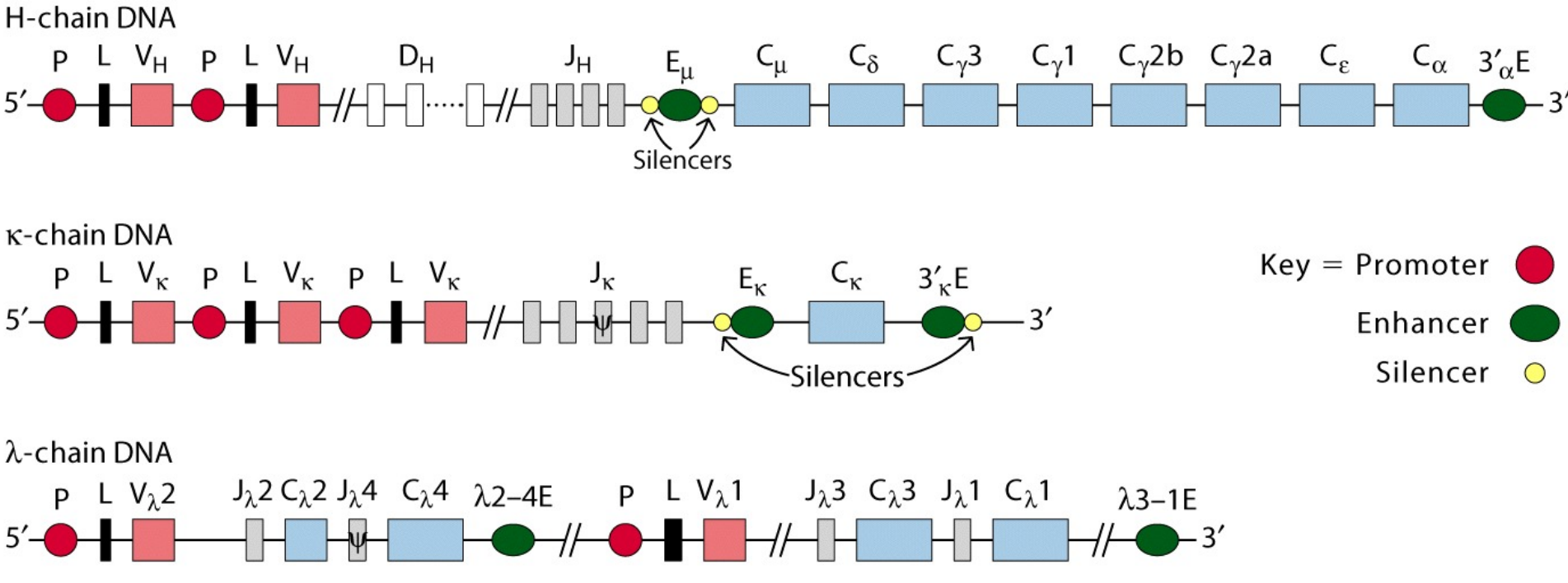
Expression of membrane forms and δ chains by alternative RN processing

Synthesis, assembly and secretion of immunoglobulins

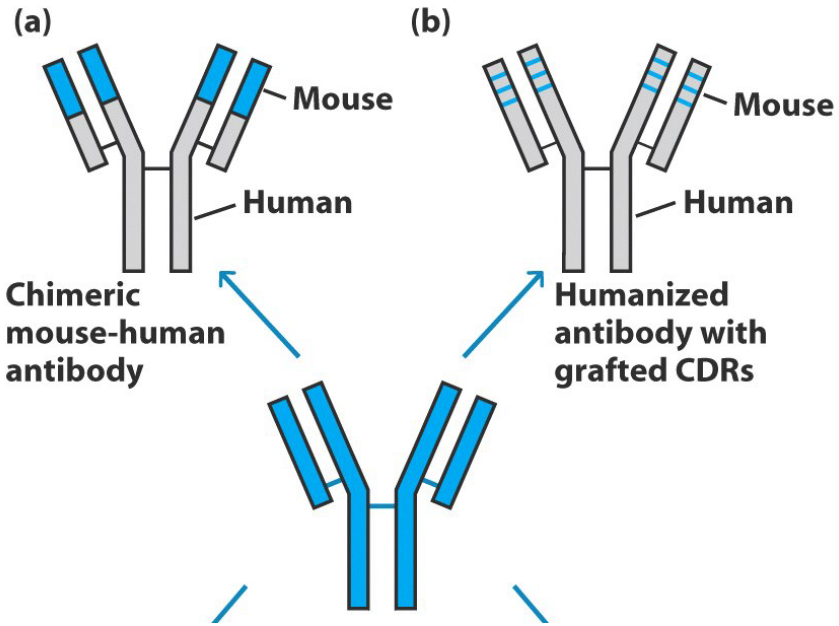


Regulation of Ig gene transcription

Each lymphocyte rearranged gene has regulatory sequences that control gene expression
Promoters: initiation sites of RNA transcription
Enhancers: upstream of downstream that transcription from the promoter sequence
Silencers: down-regulate transcription in germline cells
Gene rearrangement brings enhancer and promoter regions close together and eliminates silencer regions allowing transcription



Antibody Engineering



- Monoclonal Abs used for many clinical reasons (anti- tumor Ab, for instance)
- If developed in mice, might produce immune response when injected
    - Can be cleared in which they will not be efficient
    - Can create allergic response
- Creating chimeric Abs or humanized Abs are beneficial

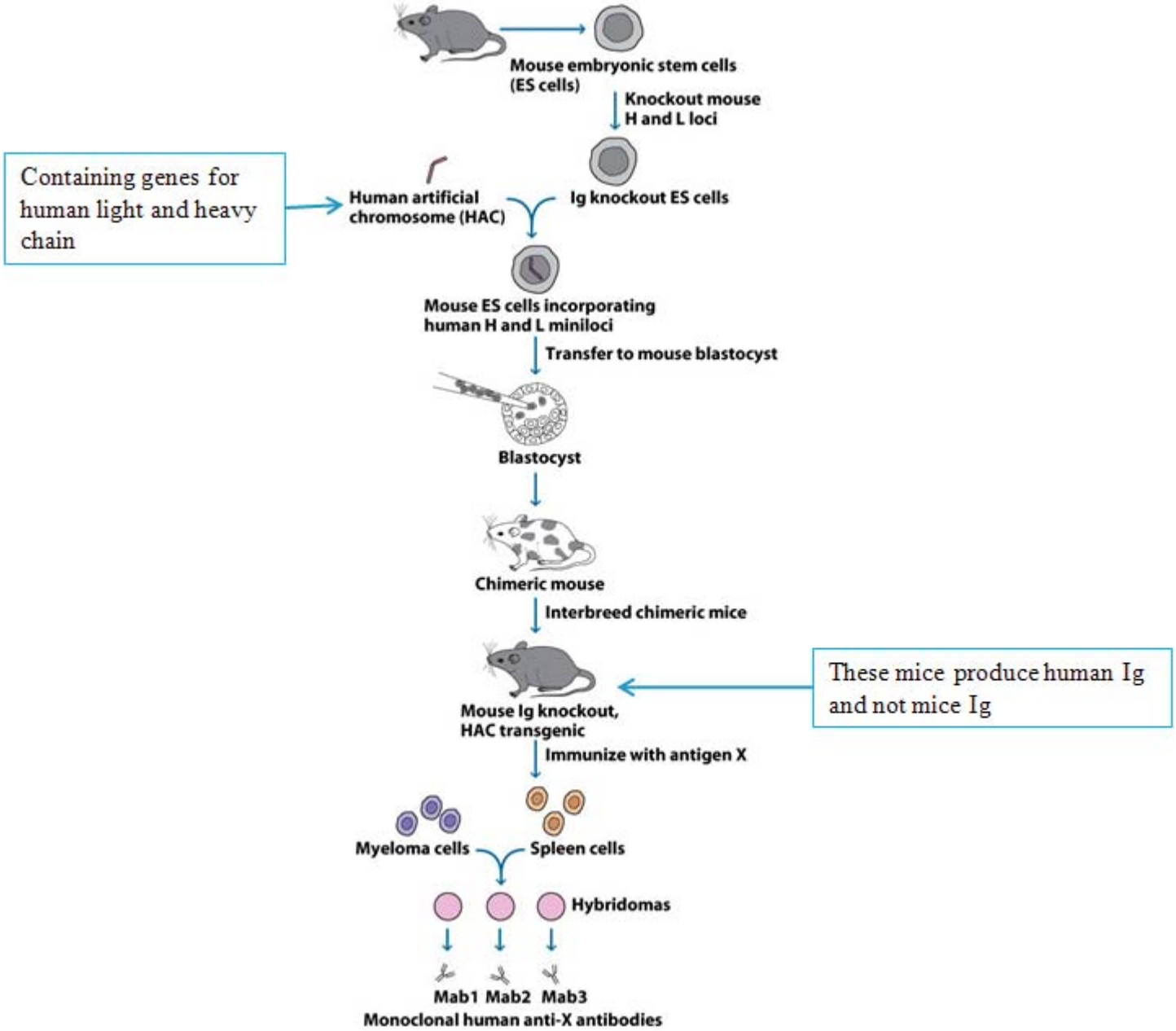


Figure 5-24
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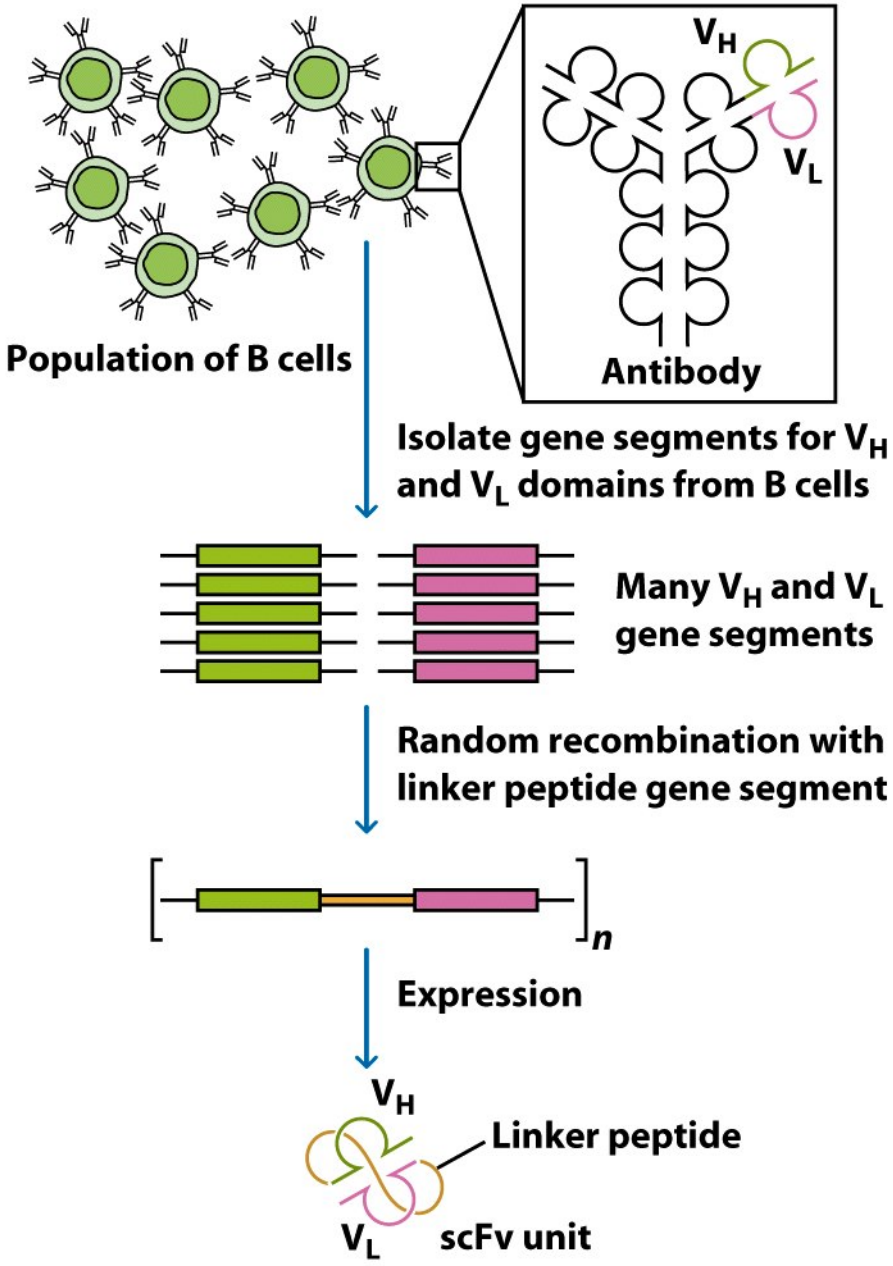

Figure 5-25a
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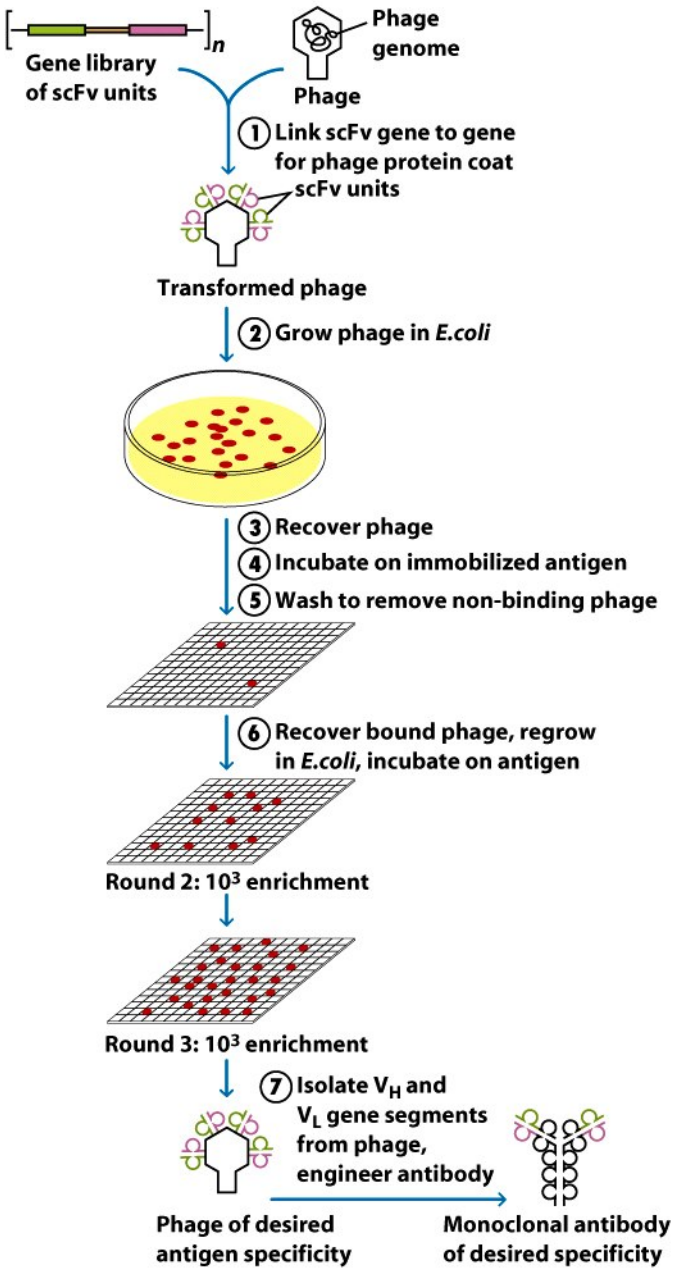

Figure 5-25b
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