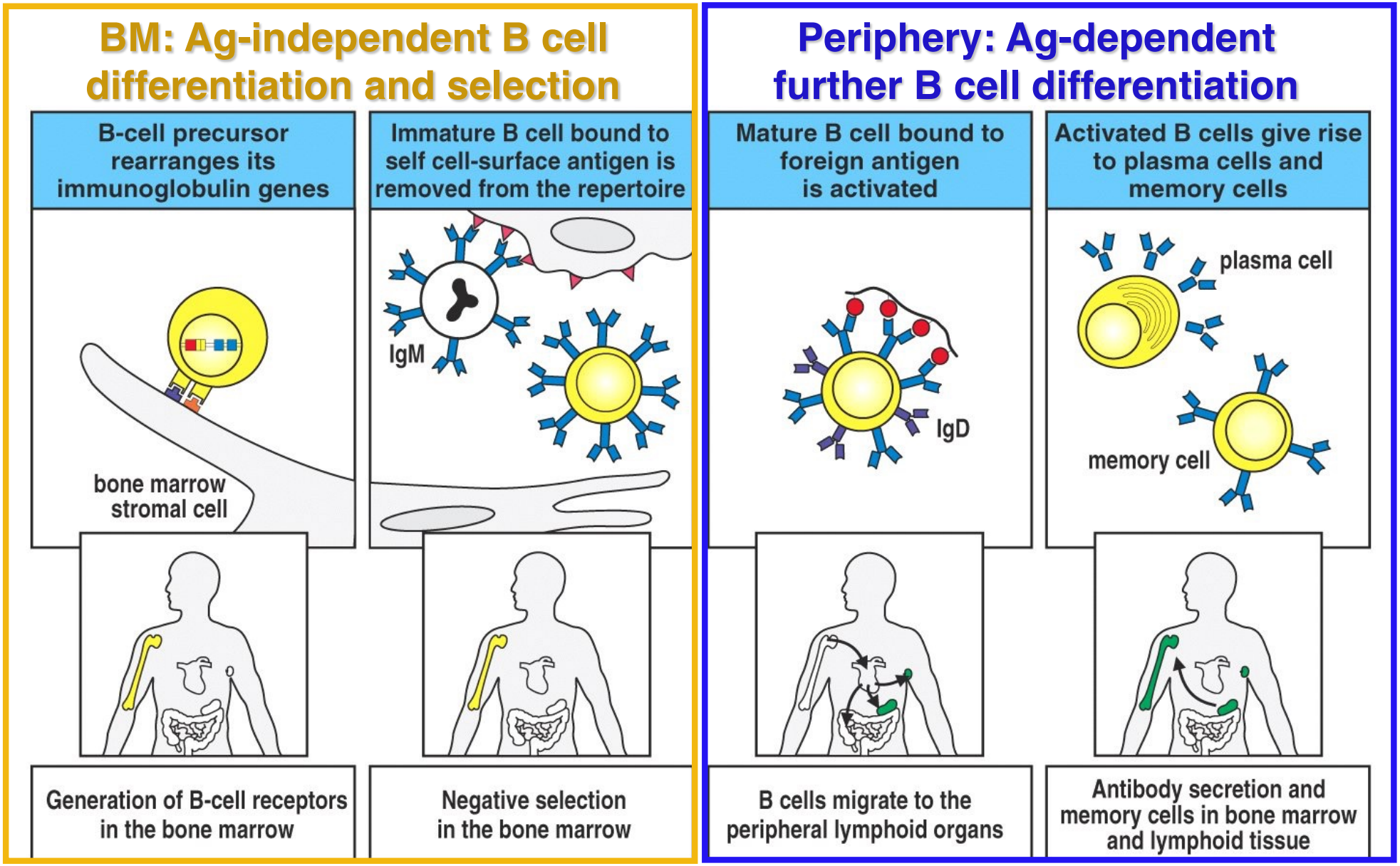
Corso di Immunologia - III anno
Prof. Paolini

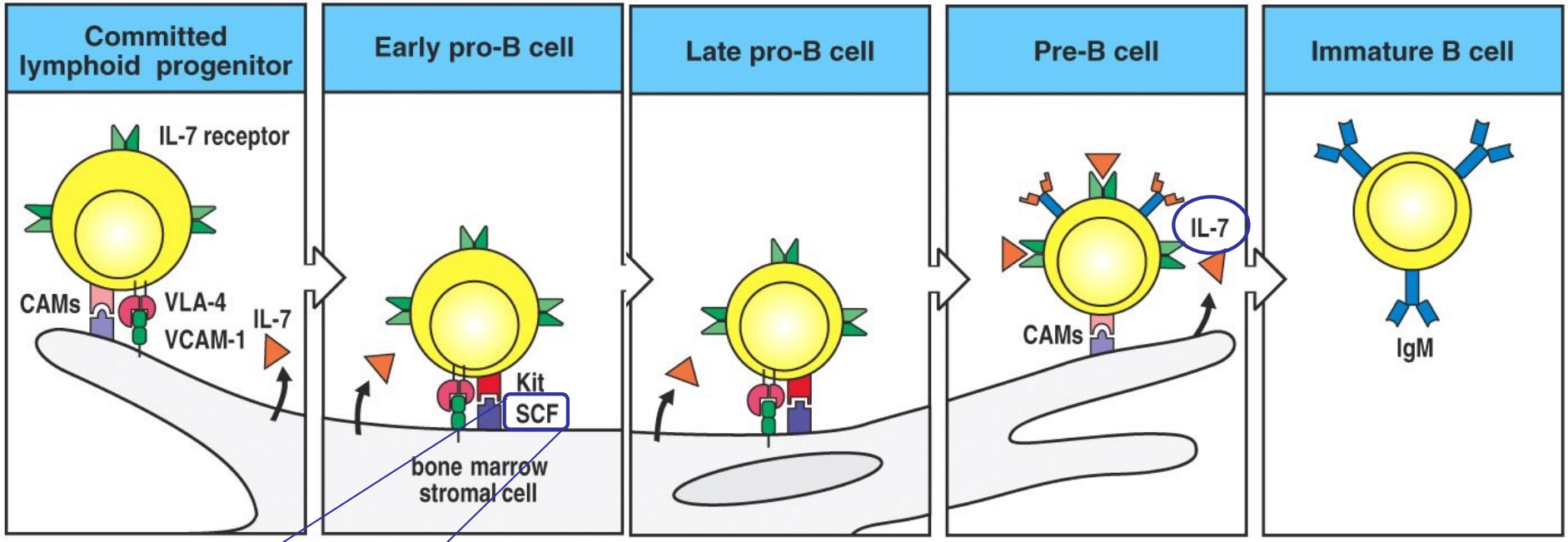
Lezione 07/11/2025

"La maturazione dei linfociti B"

B cell development



Cytokines and interaction with stromal cells guide B cell differentiation



SCF (Stem Cell Factor) or Kit ligand

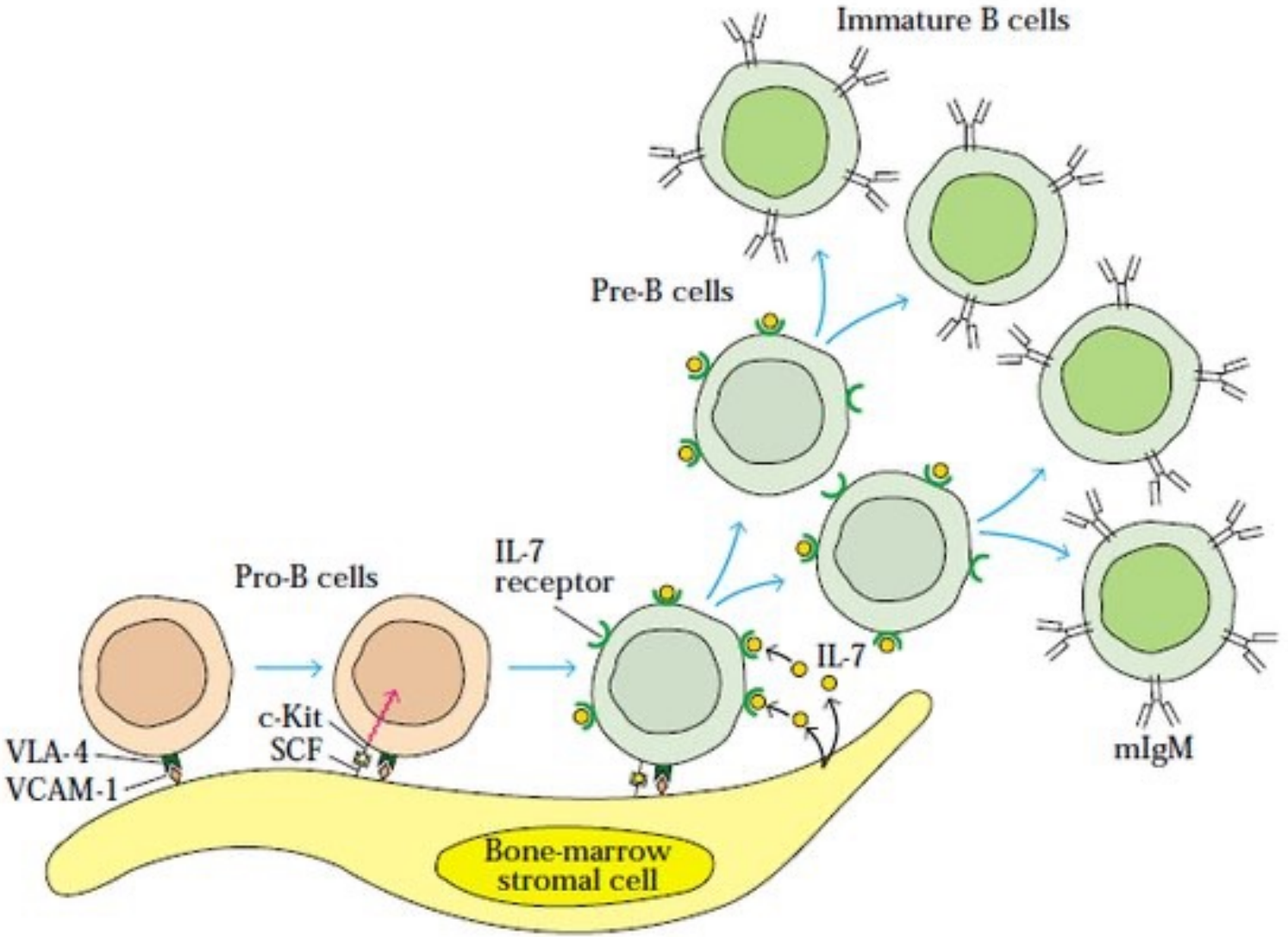
- Could be release in soluble form.
- It acts on hematopoietic stem cells that express the Kit receptor (CD117).
- The engaged receptor stimulates cell survival and promotes the proliferation of B lymphocyte progenitors.

INTERLEUKIN 7

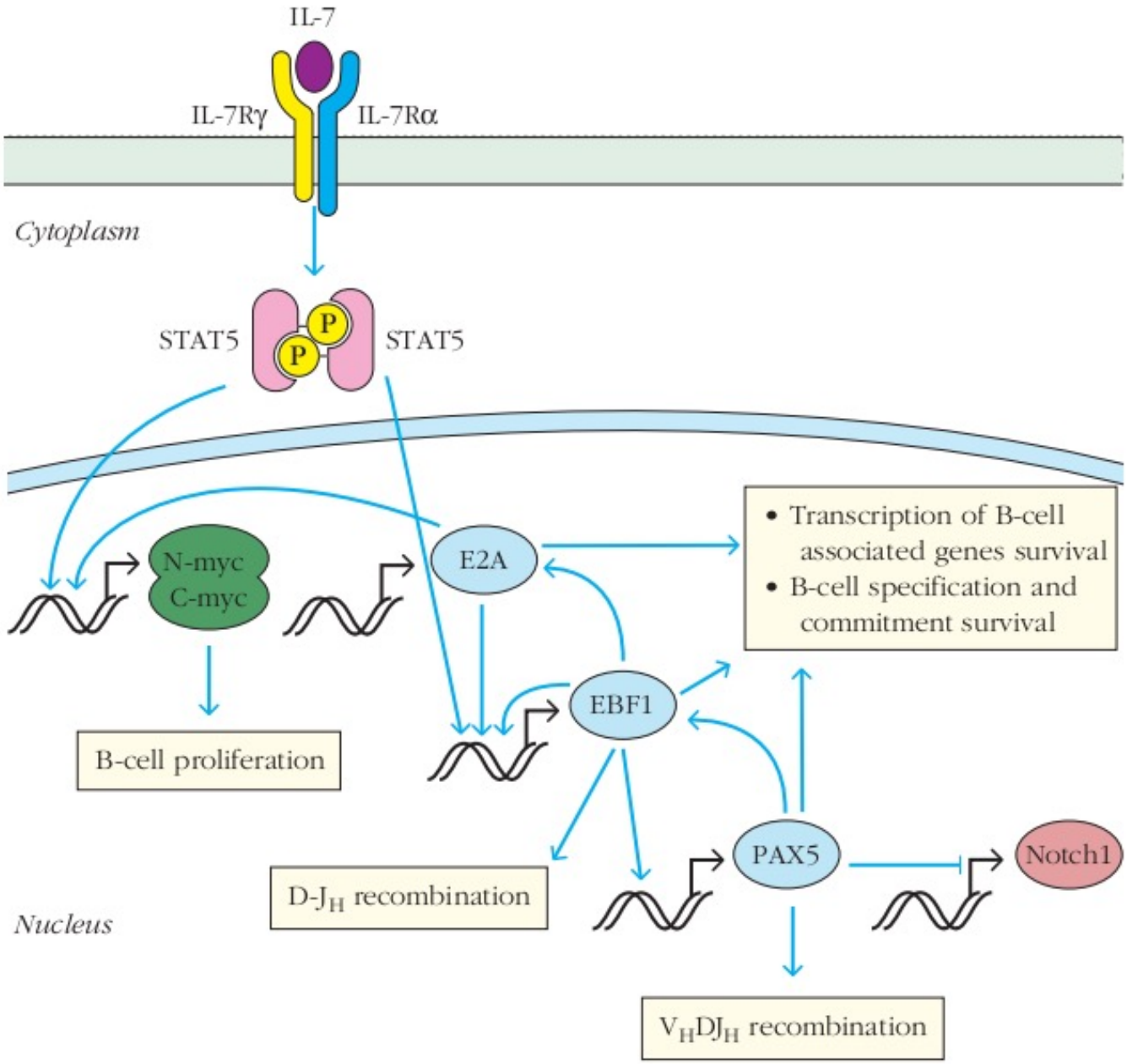
Source: Stromal cells of the bone marrow and thymus.

Main action: Proliferation of lymphoid precursors.

Pro-B-cells need IL-7 in mice

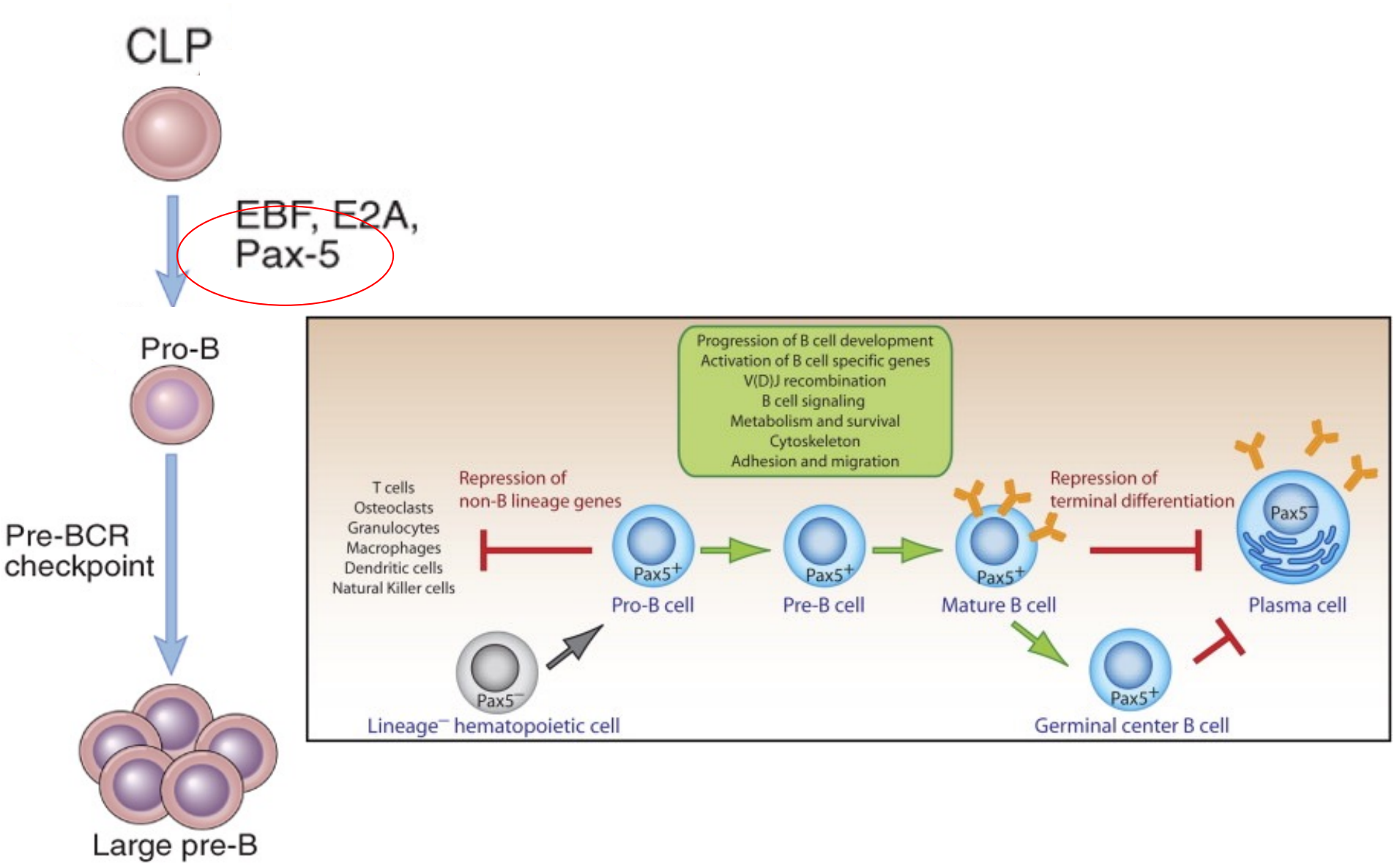


Transcription Factors in B Cell Development



Kuby Immunology – 7th Edition, Figure 10.7

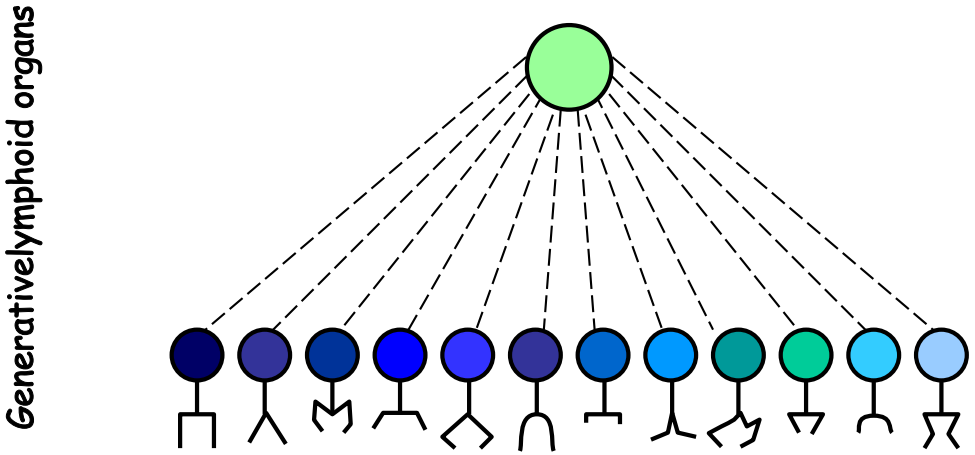
Control of B cell development by transcriptional factors



Phase 1: Generation of B cell receptor repertoire

The human genome is estimated to contain less than 50 thousand genes

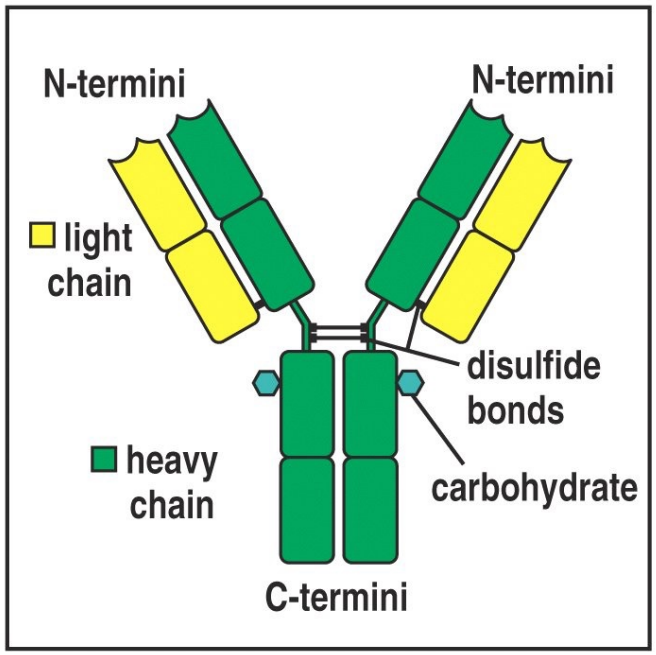
The number of antibody specificities is estimated to be 10 million or more



How can a limited number of genes encode for all these different antigen receptor plus all of the other proteins needed by the body???????

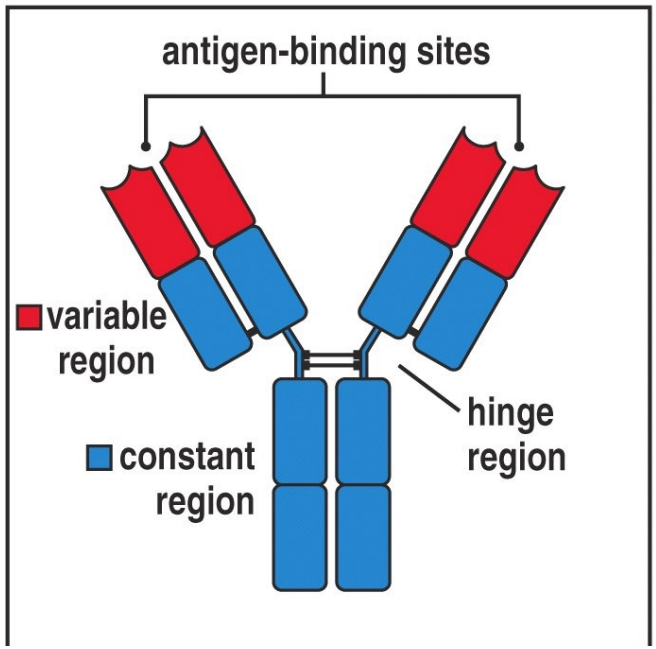
Each receptor is encoded by separate gene segments that undergo a process of random recombination!!

All B cell antigen receptors/antibodies have the same basic structure



2 identical heavy chains (green) and 2 identical light chains (yellow)

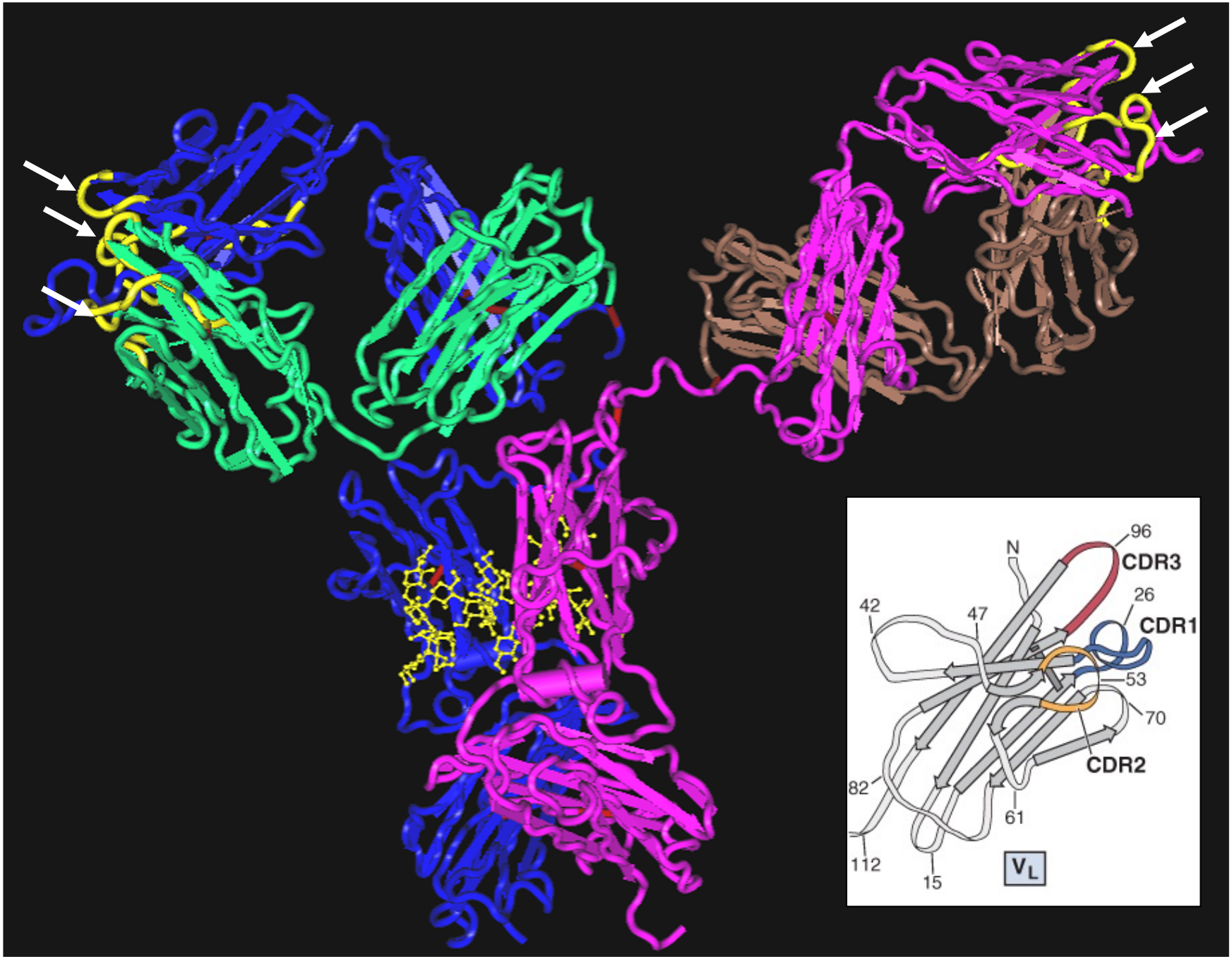
MW: 150 KD, L: ~25 KD, H: ~50 KD



The first 100 aa at the N-term vary greatly from antibody to antibody and are called variable regions (red), while only few different aa are found in the rest of the chains that are called constant region (blue)

The amino acid sequence variability in the V regions is specially pronounced in 3 hypervariable regions also called Regions that Determine Complementarity (CDRs)

The quaternary structure of the antibodies brings the 3 CDR's of the H and L chains together to form the **antigen binding site**

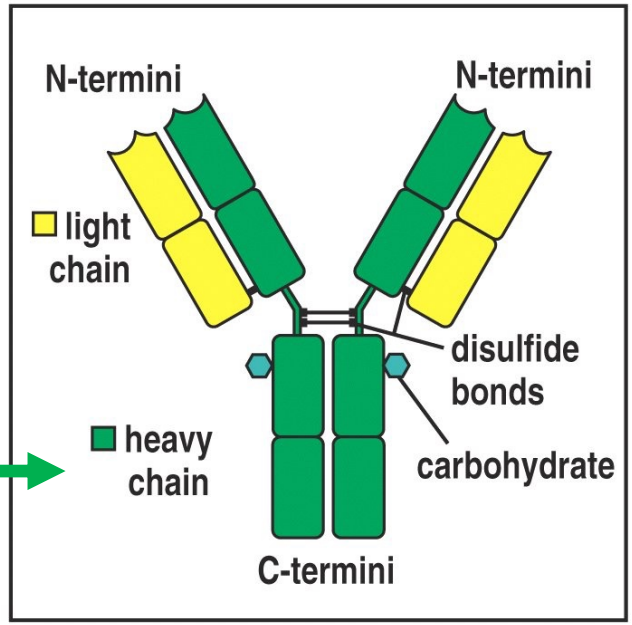


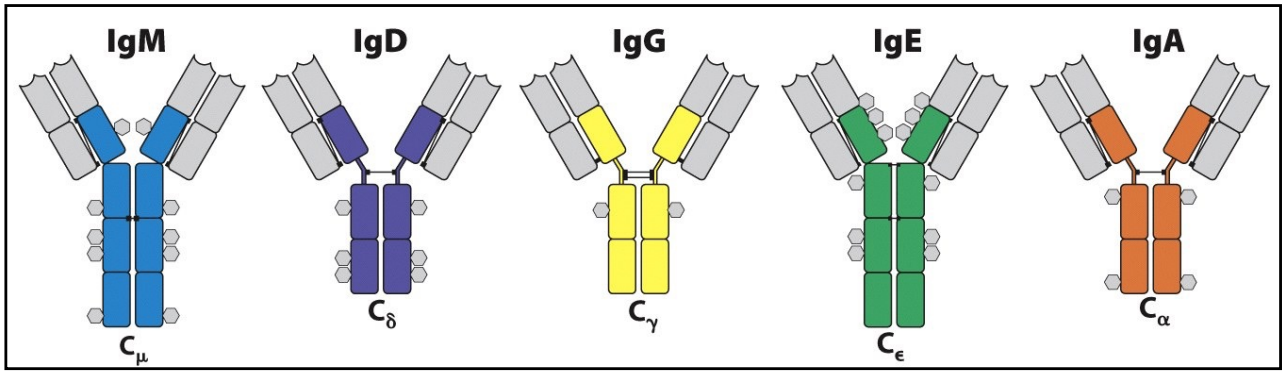
The human classes of antibody

2 different types of light chains
κ–λ

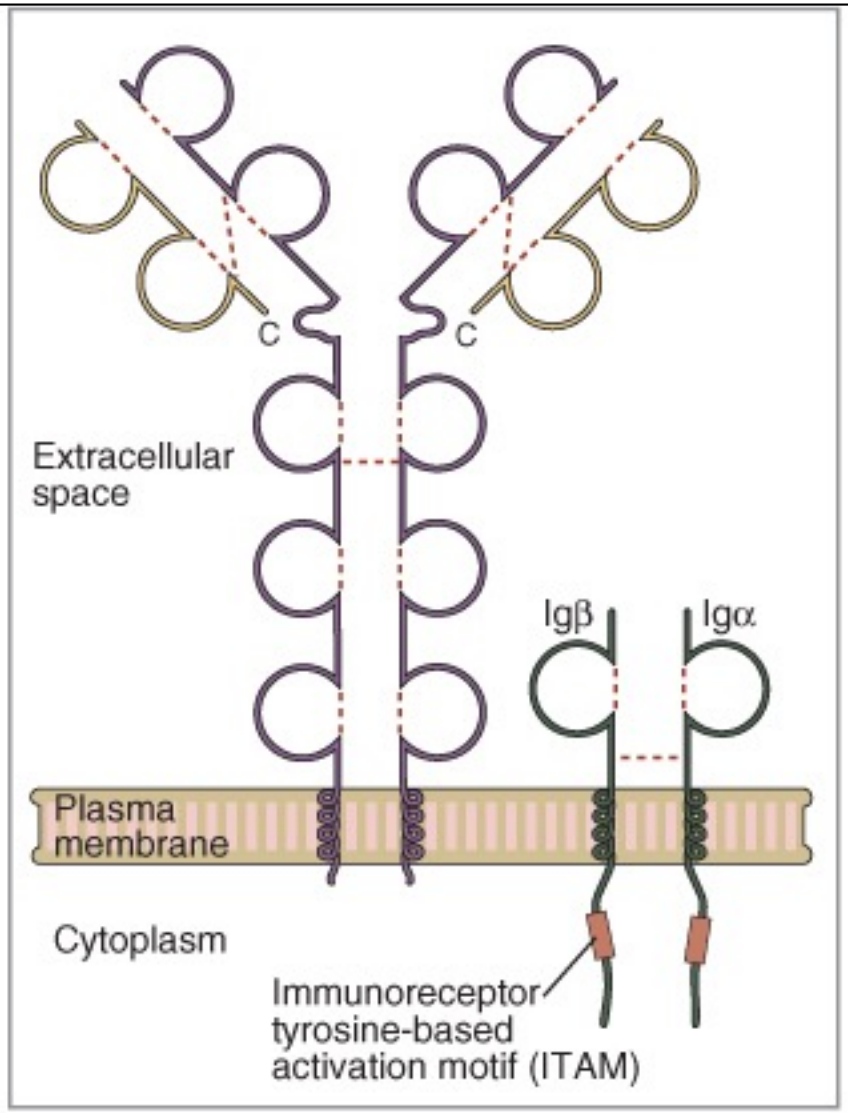
5 different types of heavy chains
γ–μ–δ–α–ε

5 classes of antibodies
IgG, IgM, IgD, IgA, IgE

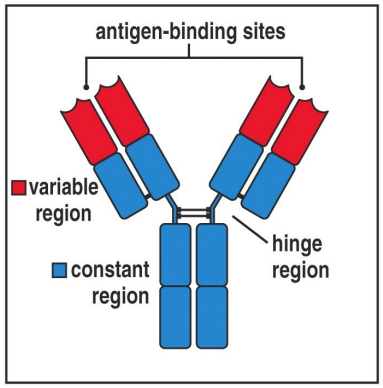




BCR complex

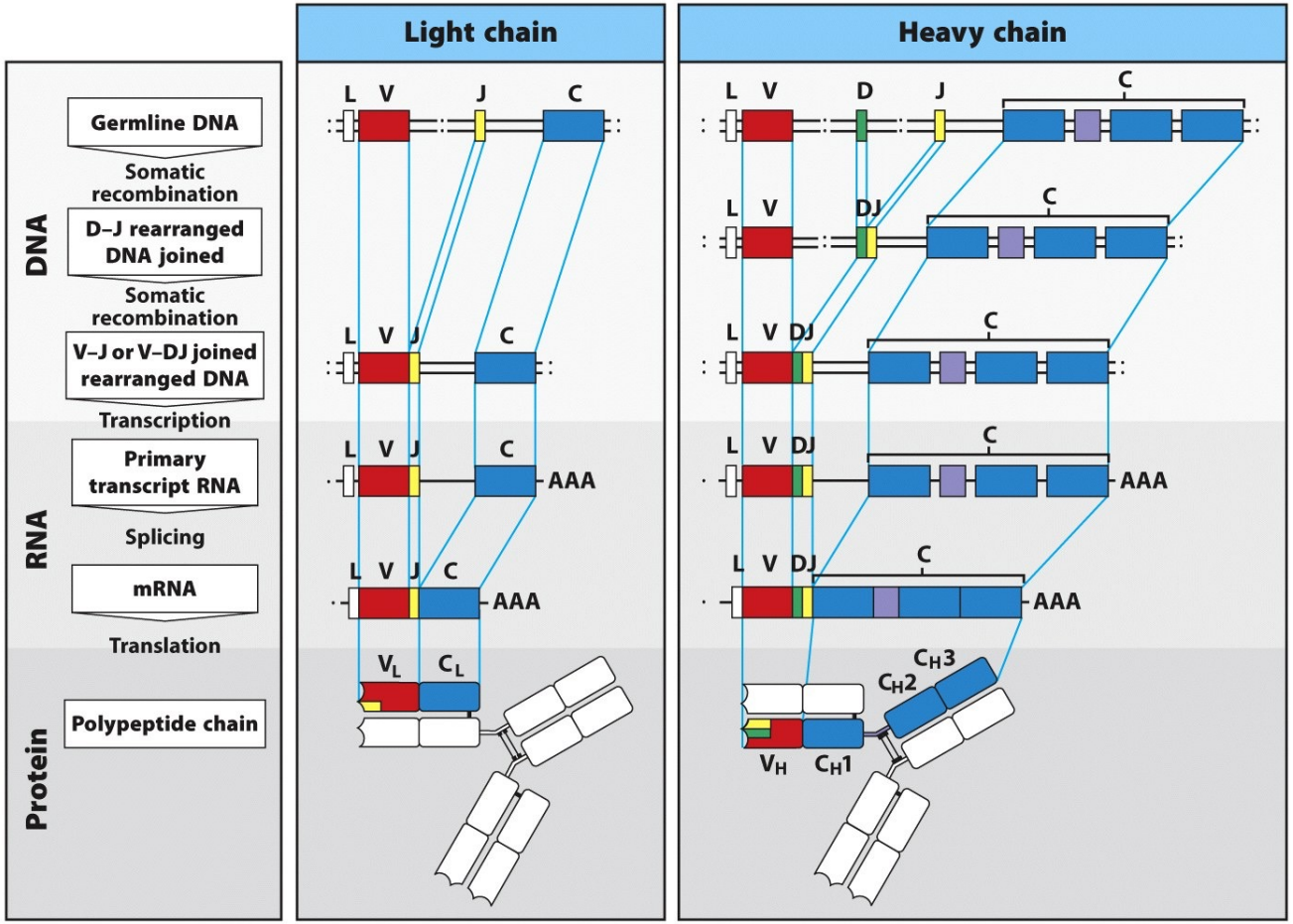


Variable and constant regions are encoded by different genes

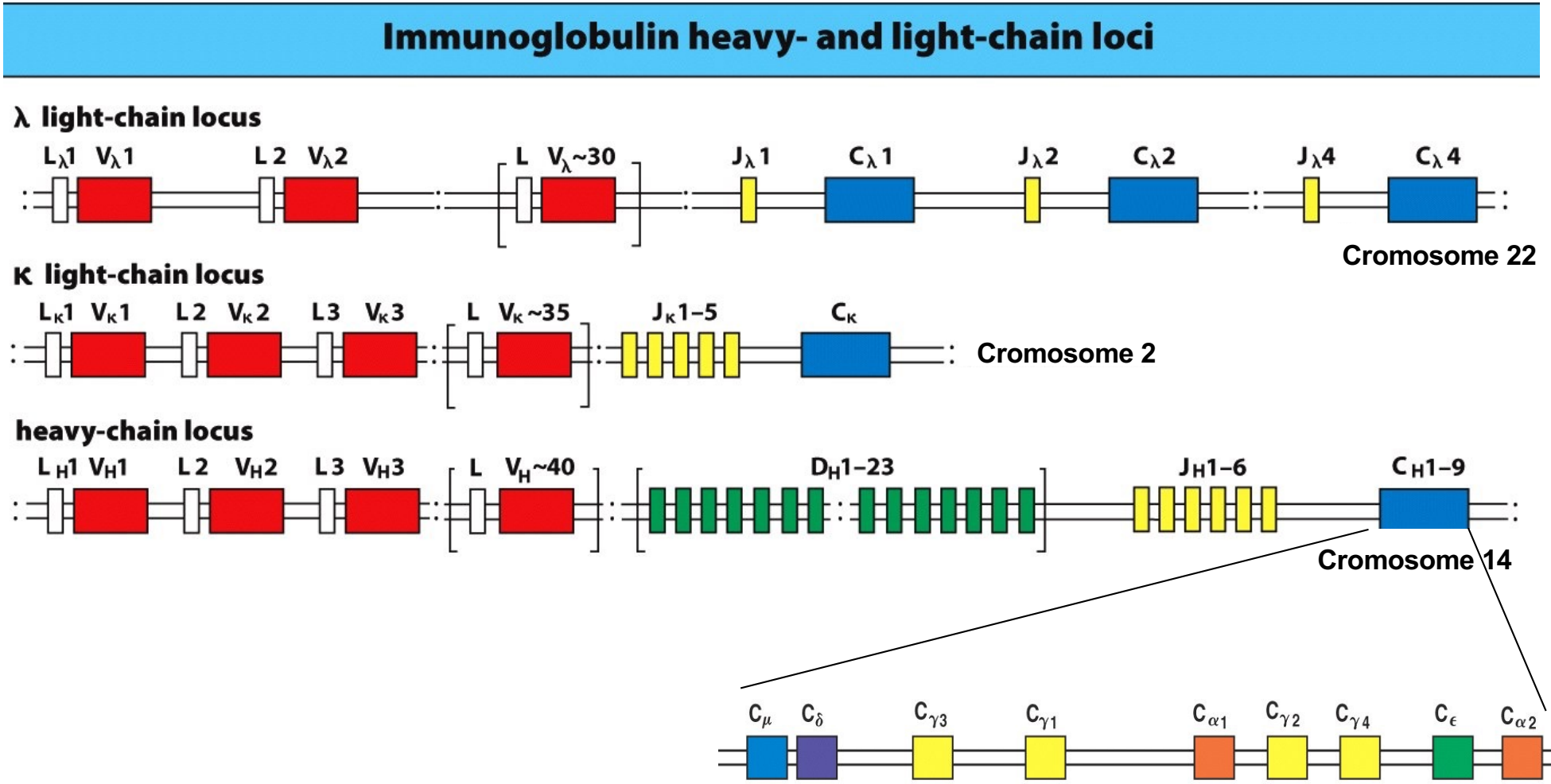


Only one gene encode the constant region of both heavy and light chain, while different gene segments encode the variable regions

CONCEPT: Heavy chain variable regions are assembled from three gene segments - V (variable), D (for diversity) and J (for joining) while light chain variable regions from two gene segments - V and J



Genomic organization of Ig genes



Starting from the 5' end of each Ig locus, there is a first cluster of V gene segments followed by a cluster of J genes.
In the H chain locus between the V and J clusters there are the D gene segments.

DNA Rearrangement Removes Sequences Between V, D and J Segments

RNA Splicing Removes Sequences Between J and C Segments

DNA

RNA

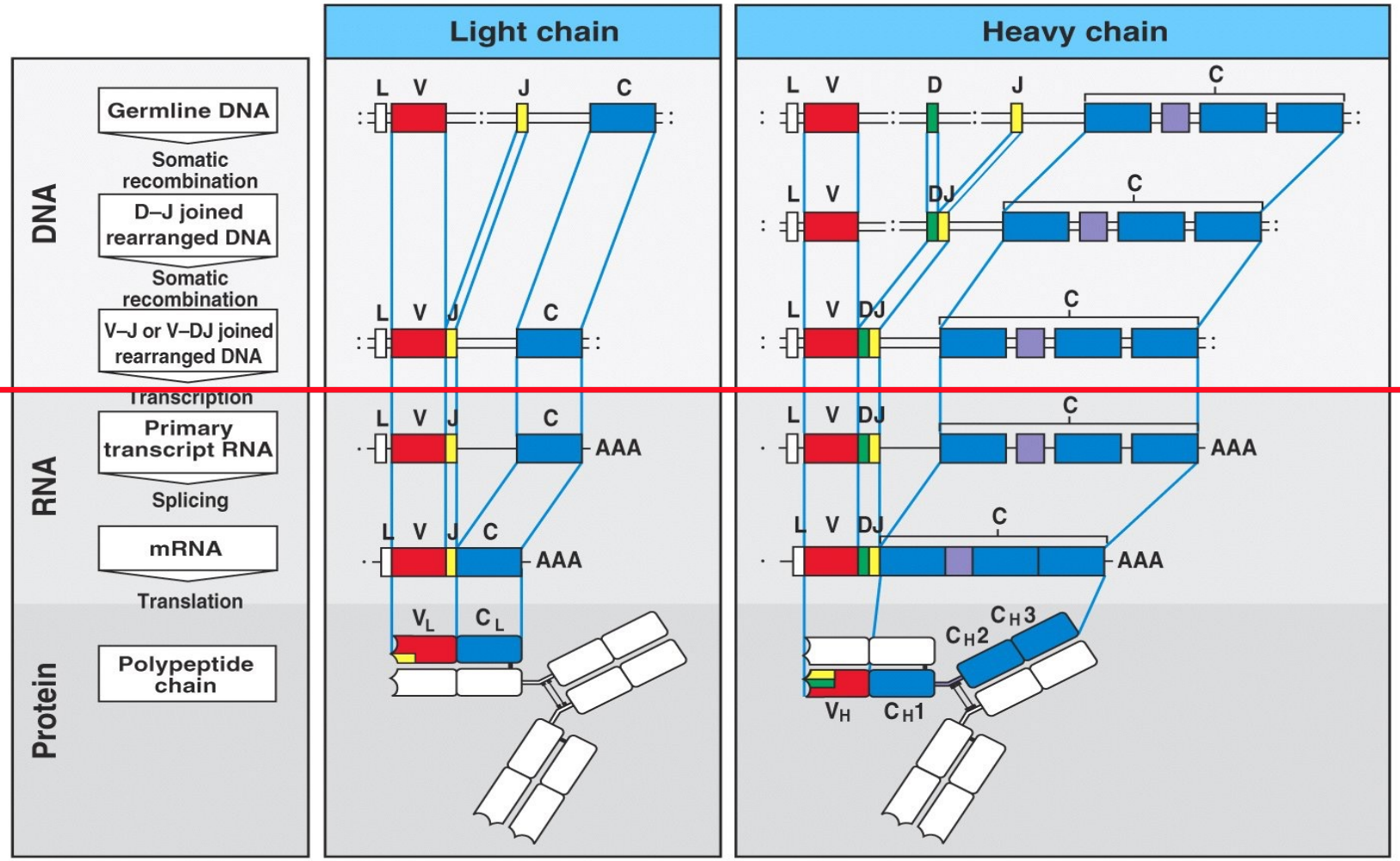
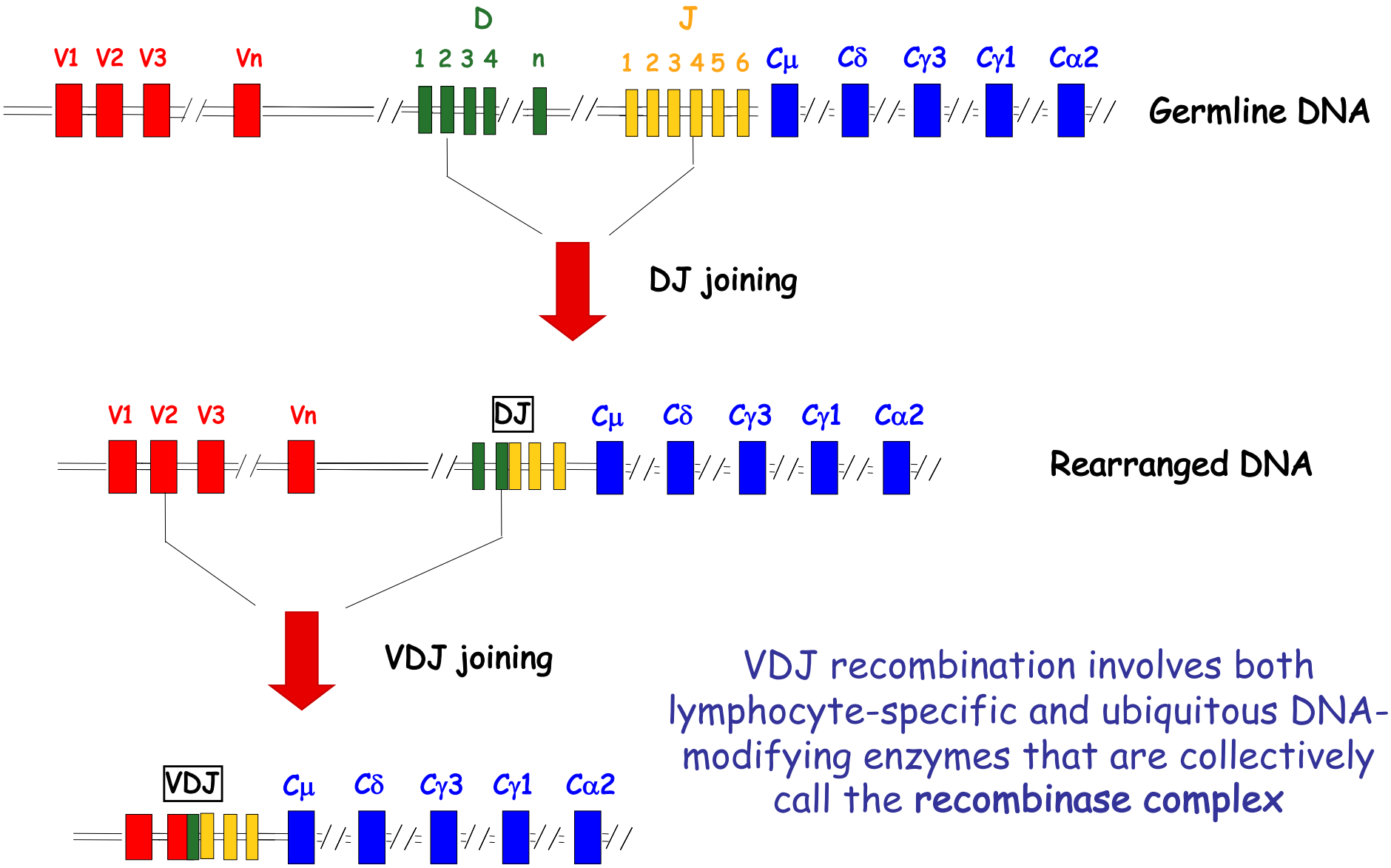


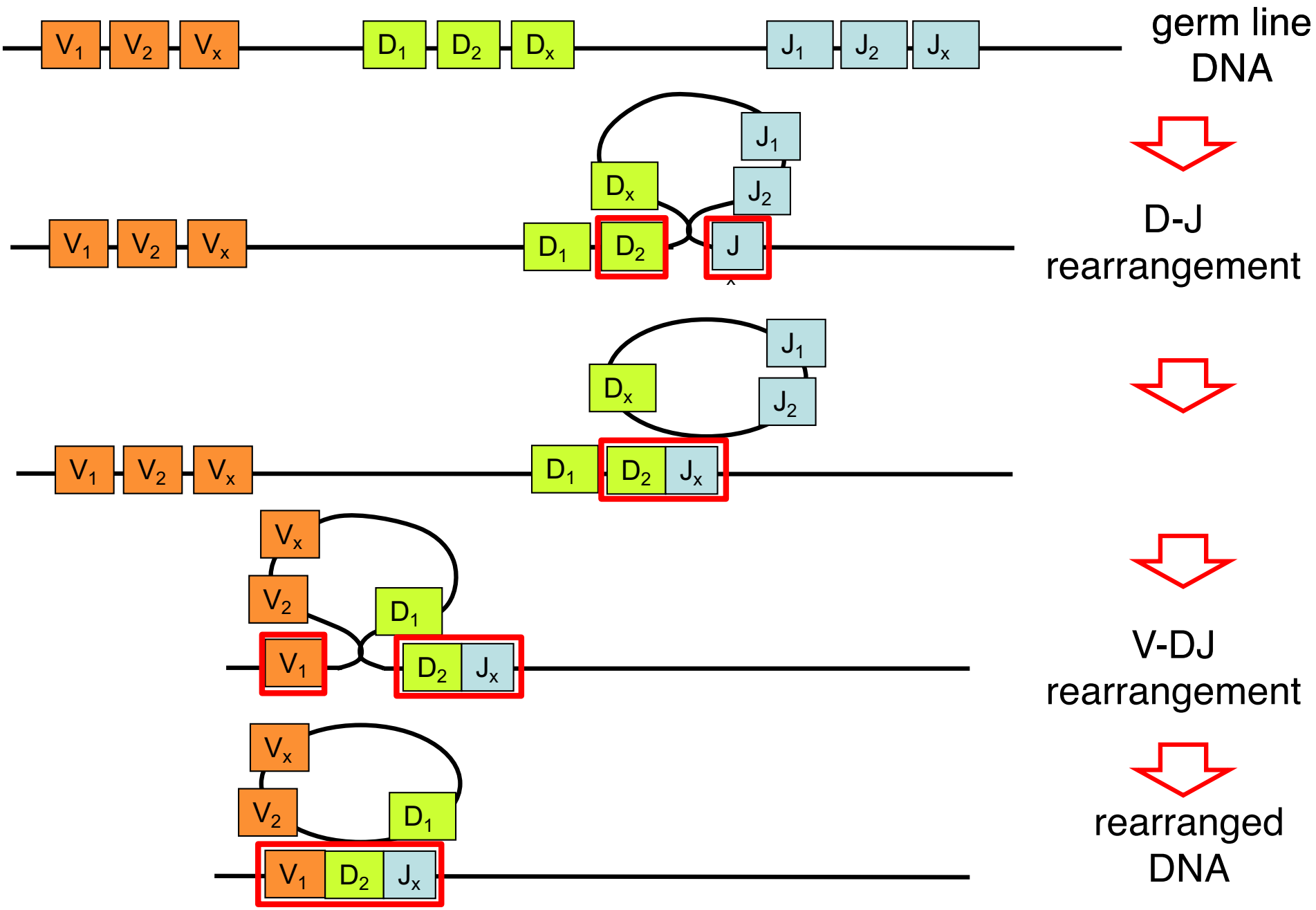
Figure 4-2 Immunobiology, 6/e. (© Garland Science 2005)

Pro-B cells: rearrangement of the heavy chain locus



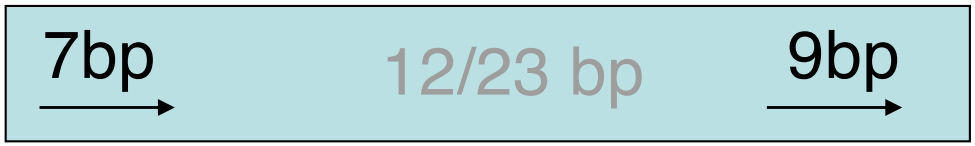
VDJ recombination involves both lymphocyte-specific and ubiquitous DNA-modifying enzymes that are collectively call the **recombinase complex**

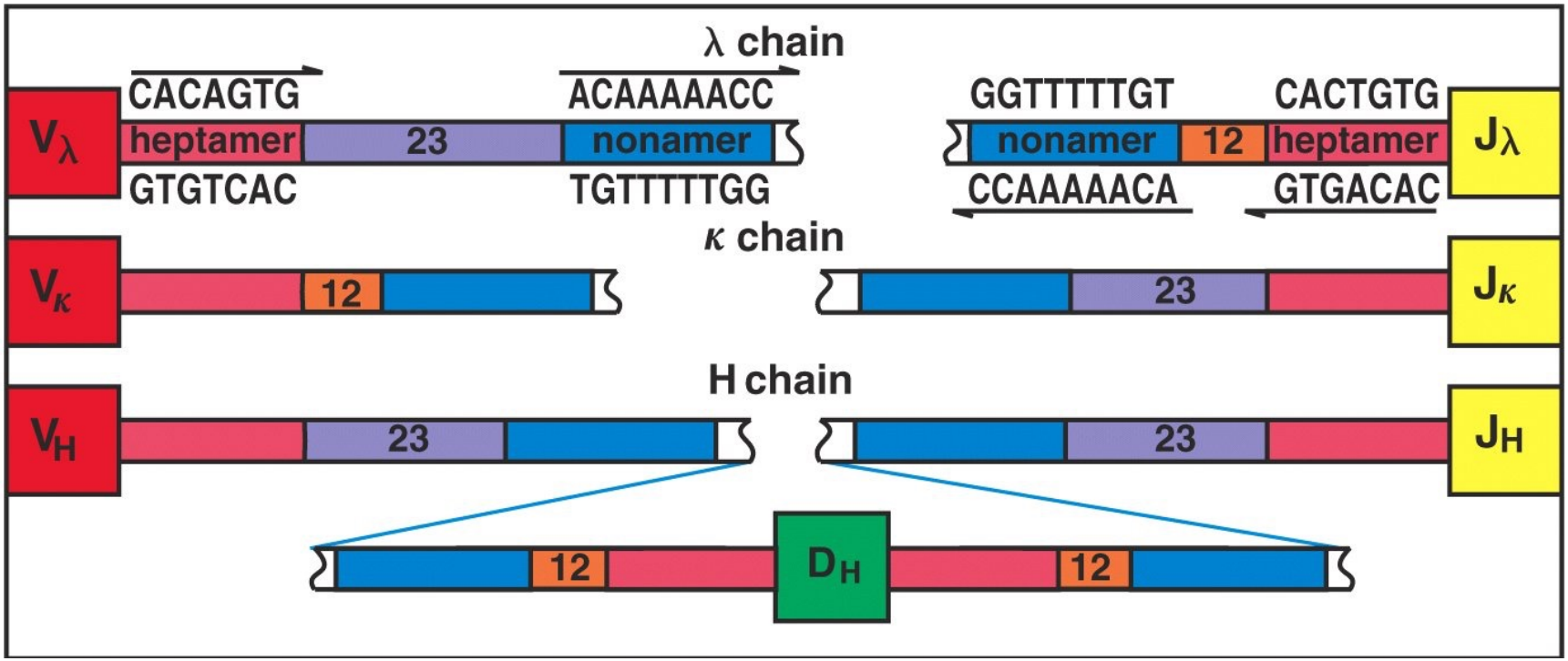
Recombinases (RAG-1 and RAG-2) recognize specific conserved DNA sequences that flank each V, D, and J gene segments and drive the rearrangiament

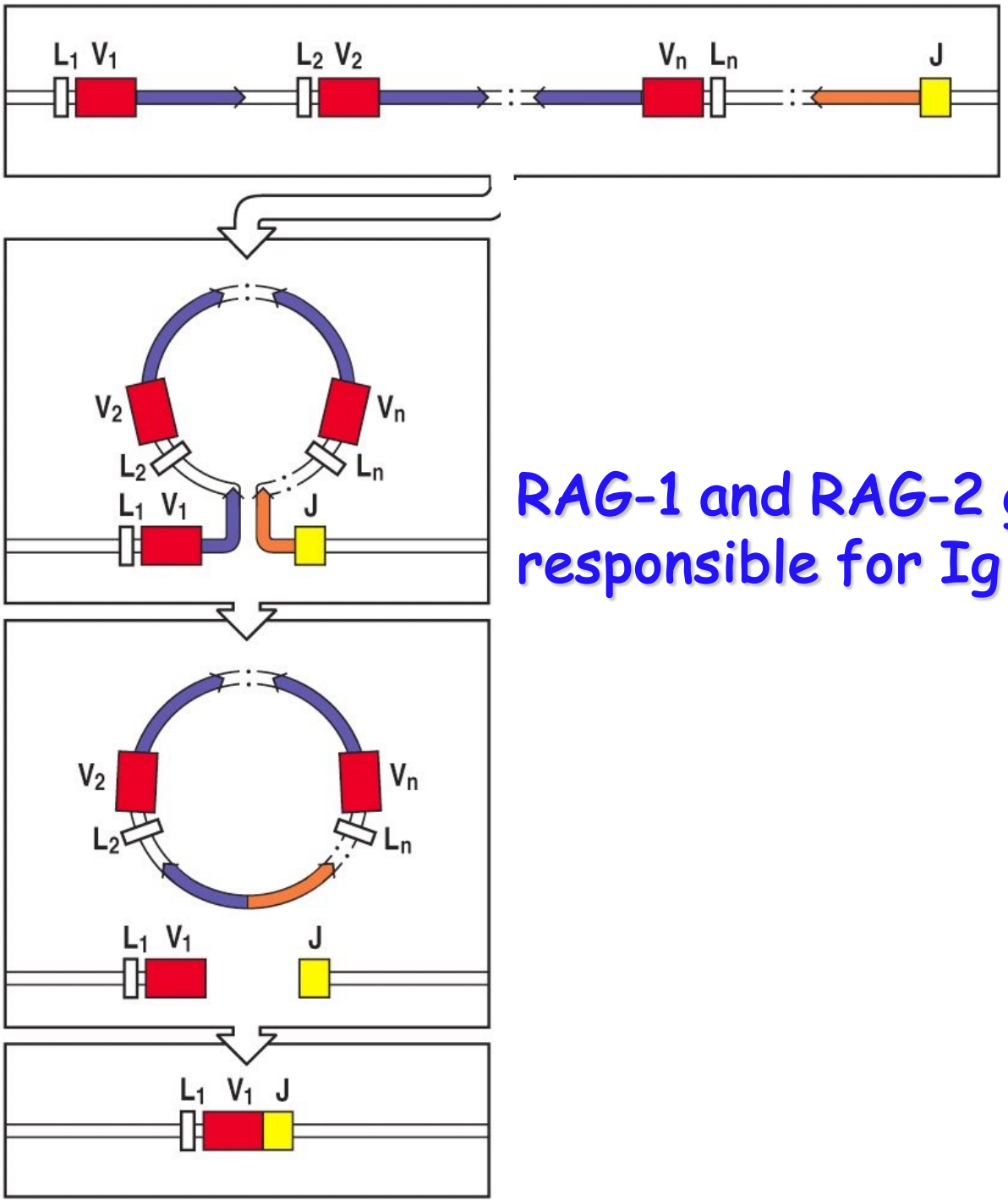


Recombination Signal Sequences (RSSs) Flank Rearranging Gene Segments

RSS=heptamer, spacer and nonamer

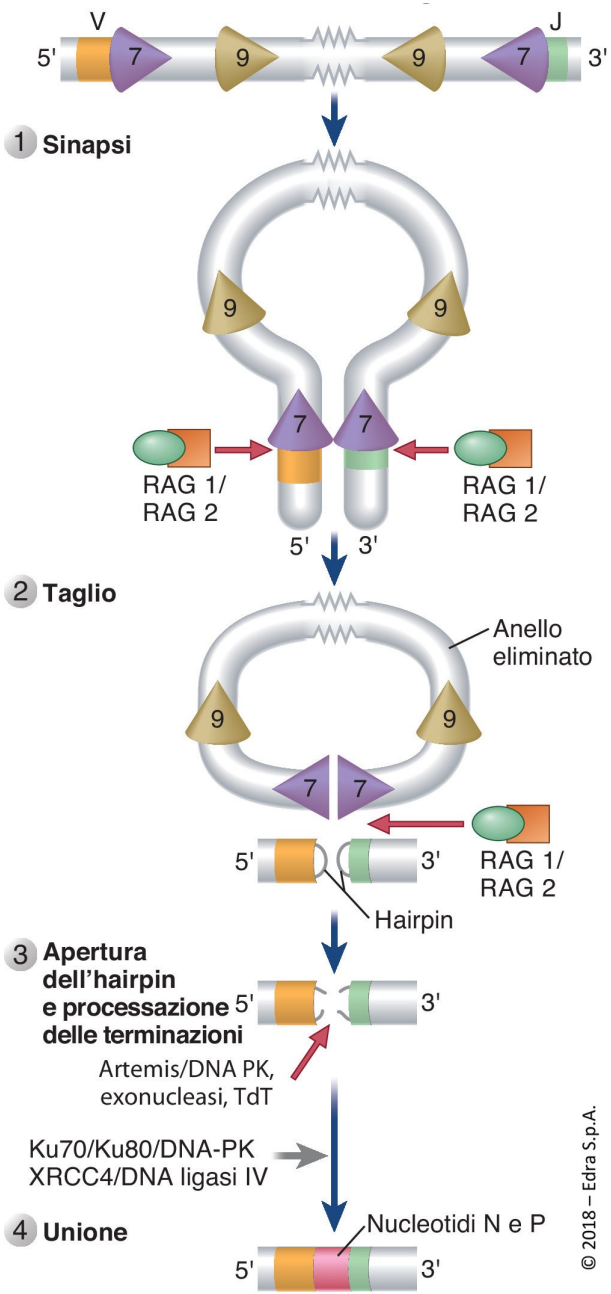






RAG-1 and RAG-2 gene products are responsible for Ig gene rearrangement

Recombination involves lymphocyte-specific enzymes and ubiquitous enzymes that modify DNA



RAG1/RAG2 cut the DNA double helix between the heptamer and the targeted gene segment.

The coding ends close together creating a hairpin structure.

Hairpins are linearized by Artemis and modified by the addition of P and N nucleotides (TdT adds N nucleotides)

The ends are joined by DNA ligases complexed with repair enzymes.

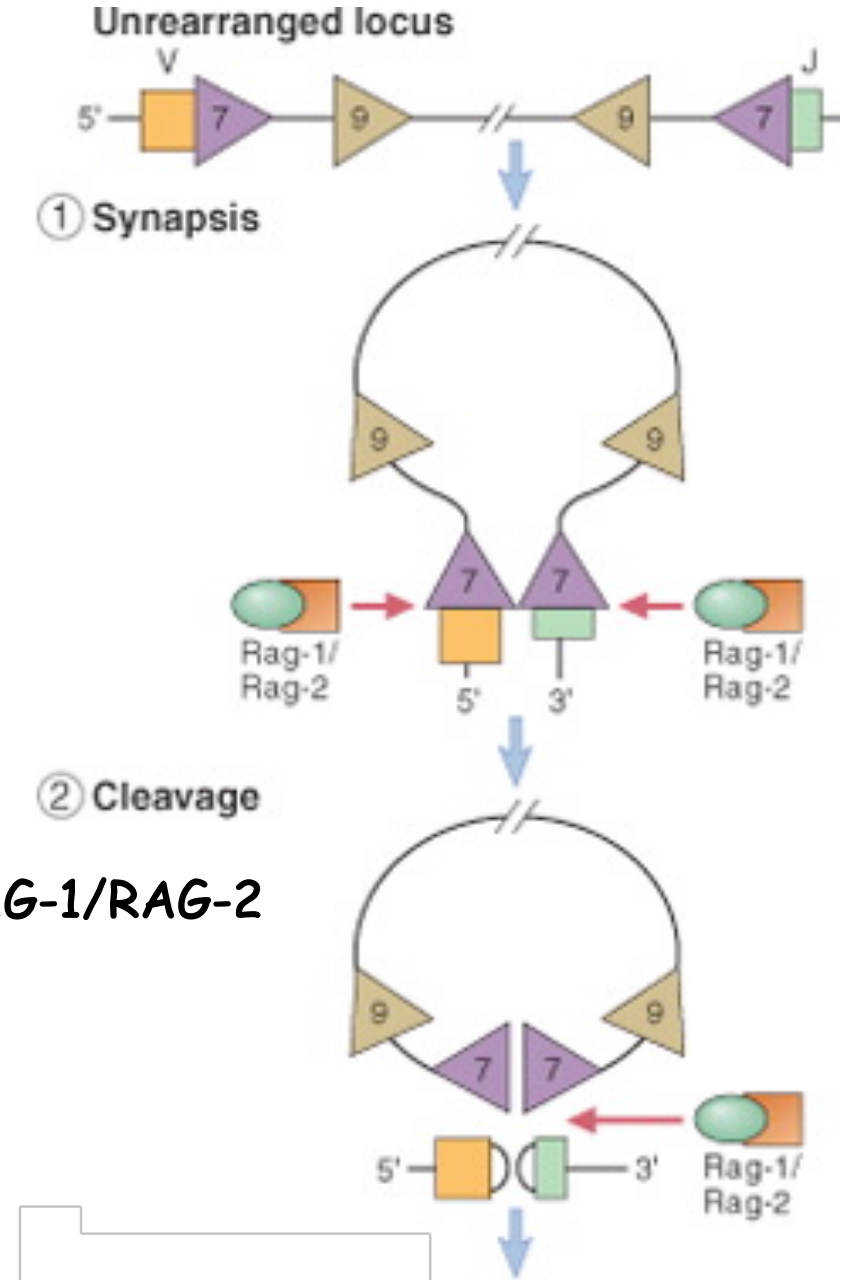
#1: Lymphocyte-Specific

Initiation of V(D)J rearrangement: RAG-dependent cleavage

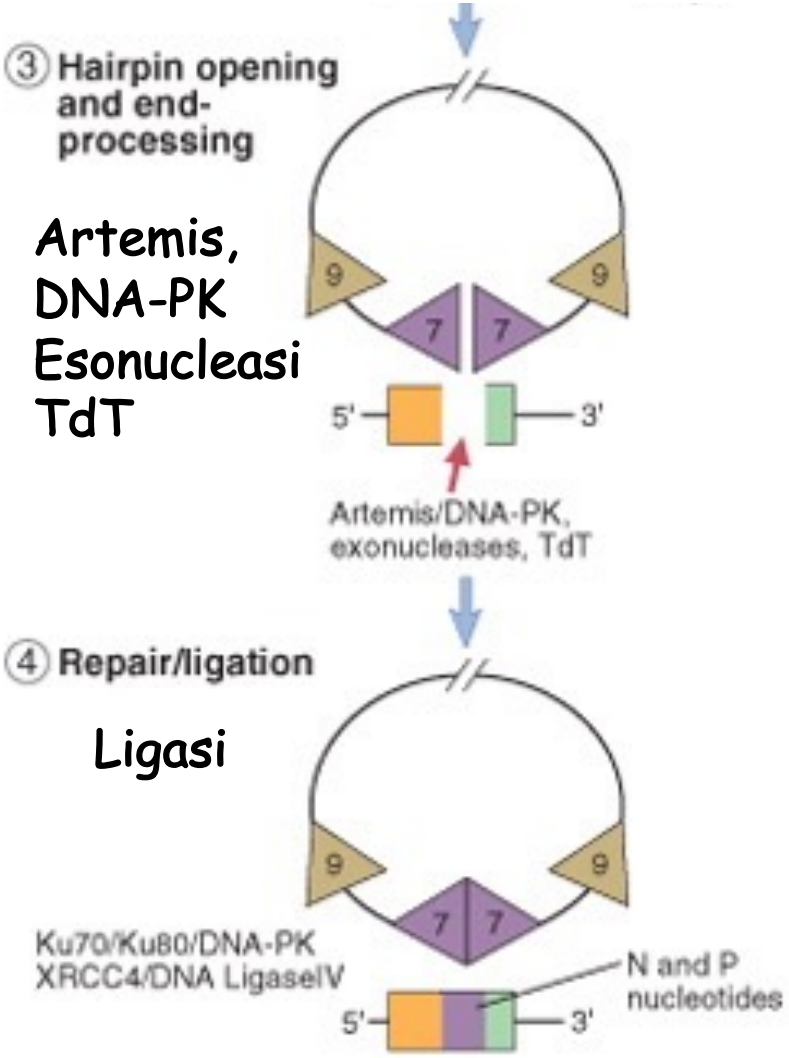
#2: Constitutive

DNA non-homologous end-joining machinery

ENZYMES INVOLVED IN THE REARRANGEMENT OF THE Ig AND TCR GENES



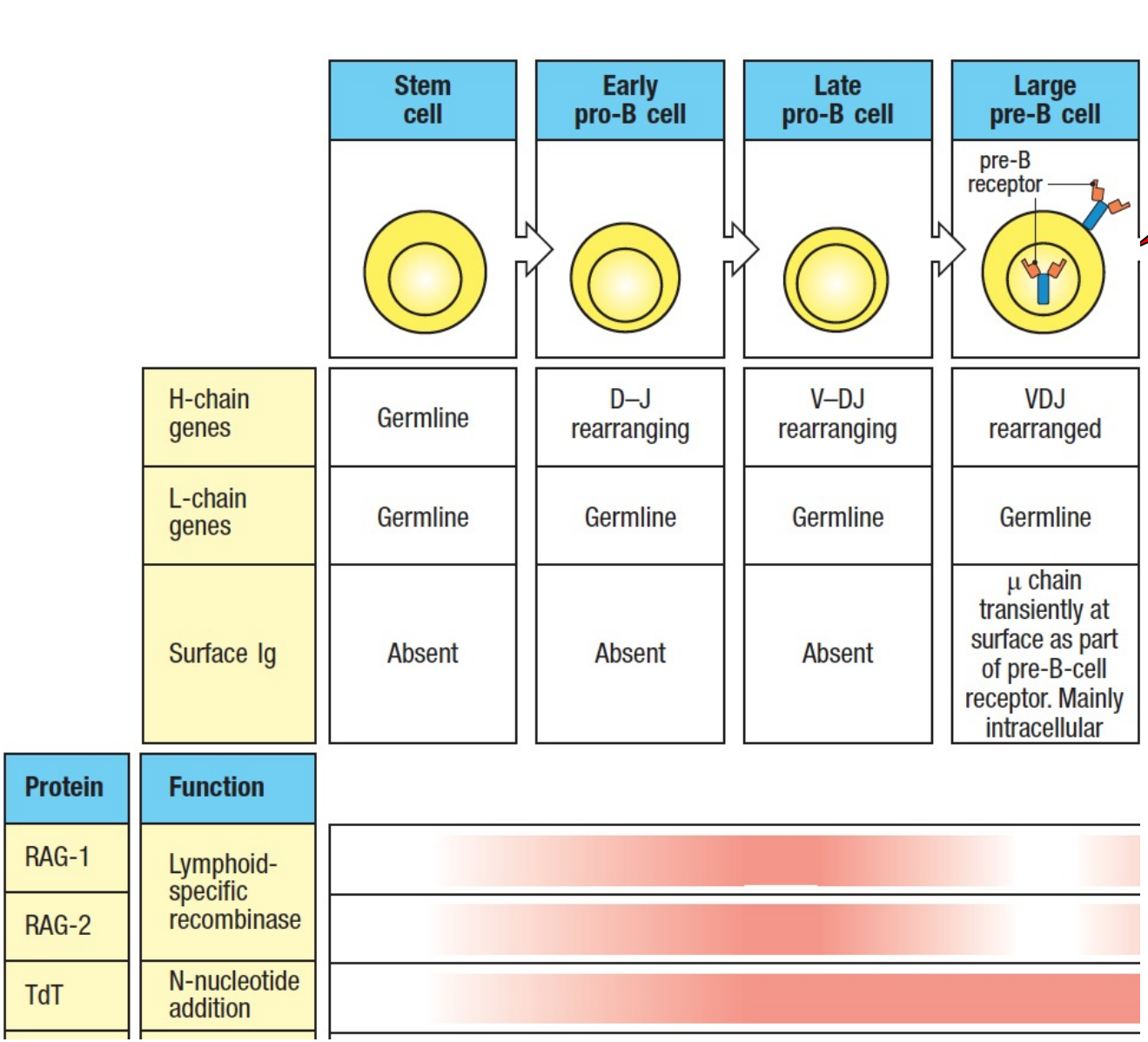
RAG-1/RAG-2



Artemis,
DNA-PK
Esonucleasi
TdT

Ligasi

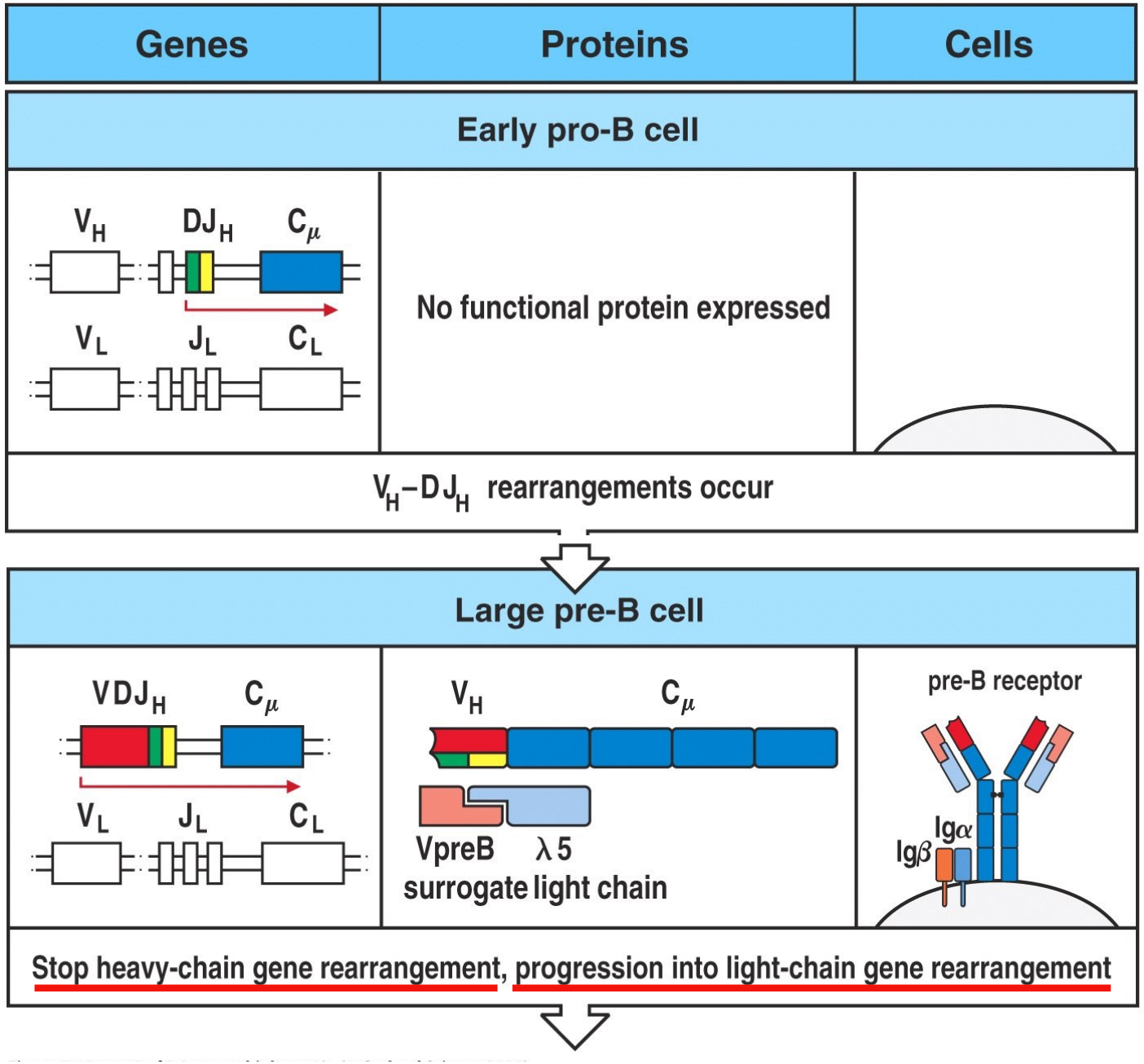
The first stages of B cell development



Check point

Successful rearrangement of the heavy-chain immunoglobulin gene segments leads to the formation of a pre-B cell receptor

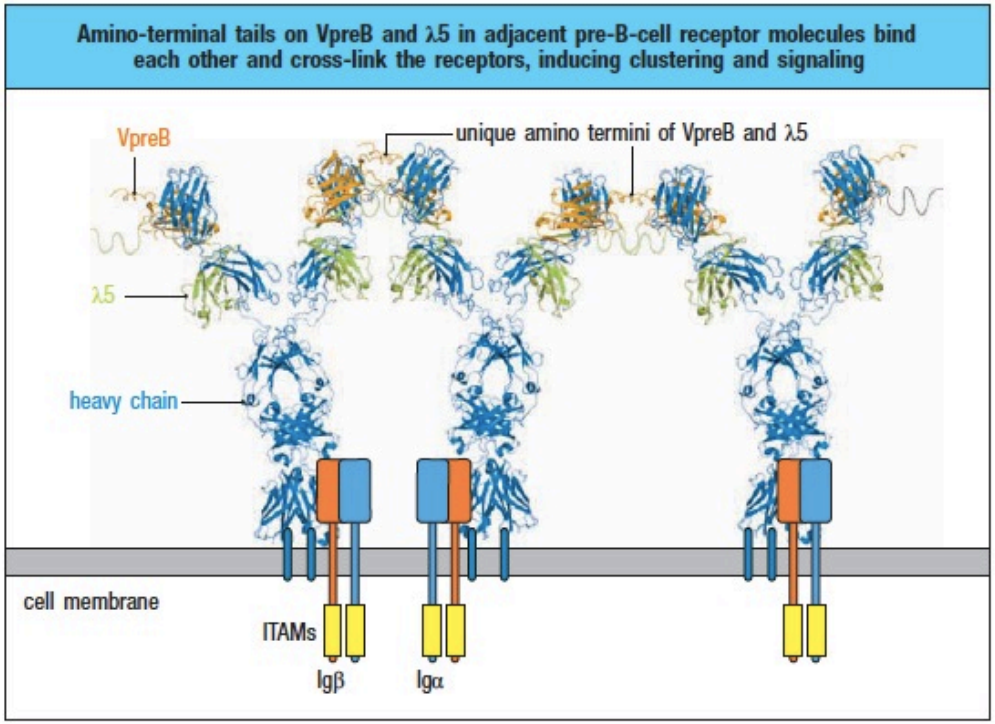
The heavy chain locus undergoes somatic recombination between pro-B and pre-B cell stage



$D_H \rightarrow J_H$

$V_H \rightarrow DJ_H$

Pre-B cells express the pre-BCR

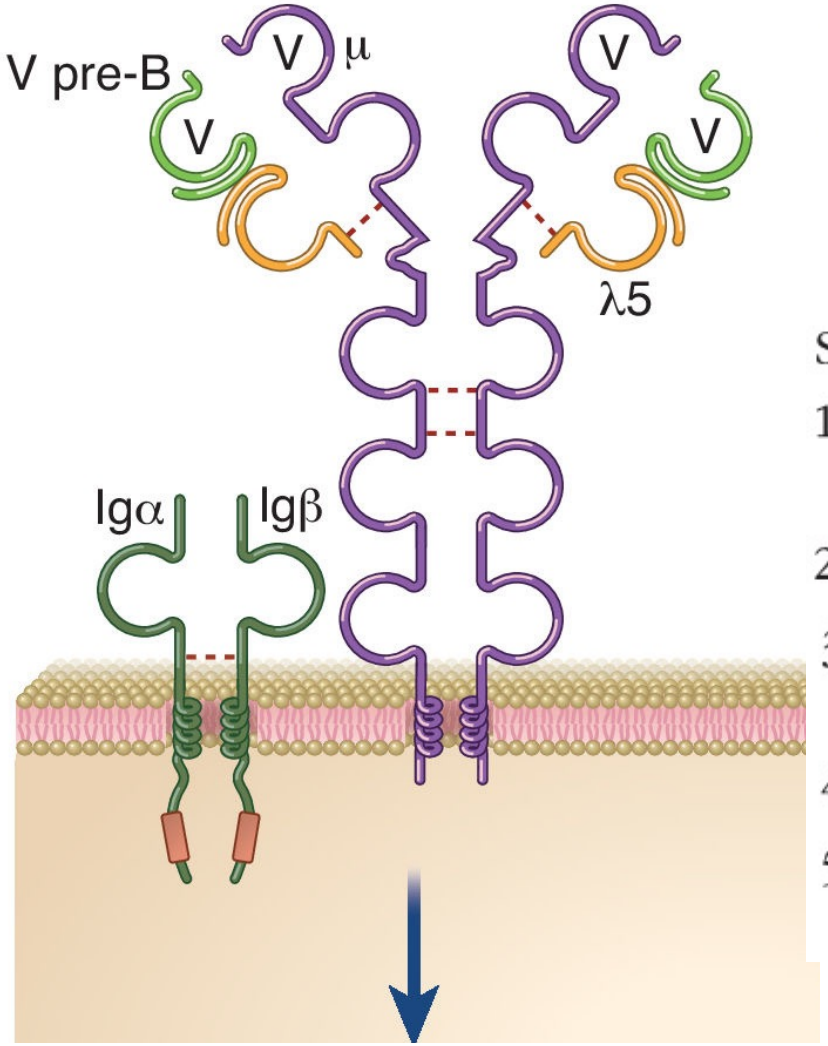


The amino-terminal regions of the VpreB and 15 surrogate chains interact with each other, favoring the spontaneous formation of pre-BCR dimers

The intracellular signal is transduced by Bruton's tyrosine kinase (Btk)

The Btk kinase generates signals required for the progression of maturation

The signal propagated by the Bruton's tyrosine kinase Btk induces:

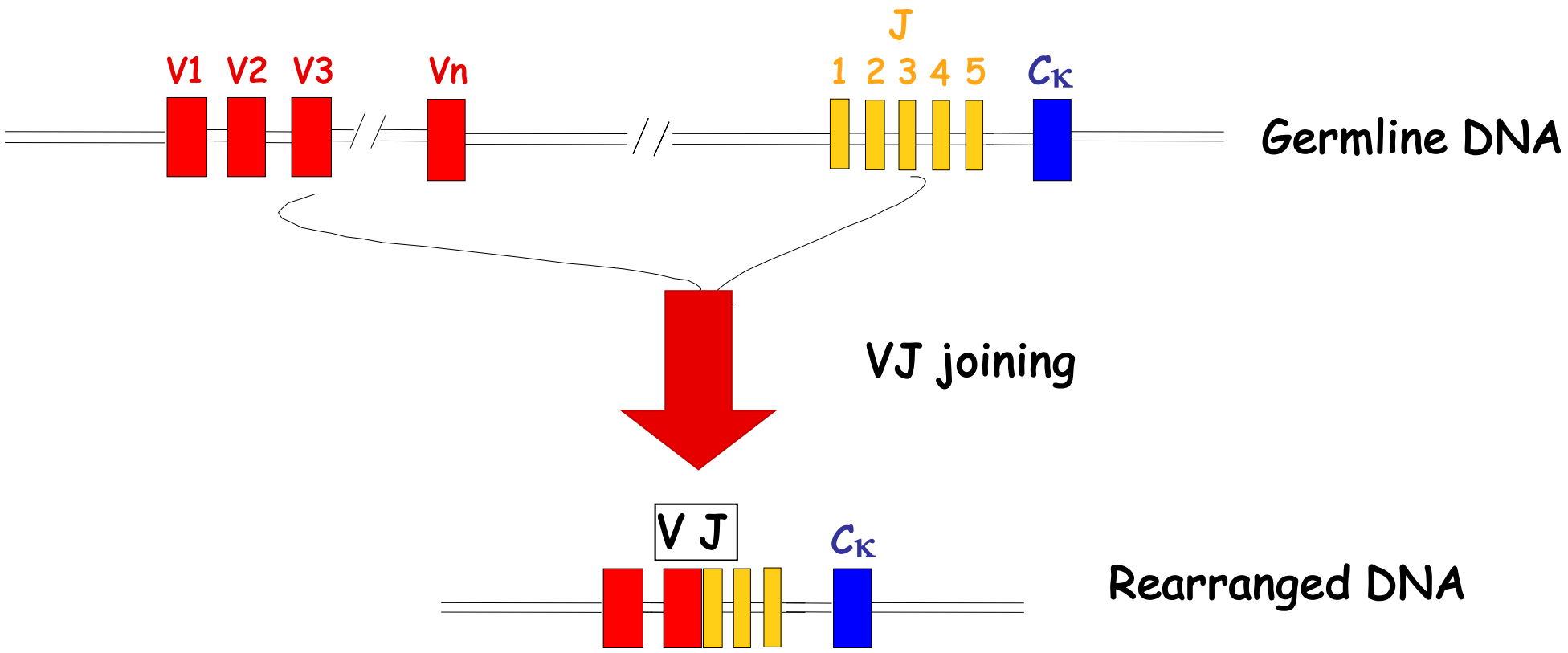


↓

Self-aggregation/cross-linking sequentially signals for:

1. ↓RAG1/2
   ↓V_H-DJ_H gene rearrangement (HC allelic exclusion)
2. Survival, proliferation
3. ↓Pre-BCR and IL-7 signals
   ↓Proliferation
4. ↑RAG1/2
5. ↑Light-chain $V_L J_L$ gene rearrangement

Pre-B cells: rearrangement of the light chain locus (locus κ)



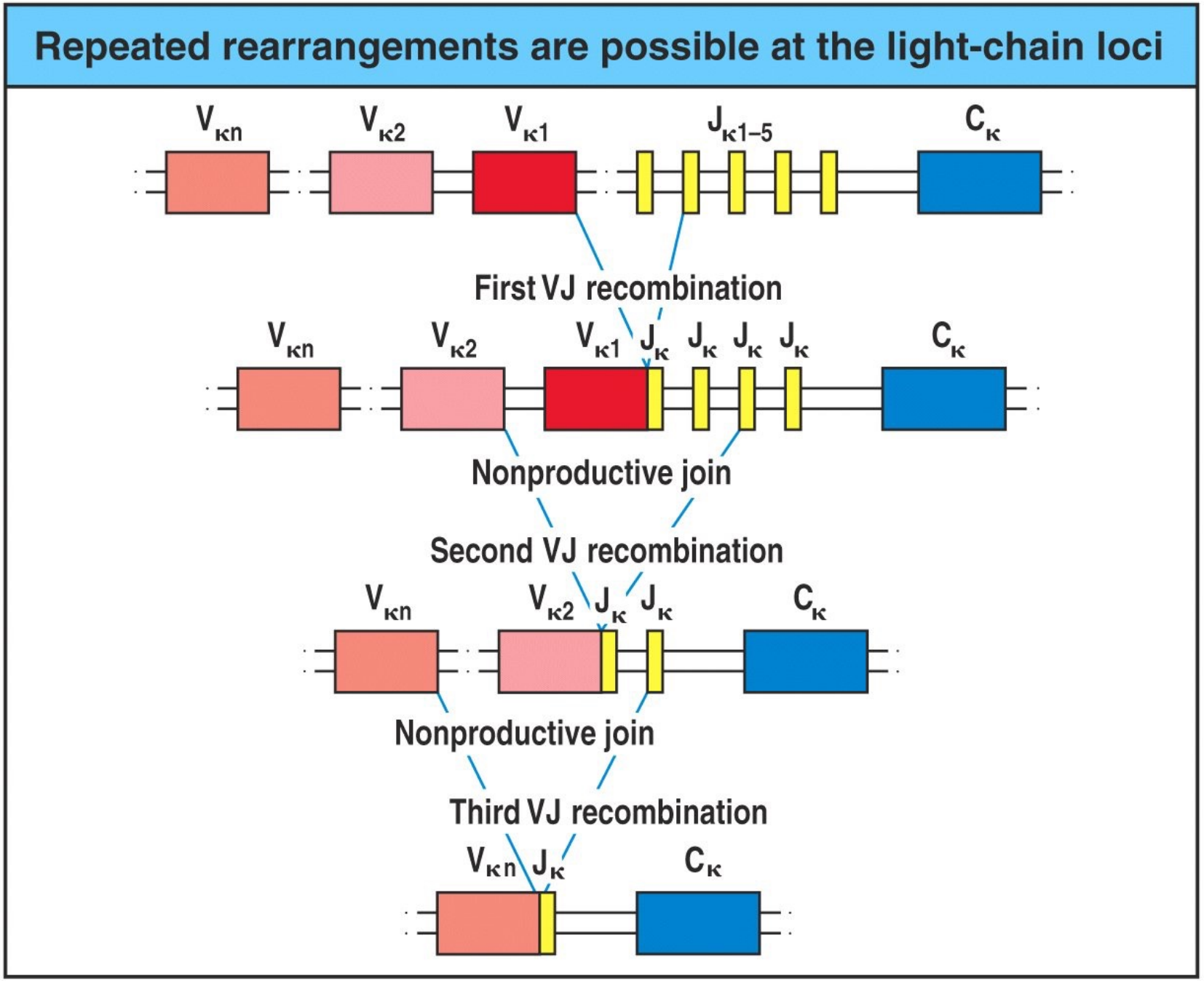
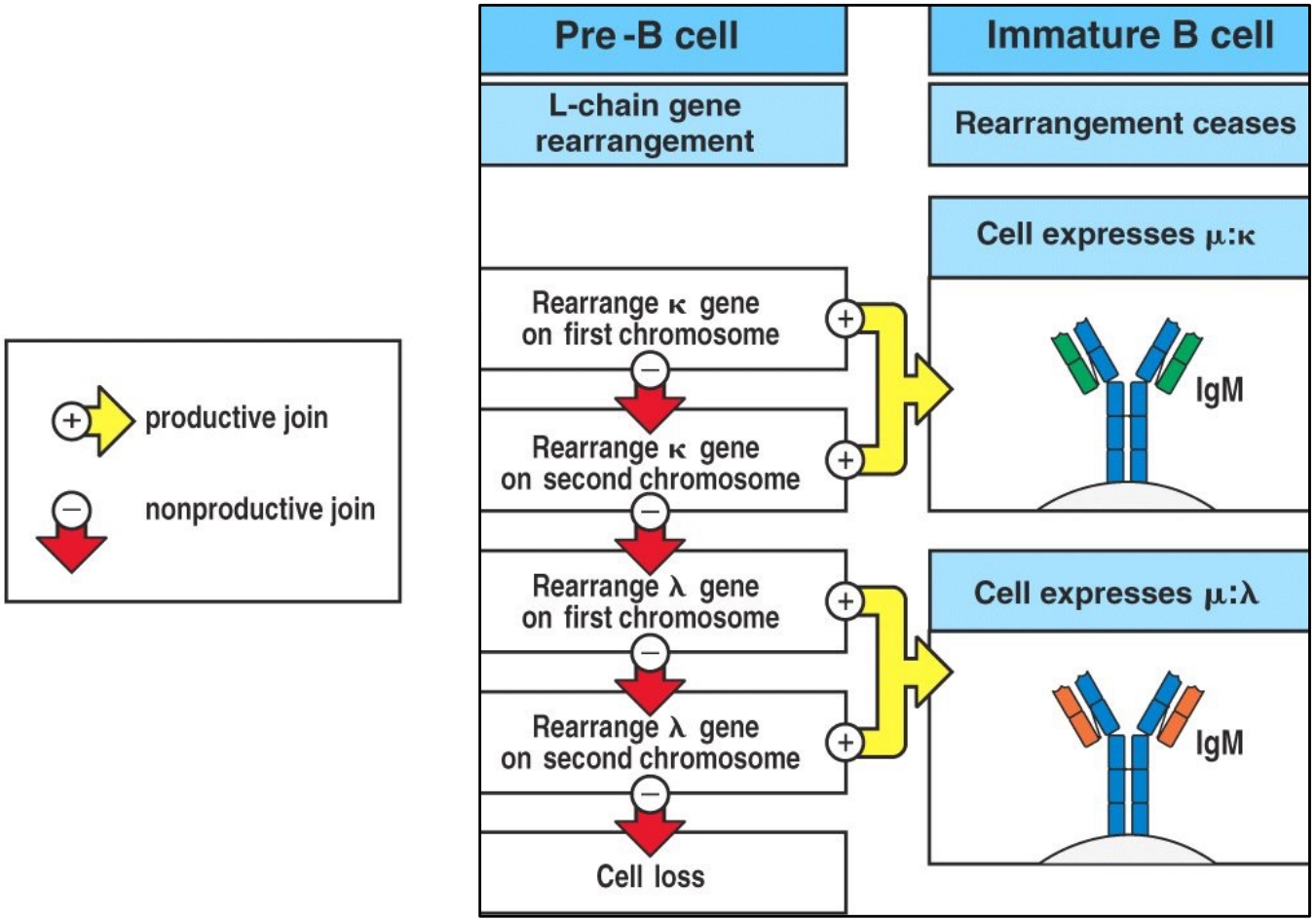


Figure 7-18 Immunobiology, 6/e. (© Garland Science 2005)

Productive rearrangement of light chain genes in the pre-B cell allows membrane expression of IgM



If the rearranged k locus failed to express a functional protein than the λ locus will rearrange

=

Each immature B cell will produce either one κ or one λ light chain from one of the inherited parental alleles

Contribution of different mechanisms to the GENERATION OF DIVERSITY of B cell antigen receptors

COMBINATORIAL DIVERSITY

- Multiple genes for the variable region V, D, J (*random selection of only one of the V, D, and J segments*)
- Combinatorial association of H-chain with L-chain

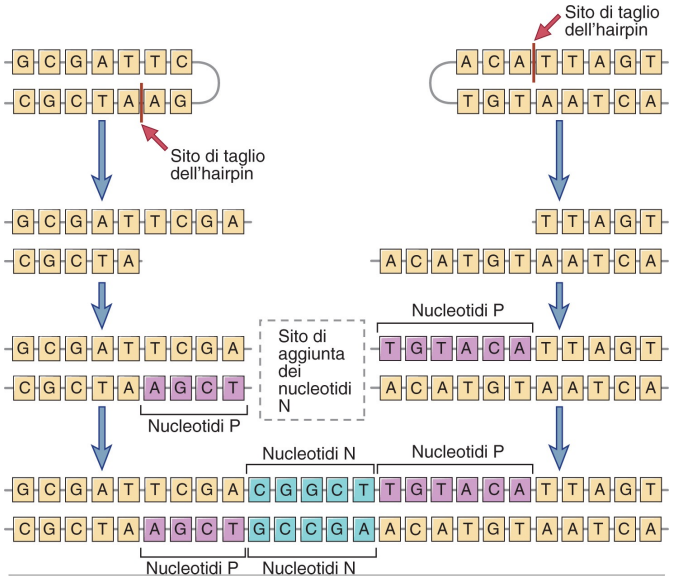
Number of functional gene segments in human immunoglobulin loci

Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	34–38	29–33	38–46
Diversity (D)	0	0	23
Joining (J)	5	4–5	6

JUNCTIONAL DIVERSITY

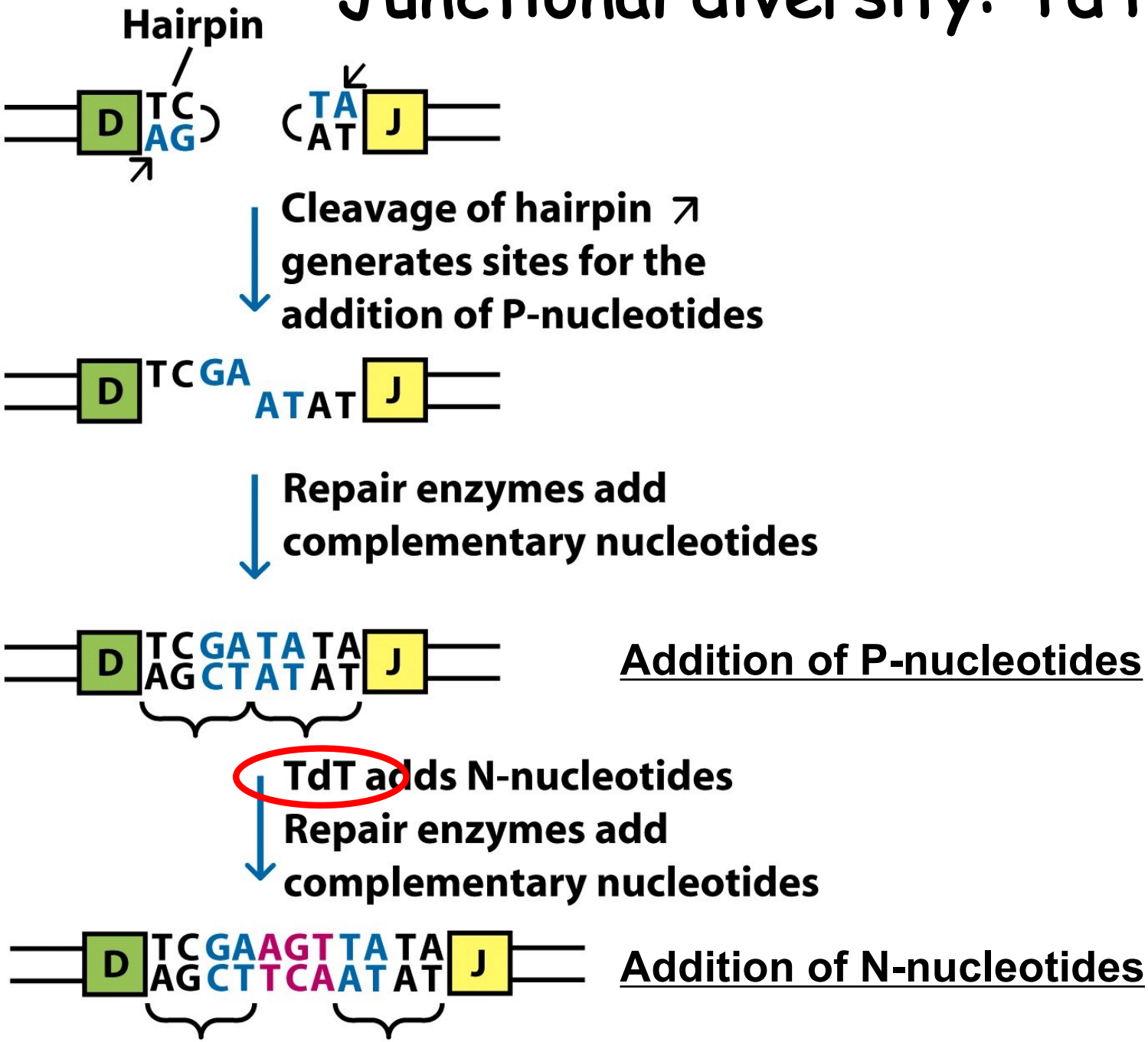
- Removal or addition of nucleotides at the
- junction point

Addition of P-nucleotides and N-nucleotides by terminal deoxynucleotidyl transferase (TdT)

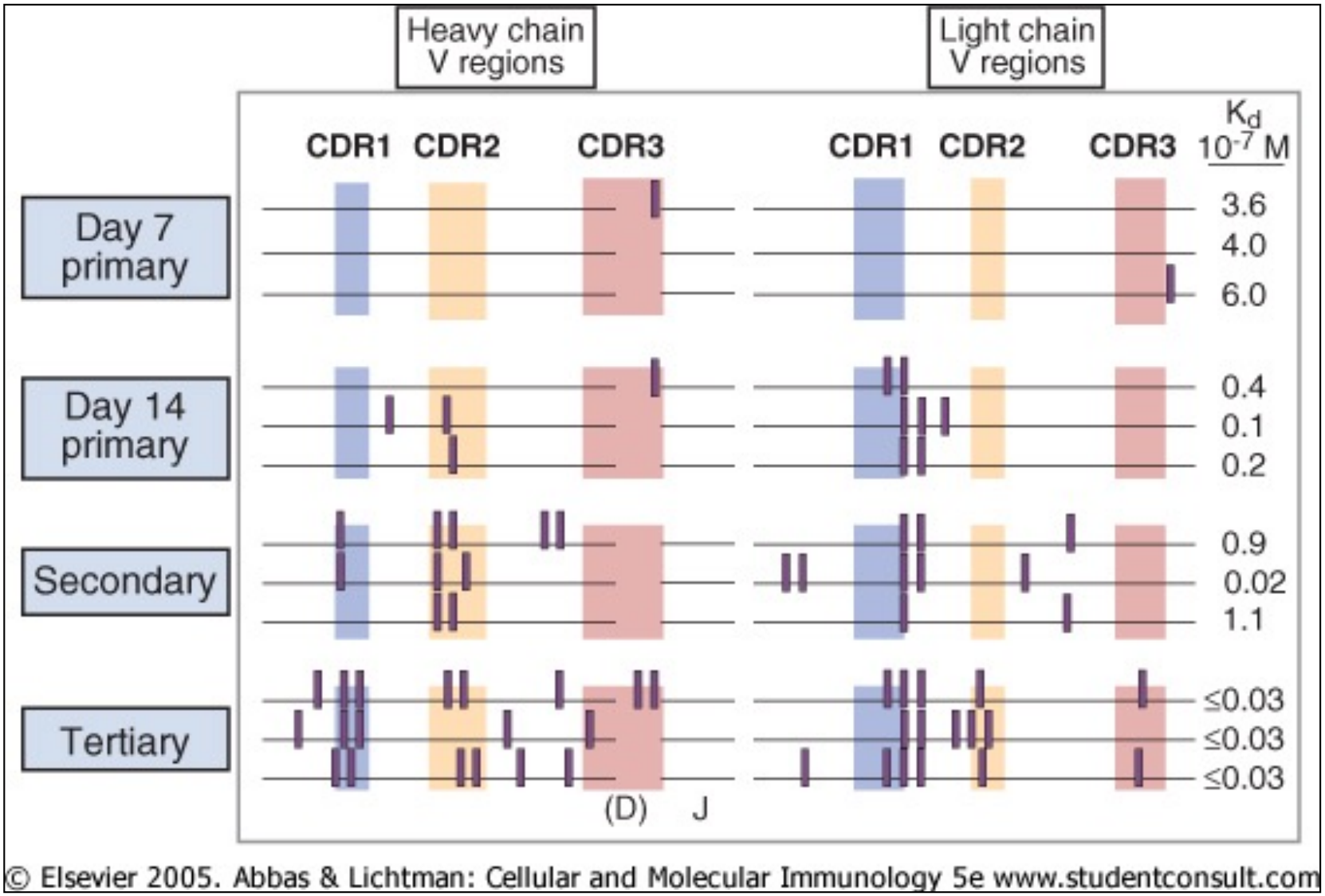


By these mechanisms a human can produce about 10^{13} different B cell receptors!!

Junctional diversity: TdT role



Ipersomatic mutations



They are activated by exposure to the antigen (secondary lymphoid organs).
The frequency of mutations increases following repeated exposure to the same antigen.

Contribution of different mechanisms to the GENERATION OF DIVERSITY of B cell antigen receptors

COMBINATORIAL DIVERSITY

- Multiple genes for the variable region V, D, J
(*random selection of only one of the V, D, and J segments*)

- Combinatorial association of H-chain with L-chain

JUNCTIONAL DIVERSITY

- Removal or addition of nucleotides at the juntion point
- Addition of P-nucleotides and N-nucleotides by terminal deoxynucleotidyl transferase (TdT)

Ipersomatic mutations

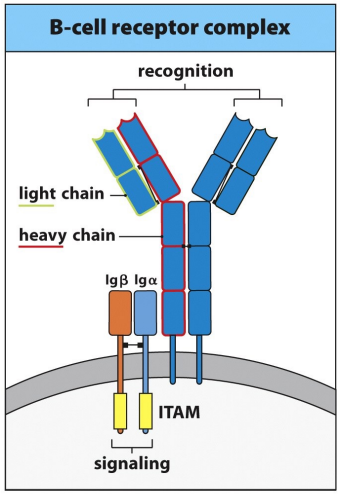
Codice OPIS assegnato al corso integrato tenuto da:

Prof. ROSSELLA PAOLINI

Biotecnologie (29887)

Modulo	Insegnamento	**Codice OPIS**
IMMUNOLOGIA I (1051487_2)	IMMUNOLOGIA (10514879)	**1BH7HMGQ**
IMMUNOLOGIA II (1051487_1)	IMMUNOLOGIA (10514879)	**MH4E21B4**

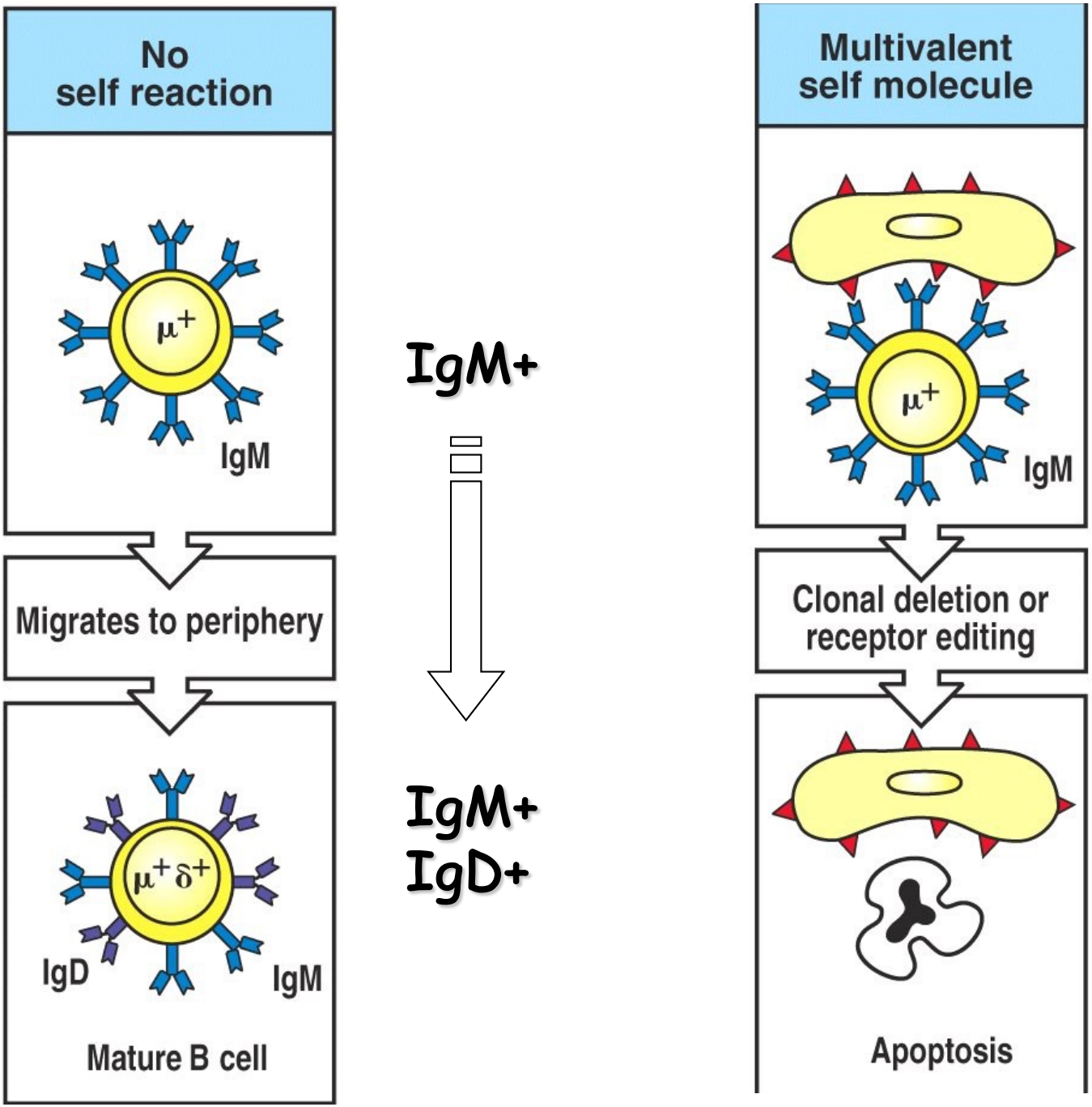
Immature B-Cells



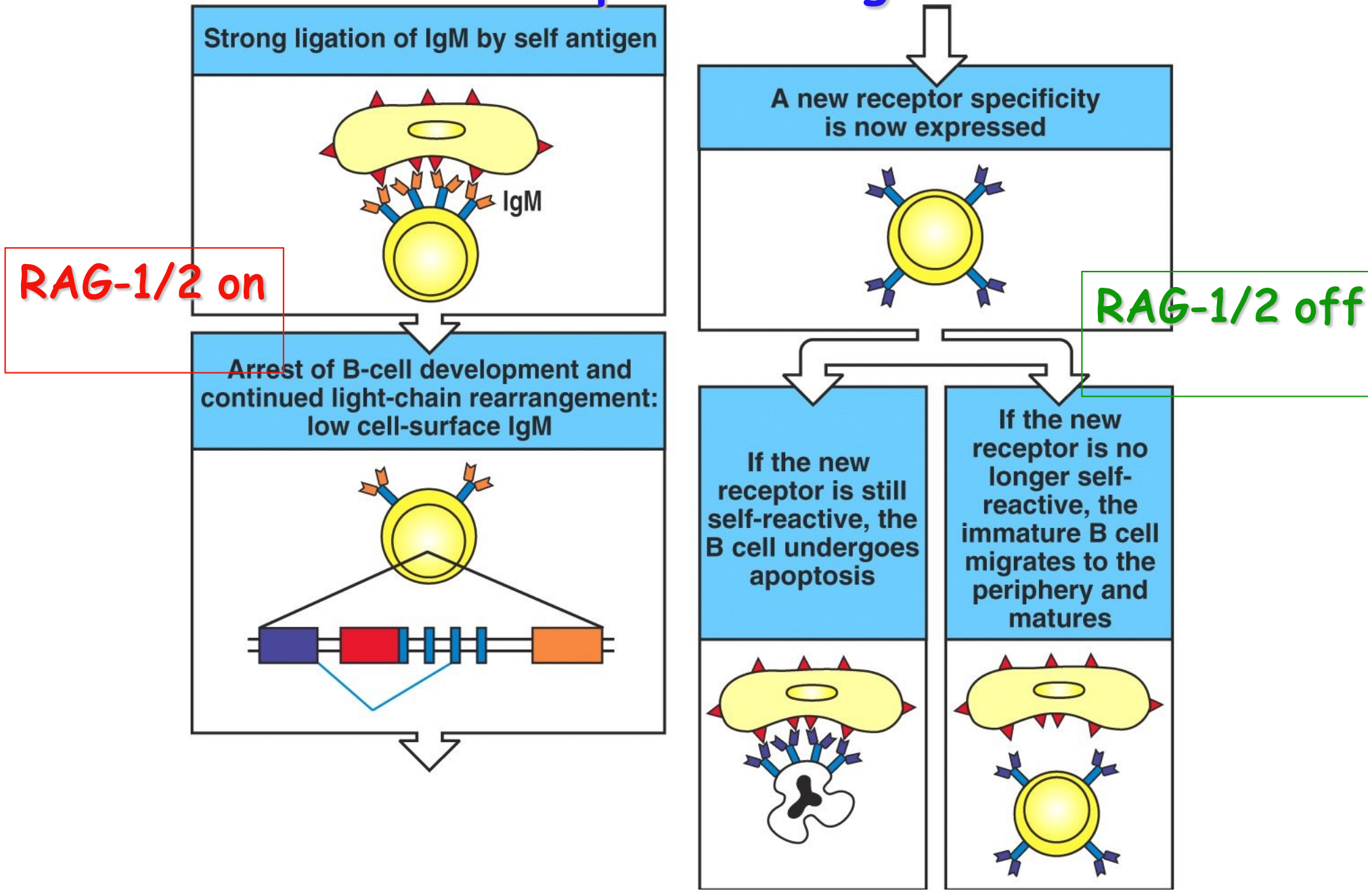
	Stem cell	Early pro-B cell	Late pro-B cell	Large pre-B cell	Small pre-B cell	Immature B cell
				pre-B receptor		IgM
H-chain genes	Germline	D–J rearranging	V–DJ rearranging	VDJ rearranged	VDJ rearranged	VDJ rearranged
L-chain genes	Germline	Germline	Germline	Germline	V-J rearranging	VJ rearranged
Surface Ig	Absent	Absent	Absent	μ chain transiently at surface as part of pre-B-cell receptor. Mainly intracellular	Intracellular μ chain	IgM expressed on cell surface

Check point

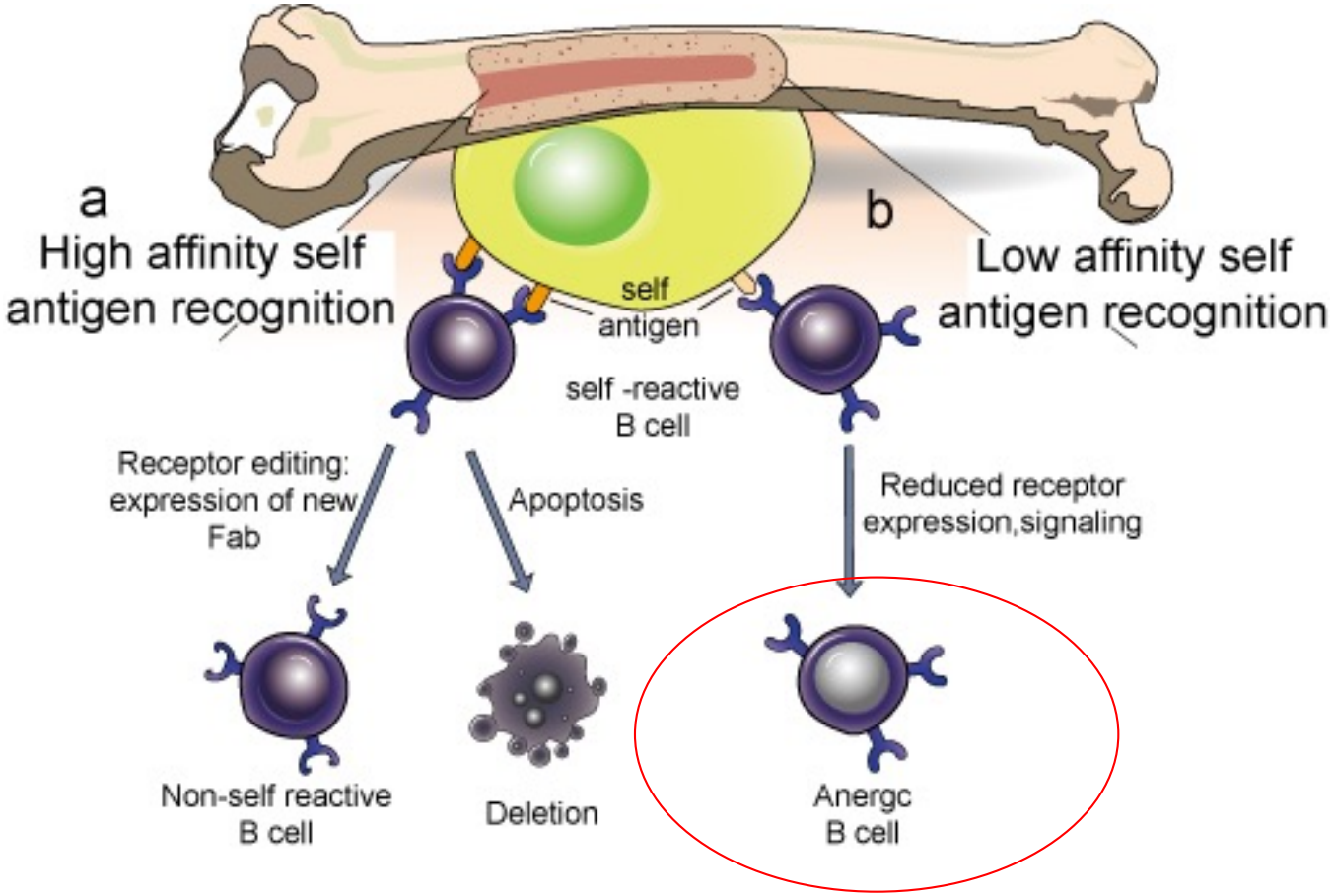
Immature B cells undergo a process of negative selection



BCR engagement at the immature stage induces receptor editing



NEGATIVE SELECTION OF AUTOREACTIVE LYMPHOCYTES



CENTRAL TOLERANCE

Immature B cells express an IgM BCR and undergo a process of negative selection

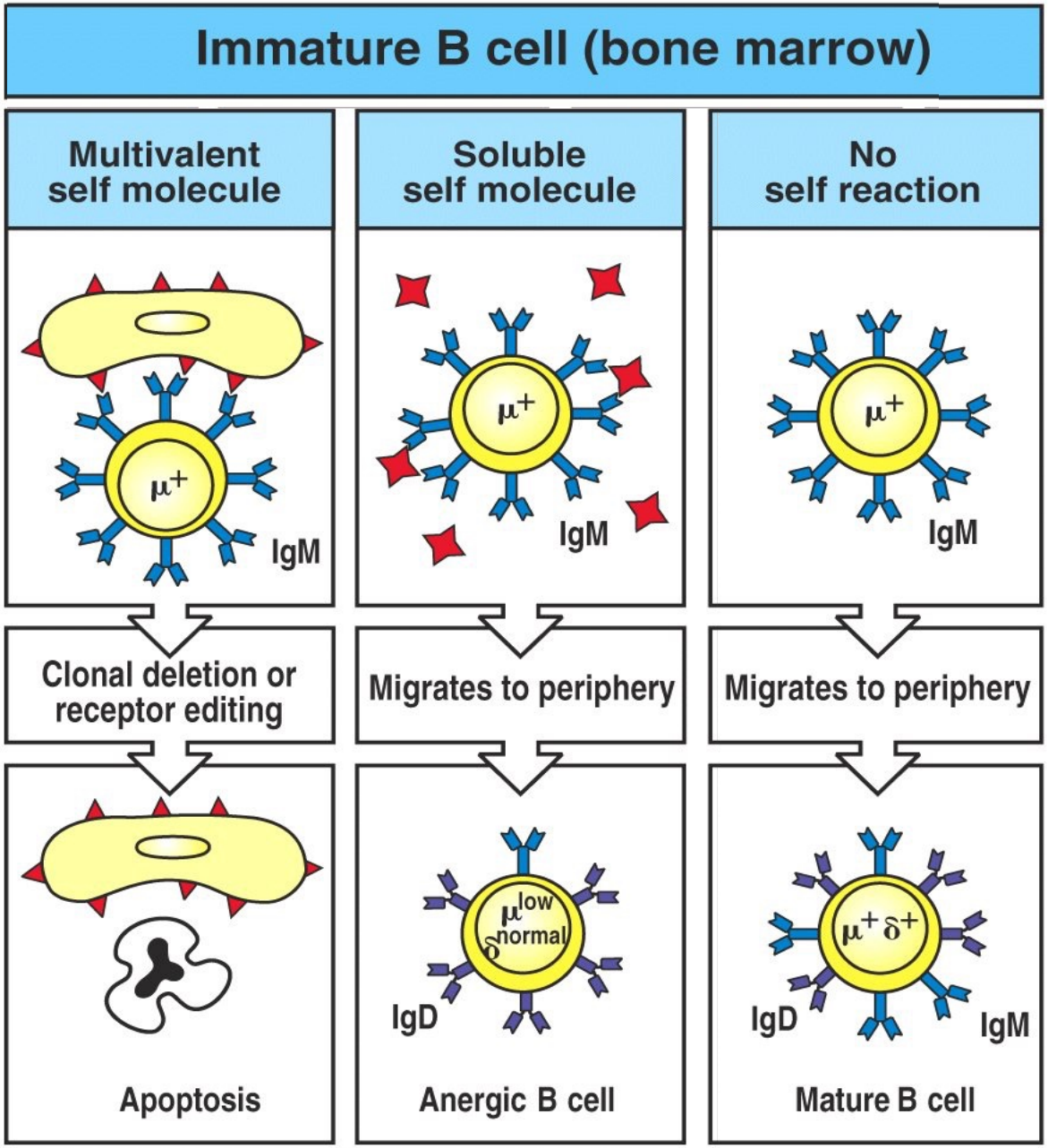
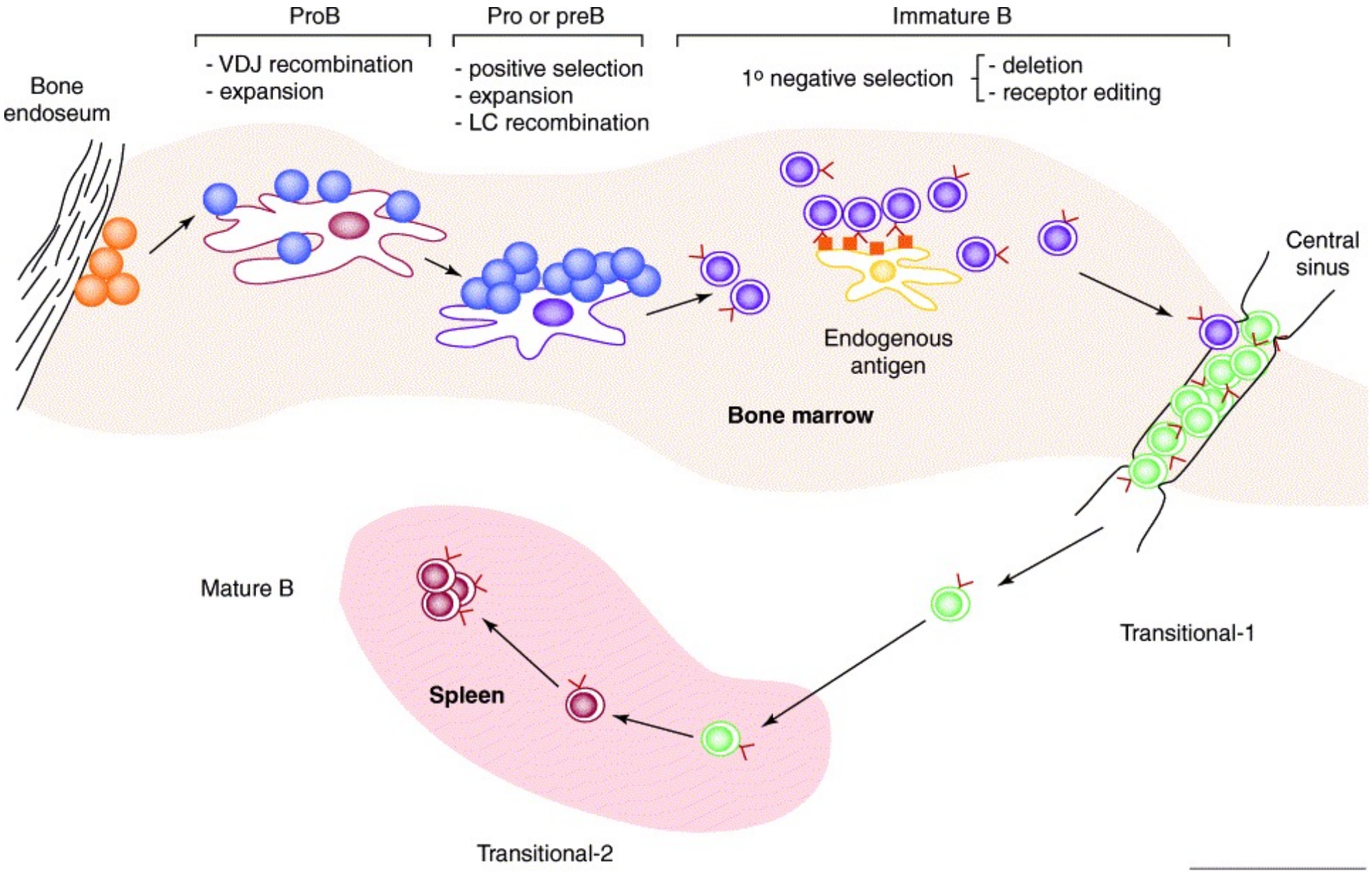


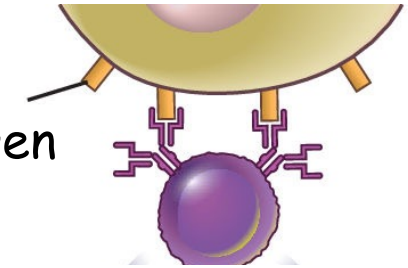
Figure 7-26 Immunobiology, 6/e. (© Garland Science 2005)

B lymphocytes that have passed selection in the bone marrow complete their maturation in the secondary lymphoid organs...

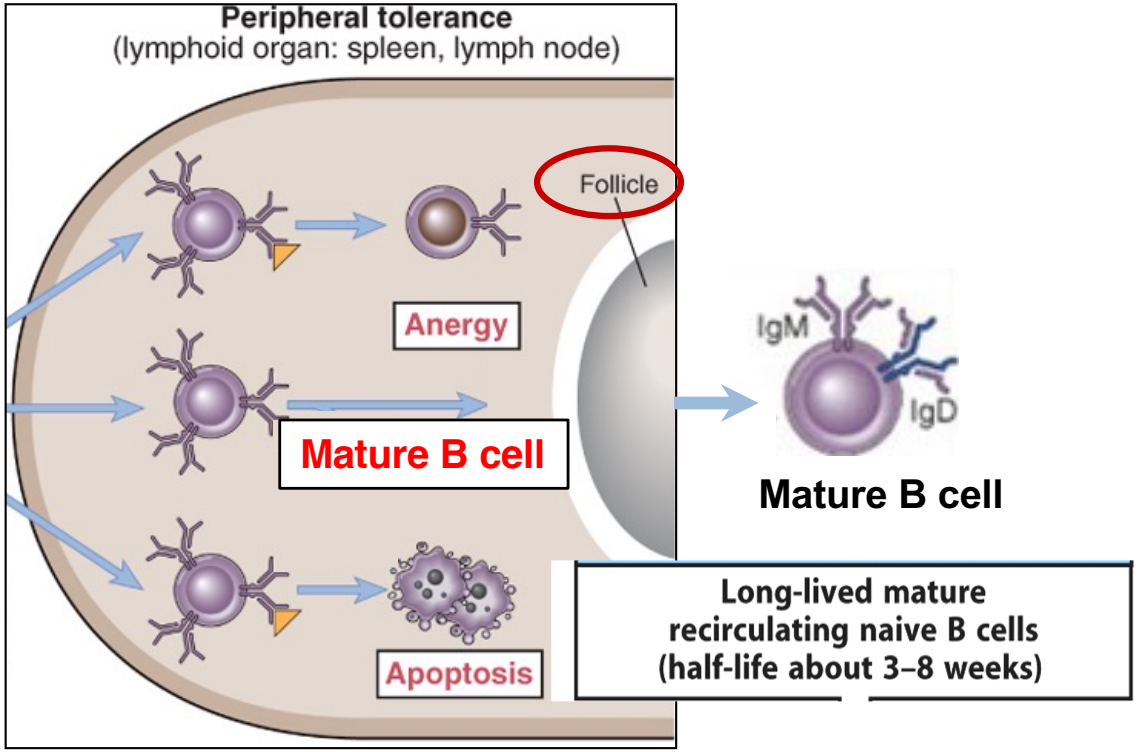


…… where they undergo a further selection process!

spleen or lymph nodes

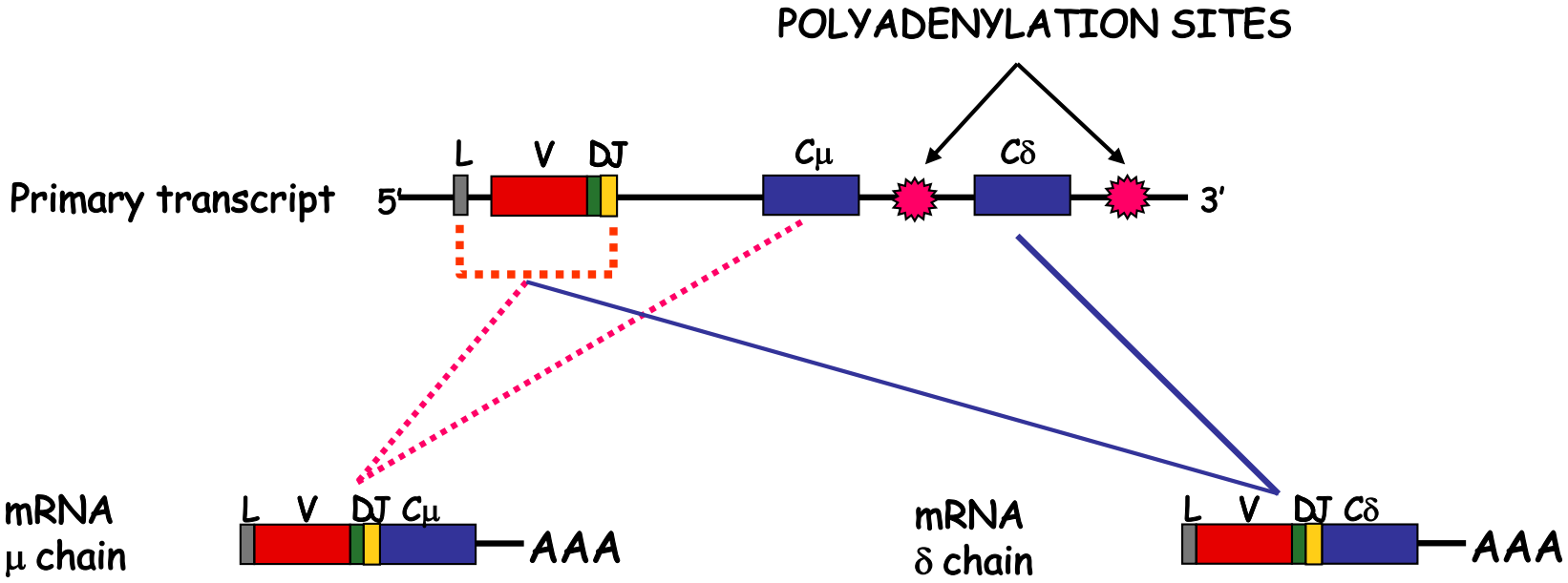


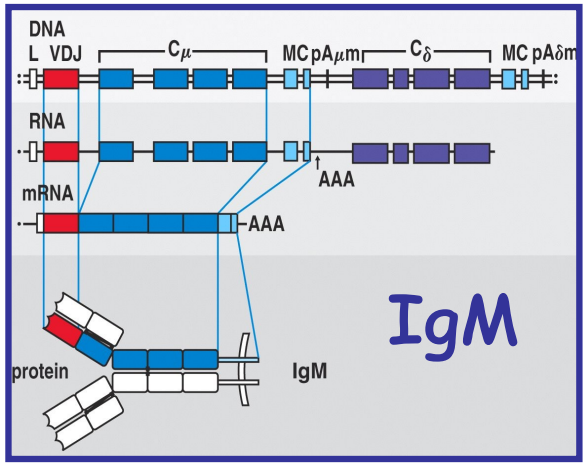
Self antigen

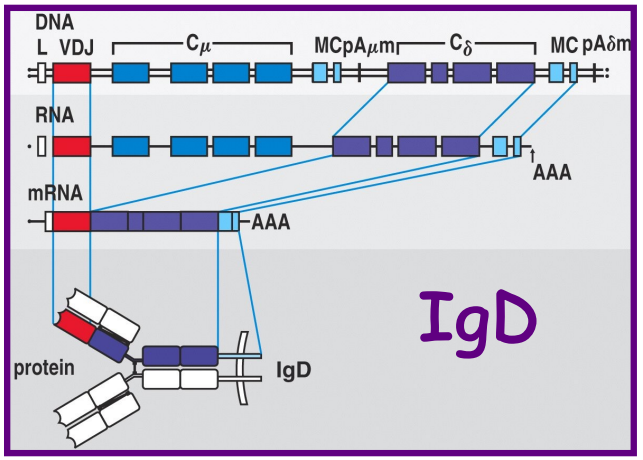


The mature B lymphocyte expresses both IgM and IgD on its membrane

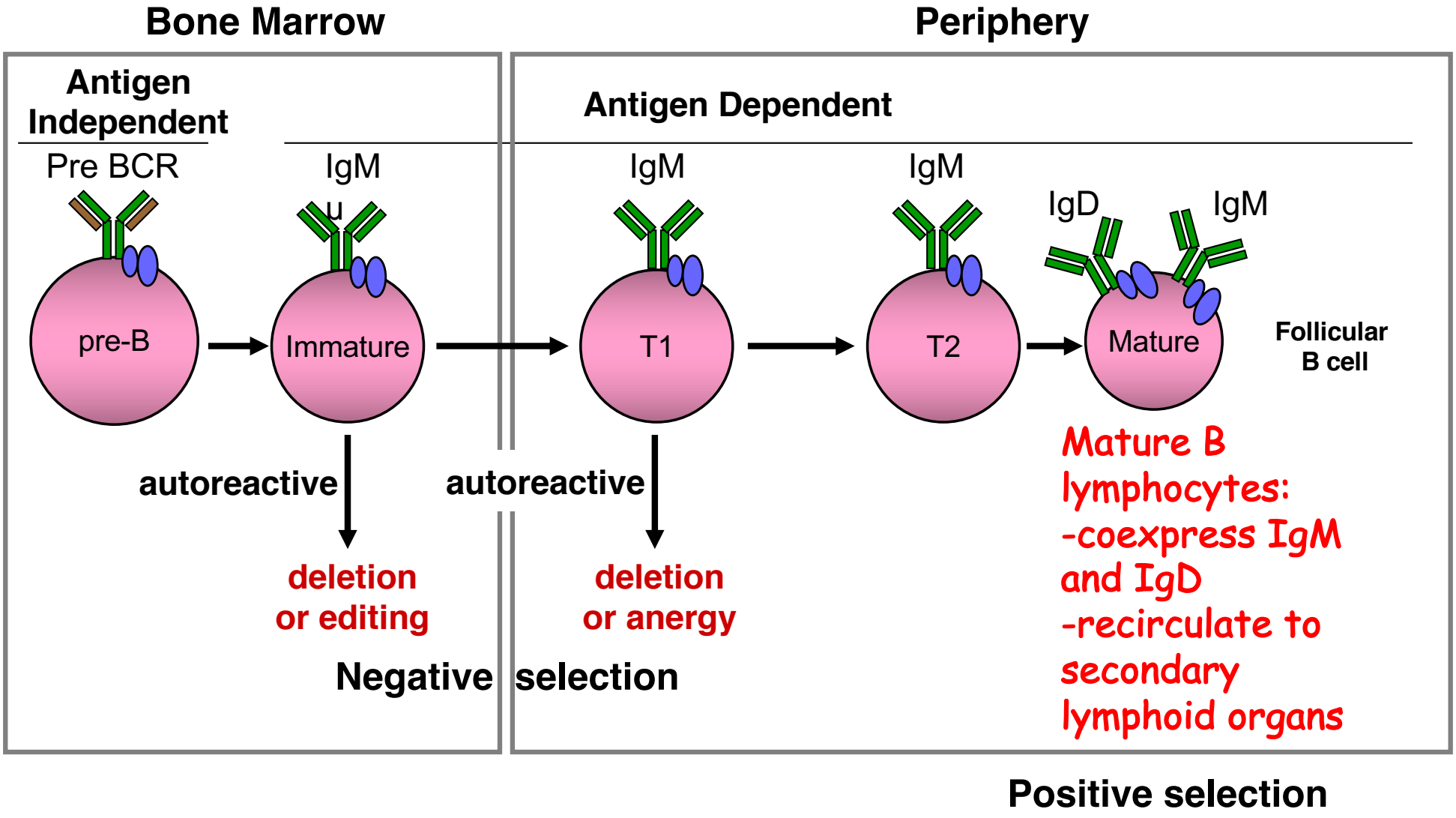
The co-expression of IgM and IgD depends on alternative splicing events





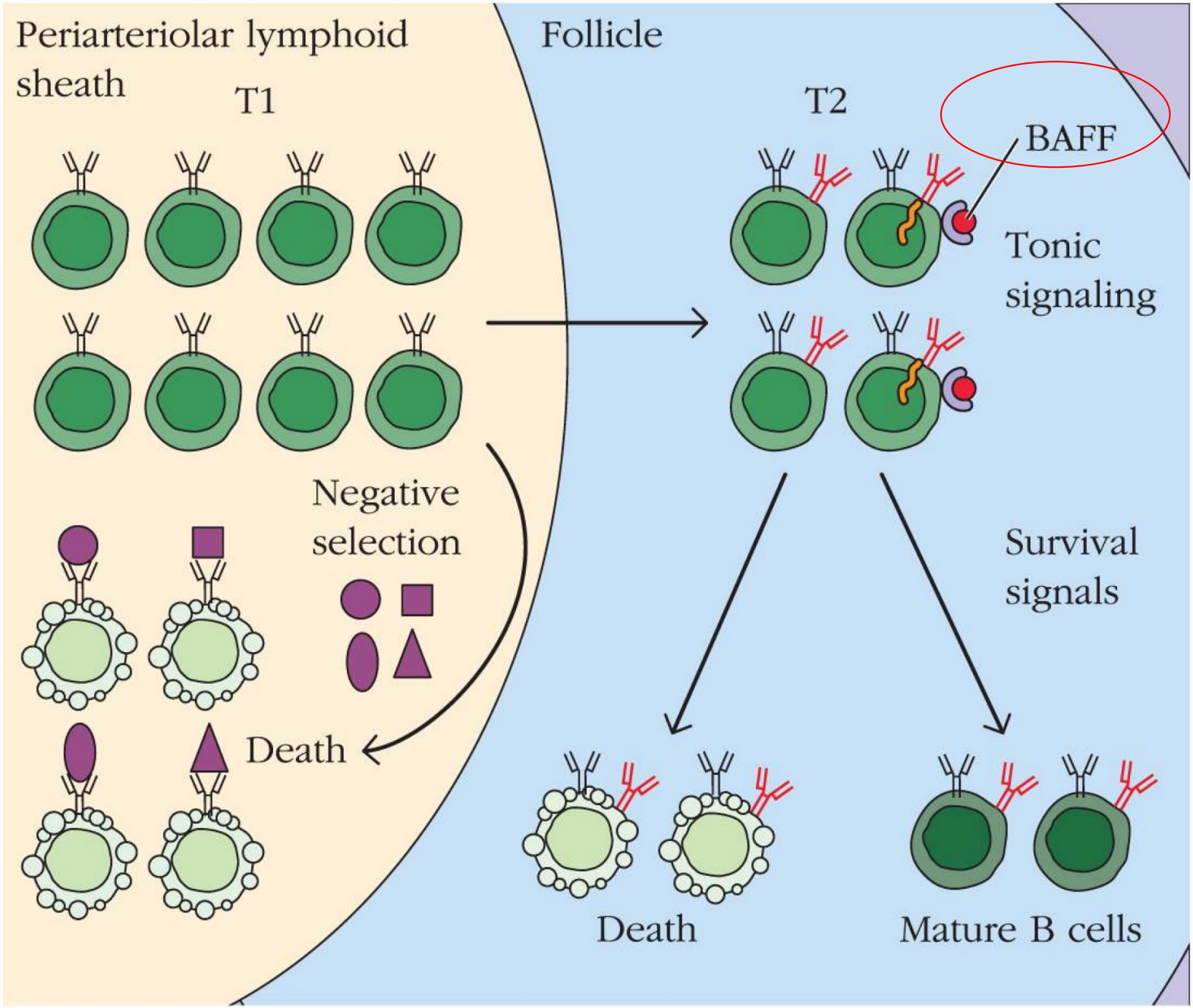


Proposal model of human B-cell development



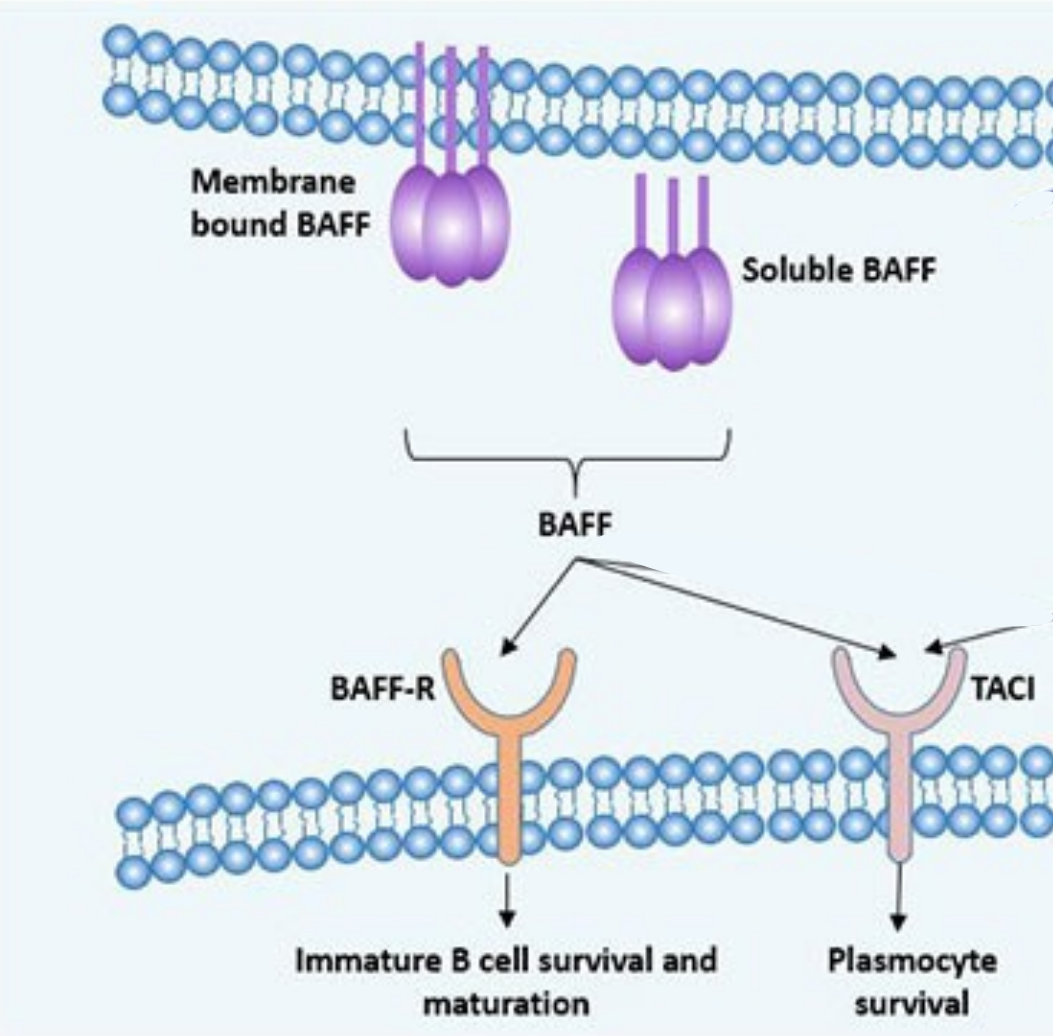
The negative selection by deleting autoreactive B cell clones will garantee the tolerance to SELF

Maturation of Transitional B Cells

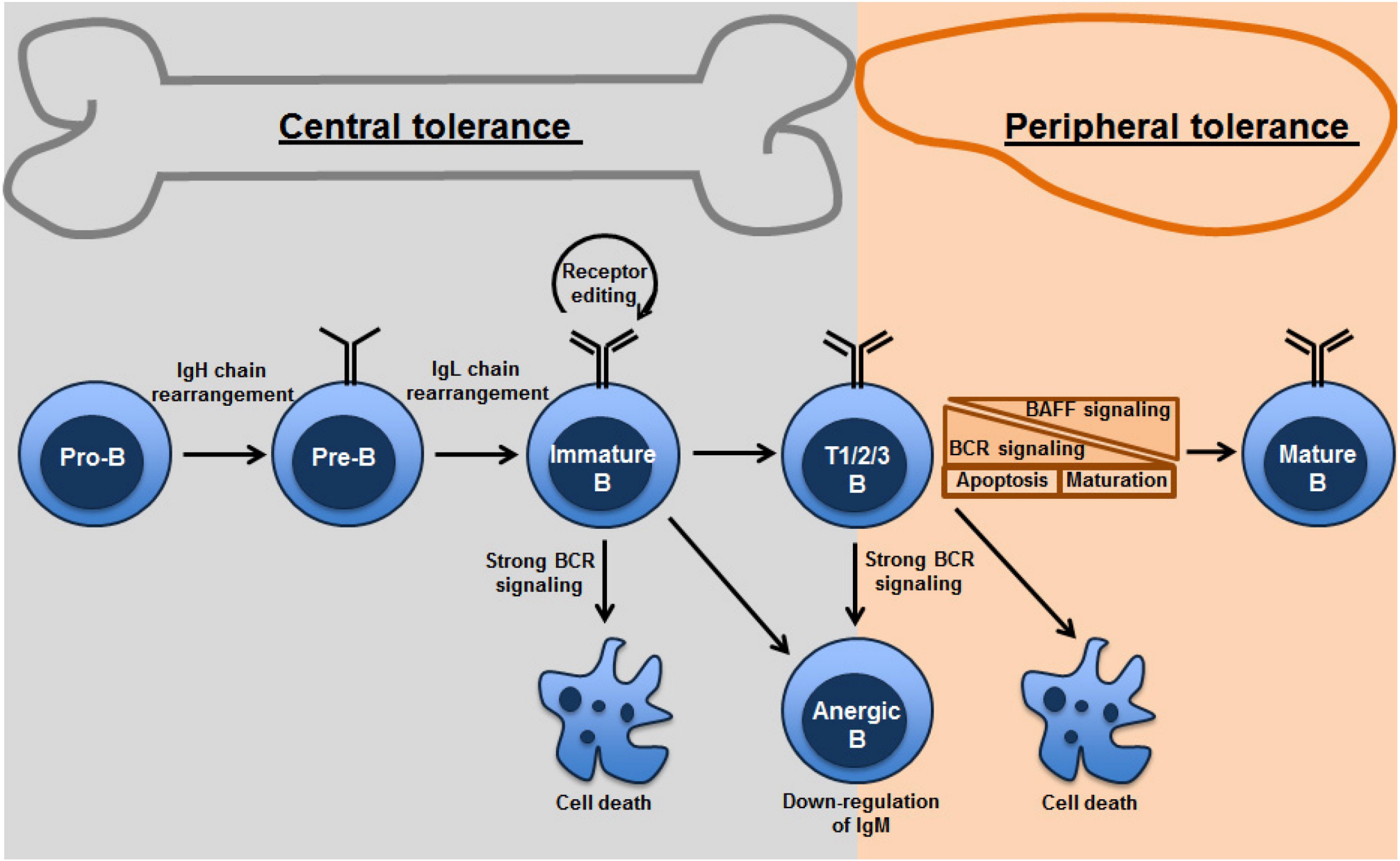


Kuby Immunology – 8th Edition, Figure 9-8

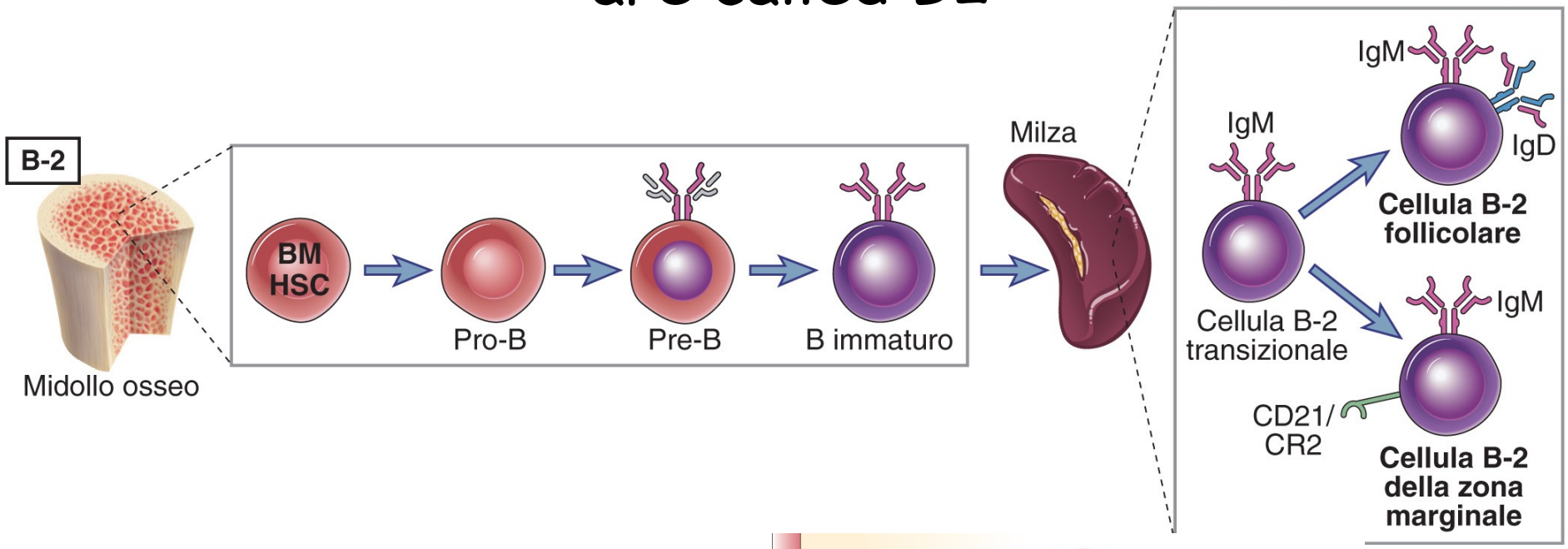
BAFF
- belongs to the TNF family
- binding to BAFF-R is required for full maturation of B lymphocytes
- BAFF-deficient mice lack mature B lymphocytes and have an excess of T2 lymphocytes



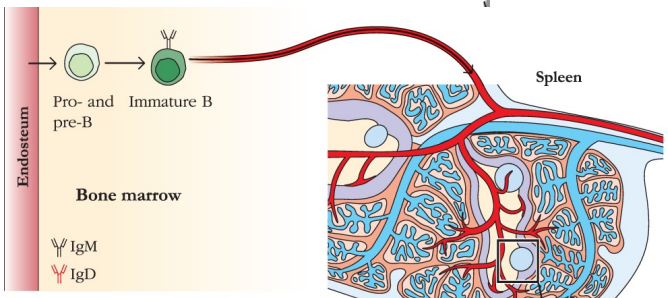
Survival of mature B lymphocytes depends on the BAFF-R receptor



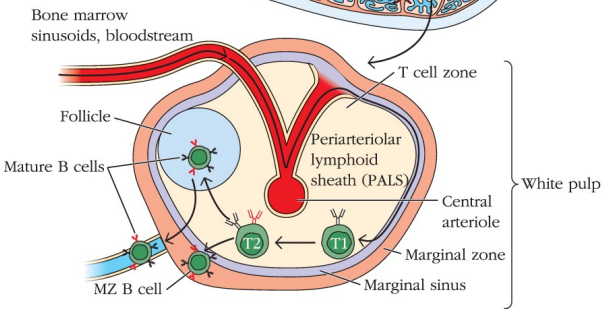
B lymphocytes generated by bone marrow precursors are called B2



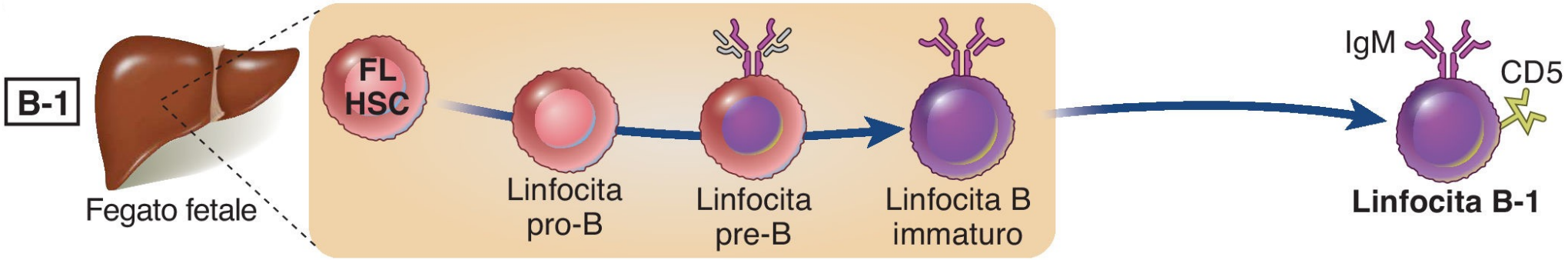
Marginal-Zone (MZ) B Cells



- MZ cells are found in white pulp outer regions of the spleen.
- Recognize protein and carbohydrate antigens and produce natural antibodies.
  - Some may be able to produce antibodies without T-cell help.
- They are characterized by low levels of IgD.



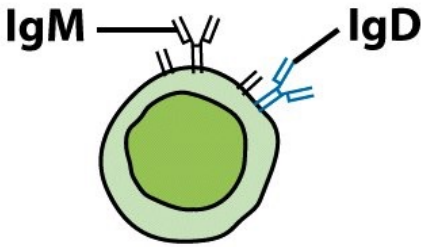
B-1 cells develop early and express a limited V gene repertoire

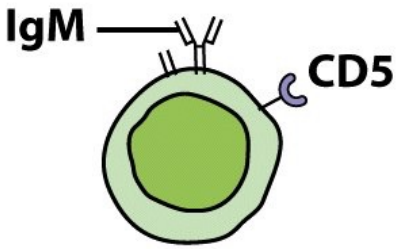


Property	B-1 cells
When first produced	Fetus
N-regions in VDJ junctions	Few
V-region repertoire	Restricted
Primary location	Body cavities (peritoneal, pleural)
Mode of renewal	Self-renewing
Spontaneous production of immunoglobulin	High

- They mature in the fetal liver
- They possess a limited receptor repertoire (less combinatorial and junctional variability, absence of TdT)
- They bind polysaccharide antigens

Properties of B cell subsets

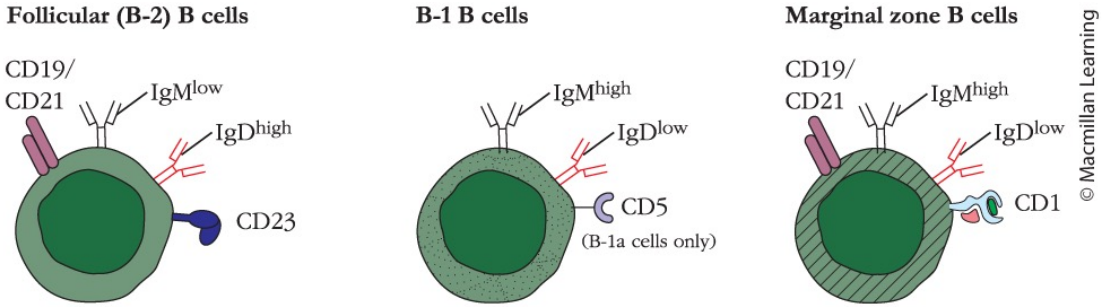




Attribute	Conventional B cells (B-2 B cells)	B-1 B cells
Major sites	Secondary lymphoid organs	Peritoneal and pleural cavities
Source of new B cells	From precursors in bone marrow	Self-renewing (division of existing B-1 cells)
V-region diversity	Highly diverse	Restricted diversity
Somatic hypermutation	Yes	No
Requirements for T-cell help	Yes	No
Isotypes produced	High levels of IgG	High levels of IgM
Response to carbohydrate antigens	Possibly	Definitely
Response to protein antigens	Definitely	Possibly
Memory	Yes	Very little or none
Surface IgD on mature B cells	Present on naive B cells	Little or none

Figure 11-5
Kuby IMMUNOLOGY, Sixth Edition

B-2, B-1 and Marginal Zone B Cells



Attribute	Follicular (B-2) B cells	B-1 B cells	Marginal zone B cells
Major sites	Secondary lymphoid organs	Peritoneal and pleural cavities	Marginal zones of spleen
Progenitors first appear in mice	HSC: on day E10.5	Progenitor: on day E9.5	From HSCs; also earlier progenitor?
Source of new B cells in adults	From HSC in bone marrow	Self-renewing (division of existing B-1 cells)	From HSCs in bone marrow, long-lived
Dependence on IL-7 and BAFF	Yes	No	Yes
V-region diversity	Highly diverse	Restricted diversity: limited V_H and V_L usage and N nucleotide addition	Somewhat restricted
Somatic hypermutation	Extensive	Some	Unclear
Requirements for T-cell help	Yes	No	Variable
Isotypes produced	High levels of IgG	Primarily IgM; some IgG	Primarily IgM; some IgG
Response to carbohydrate antigens	Possibly	Yes	Yes
Response to protein antigens	Yes	Possibly	Yes
Memory	Yes	Some	Unknown

Similarities between B- and T-Cell Development

- B- and T-cell developmental pathways share many characteristics, including:
  - the rearrangement of gene segments.
  - screening processes to avoid self-reactivity.
  - production of small subsets with discrete functions.

Differences between B- and T-Cell Development

- B- and T-cell developmental pathways also have differences, including:
  - location of maturation and screening
  - the screening processes used
    - both positive and negative selection in T-cell development
    - negative selection only in B-cell development
  - the eventual outcomes of antigen receptor stimulation
    - T cells require antigen presentation and differentiate into helper or killer subsets.
    - B cells (majority B-2 subset) require T-cell help and secrete antibodies.