B cell receptor & B cell development

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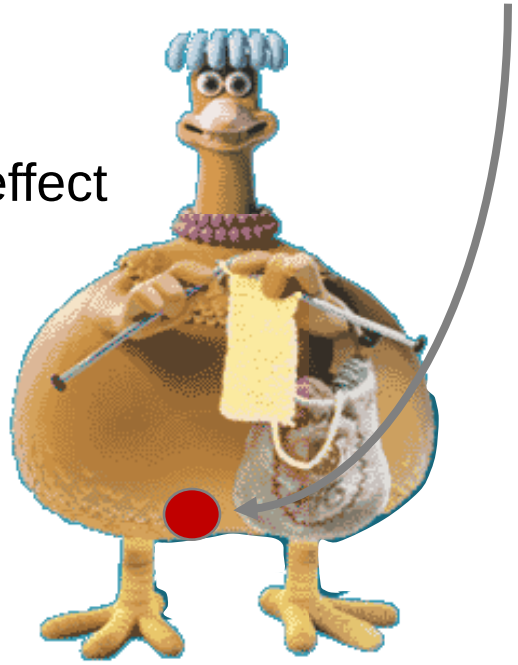
The discovery of B cell immunity

1954 - Bruce Glick, Ohio State University

Studies on the function of the bursa of Fabricius, a lymphoid organ in the cloacal region of the chicken

Bursectomy – no apparent effect

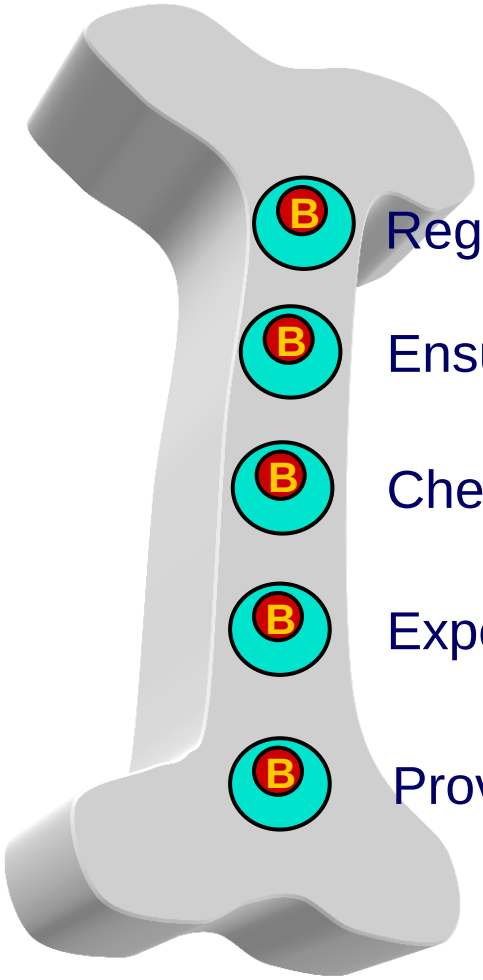


None of the bursectomised chickens made anti-Salmonella antibodies

Bursa was later found to be the organ in which antibody producing cells developed – antibody producing cells were thereafter called B cells

Mammals do not have a bursa of Fabricius

B cell development in the bone marrow



Regulates construction of an antigen receptor

Ensures each cell has only one specificity

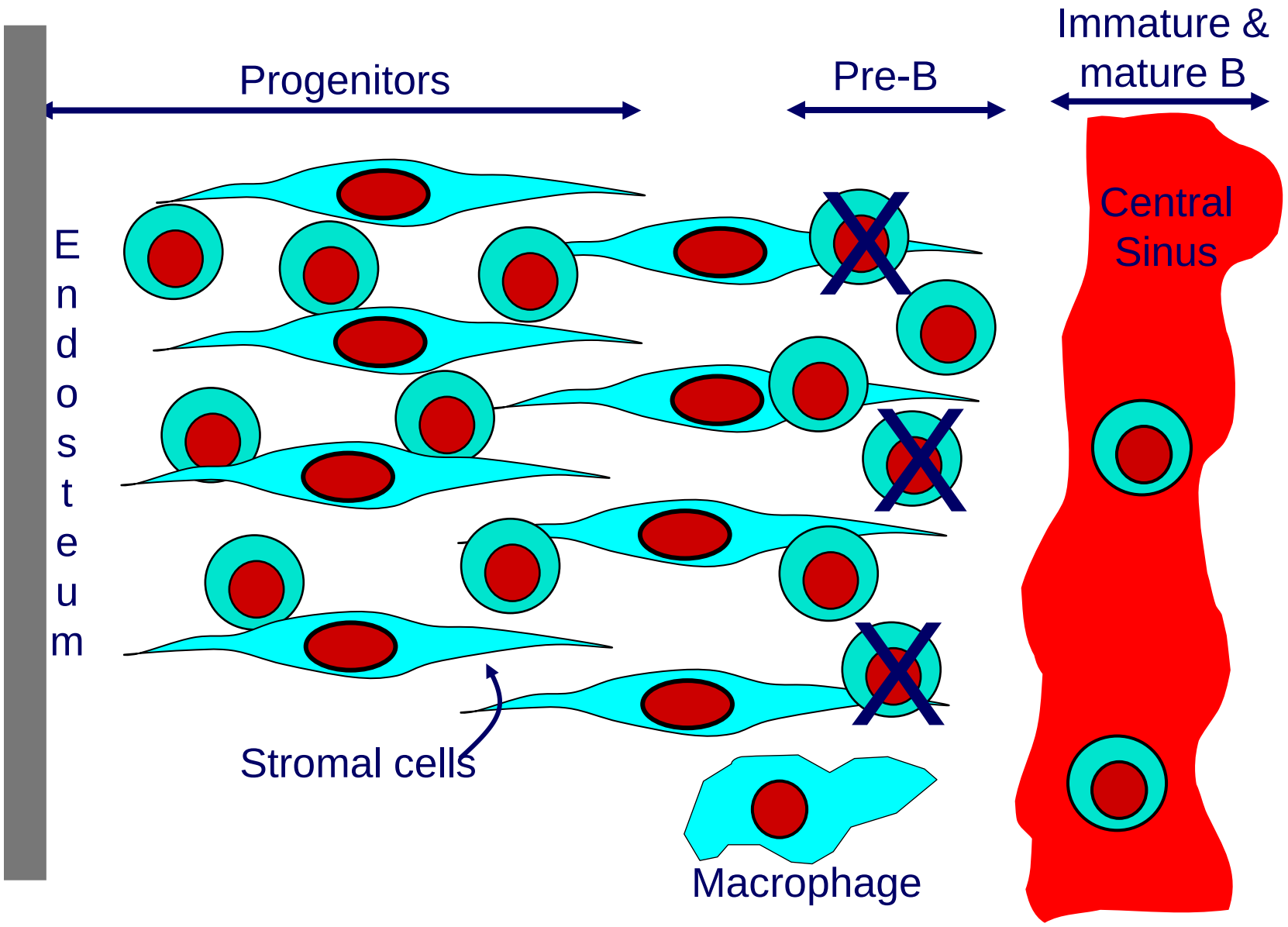
Checks and disposes of self-reactive B cells

Exports useful cells to the periphery

Provides a site for antibody production

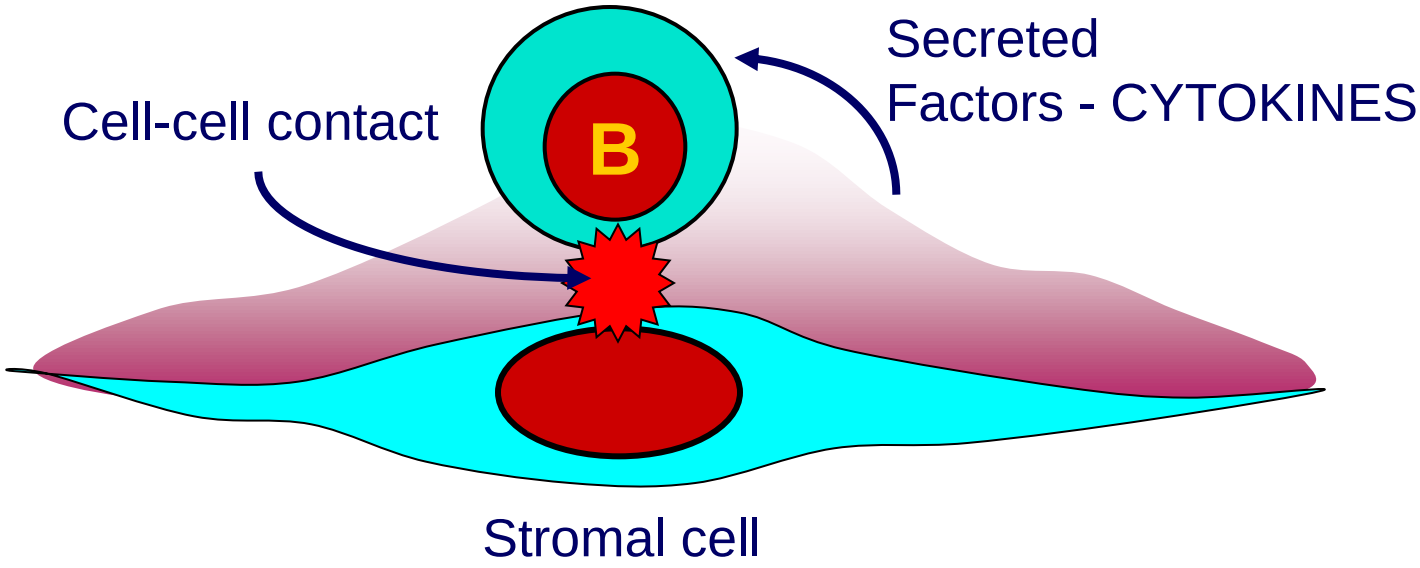
Bone Marrow provides a
MATURATION & DIFFERENTIATION MICROENVIRONMENT
for B cell development

Scheme of B Cell Development in the Bone Marrow

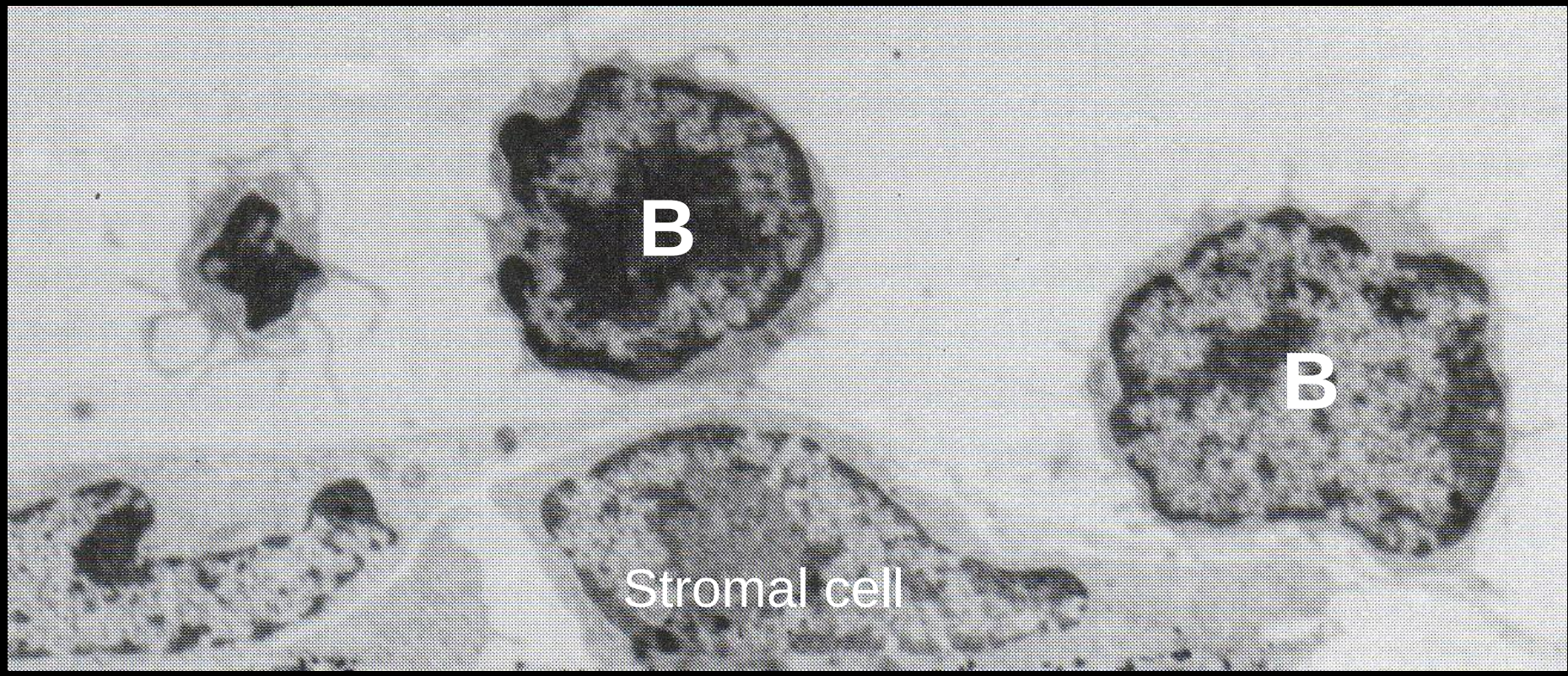


Bone marrow stromal cells nurture developing B cells

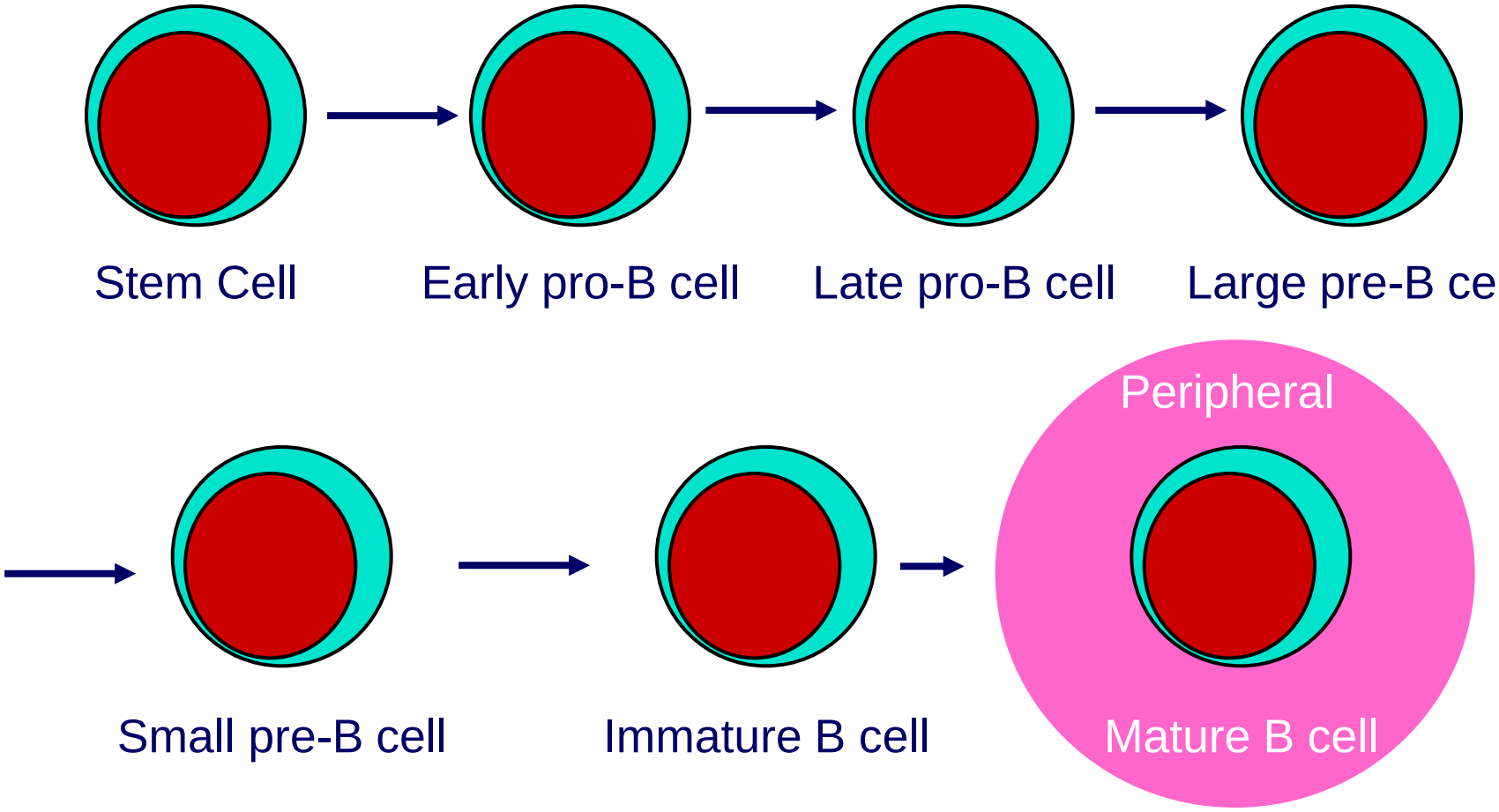
1. Specific cell-cell contacts between stromal cells and developing B cells
2. Secretion of cytokines by stromal cells



Types of cytokines and cell-cell contacts needed at each stage of differentiation are different


B
B
Stromal cell

Stages of B cell development



Each stage of development is defined by rearrangements of Heavy chain genes, Light chain genes, expression of surface Ig, expression of adhesion molecules and cytokine receptors.

Rearrangement of BCR

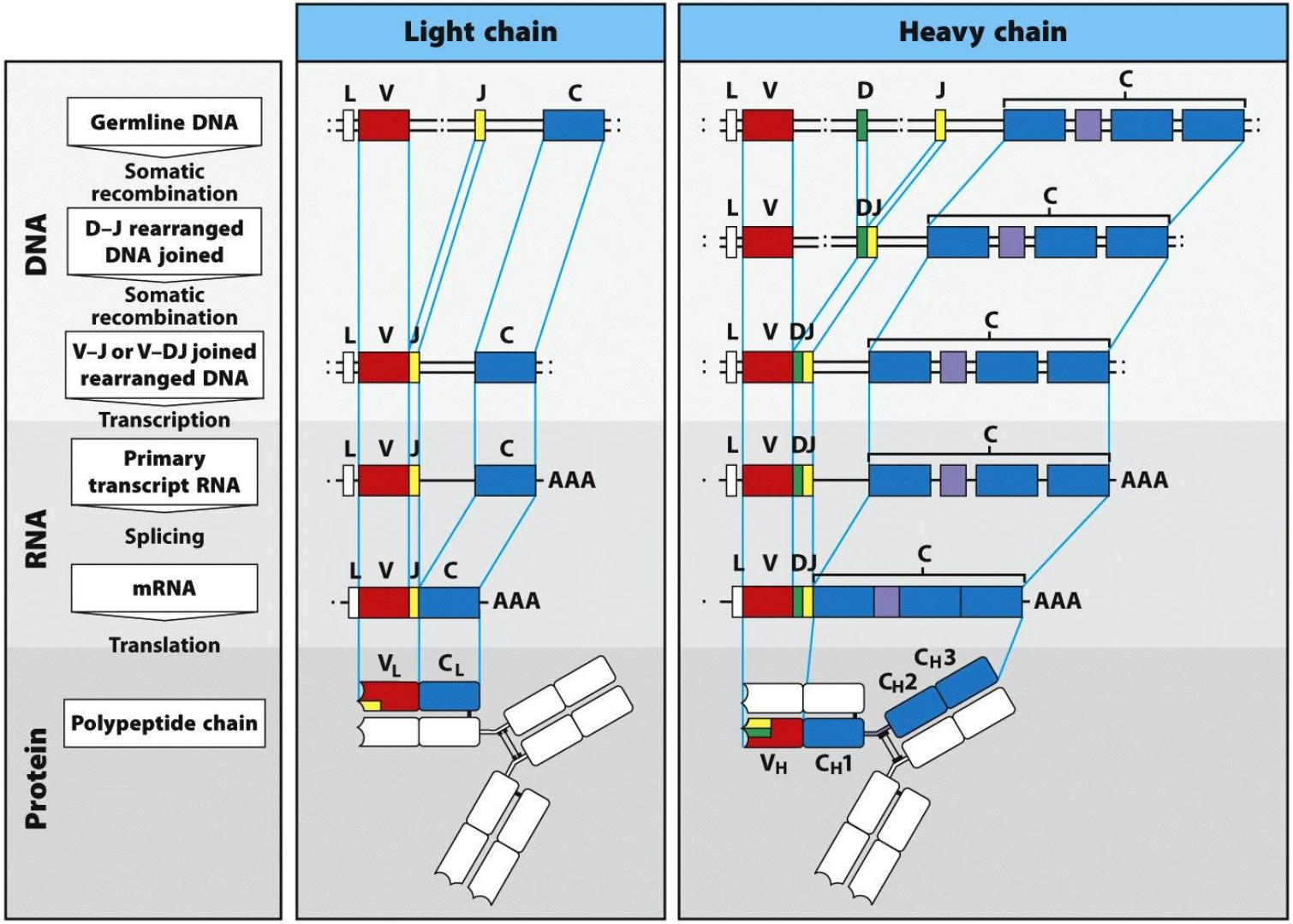
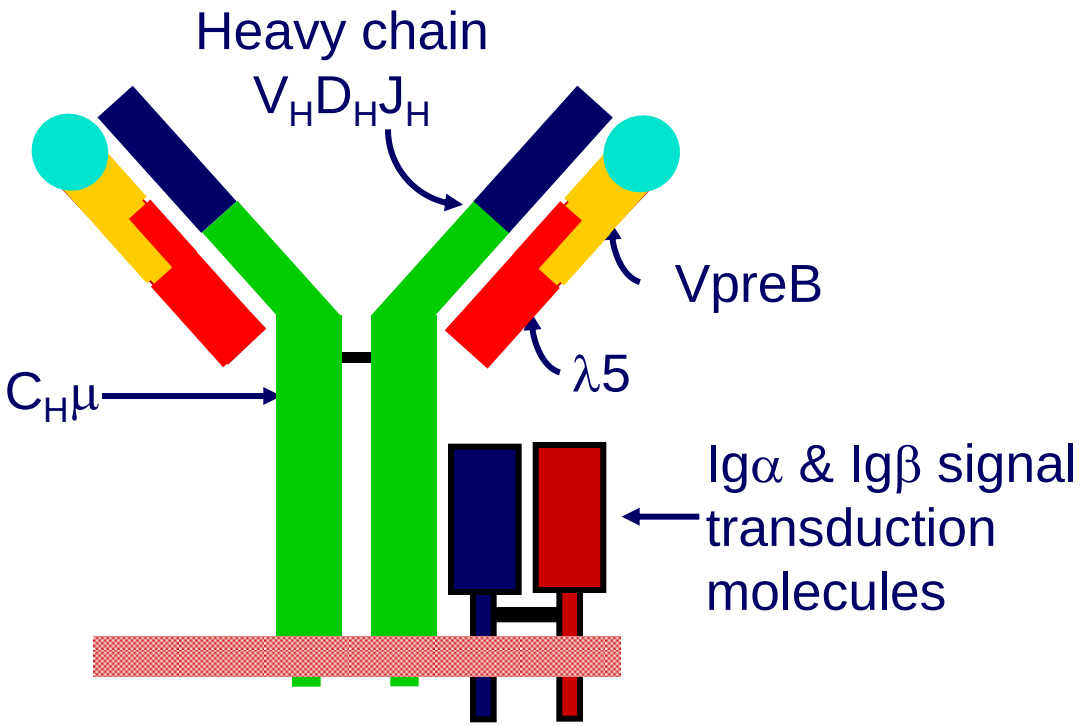


Figure 4-2 Immunobiology, 7ed. (© Garland Science 2008)

Pre-B cell receptor

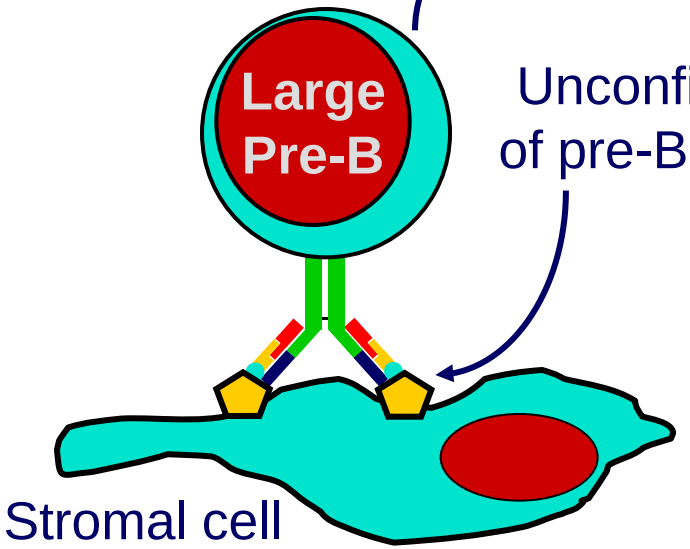


Transiently expressed when V_H-D_H-J_H-$C_H(\mu)$ is productively rearranged

VpreB-λ5, the surrogate light chain, is required for surface expression

Ligation of the pre-B cell receptor

1. Suppresses further H chain rearrangement
2. Triggers entry into cell cycle



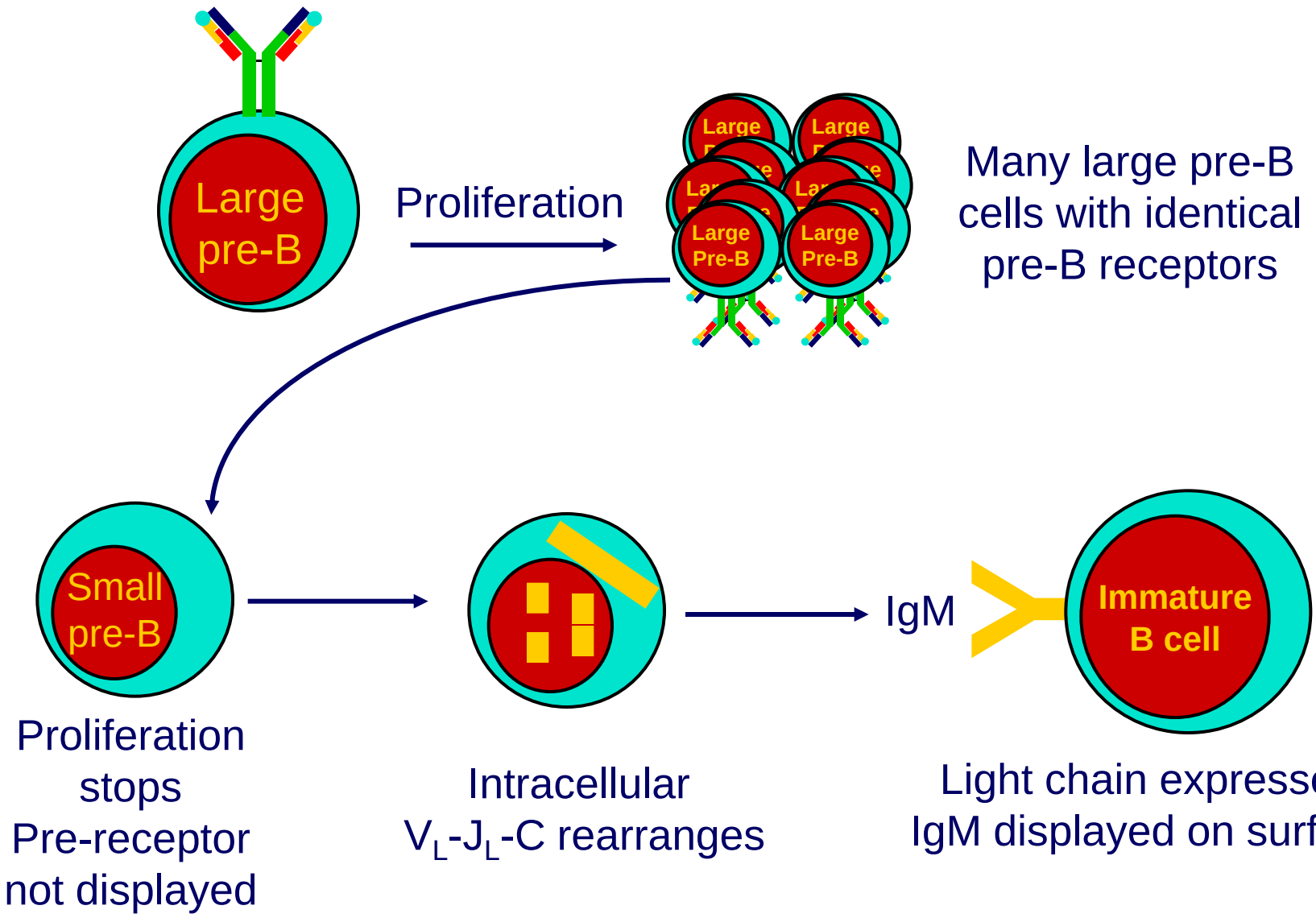
Unconfirmed ligand
of pre-B cell receptor

ALLELIC EXCLUSION

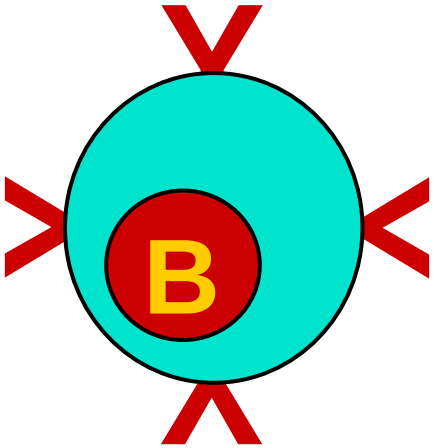
Expression of a gene on one chromosome prevents expression of the allele on the second chromosome

Ensures only one specificity of Ab expressed per cell

Ligation of the pre-B cell receptor triggers entry into the cell cycle



Acquisition of antigen specificity creates a need to check for recognition of self antigens



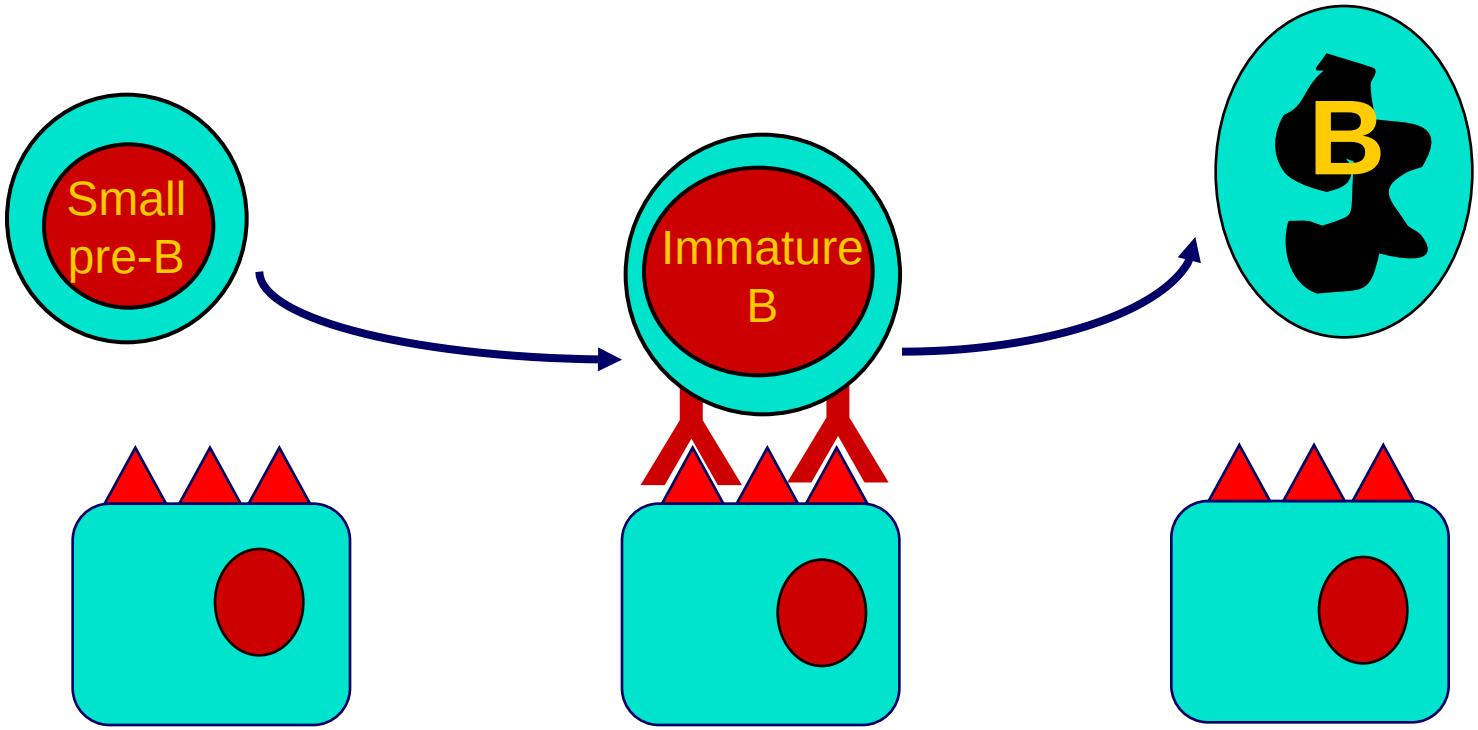
Immature B cell

Cell surface Ig expressed
Able to sense Ag environment

Can now be checked for self-reactivity

1. Physical removal from the repertoire — DELETION
2. Paralysis of function — ANERGY
3. Alteration of specificity — RECEPTOR EDITING

B cell self tolerance: clonal deletion

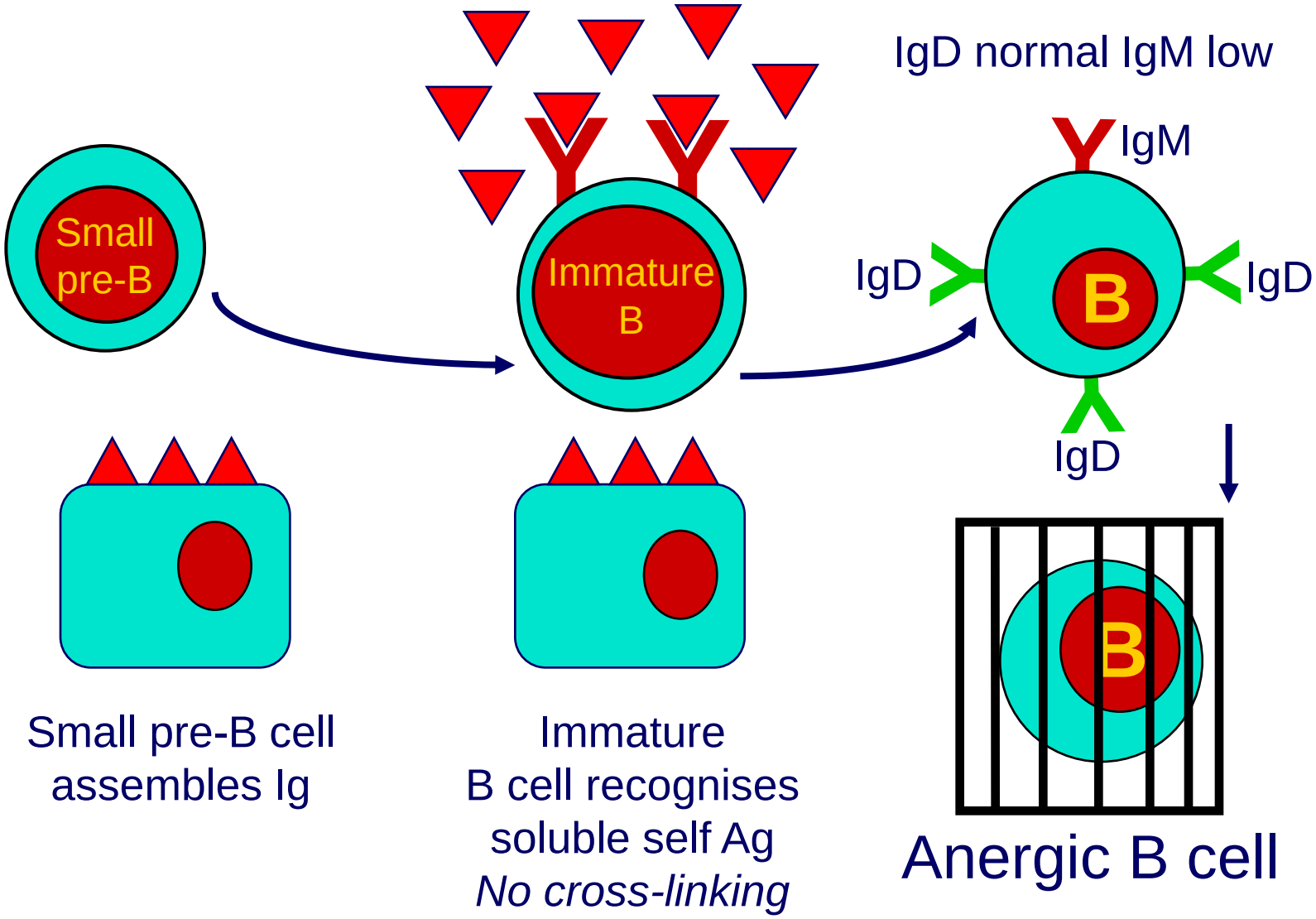


Small pre-B cell assembles Ig

Immature B cell recognises MULTIVALENT self Ag

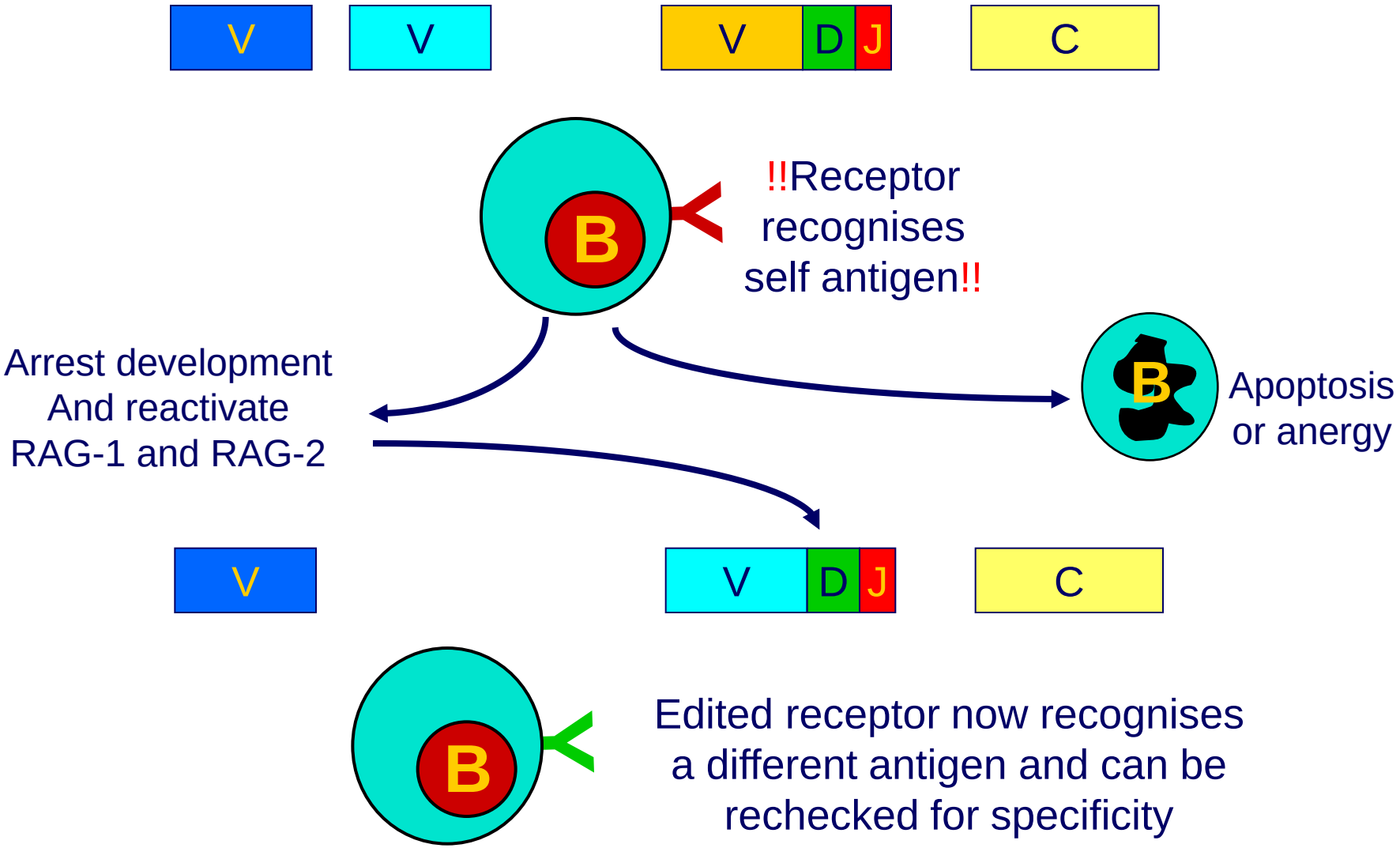
Clonal deletion by apoptosis

B cell self tolerance: anergy

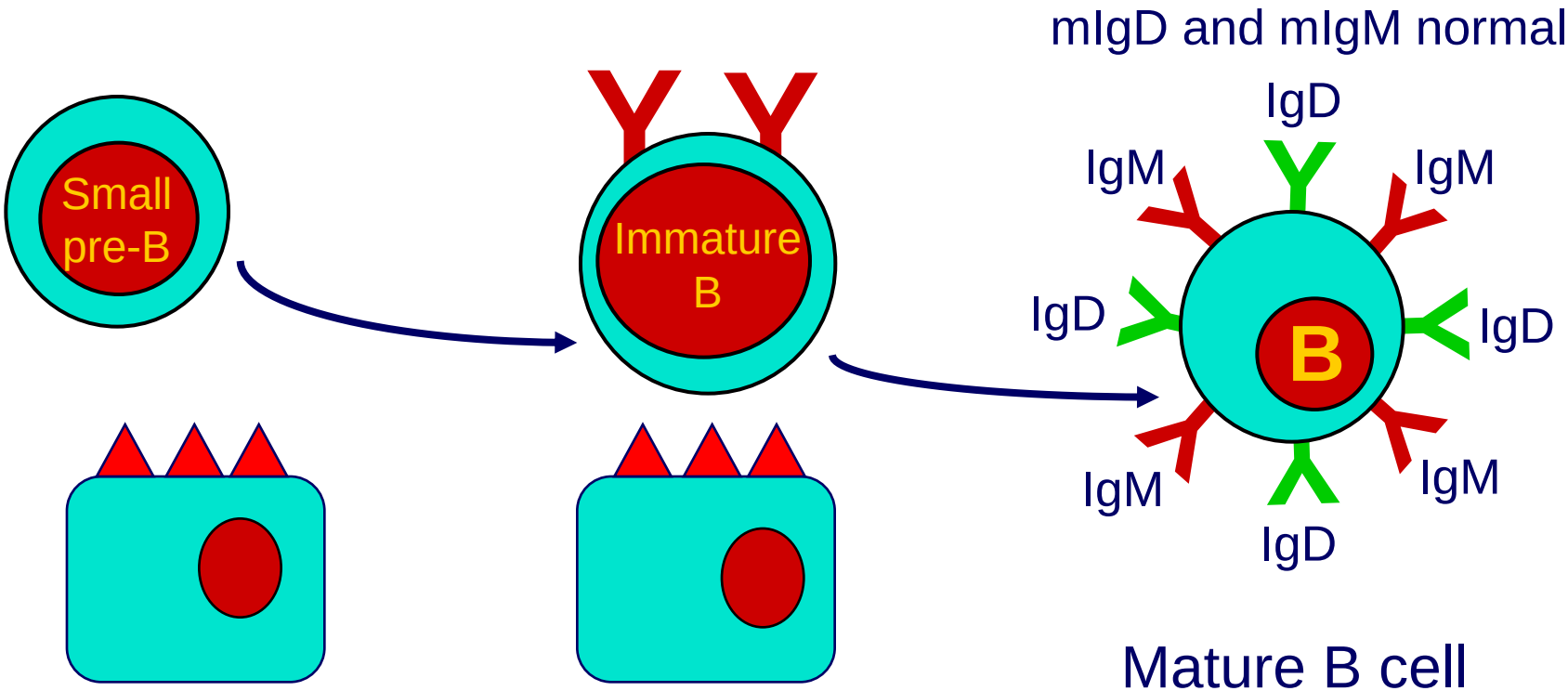


Receptor editing

A rearrangement encoding a self specific receptor can be replaced



B cell self tolerance: export of self tolerant B cells



The diversity of the BCR is generated by

- Combinatorial diversity
- Junctional diversity
- Receptor editing
- Somatic hypermutation

Combinatorial diversity

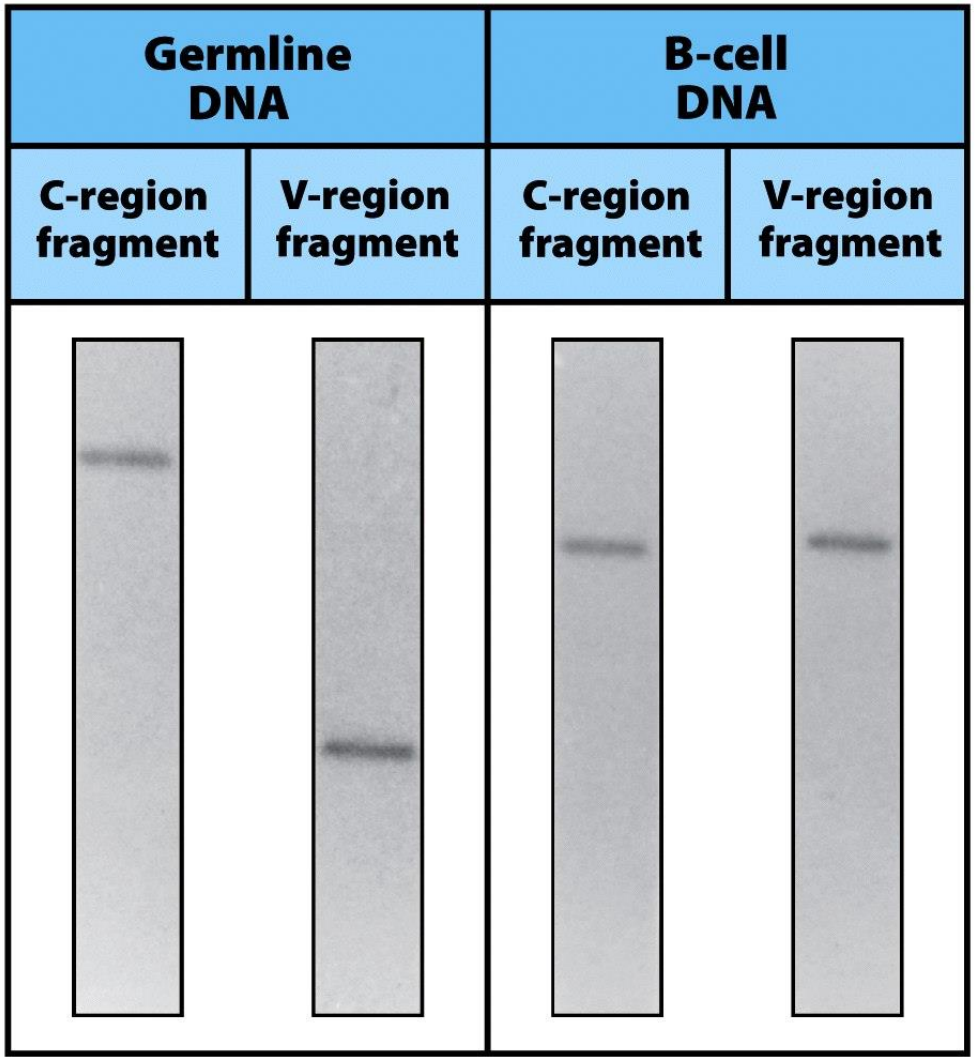


Figure 4-1 Immunobiology, 7ed. (© Garland Science 2008)

Combinatorial diversity

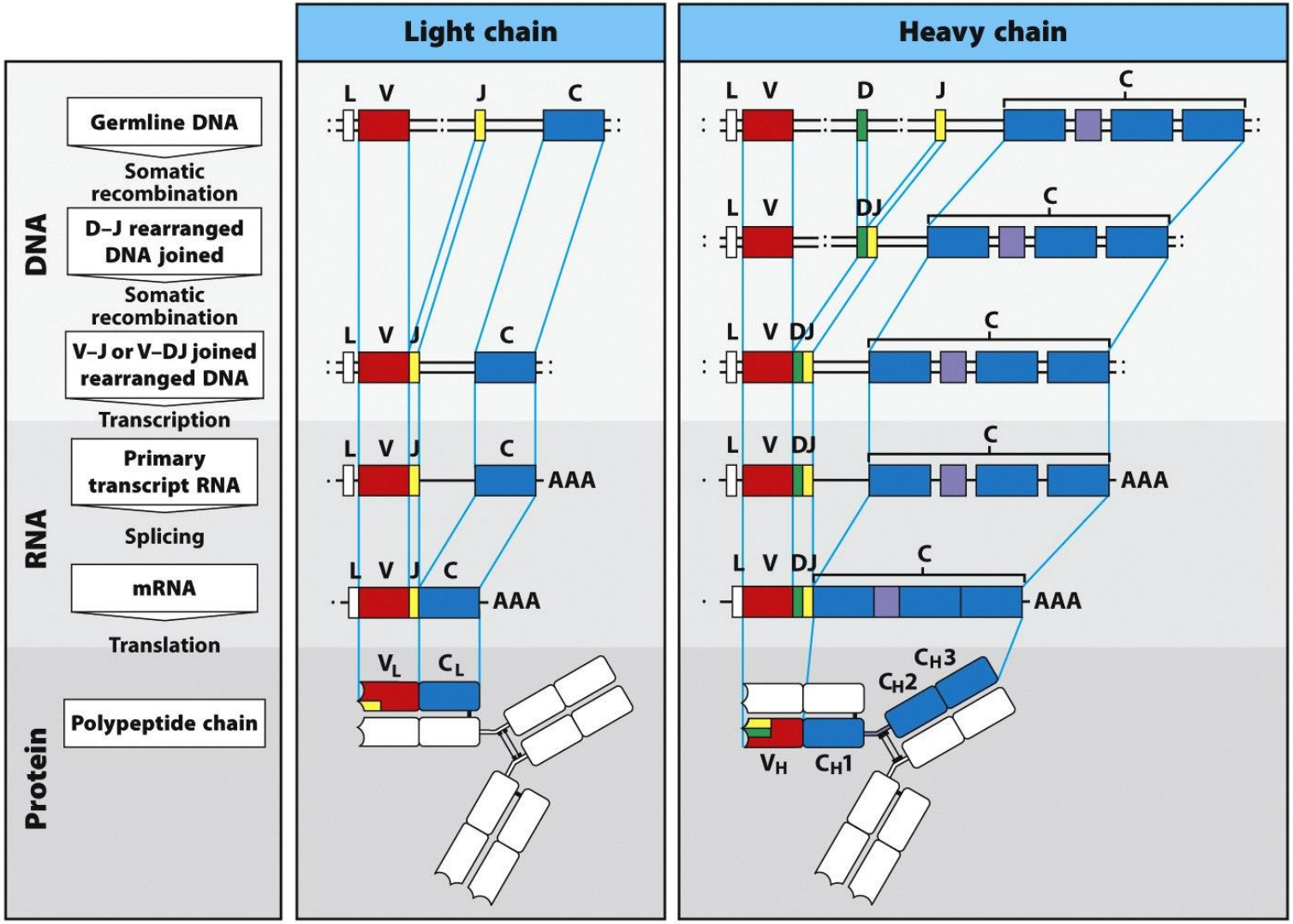


Figure 4-2 Immunobiology, 7ed. (© Garland Science 2008)

Combinatorial diversity

Number of functional gene segments in human immunoglobulin loci

Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	40	30	40
Diversity (D)	0	0	25
Joining (J)	5	4	6

Figure 4-3 Immunobiology, 7ed. (© Garland Science 2008)

Combinatorial diversity

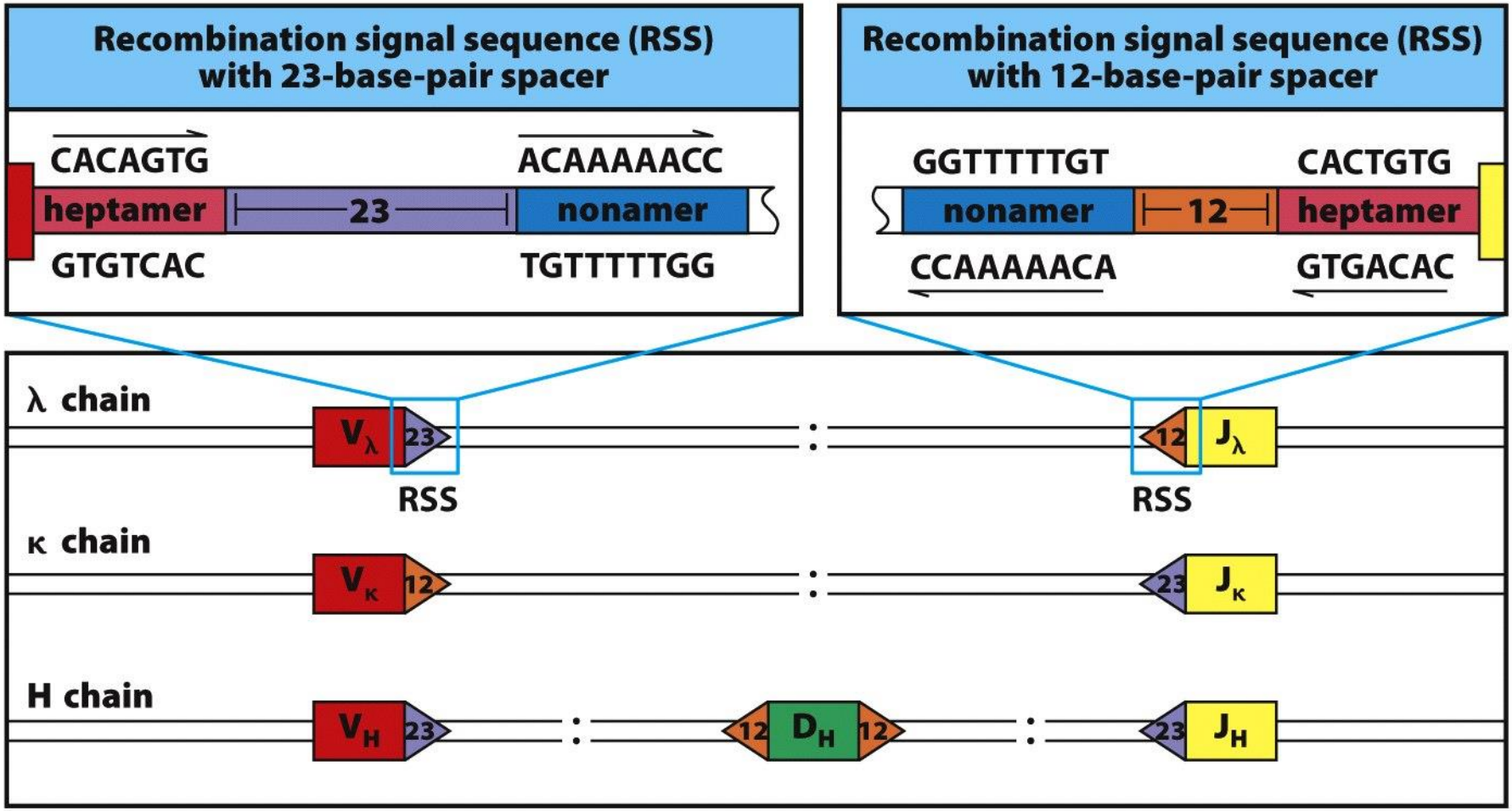


Figure 4-5 Immunobiology, 7ed. (© Garland Science 2008)

Combinatorial diversity

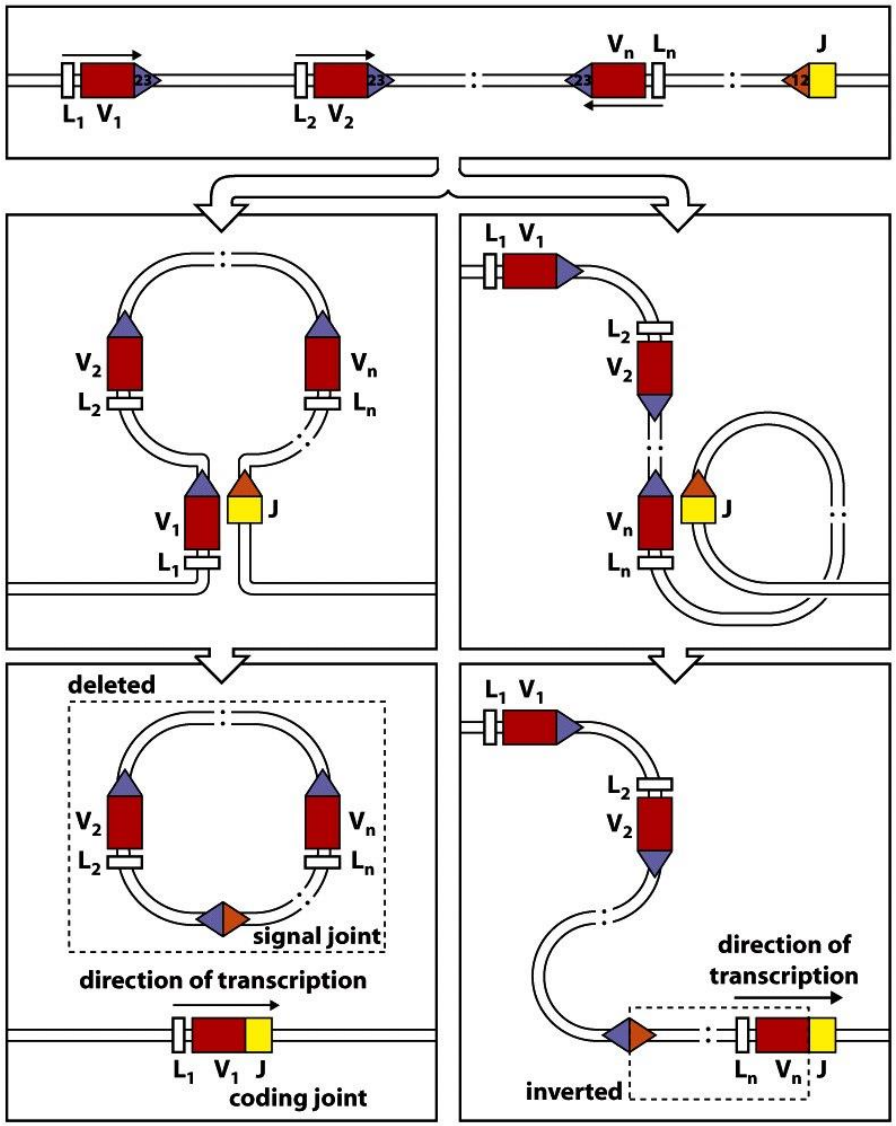


Figure 4-6 Immunobiology, 7ed. (© Garland Science 2008)

RAG（recombination activating gene）

TdT（terminal deoxynucleotidyl transferase）

Junctional diversity

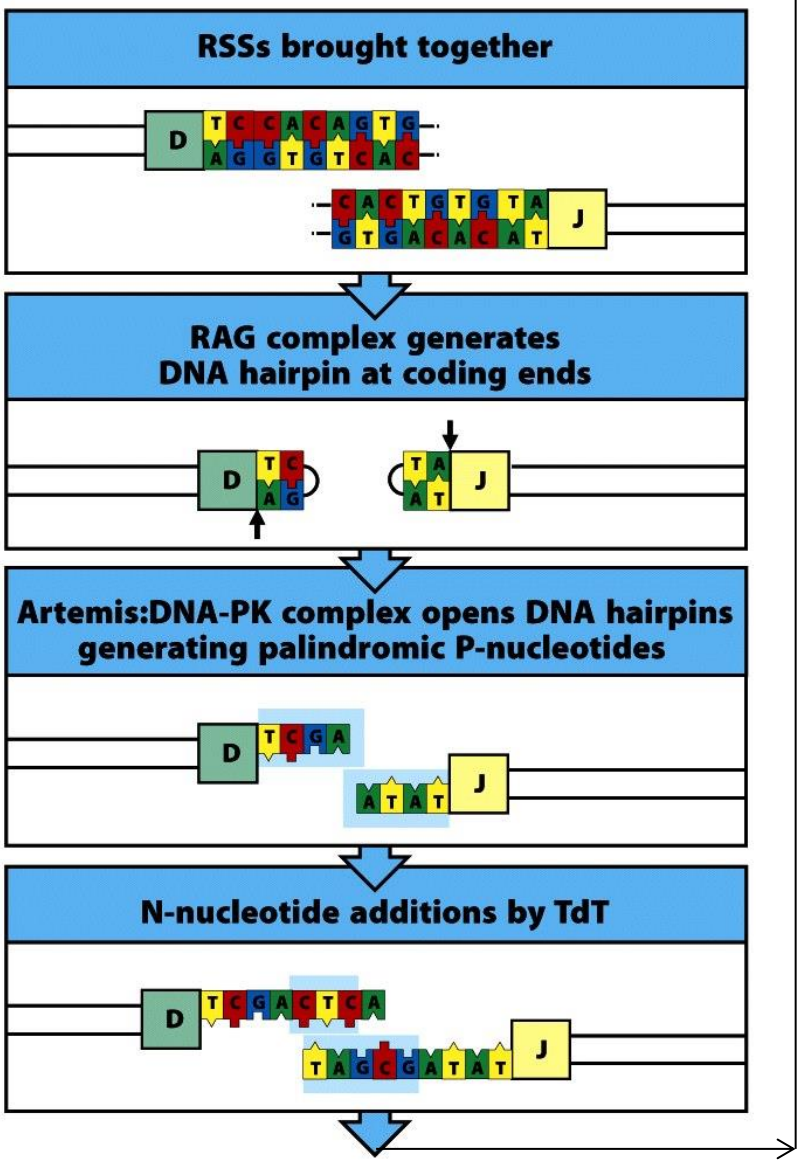


Figure 4-8 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)

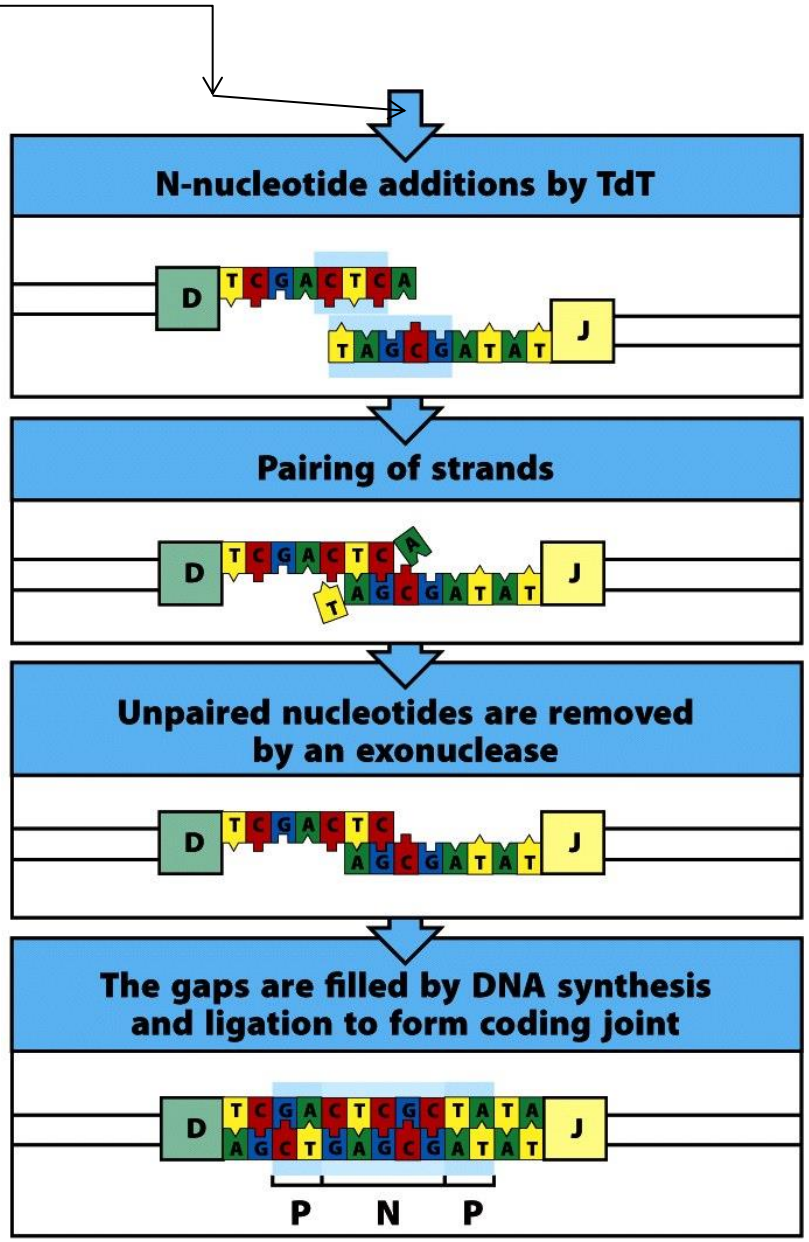
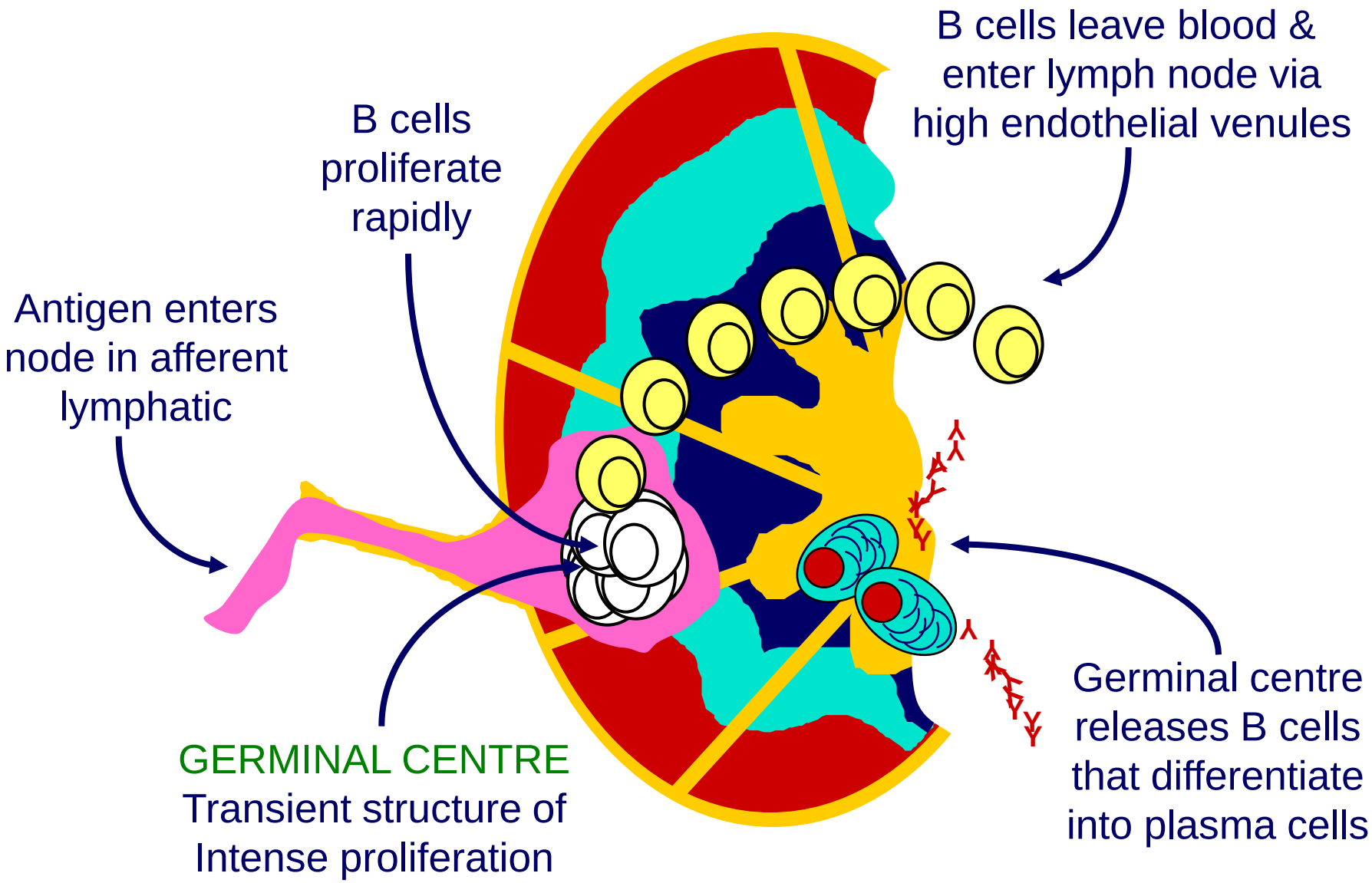


Figure 4-8 part 2 of 2 Immunobiology, 7ed. (© Garland Science 2008)

Receptor editing

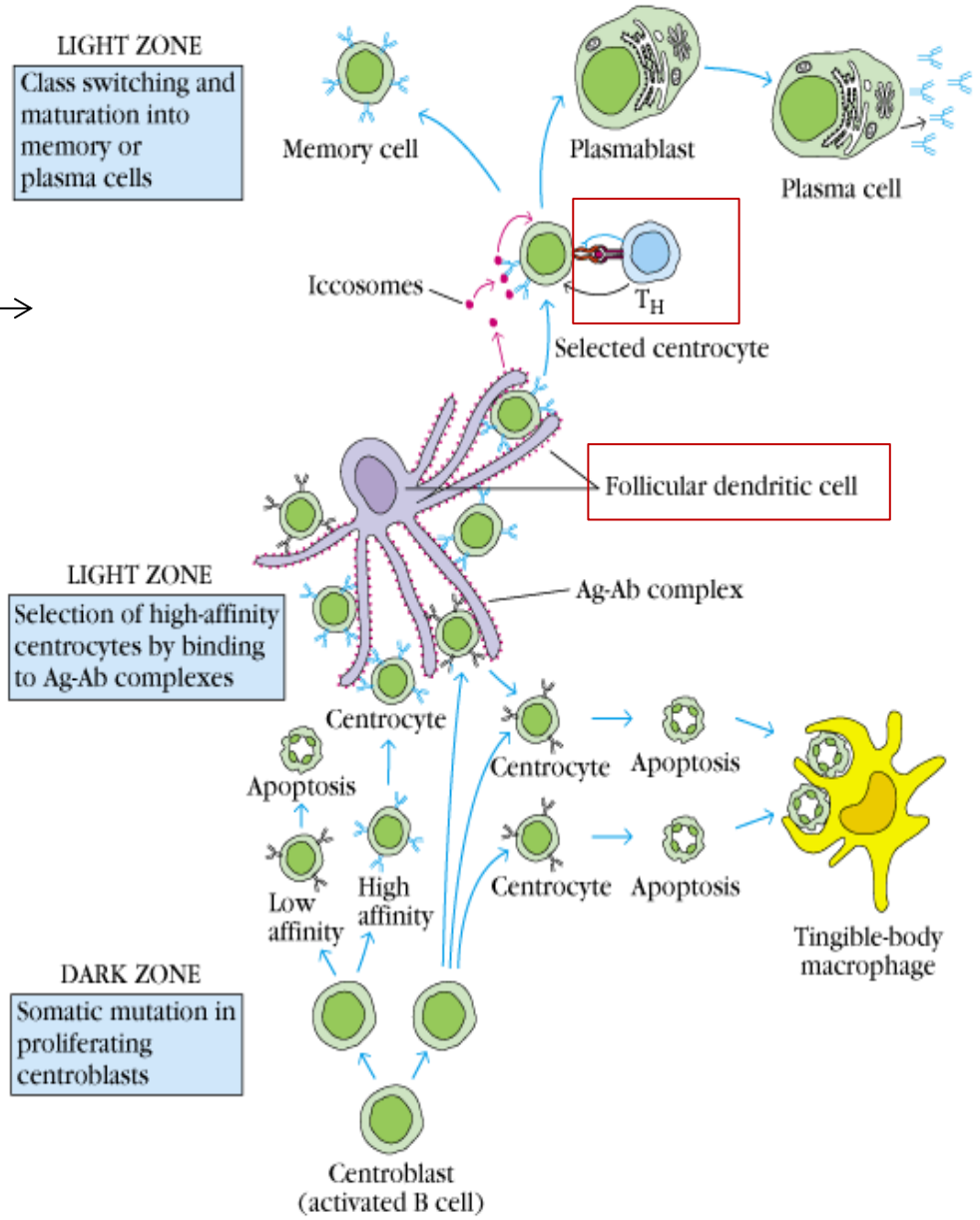
Somatic hypermutation



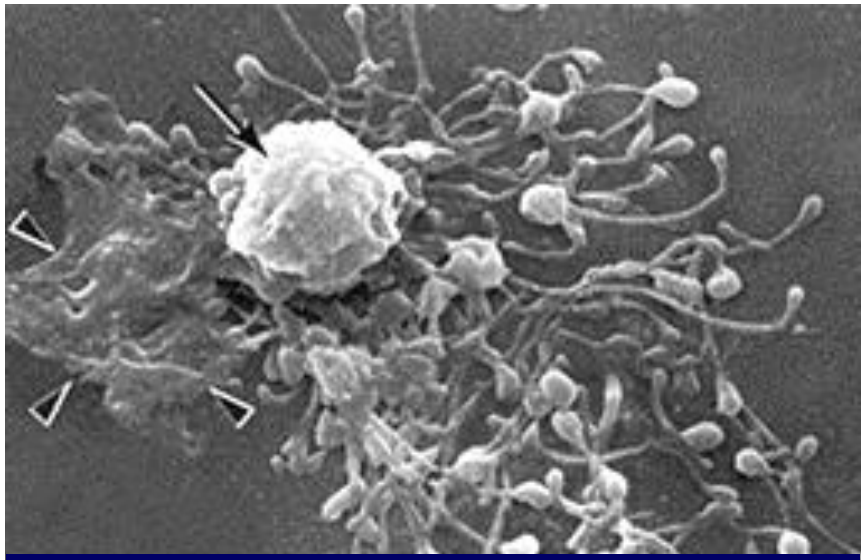
Somatic hypermutation

With the help of Tfh cell, B cell clone with high binding affinity to antigen will survive, proliferate and go through differentiation →

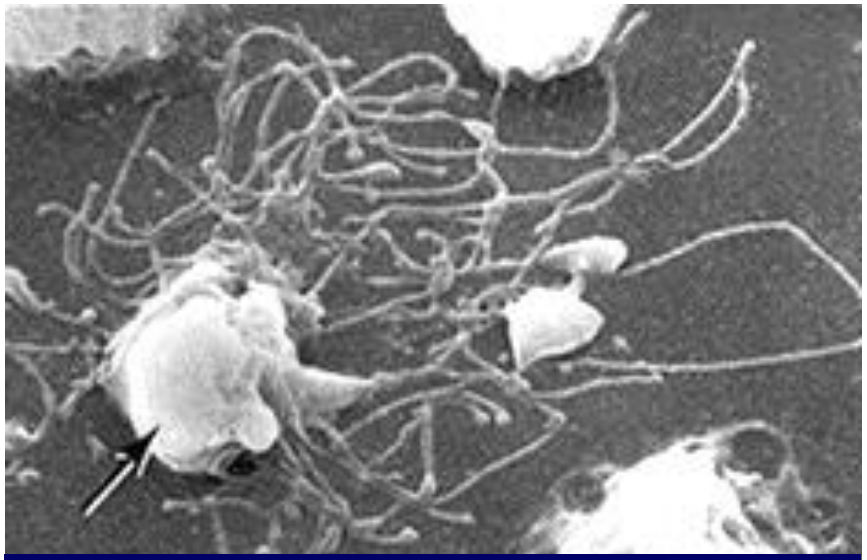
High rate of point mutation CDR region →



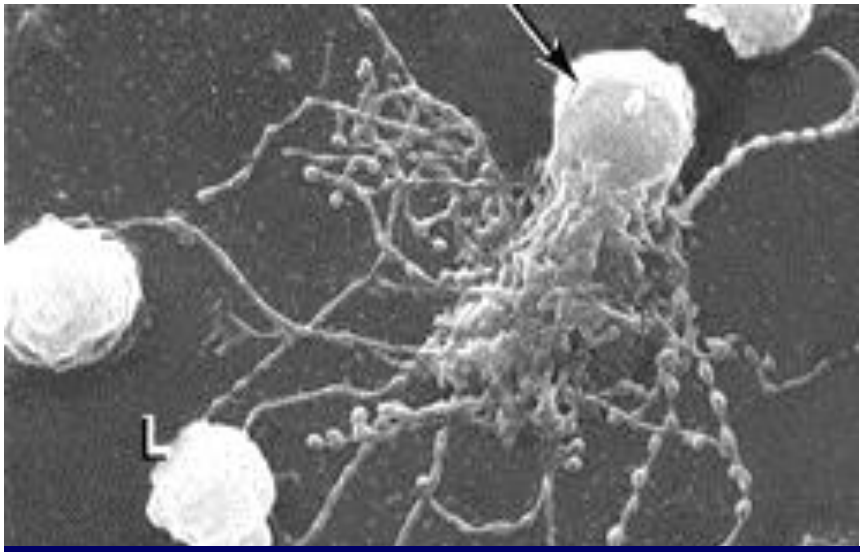
Maturation of Follicular Dendritic cells



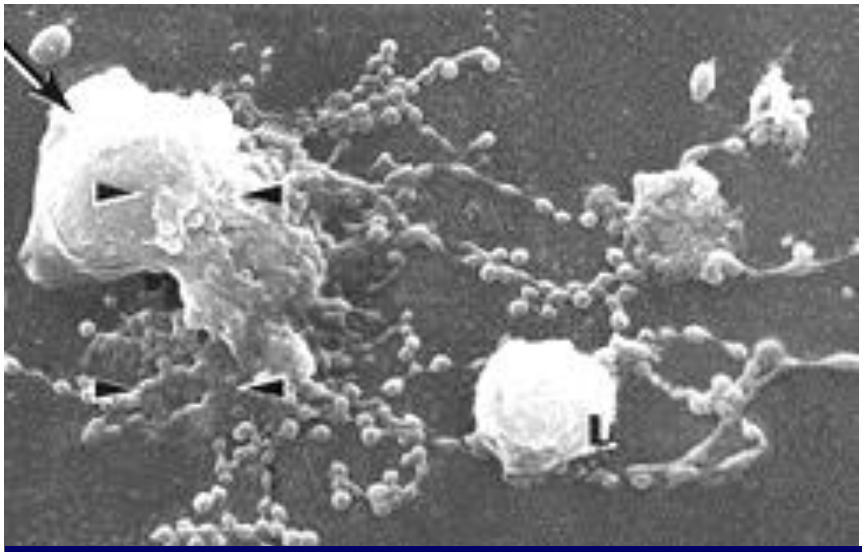
Club-shaped tips of developing dendrites



Filiform dendrites



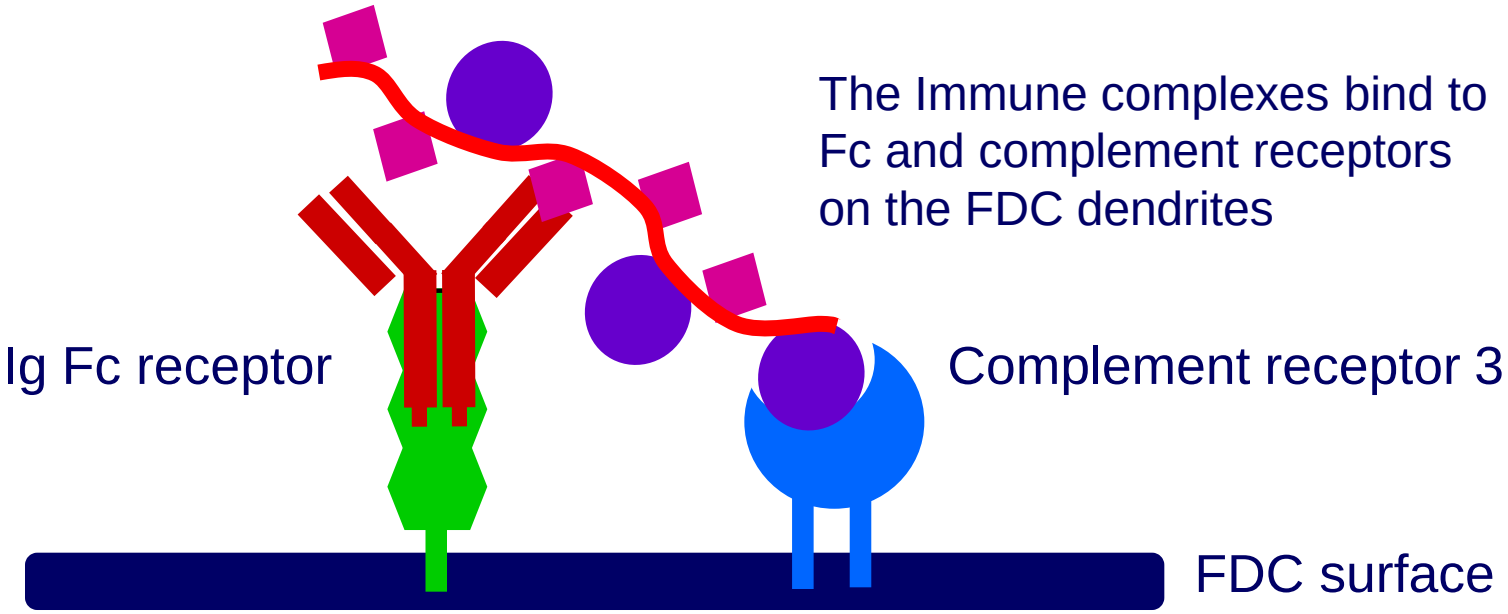
Bead formation on dendrites



Bead formation on dendrites

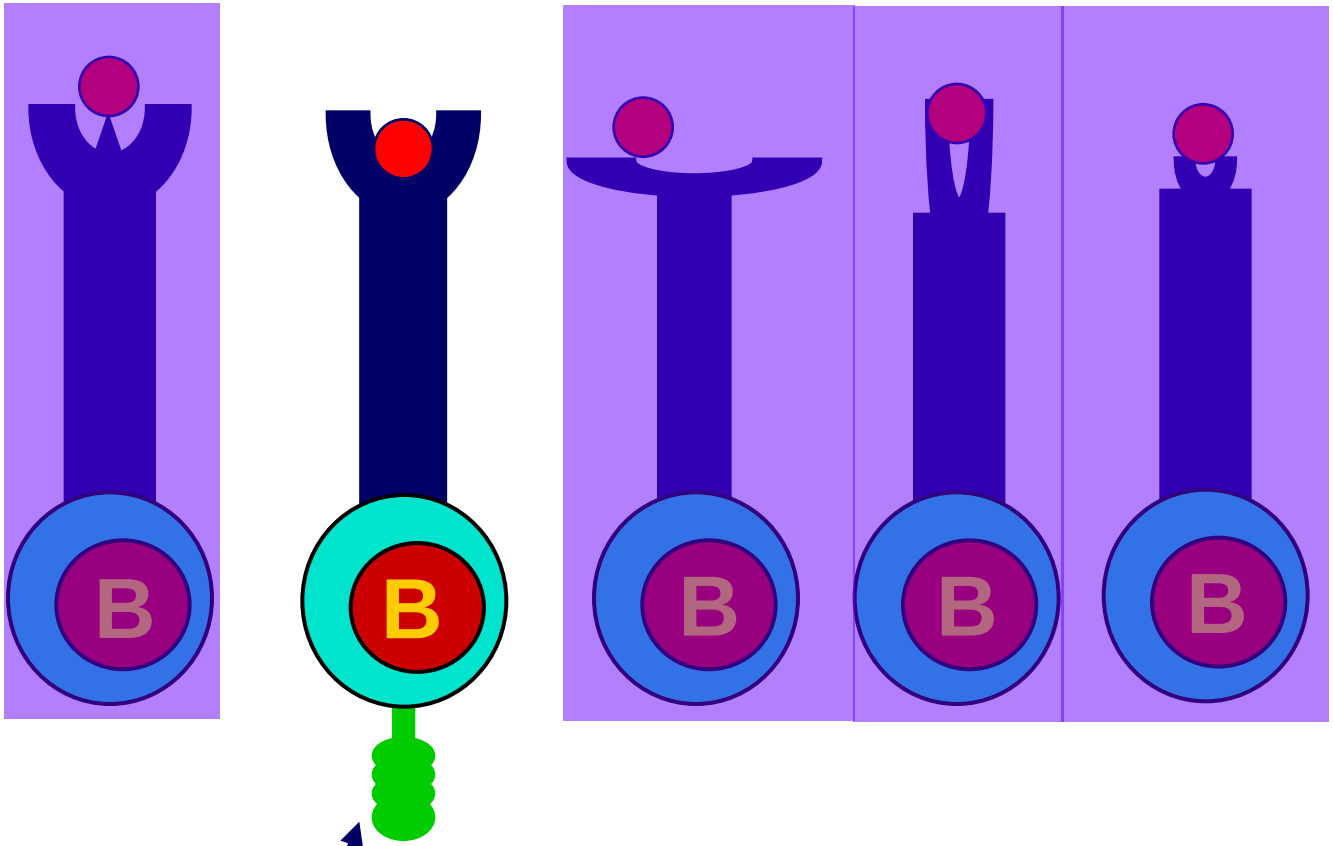
Association of antigen with FDC

Iccosomes: The immune complex



Control of Affinity & Affinity Maturation

Five B cell antigen receptors - all specific for ● , but with different affinities due to somatic hypermutation of Ig genes in the germinal centre



Only this cell, that has a high affinity for antigen can express CD40.
Only this cell can receive help from T cell
Only this cell is rescued from apoptosis i.e. clonally selected

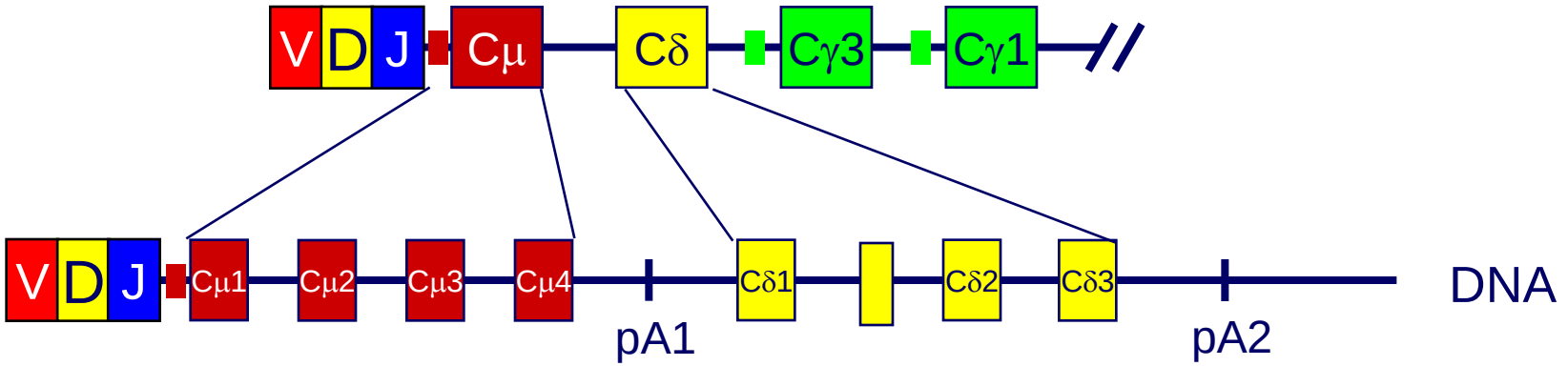
The cells with lower affinity receptors die of apoptosis by neglect

The diversity of the BCR is

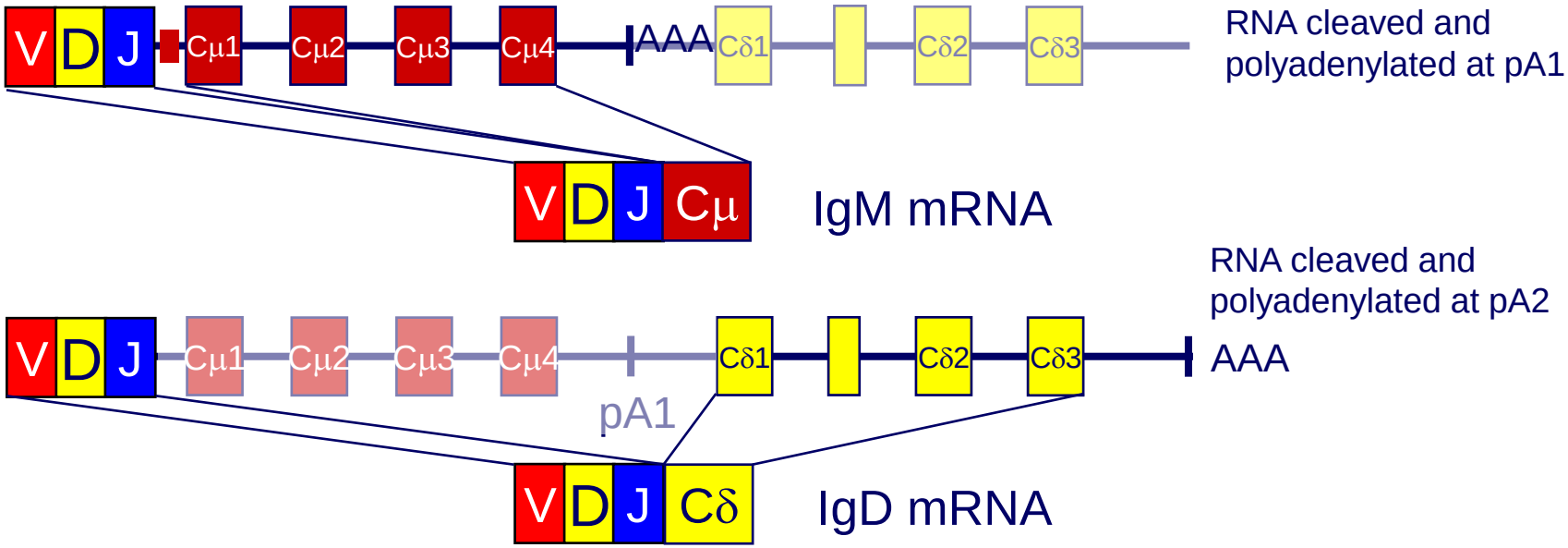
	Mouse			Human		
	H	λ	κ	H	λ	κ
Combinatorial diversity	1.4×10^4	6	1.2×10^3	1.1×10^4	1.2×10^2	2×10^21
V	300	2	300	65	30	40
D	12			27		
J	4	3	4	6	4	5
Junctional Diversity	1.3×10^5	18	3.6×10^3		3×10^7	
Join point change	+	+	+	+	+	+
Frame shift	+	+	+	+	+	+
N-region	+	-	-	+	-	-
Somatic hypermutation	+	+	+	+	+	+
Total		4.7×10^8			$\sim10^{14}$	

How can B cells express IgM and IgD simultaneously?

Splicing of IgM and IgD RNA



Two types of mRNA can be made simultaneously in the cell by differential usage of alternative polyadenylation sites and splicing of the RNA



Isotype switch

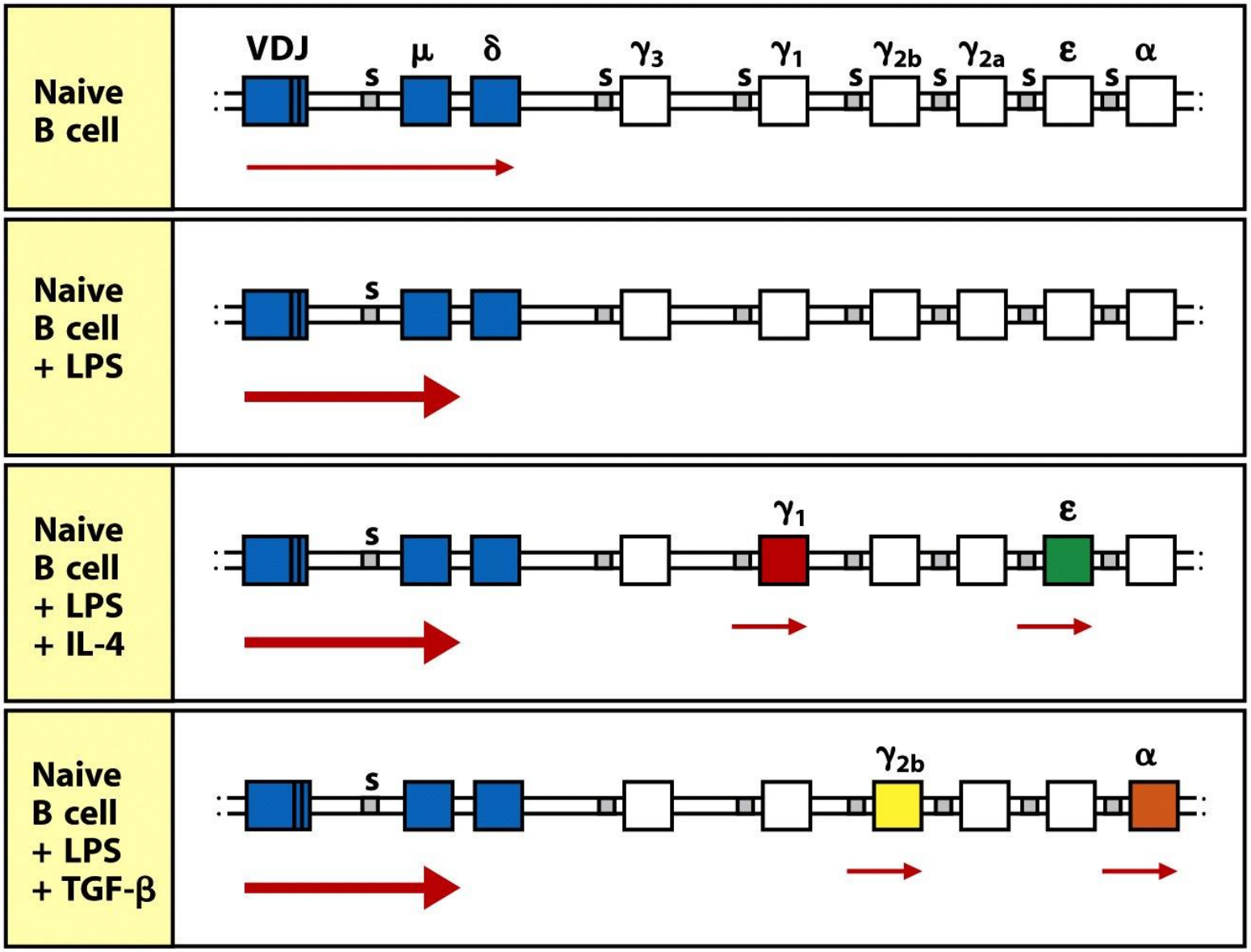
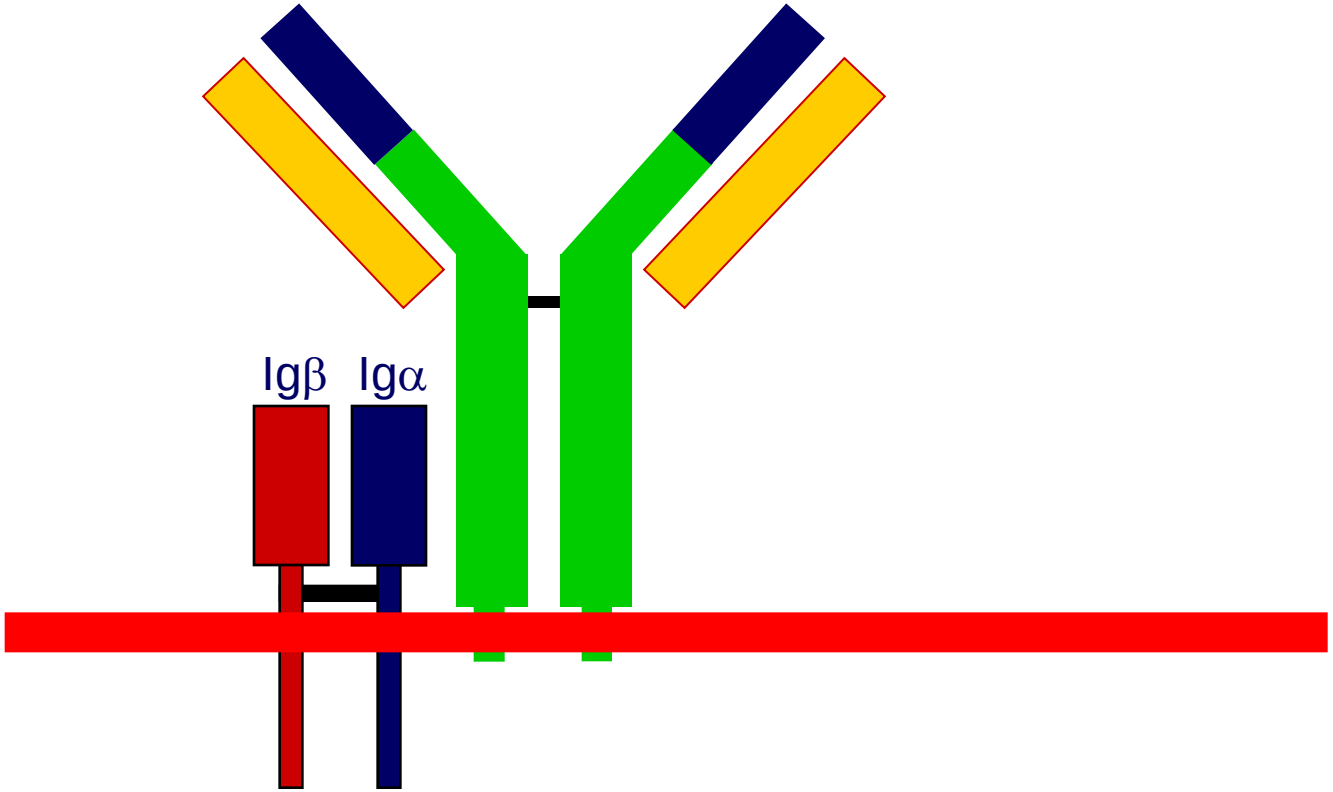


Figure 9-12 Immunobiology, 7ed. (© Garland Science 2008)

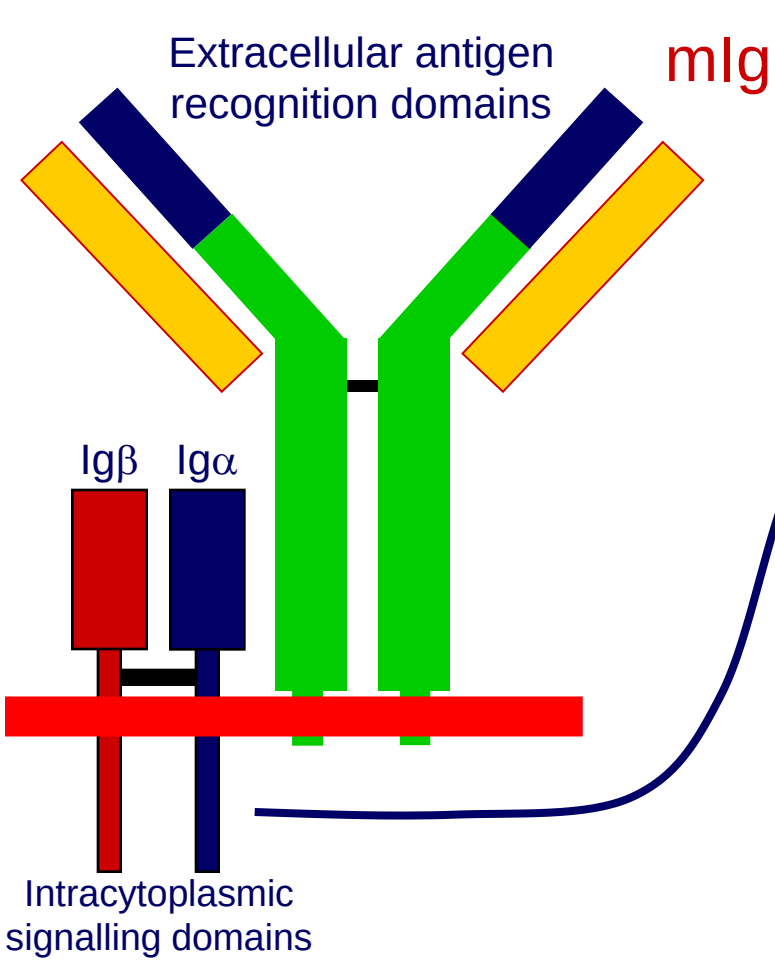
Take-home message

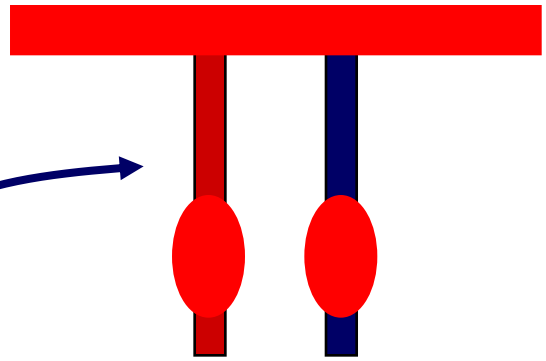
- B cells develop in adult bone marrow
- Stages of B cell differentiation are defined by Ig gene rearrangement (in variable region)
- Negative selection in the bone marrow removes B cells expressing potentially autoreactive BCRs and establishes central B cell tolerance
- BCR rearrangment happens in bone marrow before antigen encounter
- Somatic hypermutation and isotype switch occur in the germinal centers and are responsible for antibody diversification after antigen encounter
- IgM and IgD can be expressed simultaneously due to differential RNA splicing

The B cell receptor and signal transduction



Transduction of signals by the B cell receptor

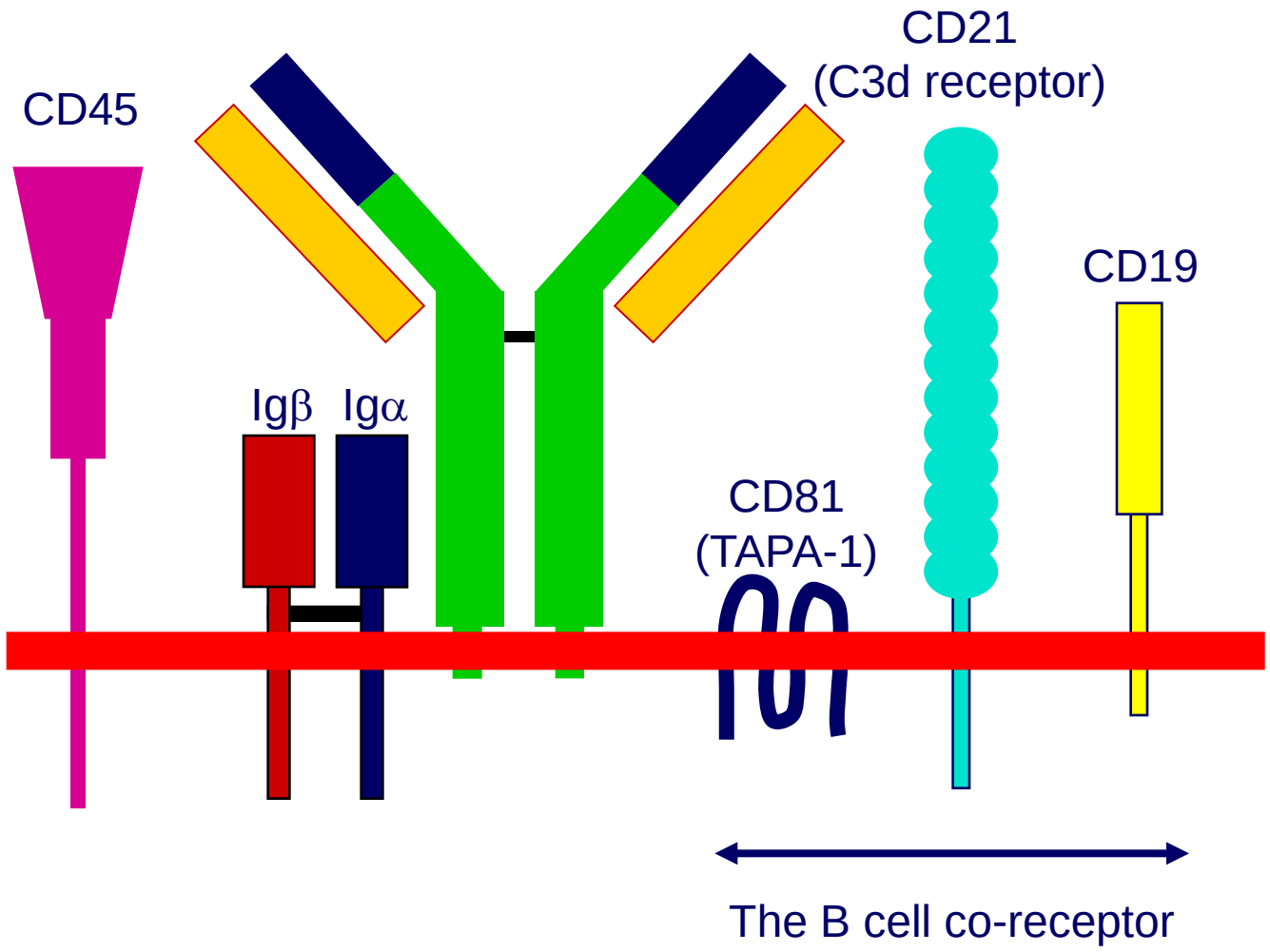




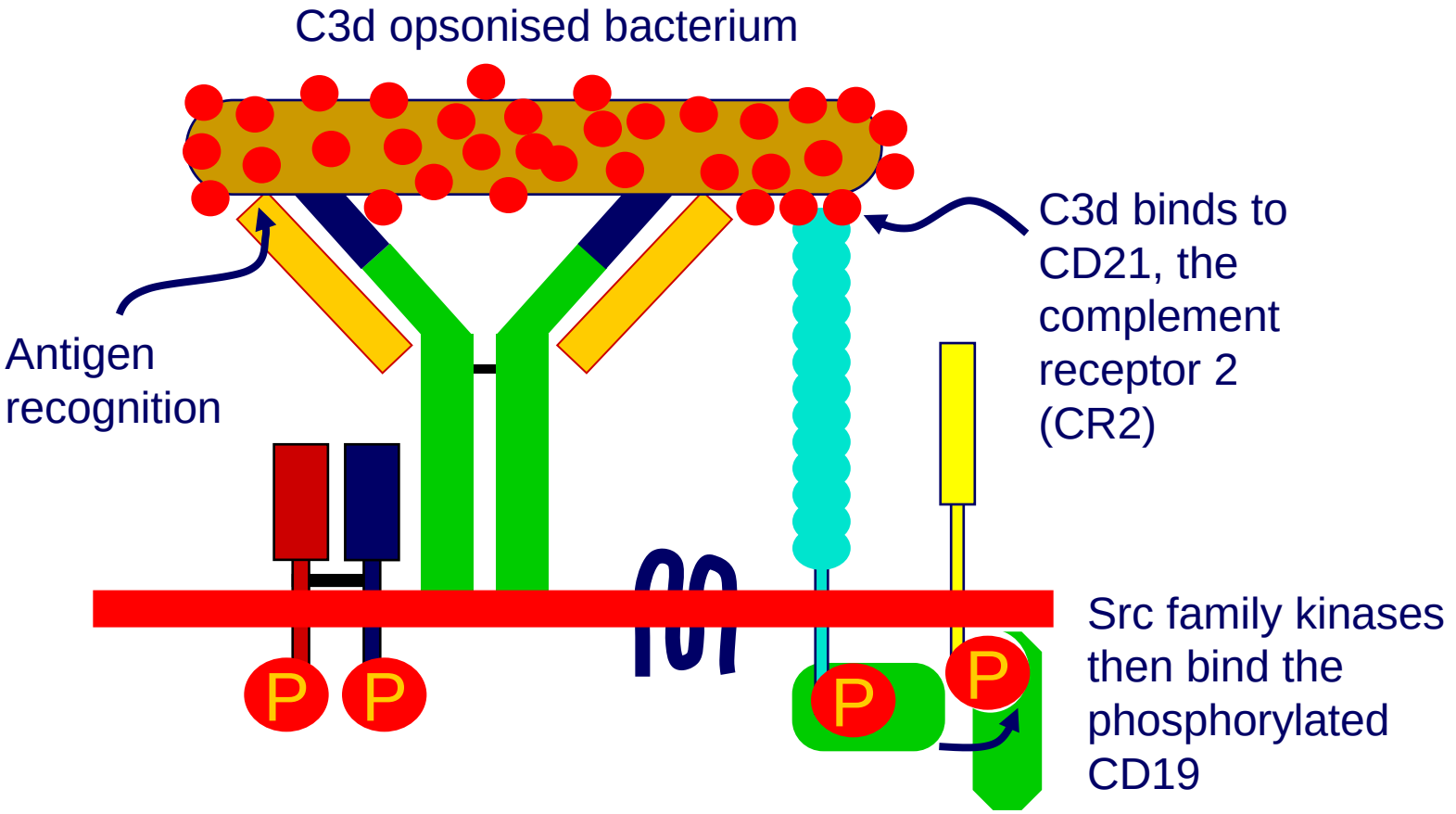
The cytoplasmic domains of the Igα and Igβ contain Immunoreceptor Tyrosine - based Activation Motifs (ITAMS) - 2 tyrosine residues separated by 9-12 amino acids - **Y**XX[L/V]X_{6-9}**Y**XX[L/V]

CD79α/79β

The B cell co-receptor



Co-receptor phosphorylation



- mIg and CD21 are cross-linked by antigen that has activated complement
- CD21 is phosphorylated and receptor-associated kinases phosphorylate CD19
- Phosphorylated CD19 activates more Src family kinases
- Ligation of the co-receptor increases B cell receptor signalling 1000 -10,000 fold

B cell activation need a second signal

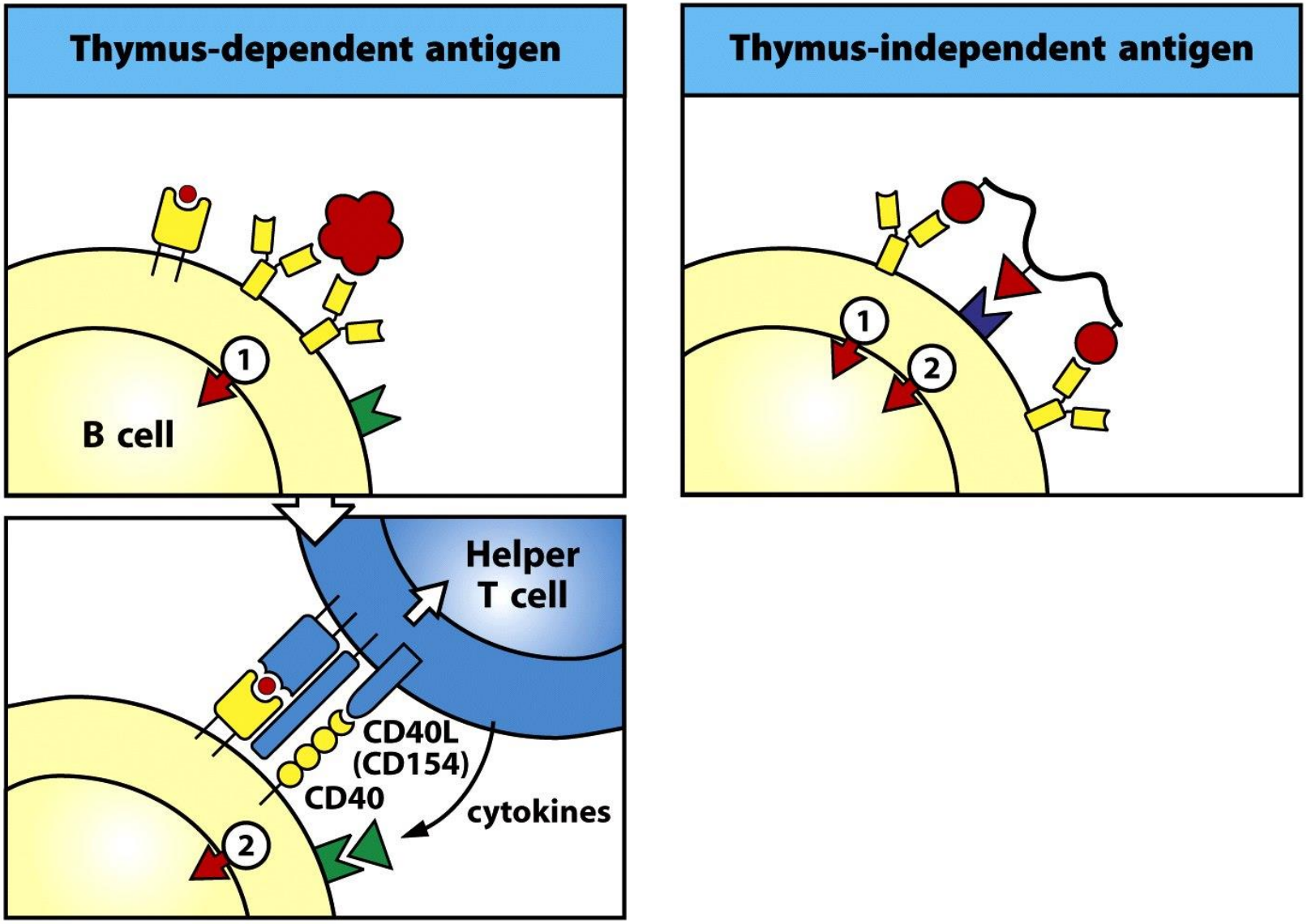


Figure 9-2 Immunobiology, 7ed. (© Garland Science 2008)

Transmission of signals from the cell surface to the nucleus

- If the B cell receive signal 1 and signal 2
- Subsequent signals that transmit signals to the nucleus are common to many different types of cell.
- The ultimate goal is to activate the transcription of genes, the products of which mediate proliferation and differentiation.

Once the B cell-specific parts of the cascade are complete, signalling to the nucleus continues via three common signalling pathways via:

1.The mitogen-activated protein kinase (MAP kinase) pathway
2. Increased in intracellular Ca2+ mediated by IP_3
3.The activation of Protein Kinase C mediated by DAG

Simplified scheme linking antigen recognition with transcription of B cell-specific genes

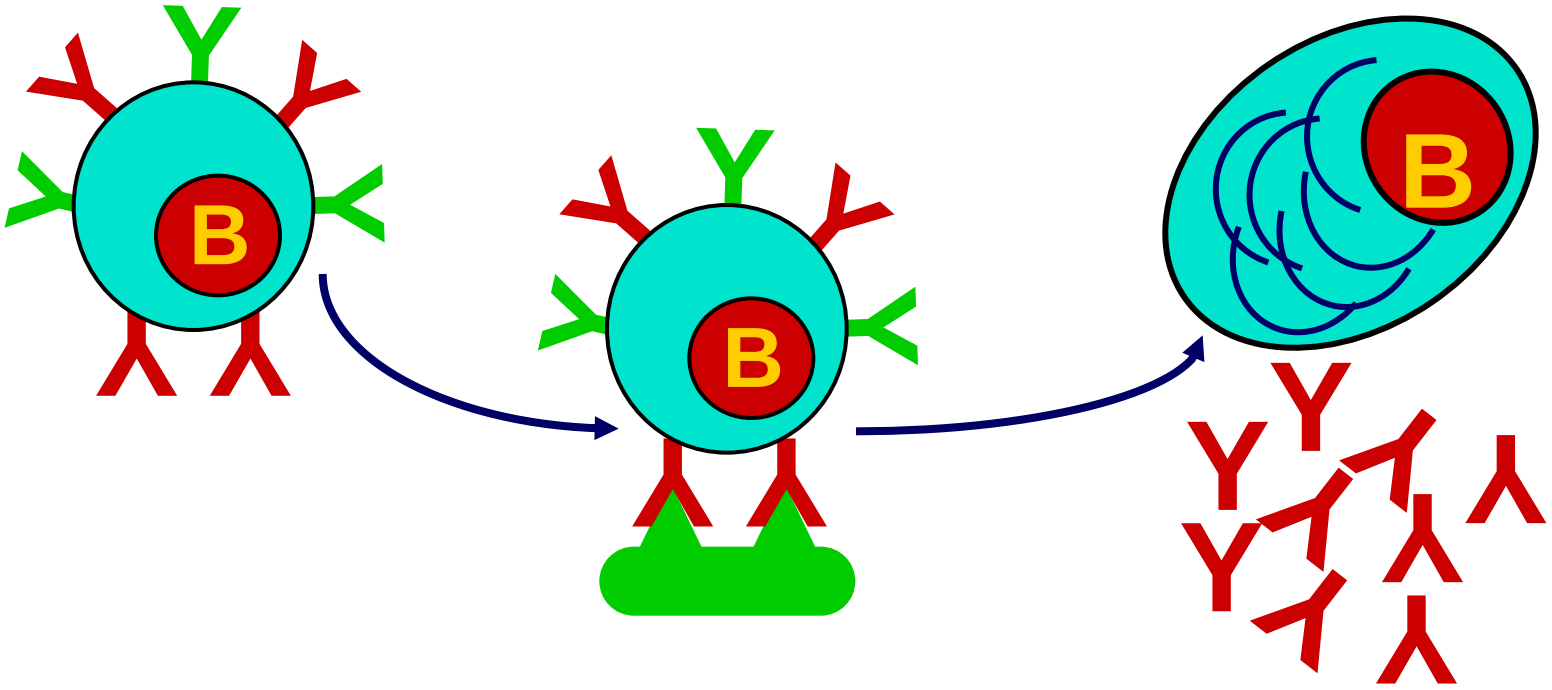
- **MAP Kinase cascade**
  Small G-protein-activated MAP kinases found in all multicellular animals - activation of MAP kinases ultimately leads to phosphorylation of transcription factors from the **AP-1** family such as **Fos** and **Jun**.

- **Increases in intracellular calcium via IP_3**
  IP_3, produced by PLC-γ, binds to calcium channels in the ER and releases intracellular stores of Ca^{++} into the cytosol. Increased intracellular [Ca^{++}] activate a phosphatase, calcineurin, which in turn activates the transcription factor **NFAT**.

- **Activation of Protein Kinase C family members via DAG**
  DAG stays associated with the membrane and recruits protein kinase C family members. The PKC, serine/threonine protein kinases, ultimately activate the transcription factor **NFκB**

The activated transcription factors AP-1, NFAT and NFκB induce B cell proliferation, differentiation and effector mechanisms

Differentiation in the periphery

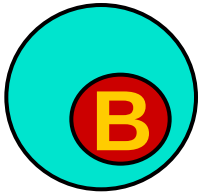
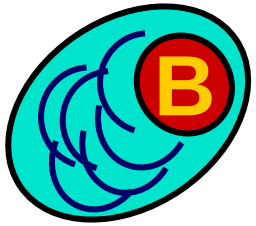


Mature peripheral B cell

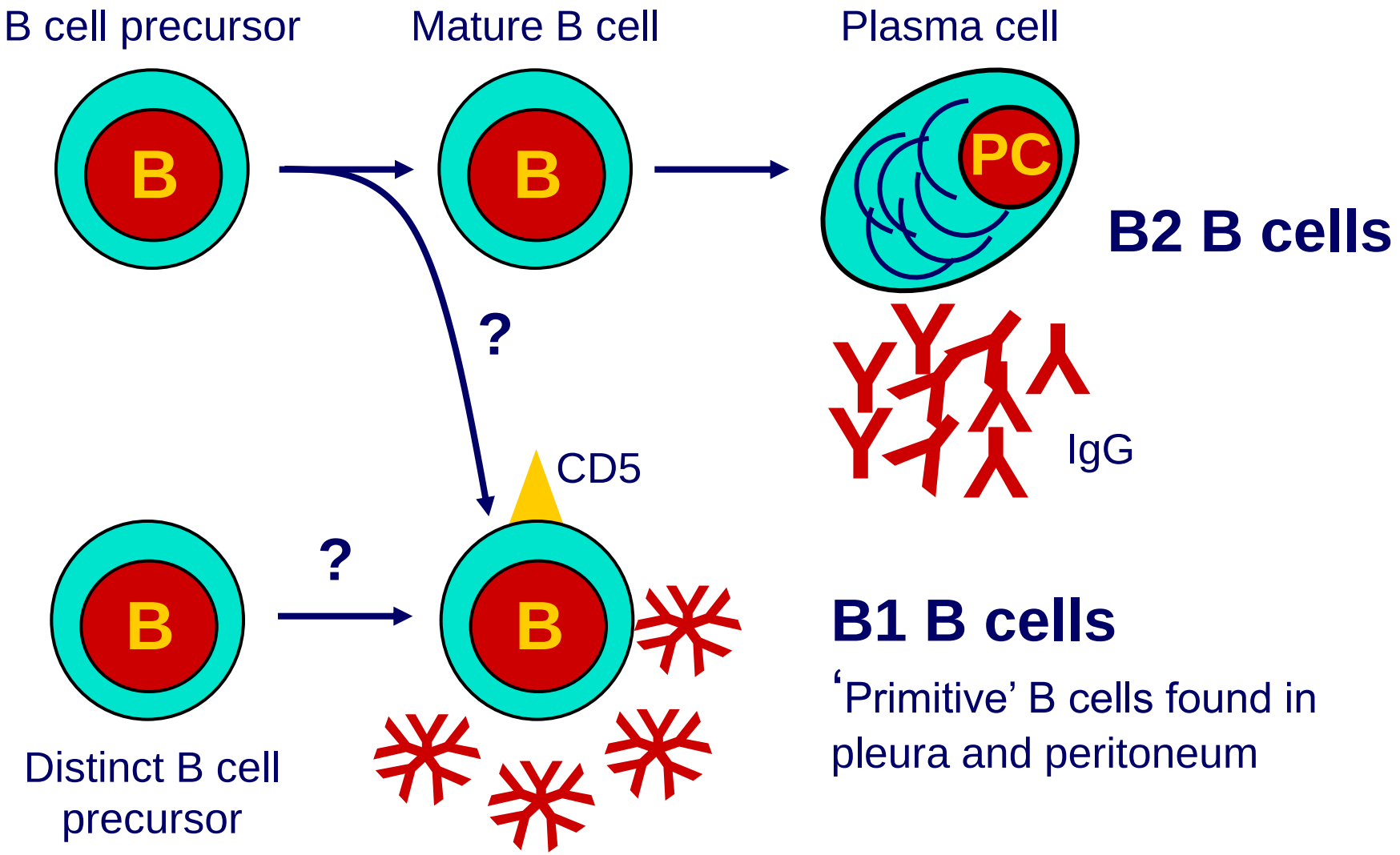
B cell recognises non-self antigen in periphery

Ig-secreting plasma cell

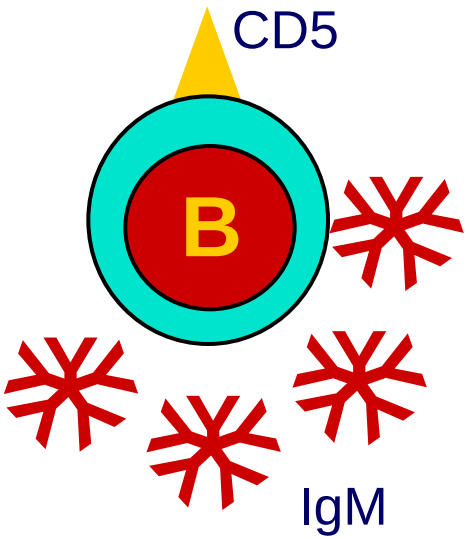
Plasma cells

	Surface Ig	Surface MHC II	High rate Ig secretion	Growth	Somatic hypermut'n	Isotype switch
 Mature B cell	High	Yes	No	Yes	Yes	Yes
 Plasma cell	Low	No	Yes	No	No	No

Two B cell lineages



B-1 B Cells



Peritoneal and pleural cavity

BCR uses a distinctive & restricted range of V regions

Recognises repeating epitope Ag such as polysaccharides

Can make Ig without T cell help

NOT part of adaptive immune response:

- No memory induced
- No increase of binding affinity
- No isotype switch

Comparison of B-1 and B-2 B cell properties

Property	B-1 cells	B-2 cells
V region repertoire	Restricted	Diverse
Location	Peritoneum/pleura	Everywhere
Renewal	Self renewal in situ	Bone marrow
Spontaneous Ig production	High	Low
Isotypes	IgM	IgM/G/A/D/E
Carbohydrate specificity	Yes	Rarely
Carbohydrate specificity	Yes	Rarely
Protein specificity	Rarely	Yes
Need T cell help	No	Yes
Memory development	No	Yes

T Dependent & Independent Antigens

	T Dependent Antigens	TI-1 Antigens	TI-2 Antigens
Induces response in babies	Yes	Yes	No
Induces response in athymia	No	Yes	Yes
Primes T cells	Yes	No	No
Polyclonally activates B cells	No	Yes	No
Requires repeating epitopes	No	No	Yes

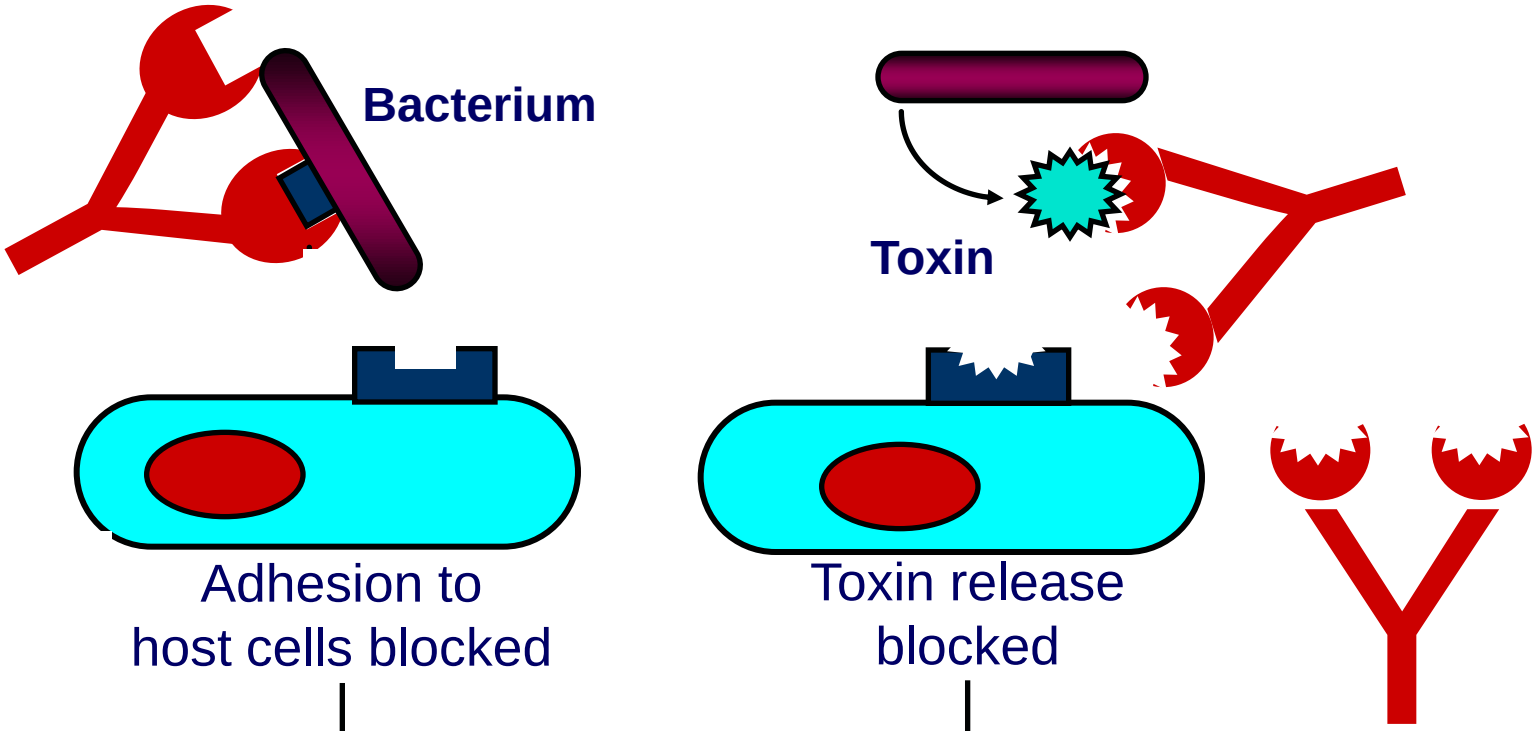
TD: Activate B-1 and B-2 B cells
TI-1: Activate B-1 and B-2 B cells
TI-2: Activate only B-1 B cells

Examples

TD: Diptheria toxin, influenza heamagglutinin, *Mycobacterium tuberculosis*
TI-1: Bacterial lipopolysaccharides (LPS), *Brucella abortis*
TI-2: Pneumococcal polysaccharides, *Salmonella* polymerised flagellin

Immune effector mechanisms against extracellular pathogens & toxins

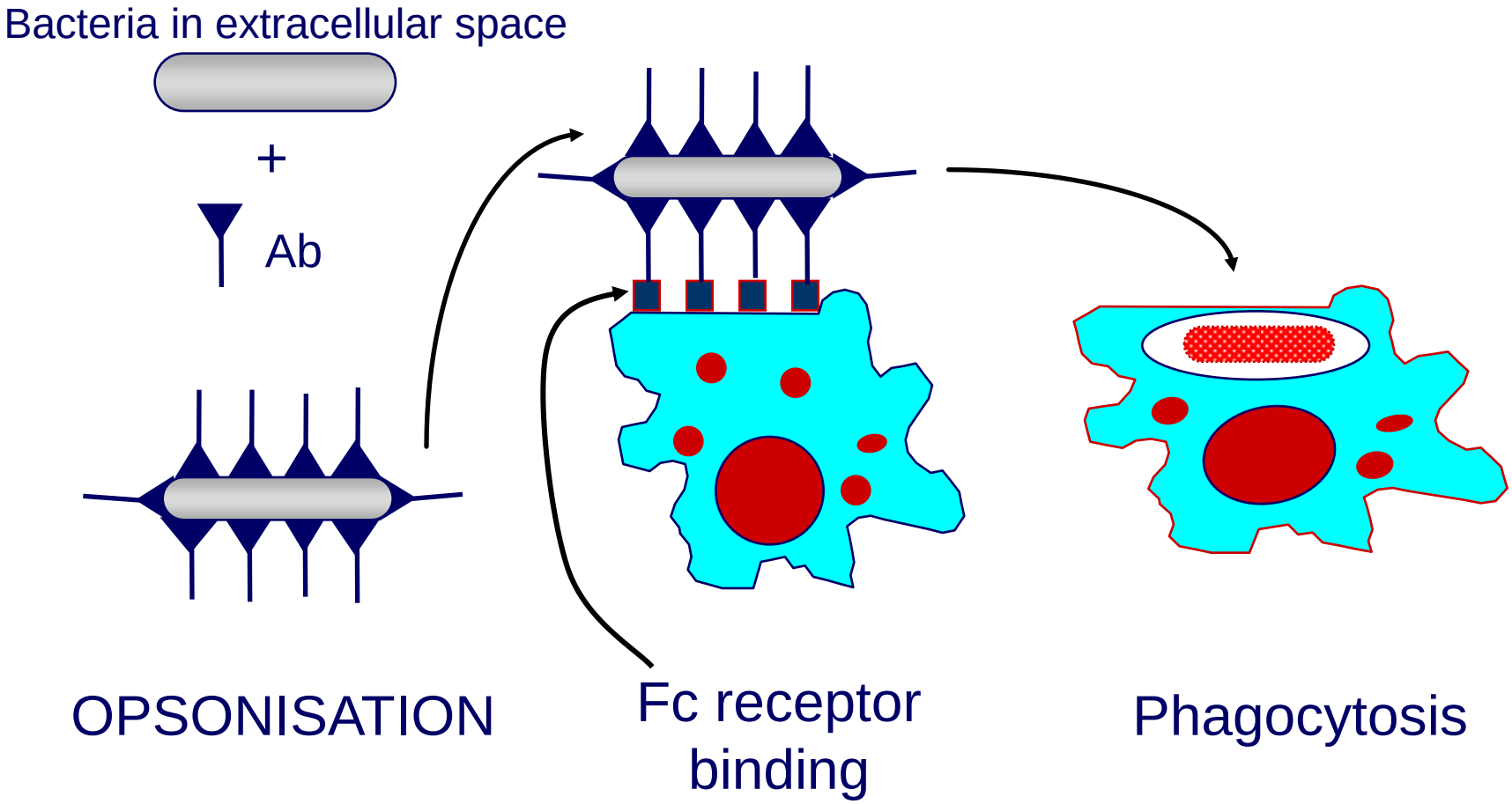
NEUTRALISATION



NEUTRALISING ANTIBODIES

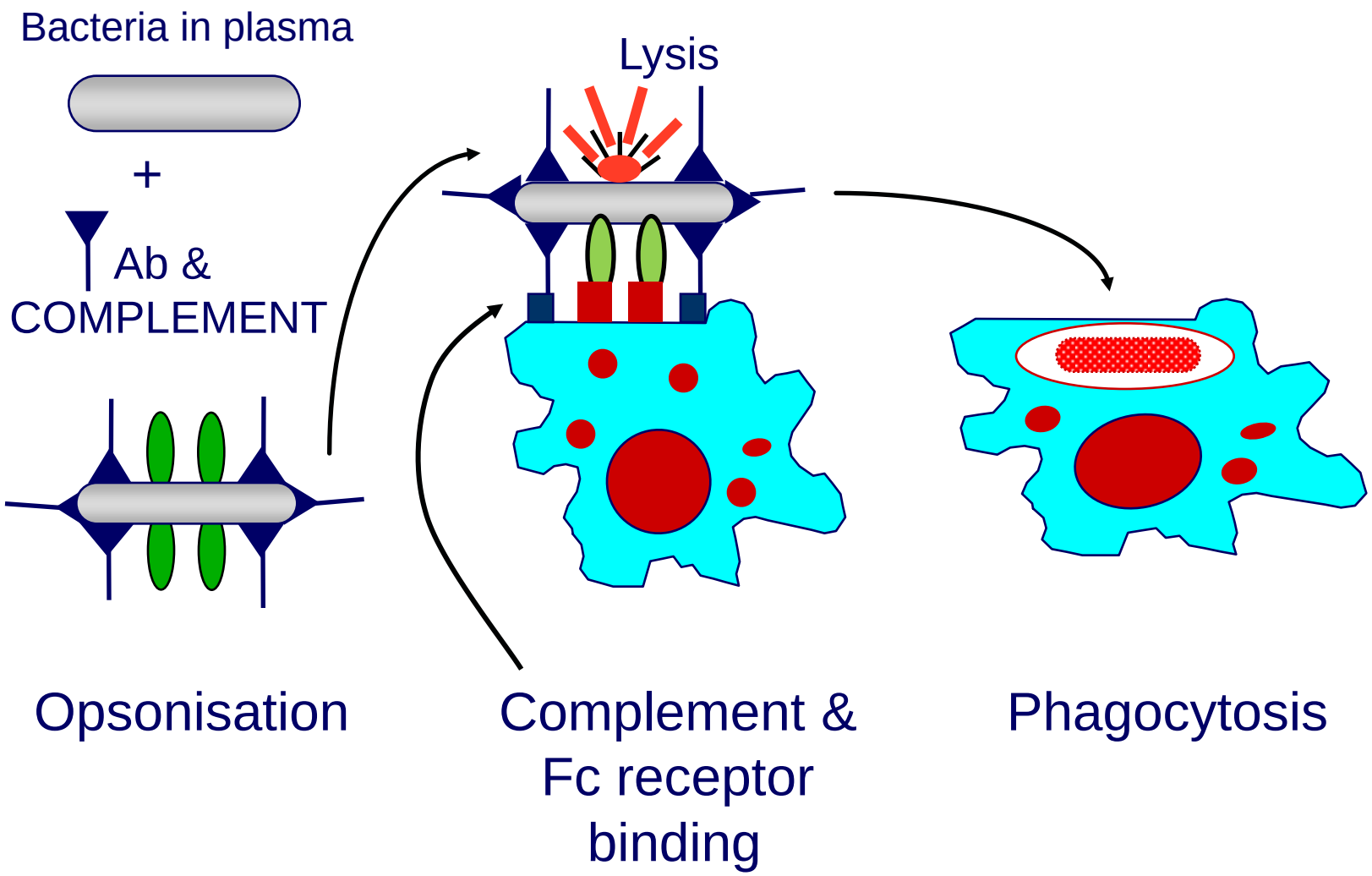
Effector mechanisms against extracellular pathogens

OPSONISATION



Effector mechanisms against extracellular pathogens

COMPLEMENT Activation



B cells can also

- ✓ Function as APC cells
- ✓ Produce cytokine to regulate immune response