

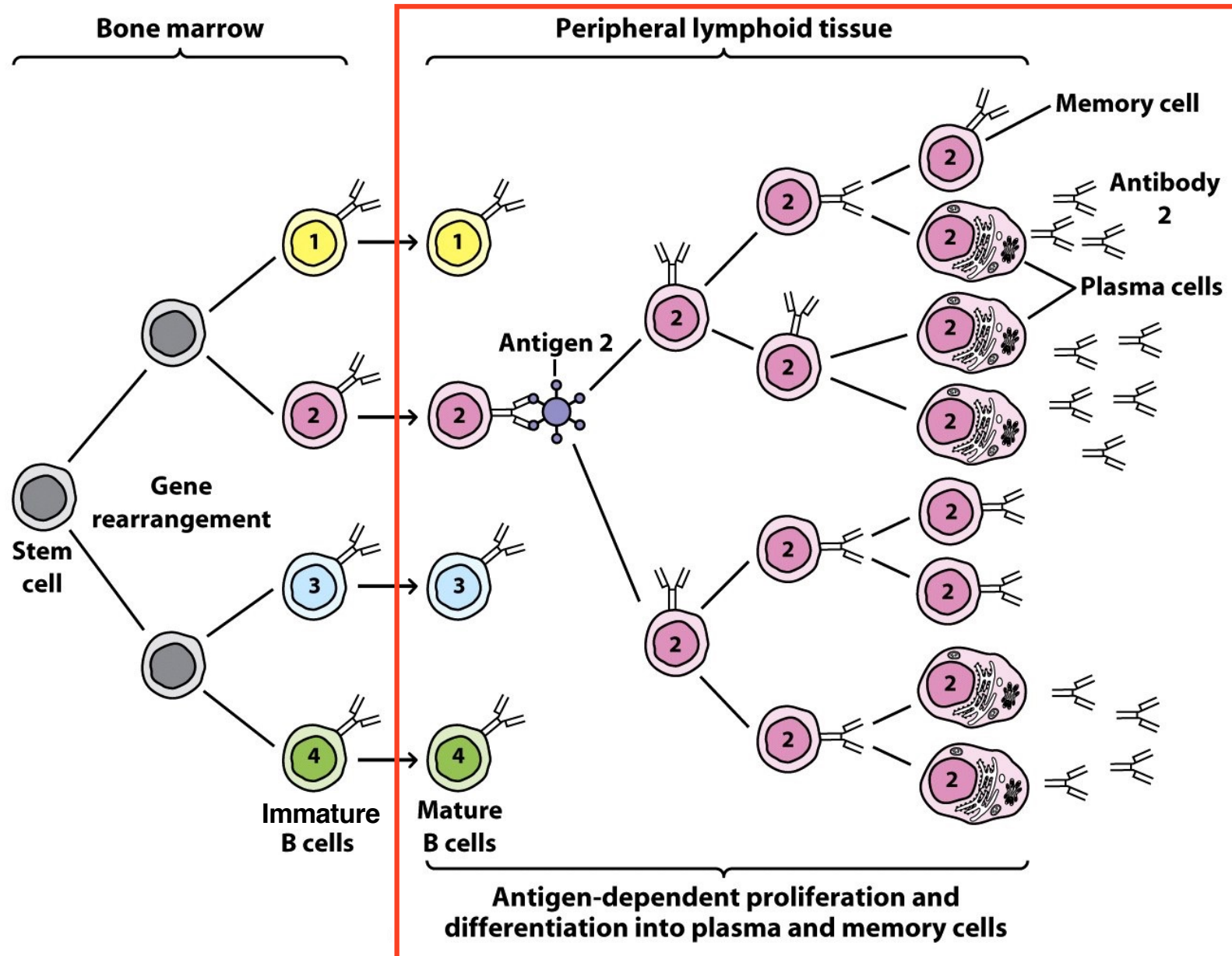
Corso di Immunologia - III anno
Prof. Paolini

Lezione 14/11/2025

"L'attivazione dei linfociti B"

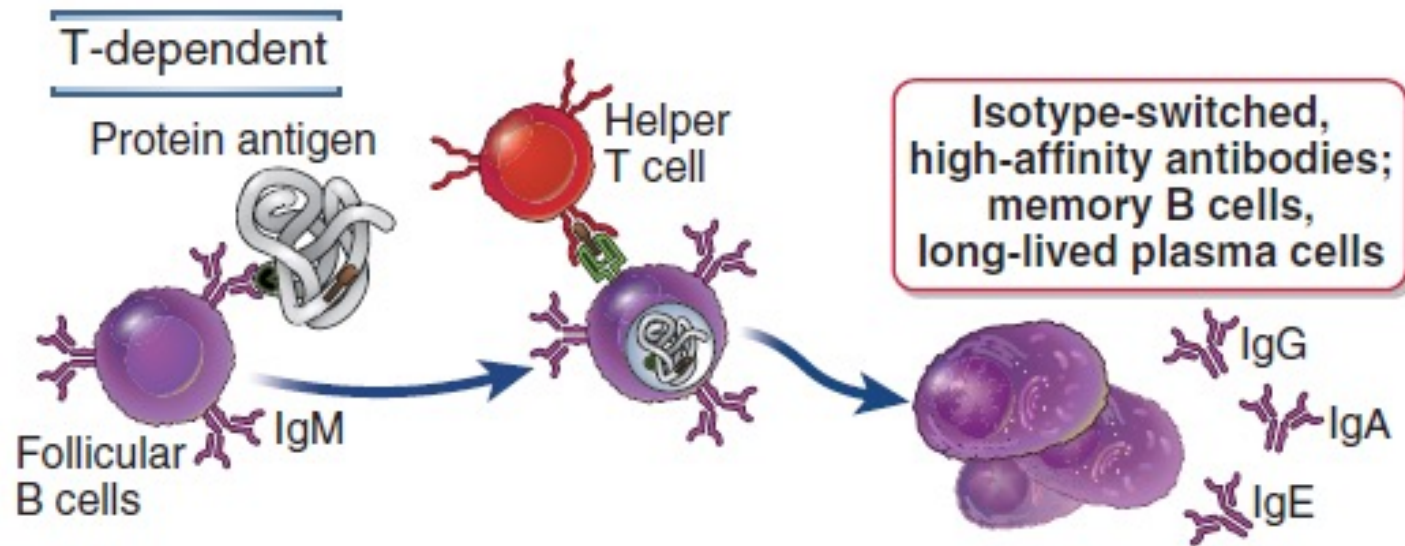
**Il materiale presente in questo documento viene distribuito
esclusivamente ad uso interno e per scopi didattici.**

The big moment: B cell response to foreign antigen

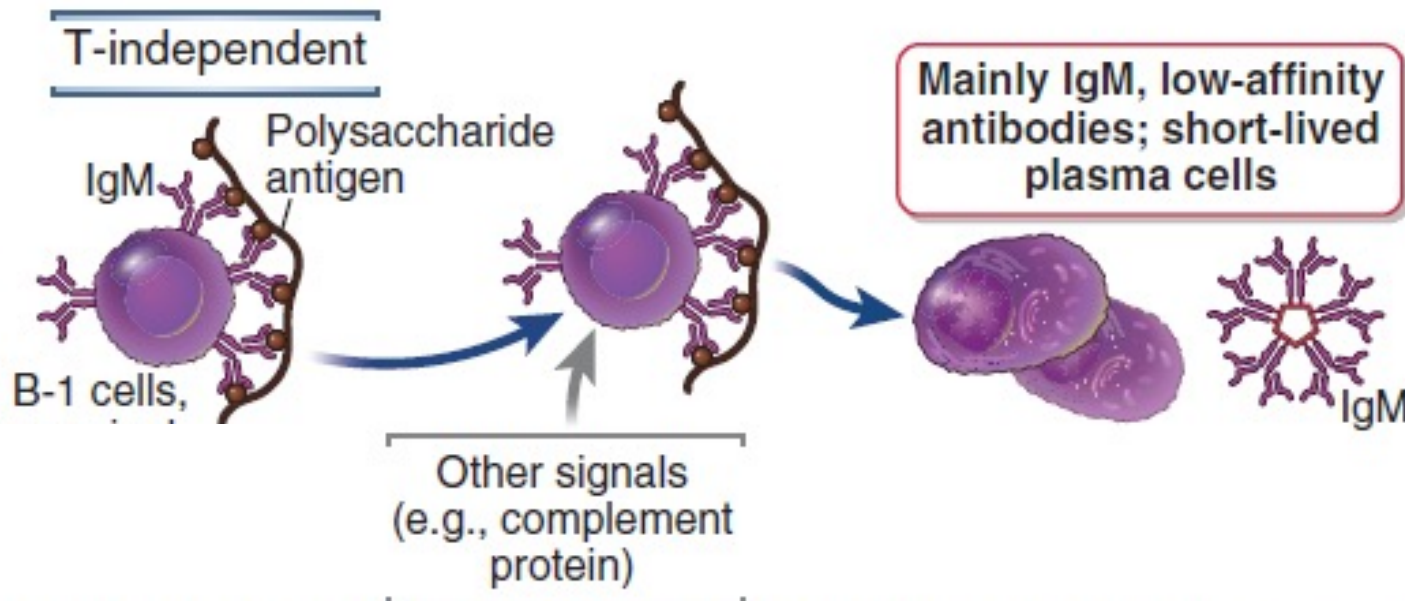


T-dependent and T-independent antibody responses

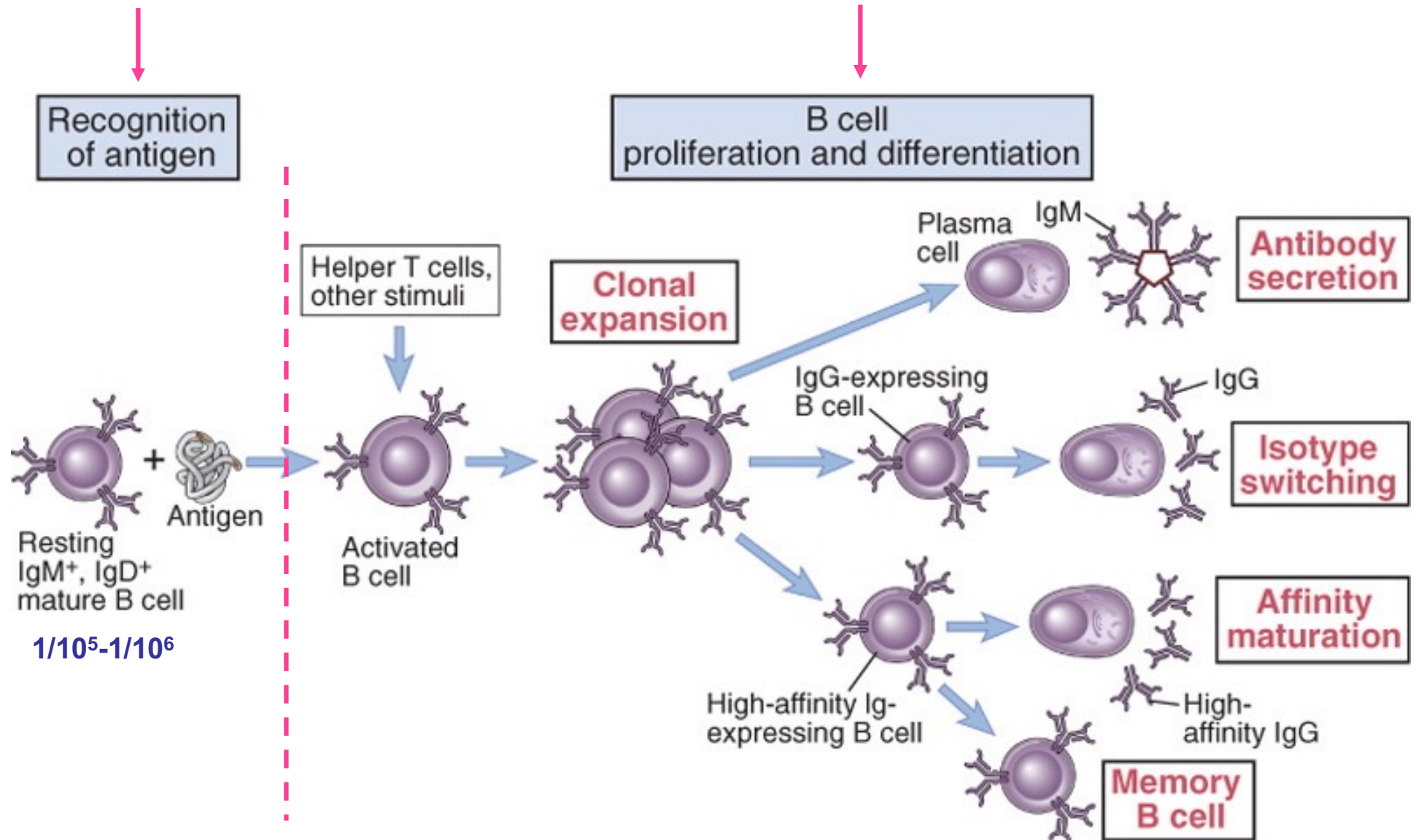
B-2



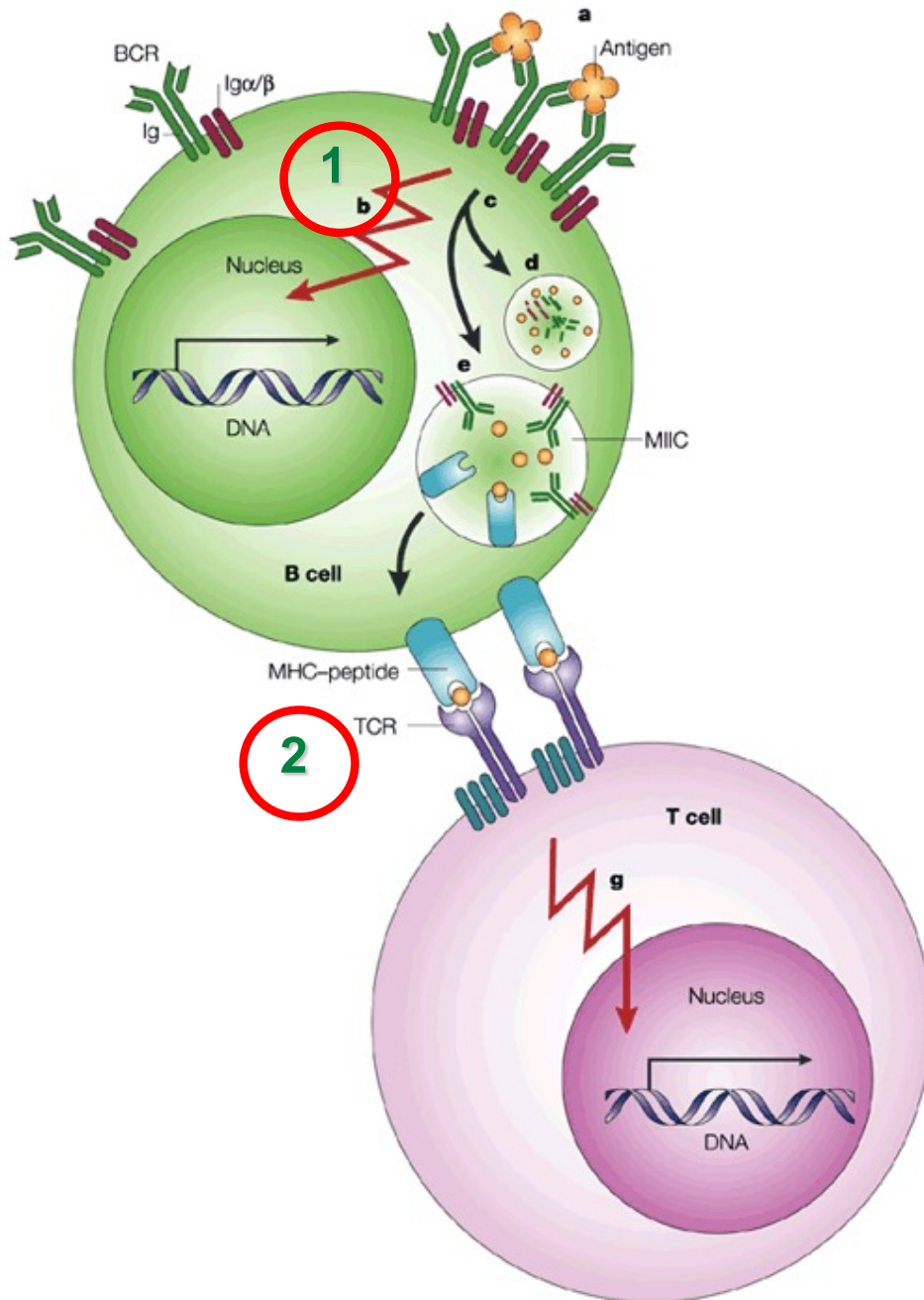
B-1



B cell response to proteins antigens

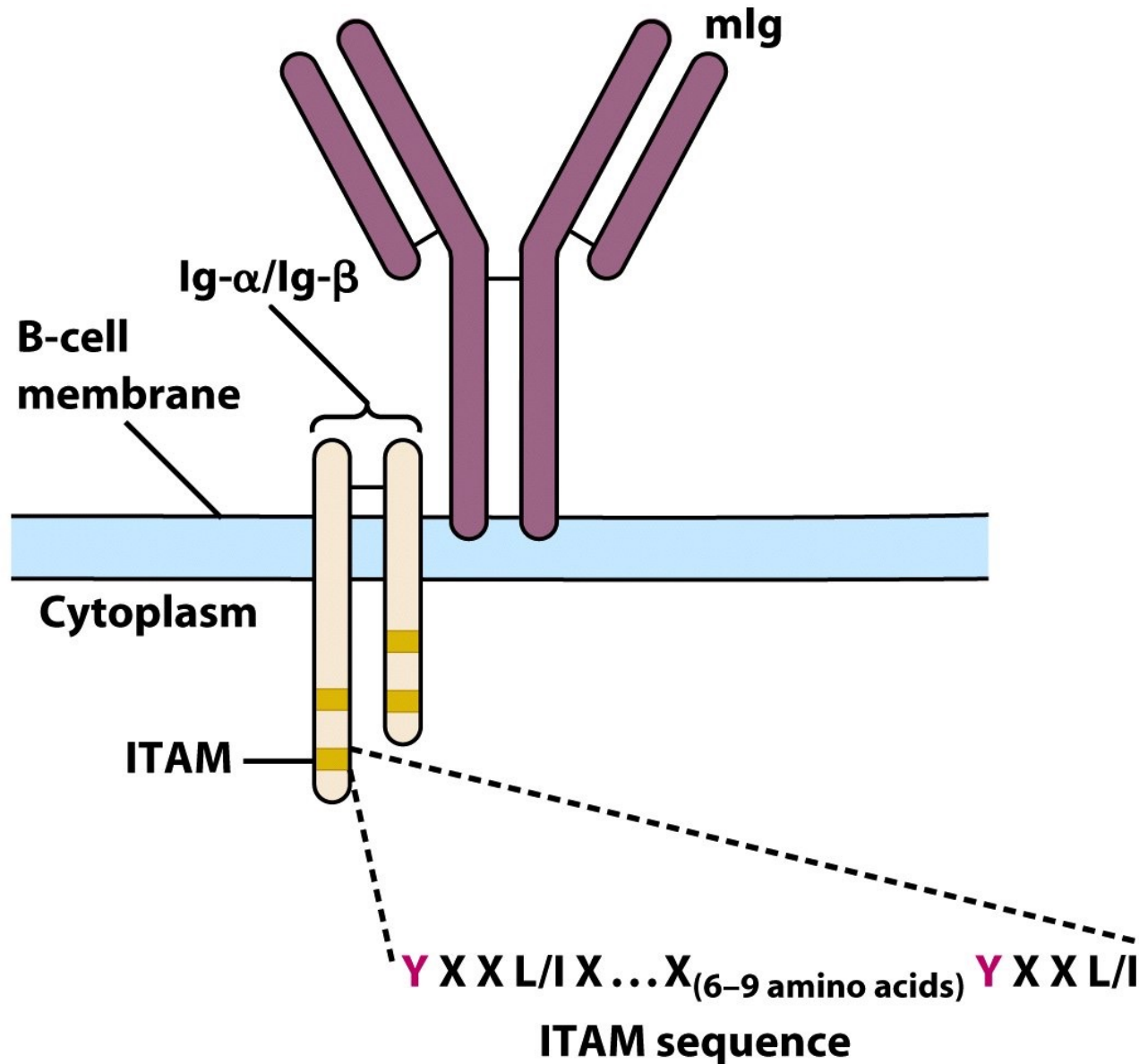


Two Signal Model for B Cell Activation

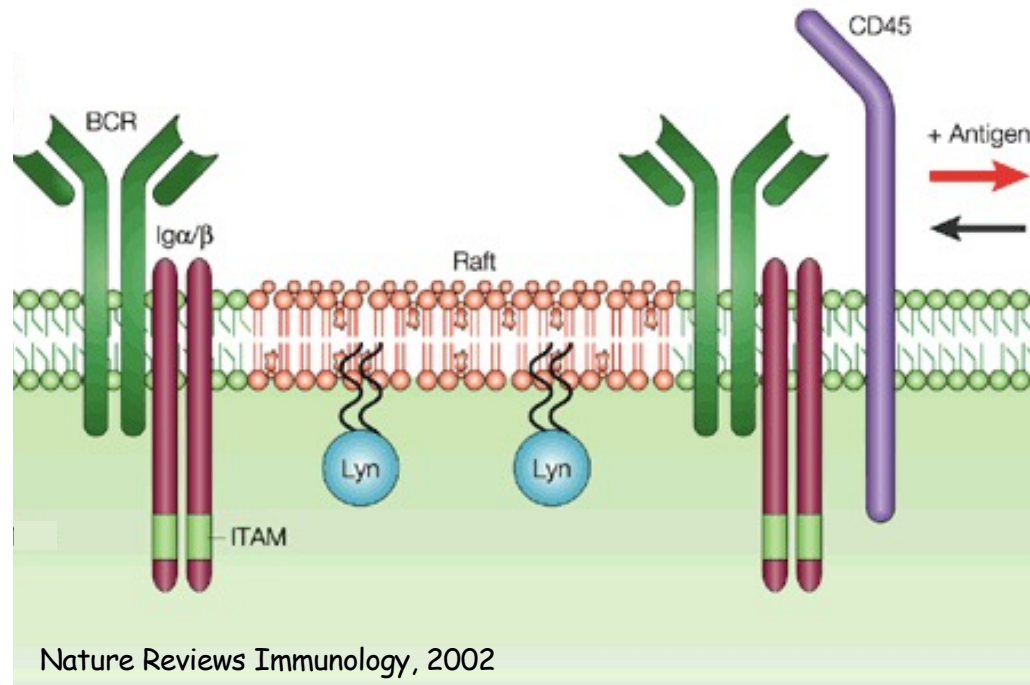


1. BCR engagement and signal transduction
2. BCR/Ag endocytosis, Ag processing and presentation to T cells

B CELL RECEPTOR (BCR) and signal transduction



The Initial Stages of Signal Transduction by an Activated B Cell Receptor Signal 1



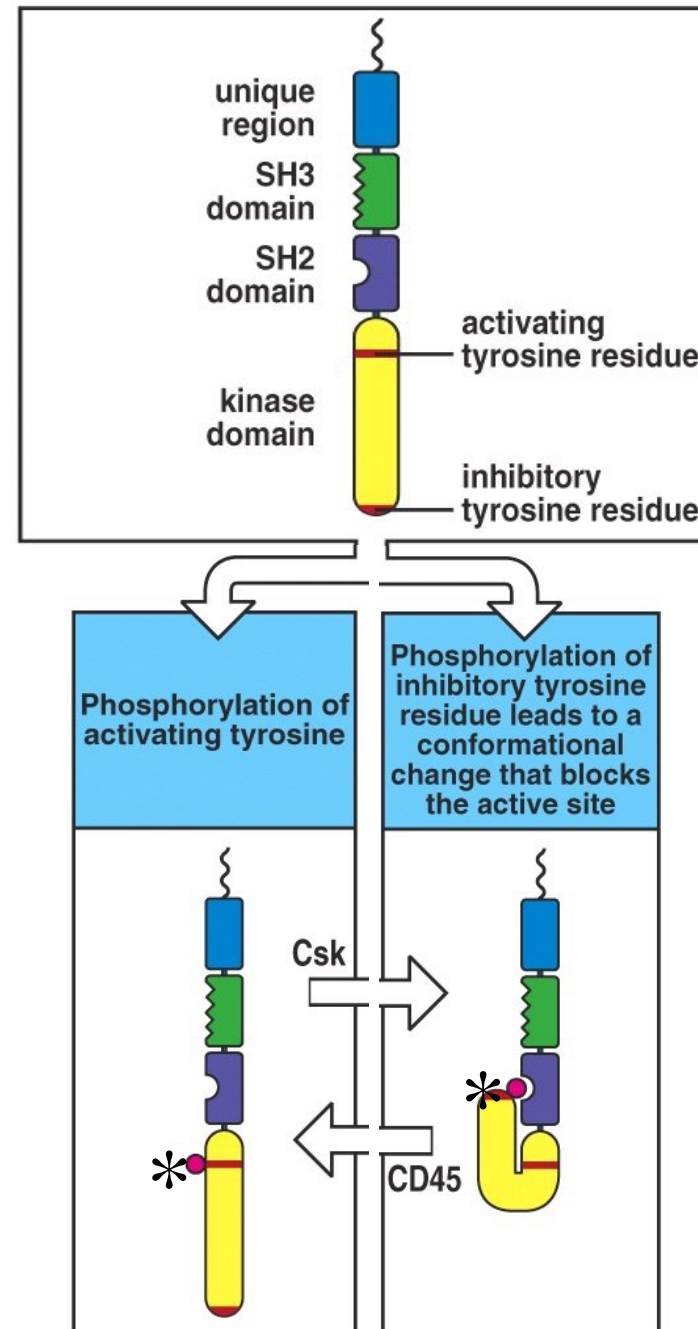
In resting B cells, the Src-family kinases, such as Lyn, are concentrated into lipid rafts, while BCR is excluded from rafts

The Src family tyrosine kinase **lyn** initiates signal transduction in B cells

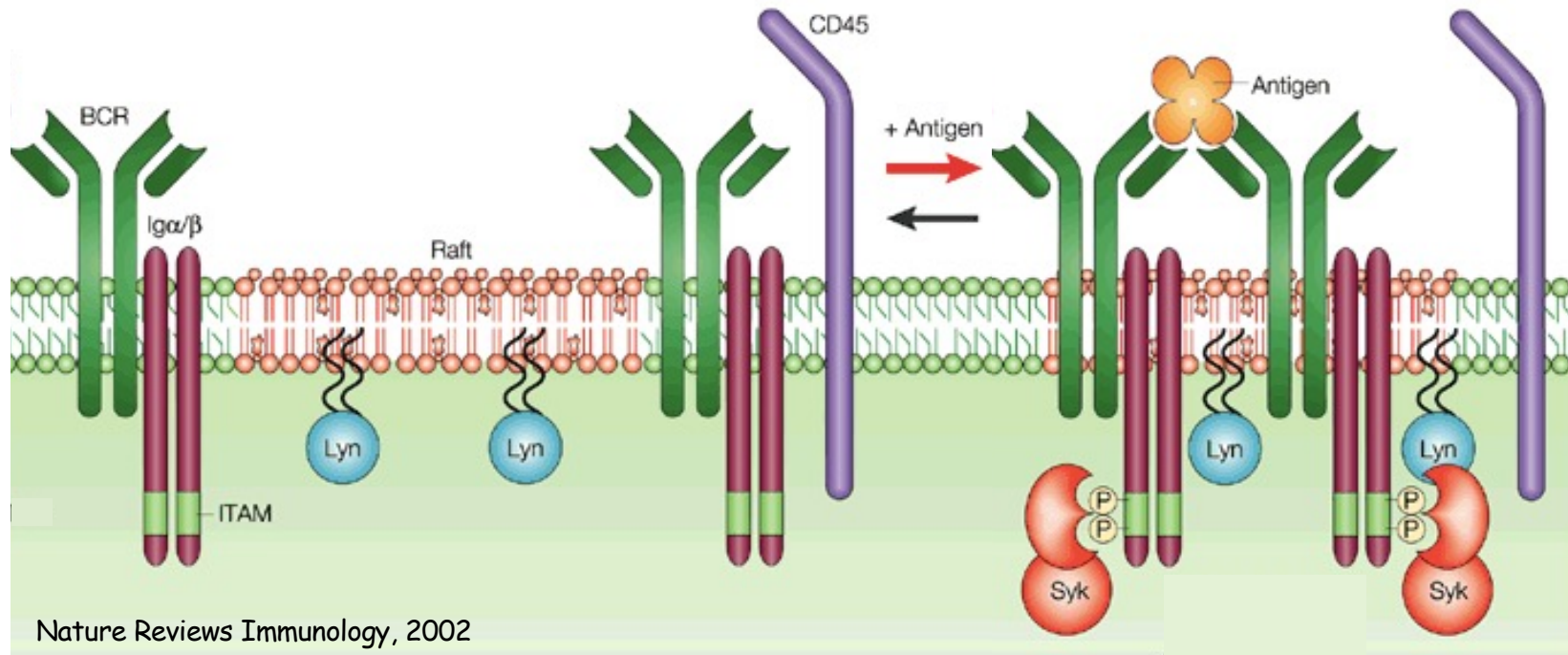
Their activity is regulated by inhibitory and activating tyrosine residues

Csk= tyrosine kinase

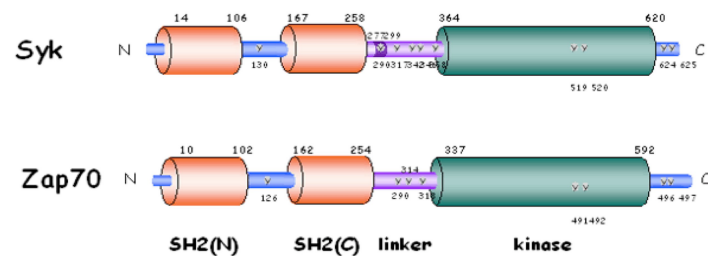
CD45= tyrosine phosphatase



The Initial Stages of Signal Transduction by an Activated B Cell Receptor Signal 1

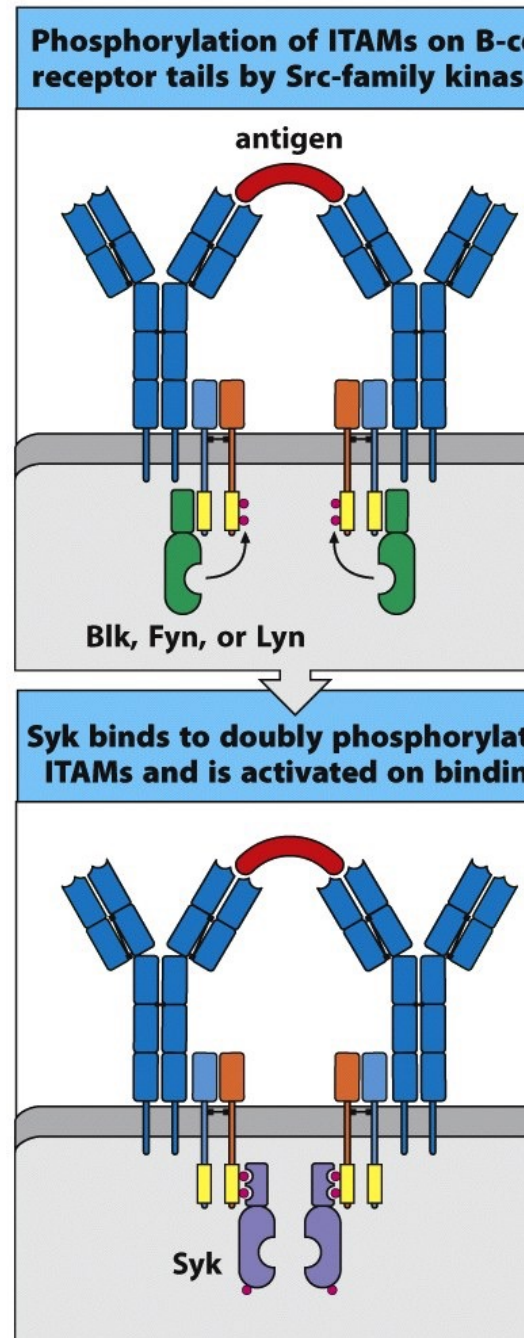


In resting B cells, the Src-family kinases, such as Lyn, are concentrated into lipid rafts, while BCR is excluded from rafts

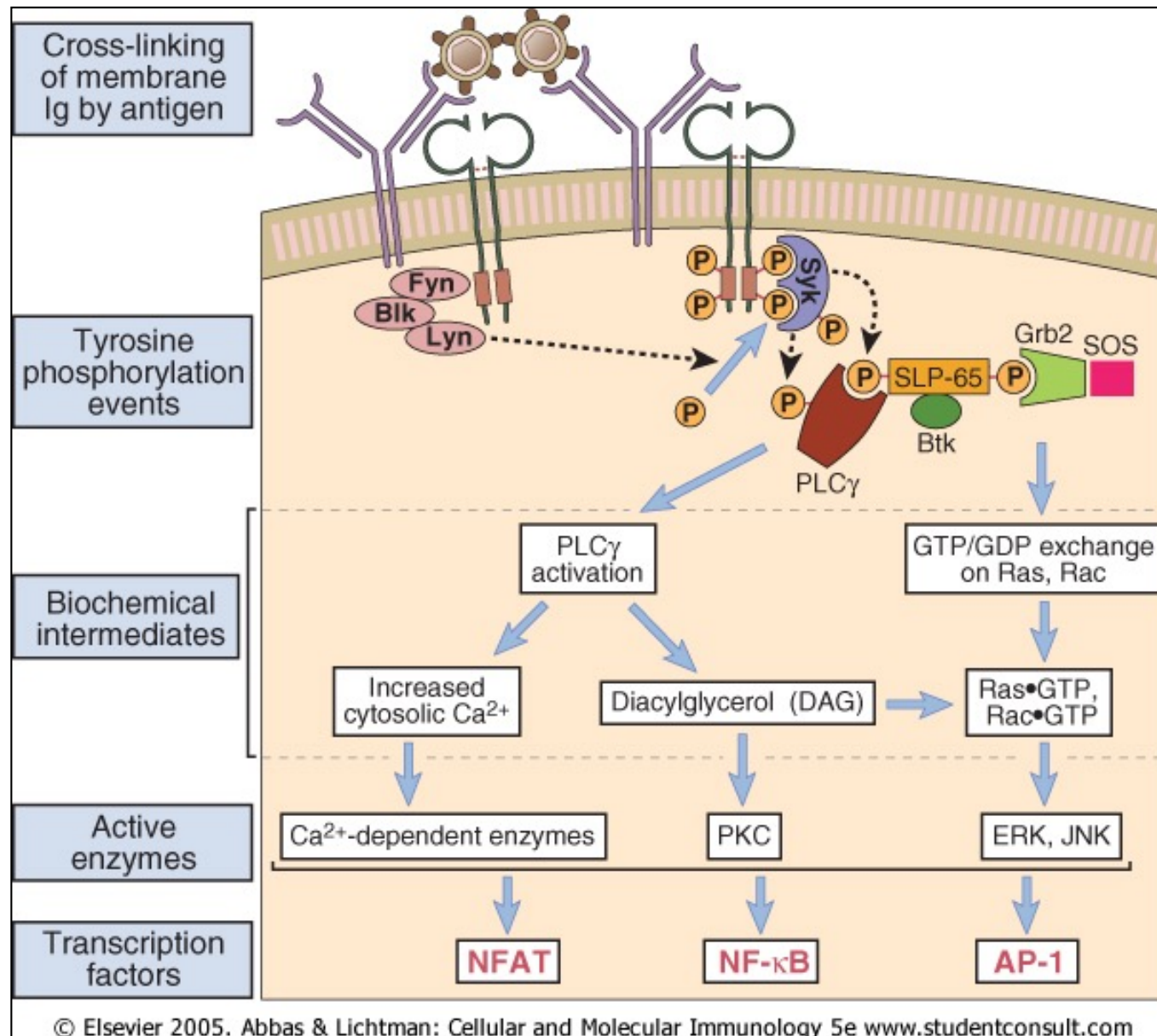


Upon antigen cross-linking, BCRs translocate into rafts and associate with Lyn which phosphorylates ITAMs, recruiting Syk and initiating signalling cascades

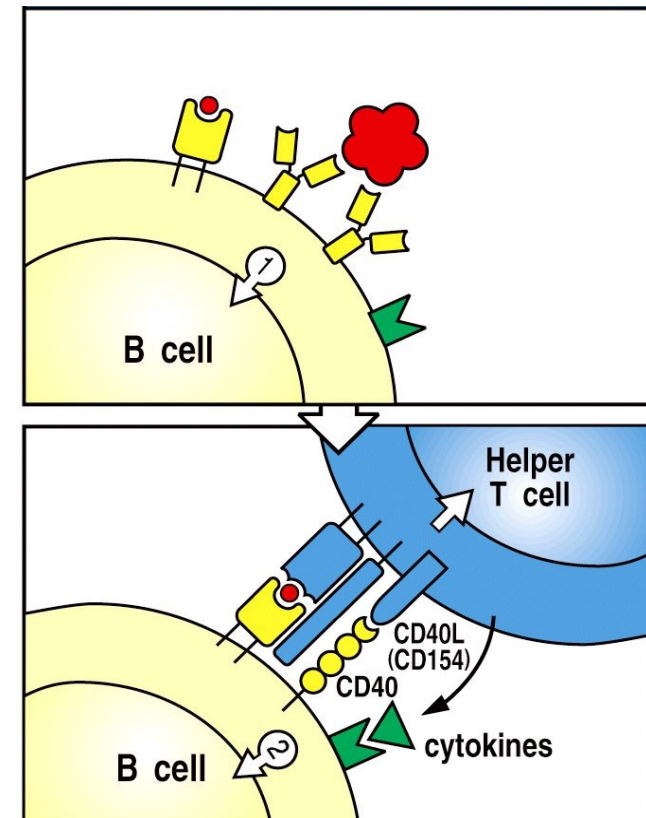
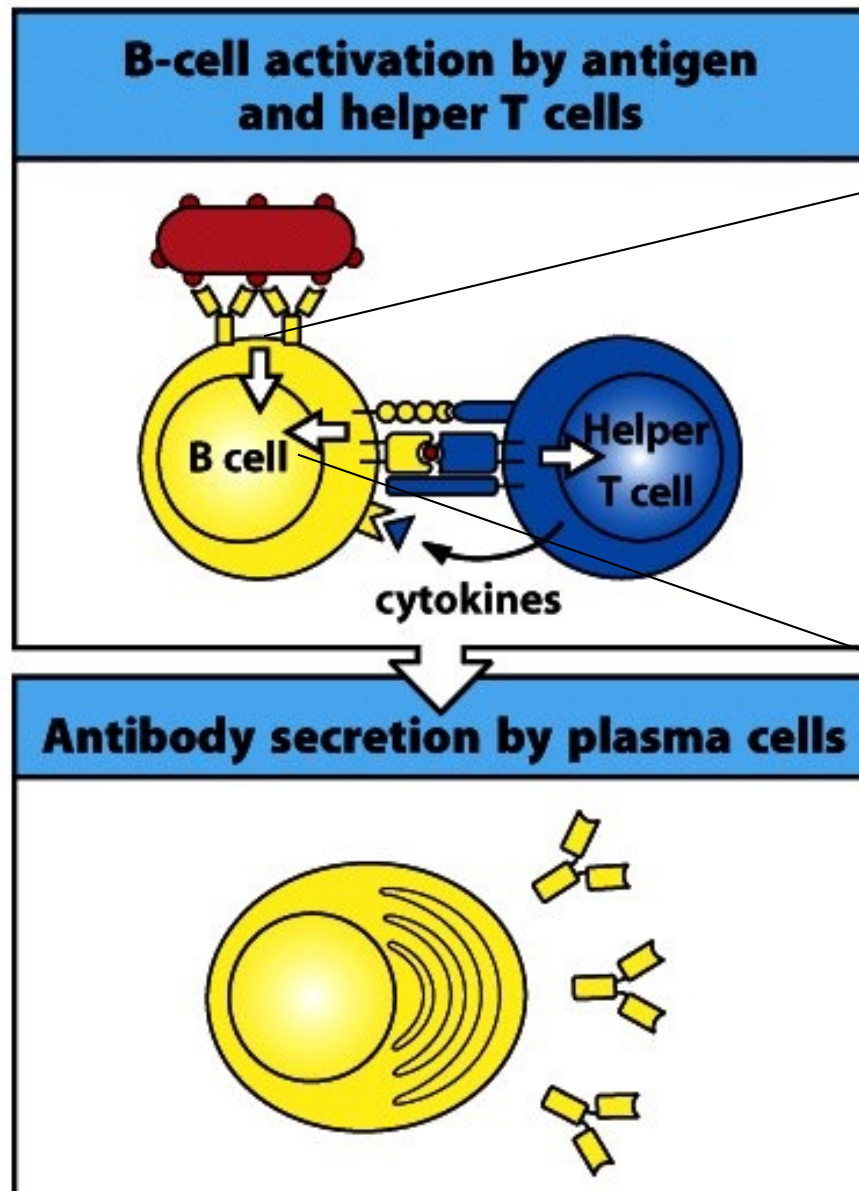
The Initial Stages of Signal Transduction by an Activated B Cell Receptor



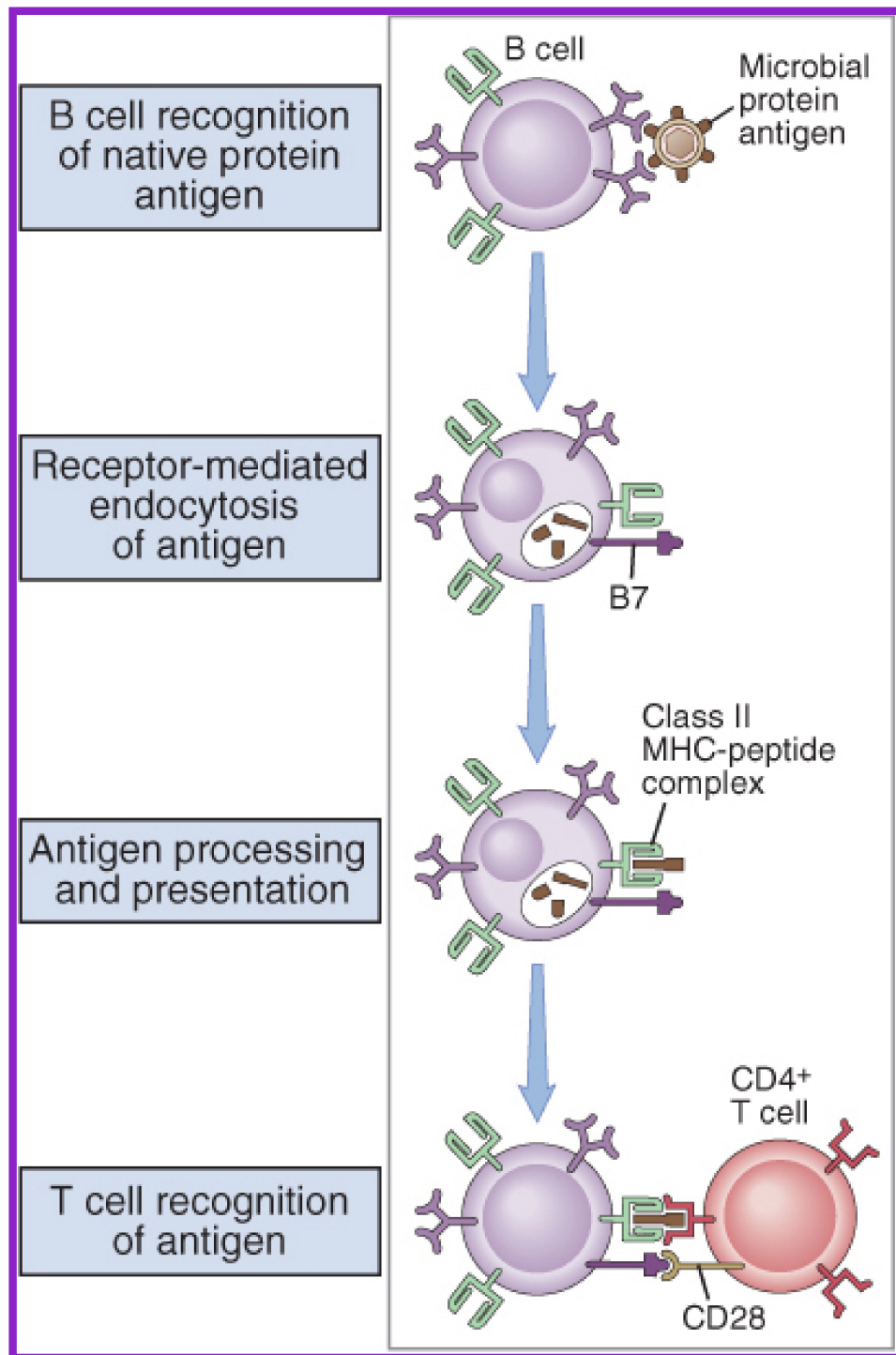
Antigen receptor-mediated signal transduction in B cells



B cell activation to a protein antigen requires T cell help



two signals are required for full B cell activation!



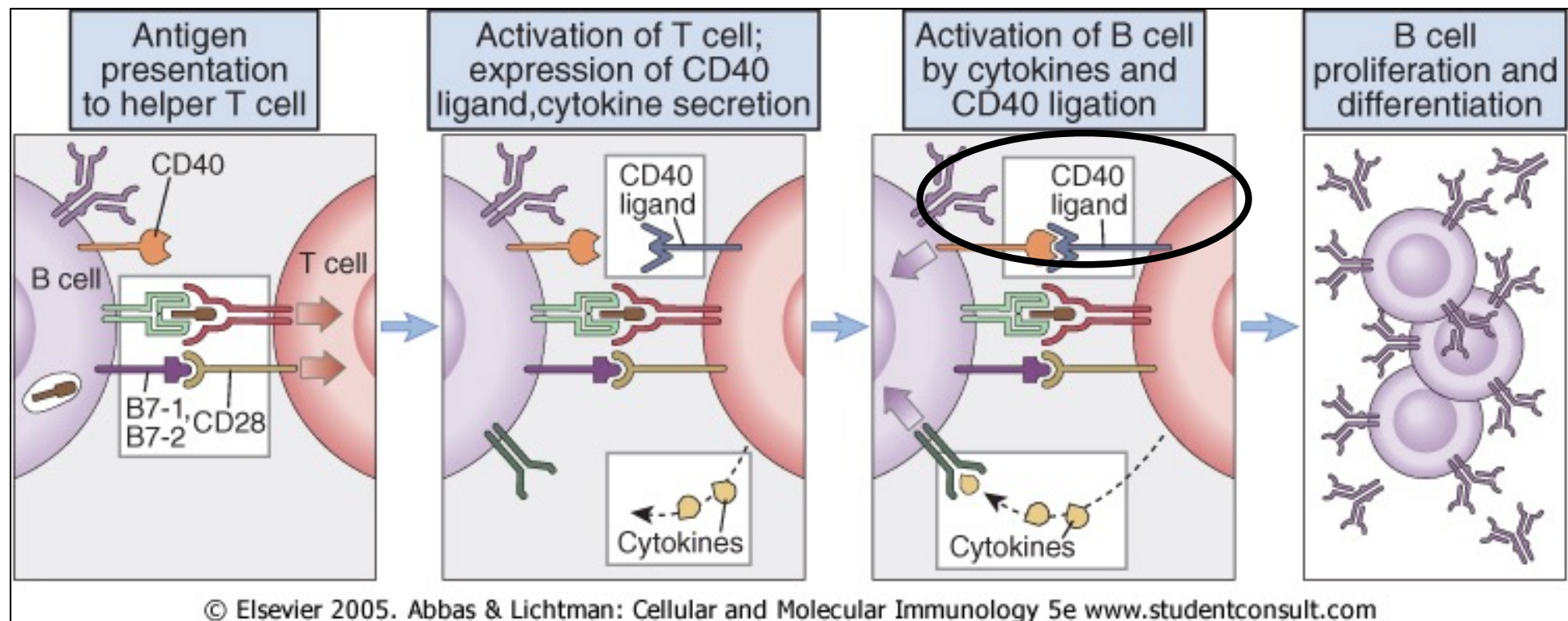
**B cells present antigen
to CD4+ helper T cells..**

30-40 minutes

1-6 hours

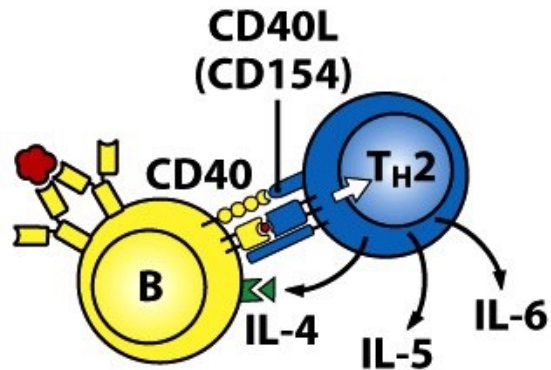
**... to start a dialogue
leading to a coordinate
activation of both cells**

Helper T cells provide the second signal to the B cell

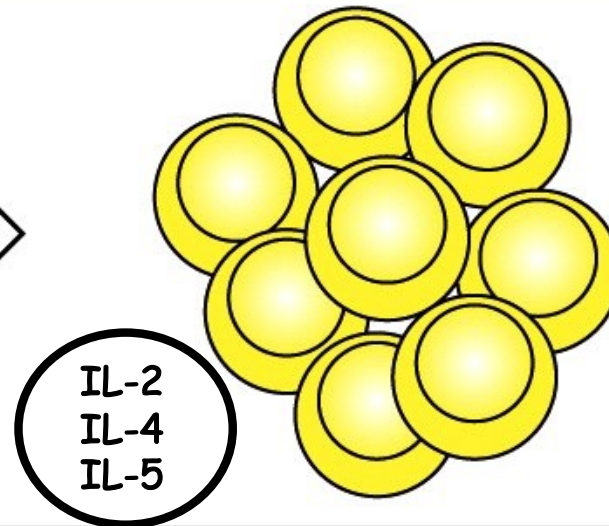


CD40 signaling and cytokines promote B lymphocyte proliferation

Antigen recognition induces expression of effector molecules by the T cell, which activates the B cell



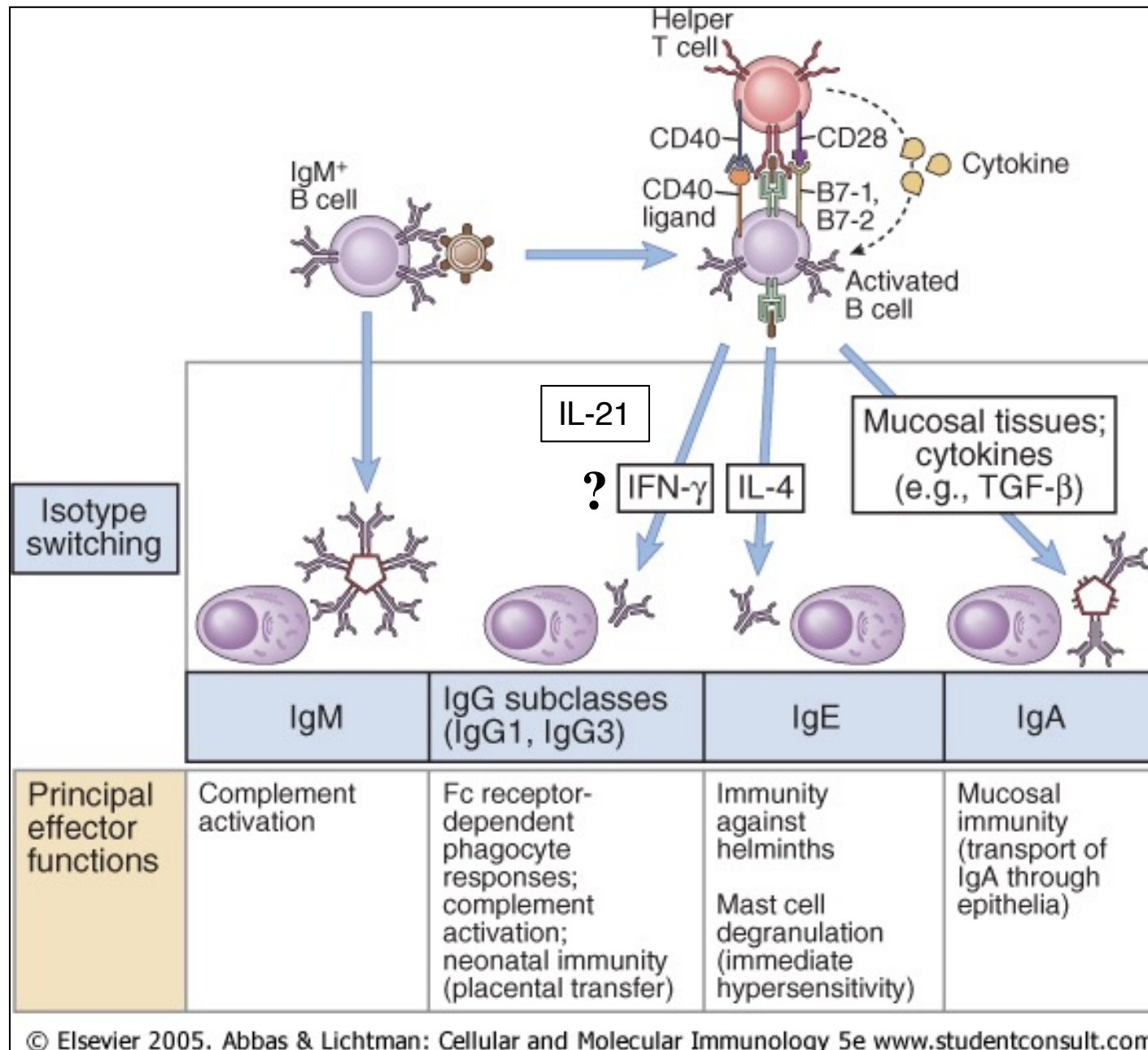
B-cell proliferation



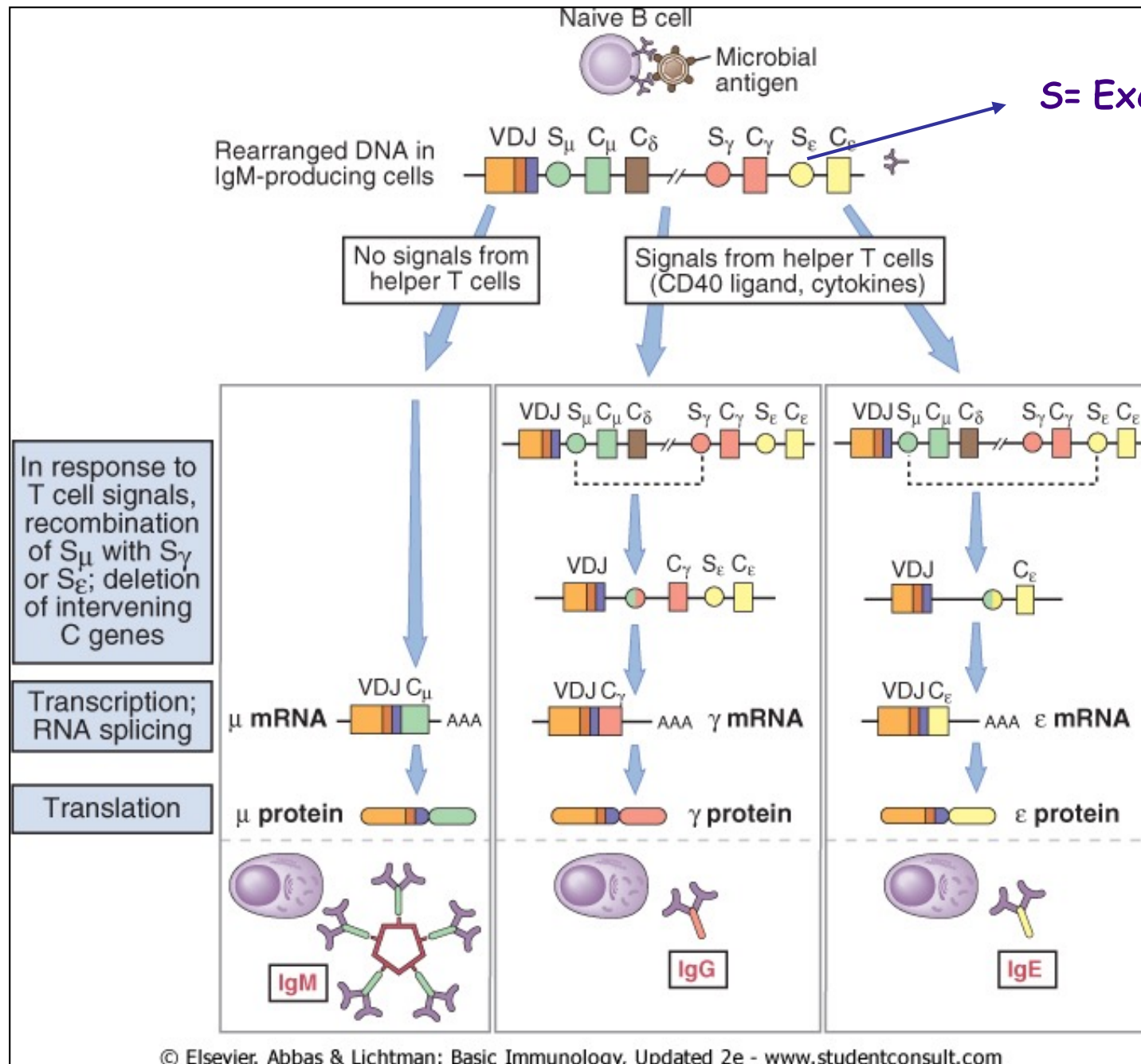
CYTOKINES PLAY TWO MAIN FUNCTIONS IN THE ANTIBODY RESPONSE

- They provide amplification circuits, enhancing B lymphocyte proliferation
- They regulate isotype switching

Isotype switching of immunoglobulin heavy chains



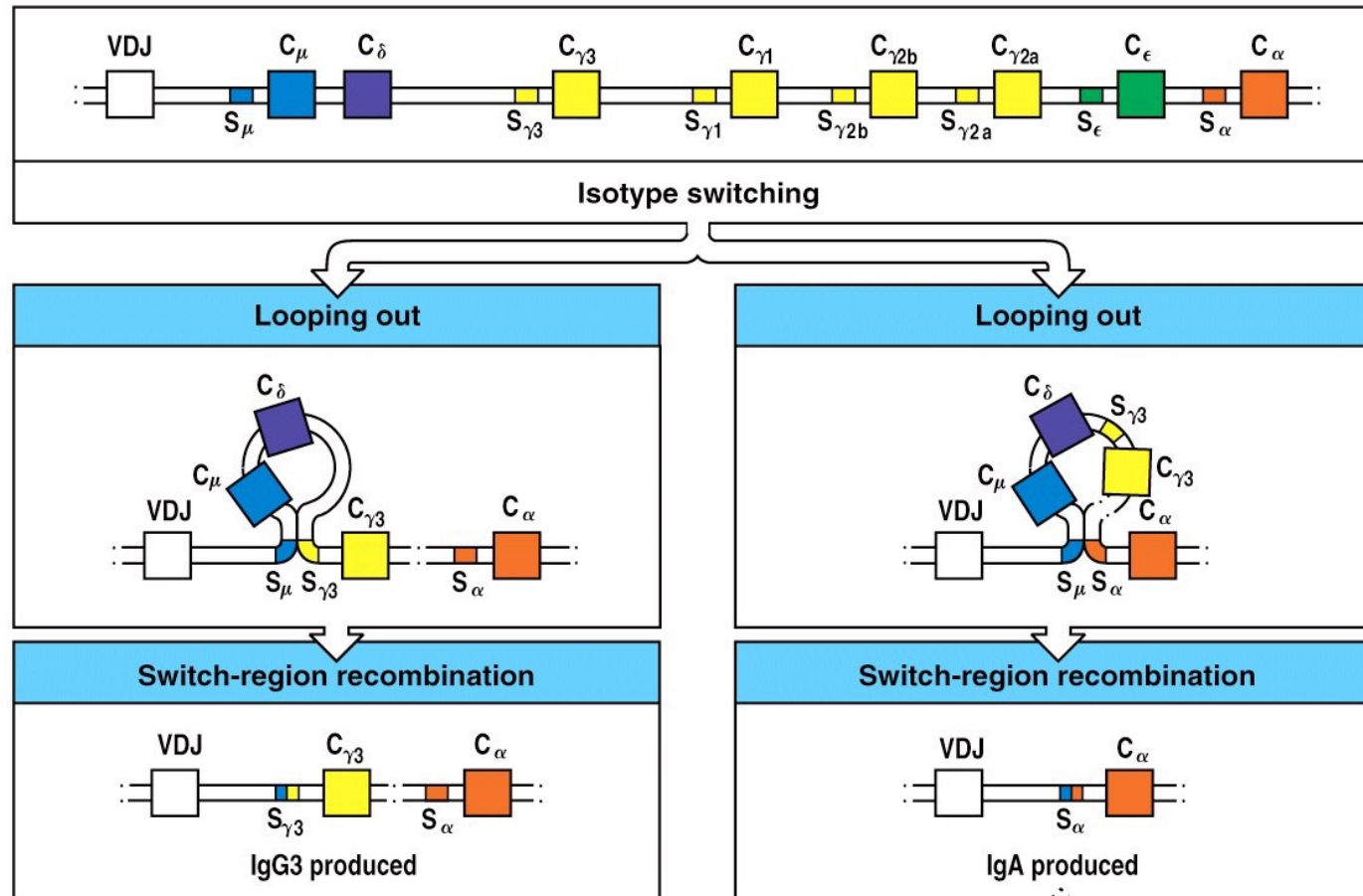
Isotype switching: recombination of heavy chain C genes



S = Exchange sequences

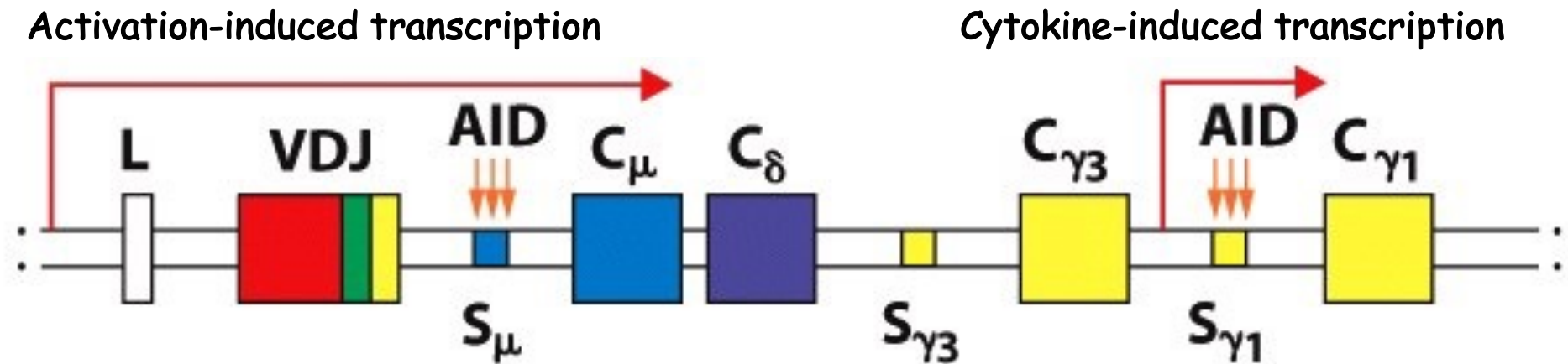
Isotype switching is regulated by recombination events promoted by exchange sequences (S)

Isotype switch enables production of different Ig classes



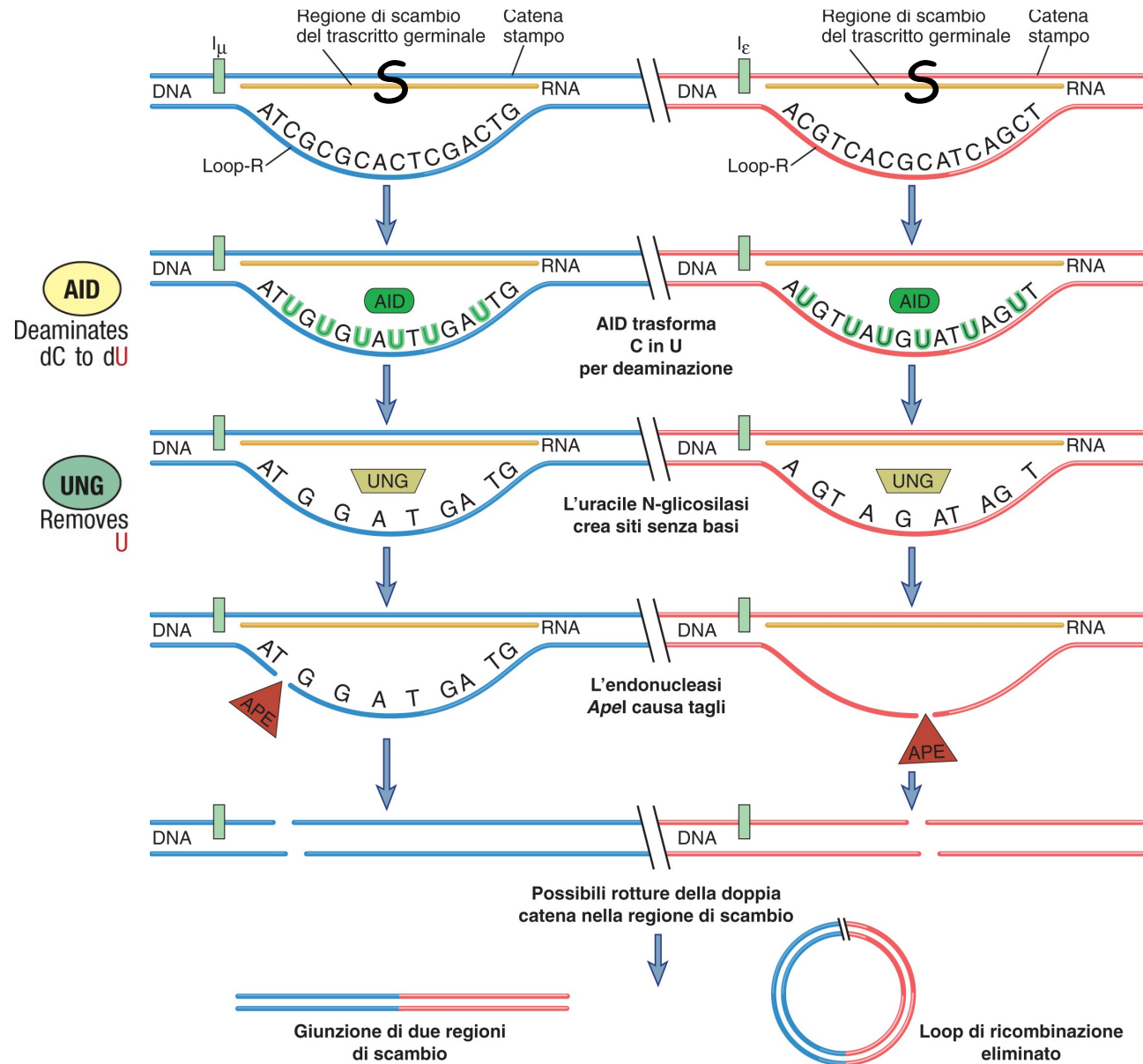
Switch recombination differs in respect to VDJ recombination: it happens after antigen stimulation in secondary lymphoid organs; it uses different flanking region and different recombination enzymes; it is dependent on helper T cells

The S regions (1-10kb) contain numerous tandemly repeated CG-rich DNA sequences and are found upstream of every C gene except the C δ gene



Transcription makes the S regions accessible to the enzyme AID (Activation Induced Deaminase)

AID MECHANISM OF ACTION (Activation-Induced Deaminase)



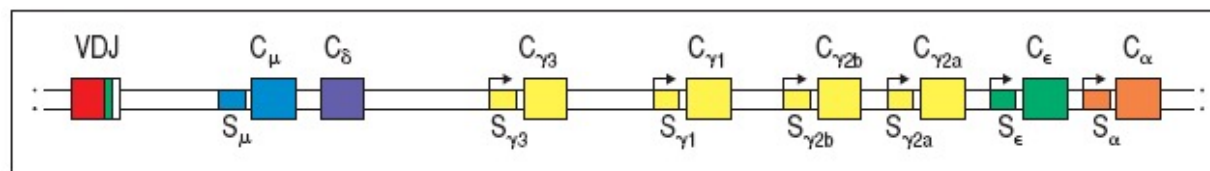
Transcription begins upstream of the S region and single-stranded DNA loops are formed at which **AID** can act.

AID catalyzes the deamination of cytosine to uracil.

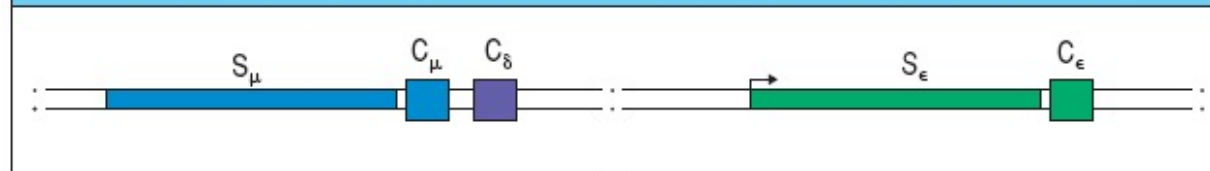
UNG (Uracil N-Glycosylase) removes uracil, generating sites devoid of nitrogenous bases..

.. recognized by the **APE** endonuclease which generates cuts

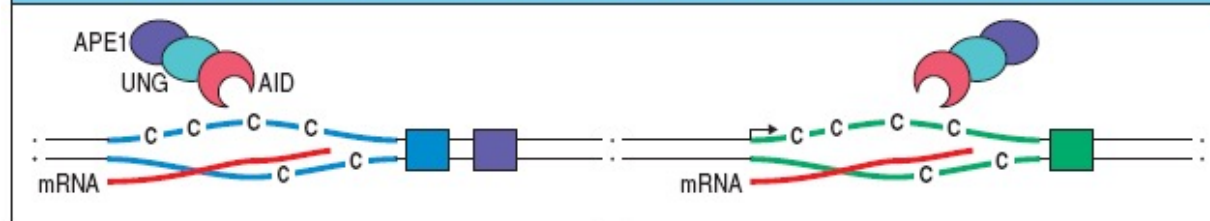
After the polymerase **AID**, **UNG** and **APE** also create cuts on the second strand



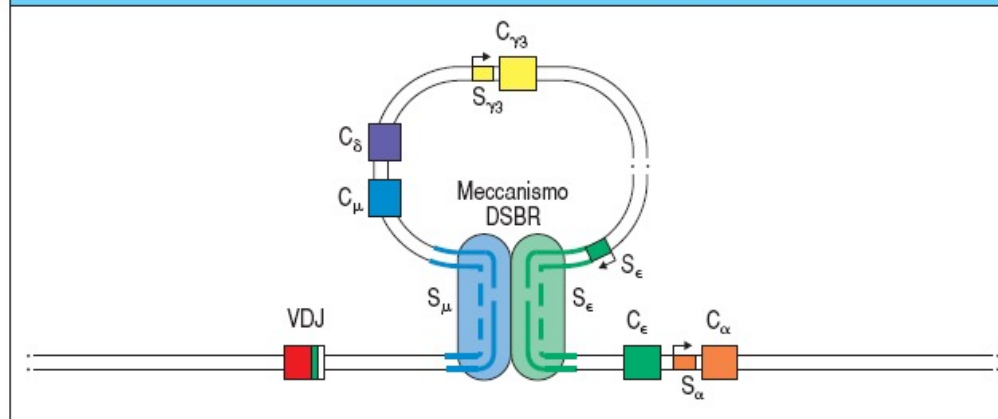
La trascrizione nella regione di scambio è iniziata dall'attivazione del promotore a monte



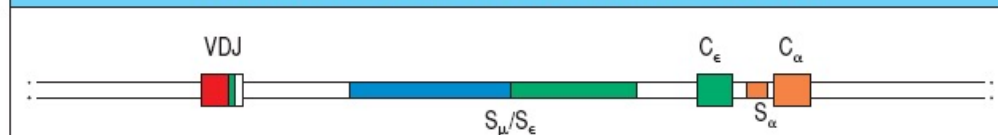
AID, UNG, e APE1 introducono intaccature in gruppi su entrambi i filamenti di DNA



Il meccanismo DSBR unisce le due regioni di scambio e taglia le sequenze interposte



La regione costante selezionata è ora posta vicino alla regione VDJ



MOLECULAR MECHANISMS OF ISOTYPE EXCHANGE

Further rearrangement may occur

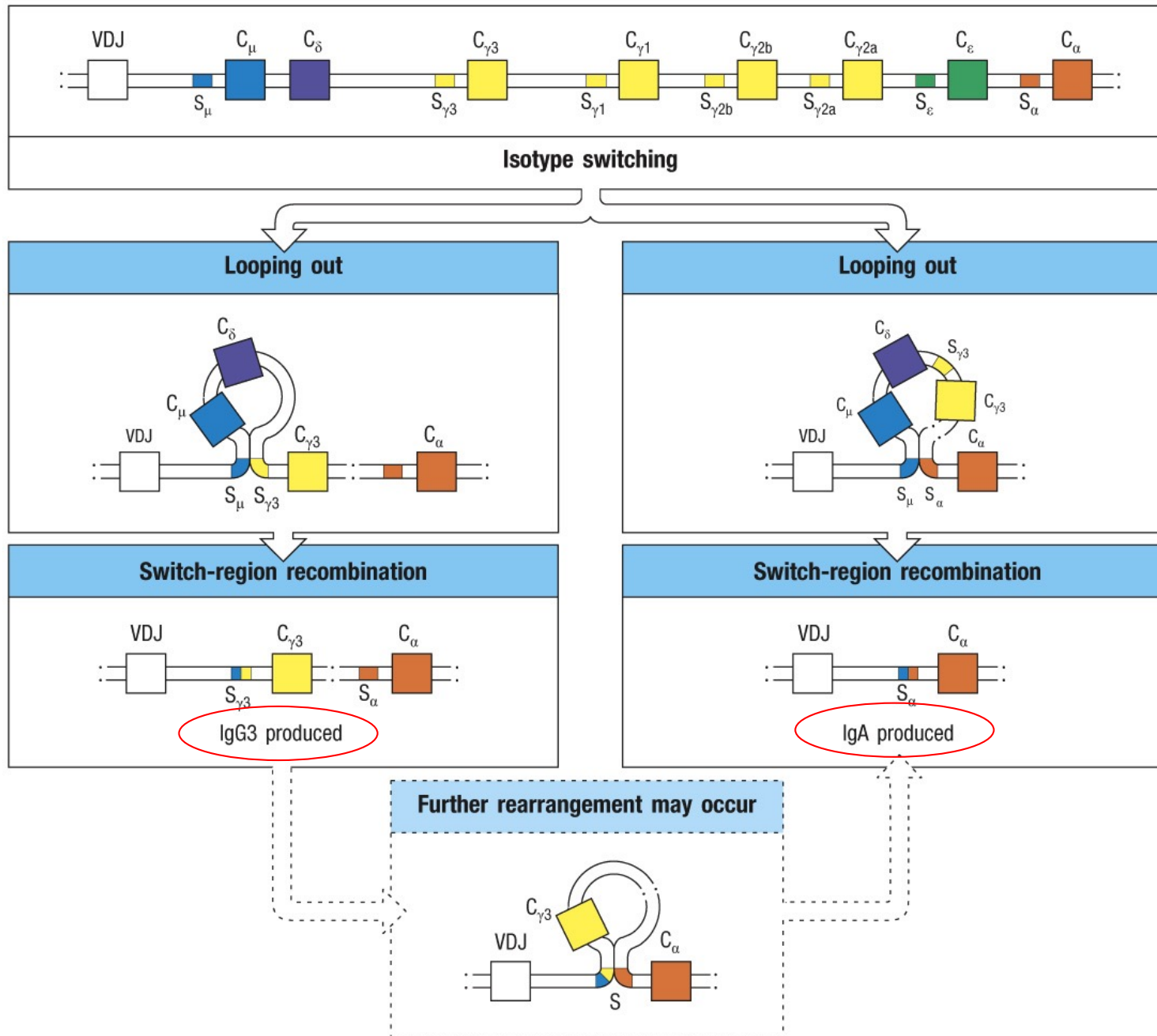
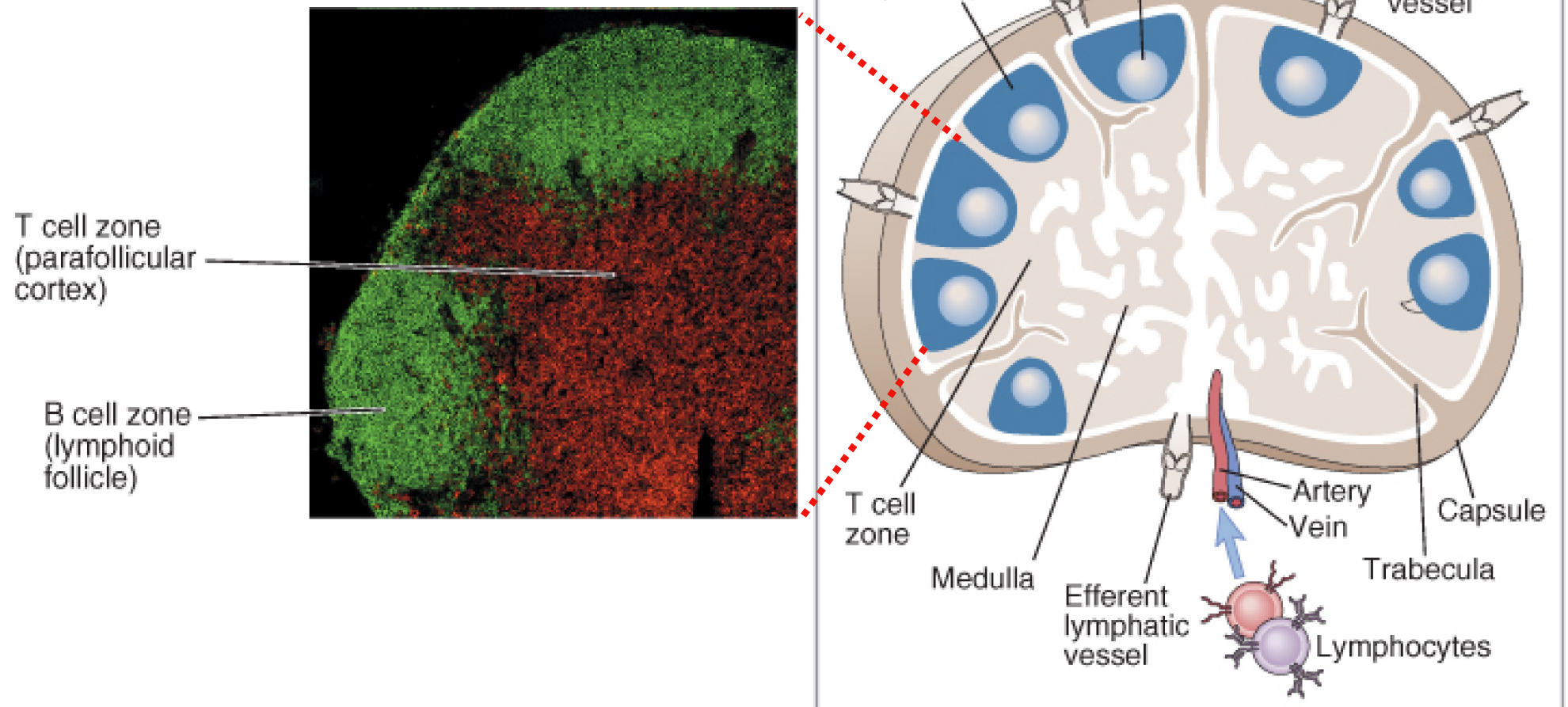


Figure 2.2 Case Studies in Immunology, 7th ed. (© Garland Science 2016)

Where and how B cells and T cells specific for different region of the same protein antigens find one another?

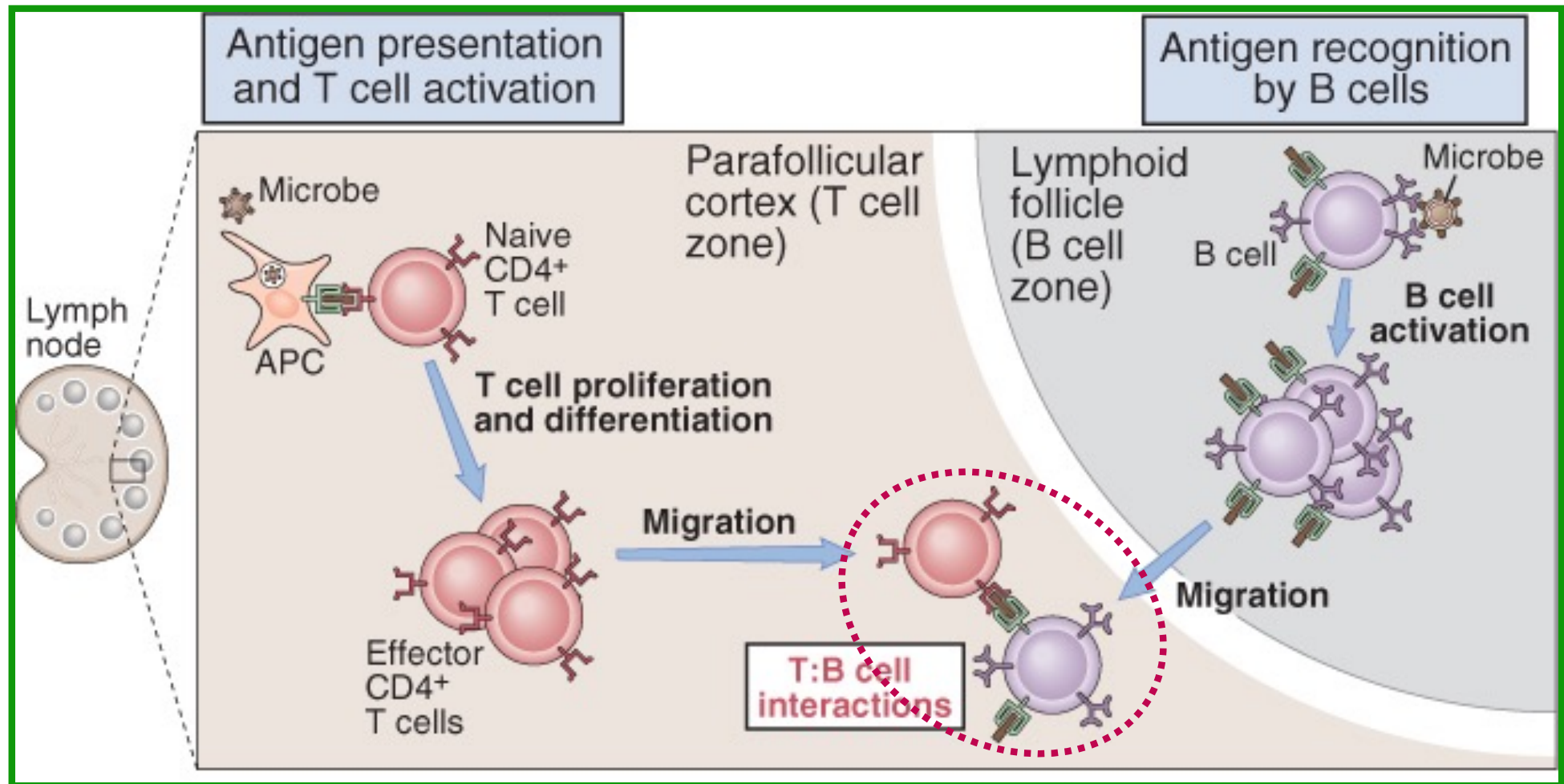
Lymph Node - Where it Happens

Organization of a lymph node:



- **CCR7** is the receptor expressed by T cells and binds the chemokines CCL19 and CCL21
- **CXCR5** is the receptor expressed by B cells and binds the chemokine CXCL13

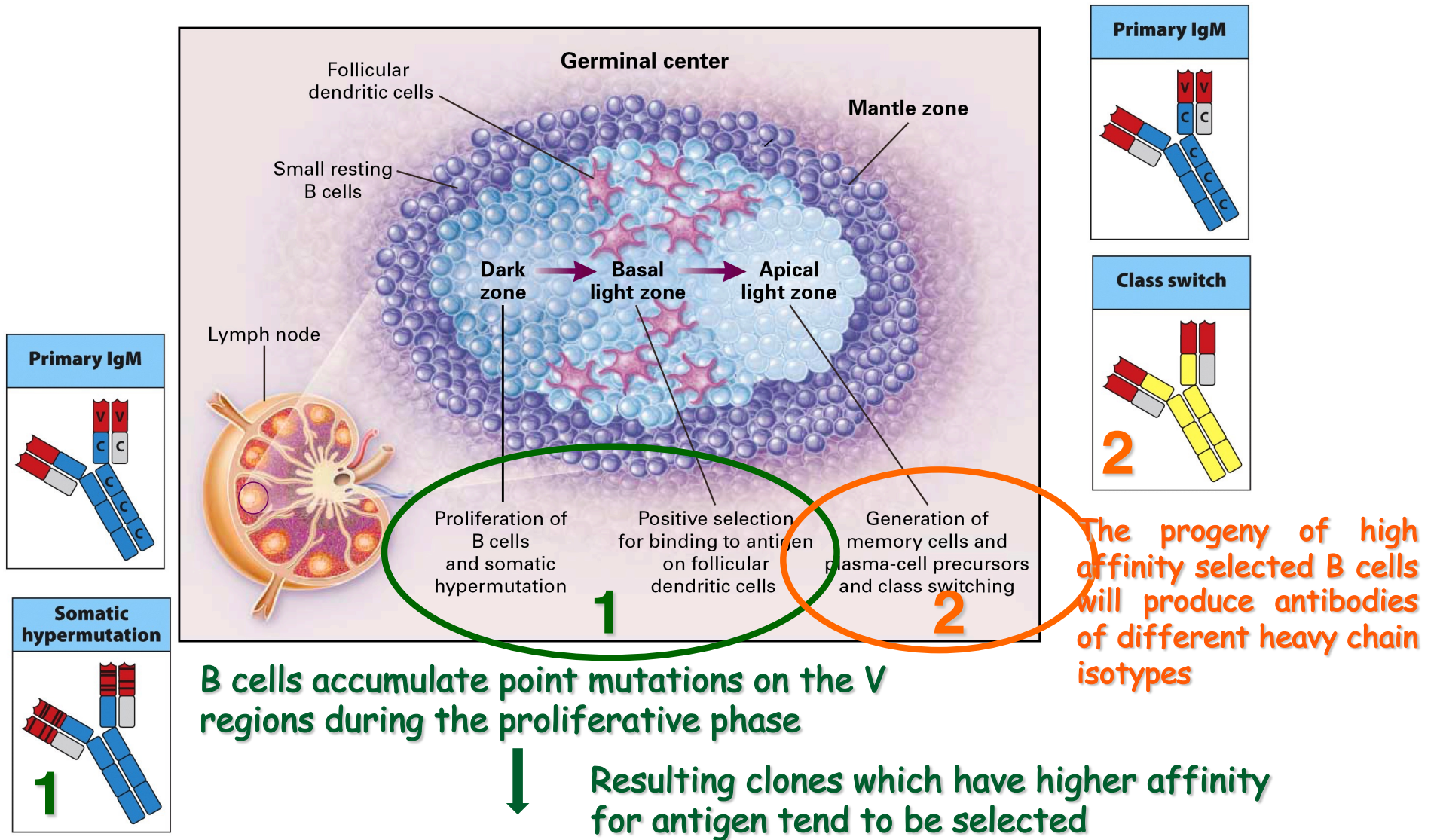
Antigen specific T and B cells meet at the border between the T-dependent and the B-dependent areas



This initial T and B cell interaction at the border area results in the production of low level of low affinity antibodies

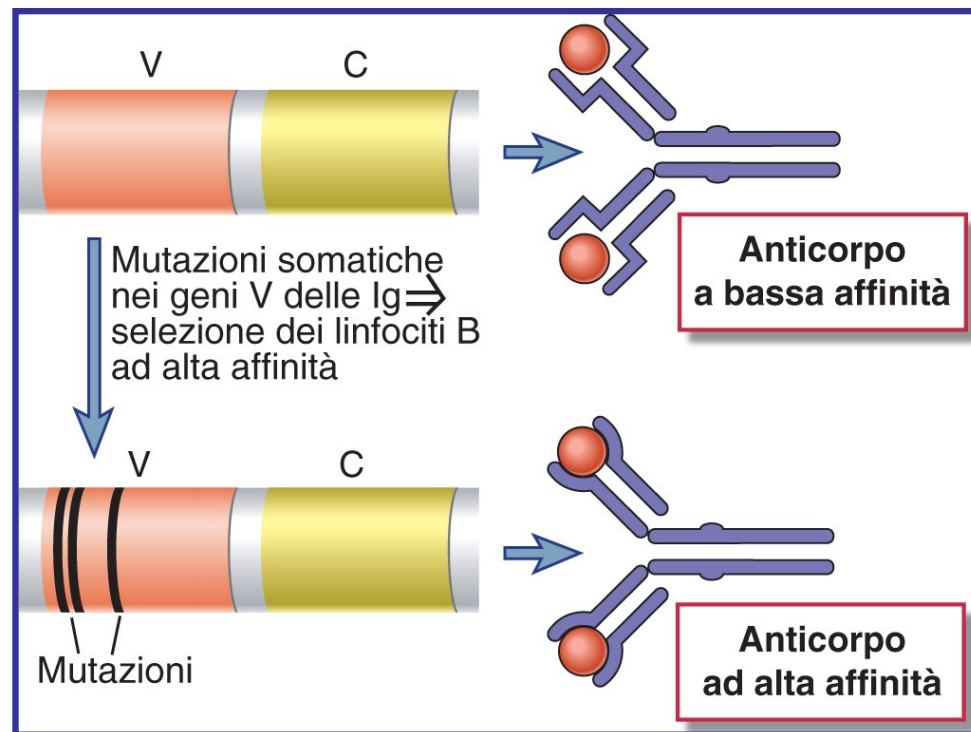
Germinal centers (GC) and the repertoire shaping

Somatic hypermutation/affinity maturation and class switching

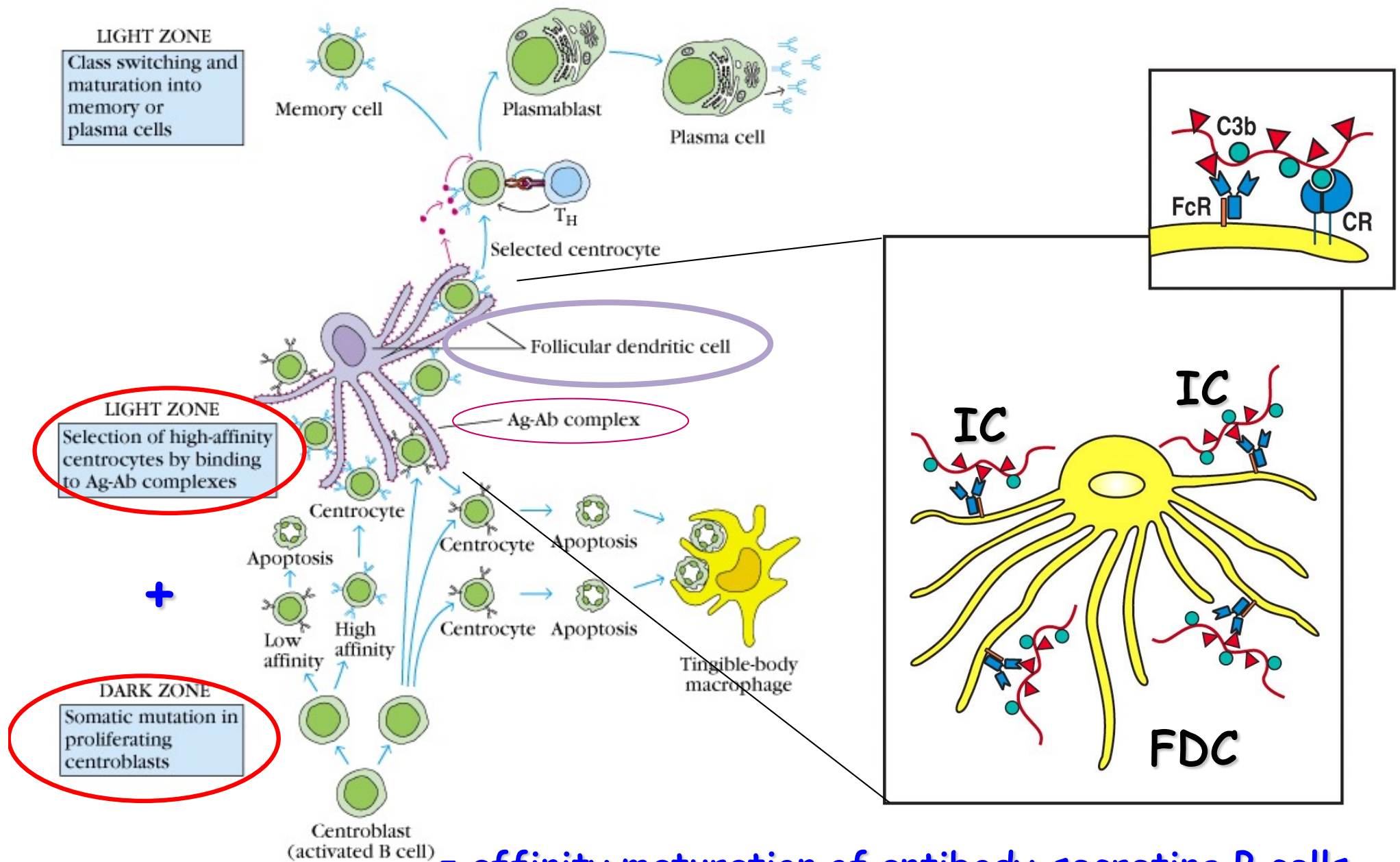


L'IPERMUTAZIONE SOMATICA

- Avviene durante le divisioni mitotiche del linfocita B attivato dall'antigene (centroblasta)
- Le mutazioni non sono distribuite in maniera casuale, ma concentrate nei geni riarrangiati V(D)J
- Il risultato è la formazione di centroцитi con recettori dotati di affinità diverse per l'antigene
- Tra questi verranno selezionati quelli più affini per l'antigene (maturazione dell'affinità)

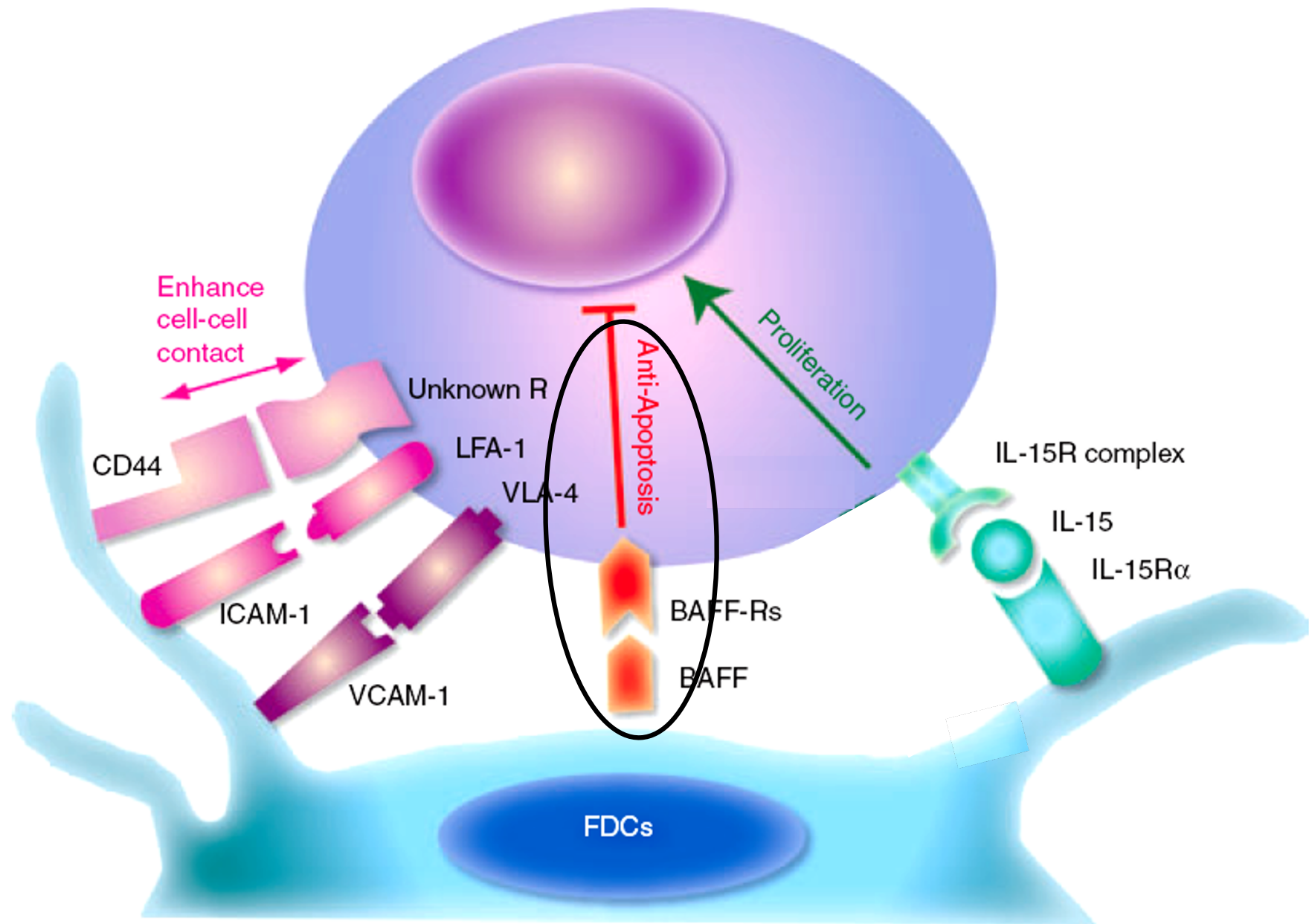


Selection of high-affinity B cells in germinal center

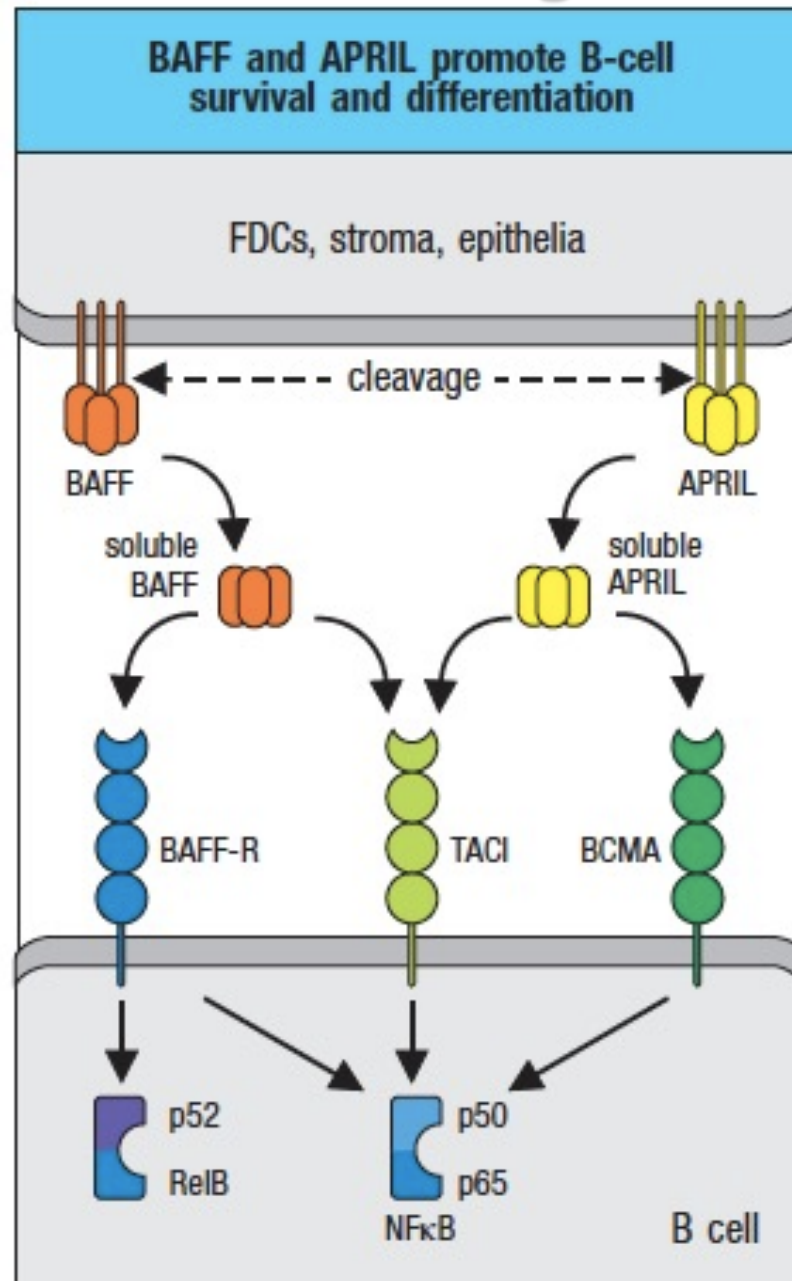


= affinity maturation of antibody-secreting B cells

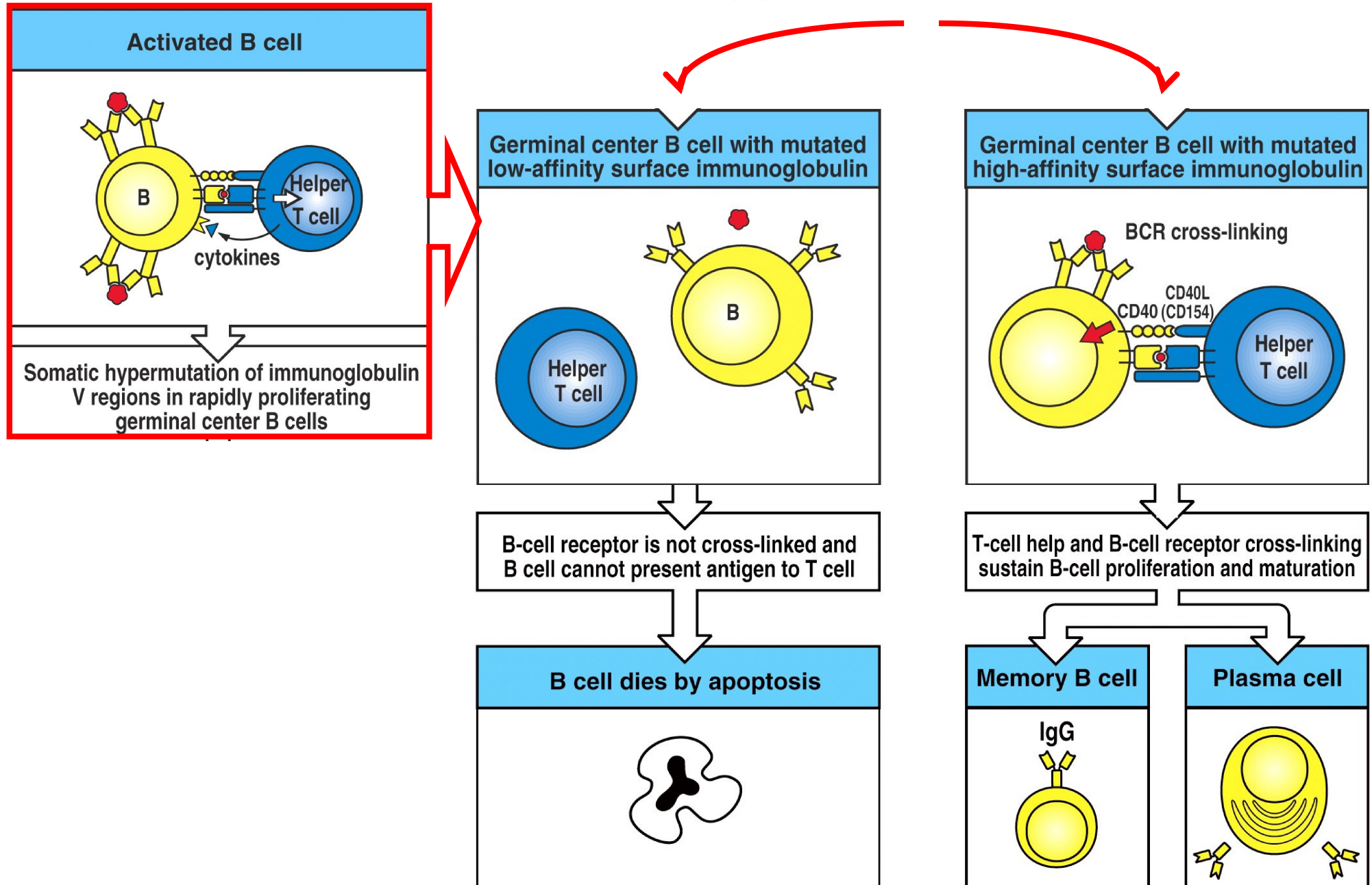
Follicular dendritic cells (FDCs) promote the proliferation and survival of selected B lymphocytes



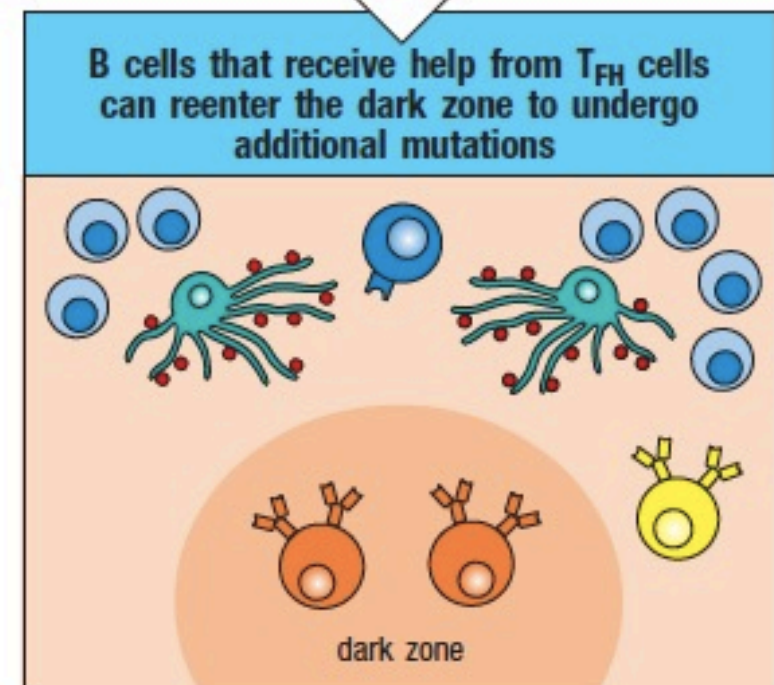
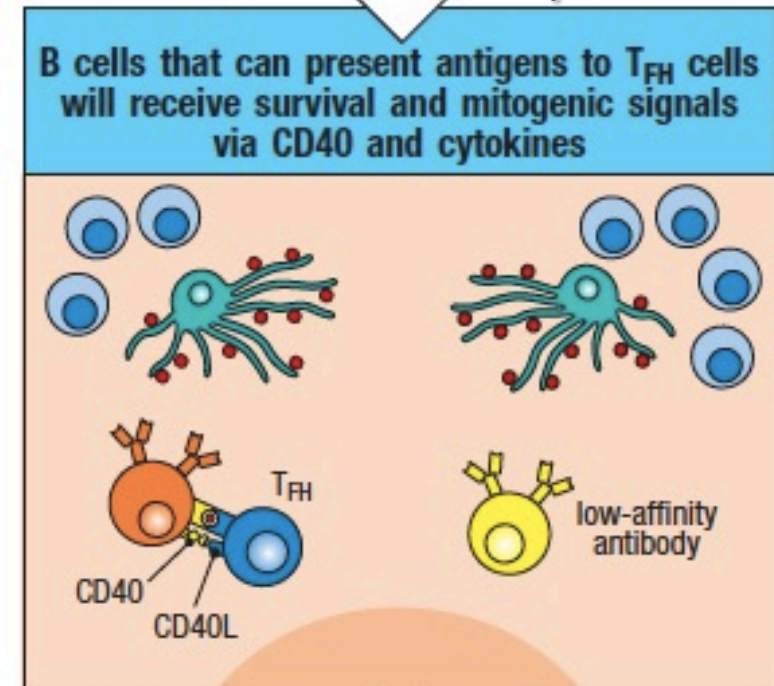
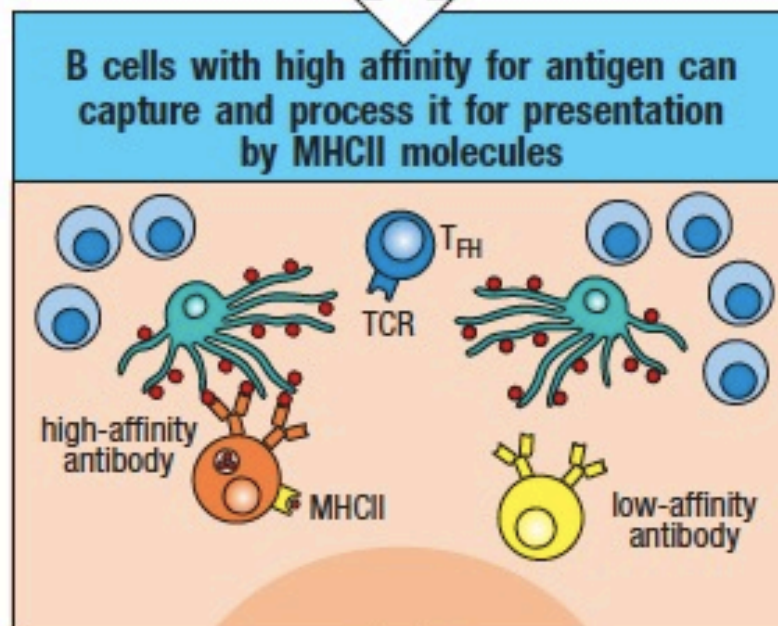
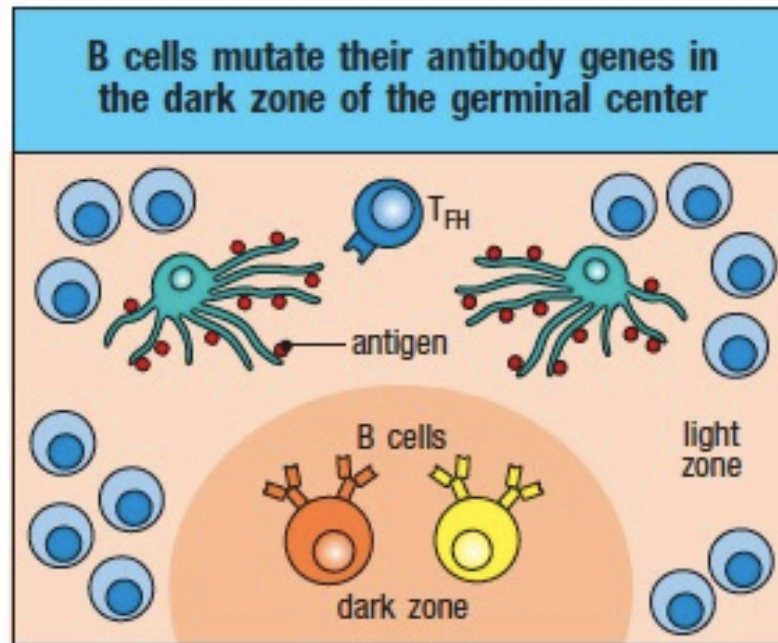
Follicular dendritic cells provide survival signals



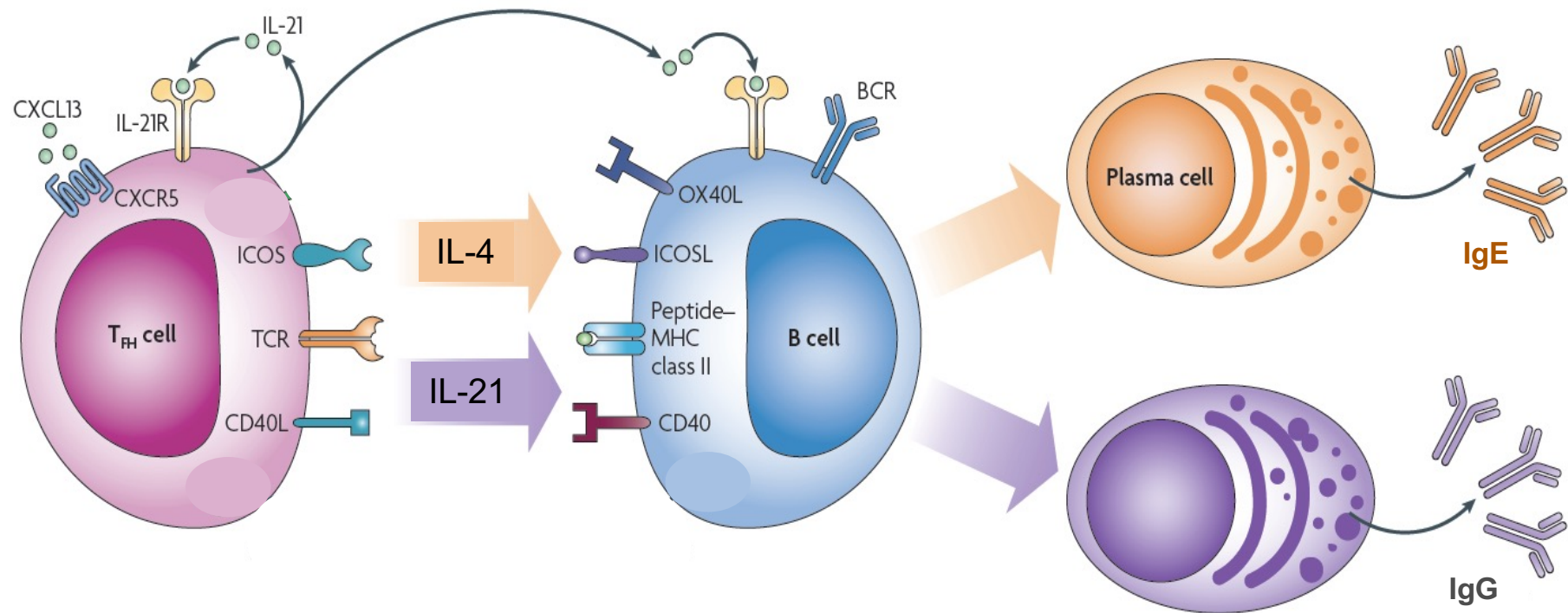
B cells with a mutated BCR need T cell help to survive and differentiate



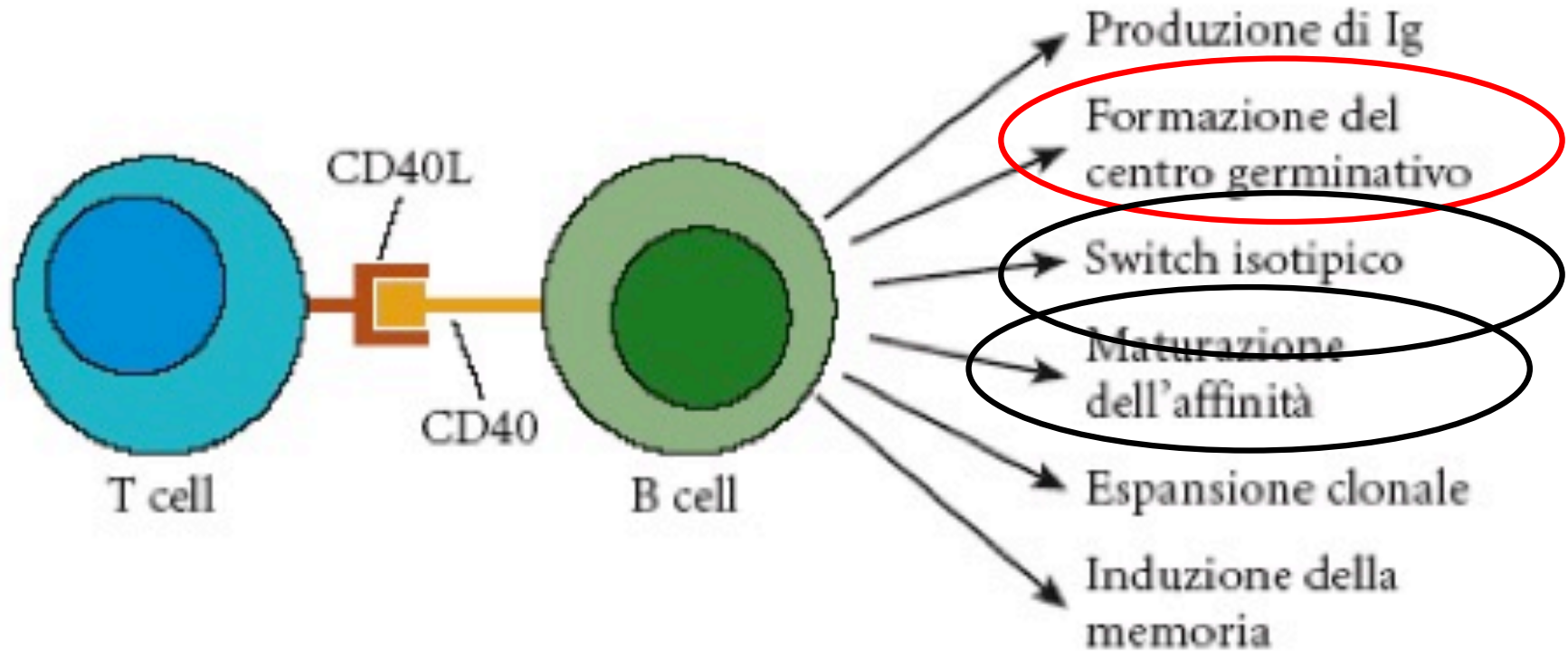
The maturation of affinity is the result of repeated mutation and selection events



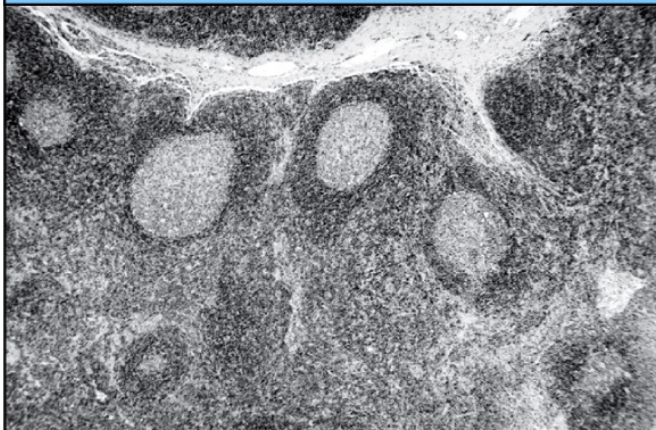
Isotype switching begins in extrafollicular foci and continues in the GCs by T_{FH}



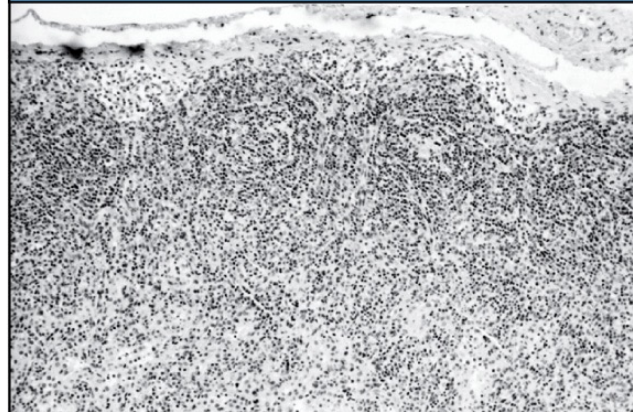
FUNCTIONAL CONSEQUENCES OF THE SIGNAL TRANSDUCED IN B LYMPHOCYTES BY CD40



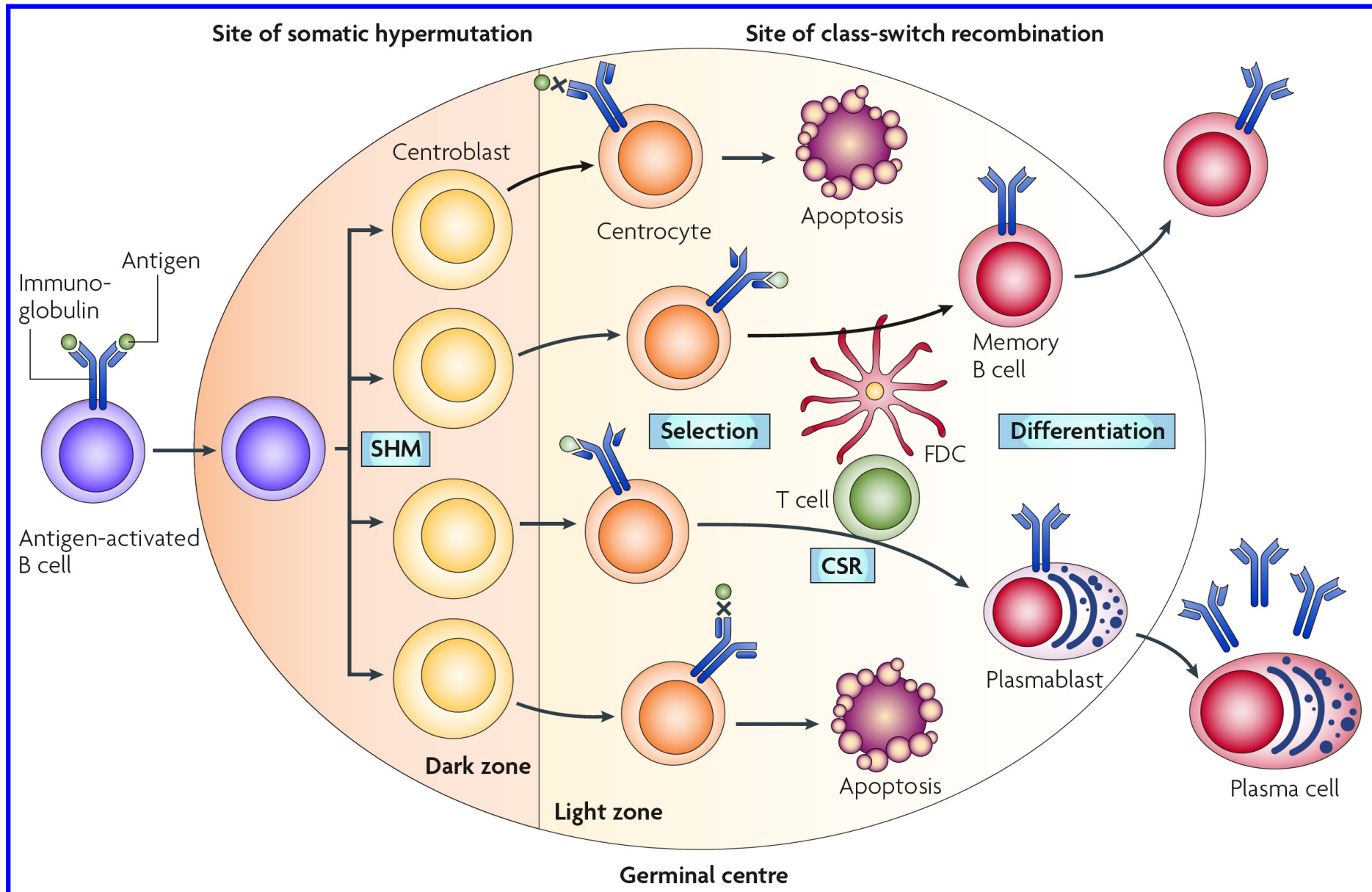
Lymph node with germinal centers



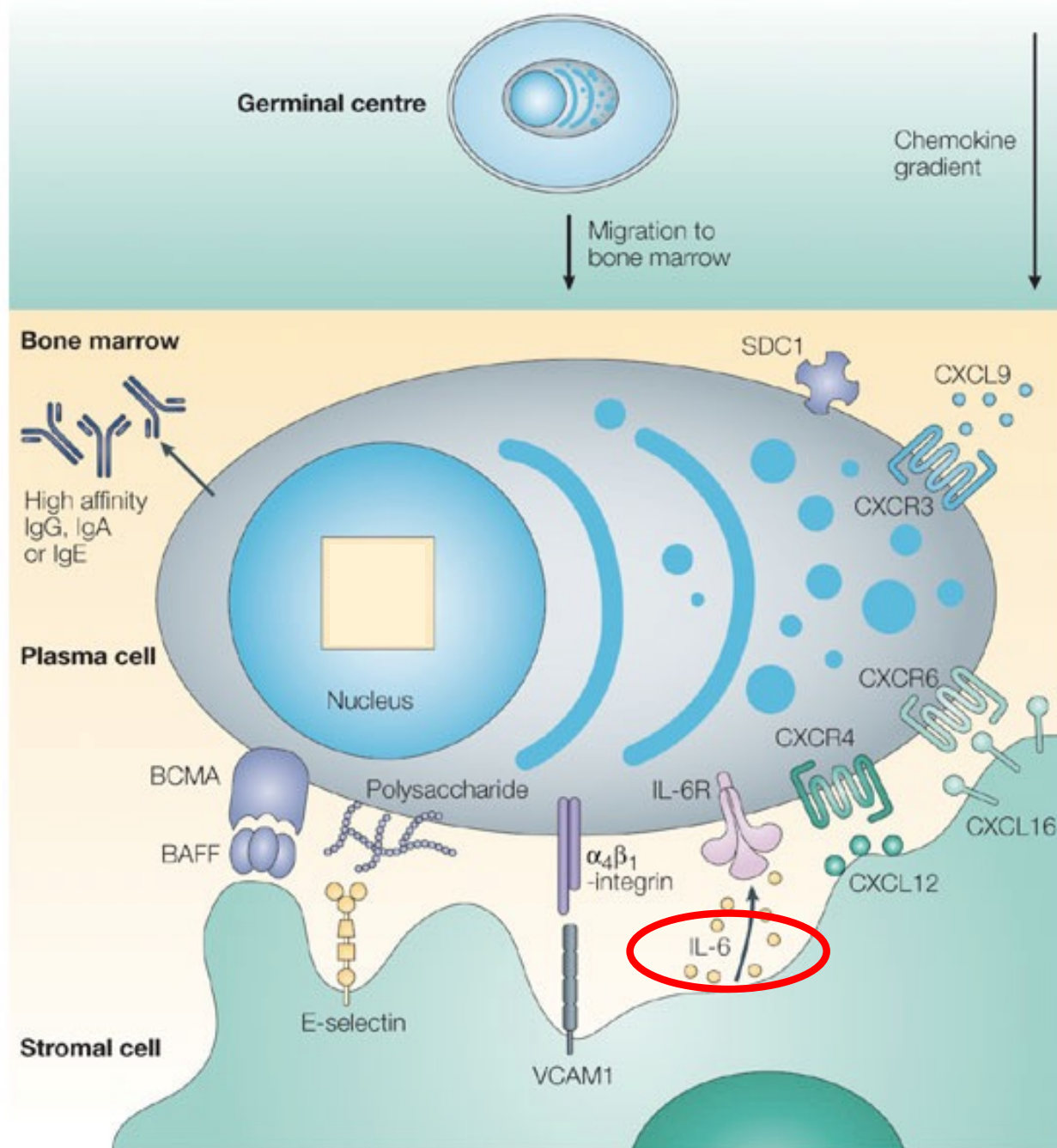
Lymph node from a patient with CD40L deficiency (no germinal centers)



The germinal center reaction

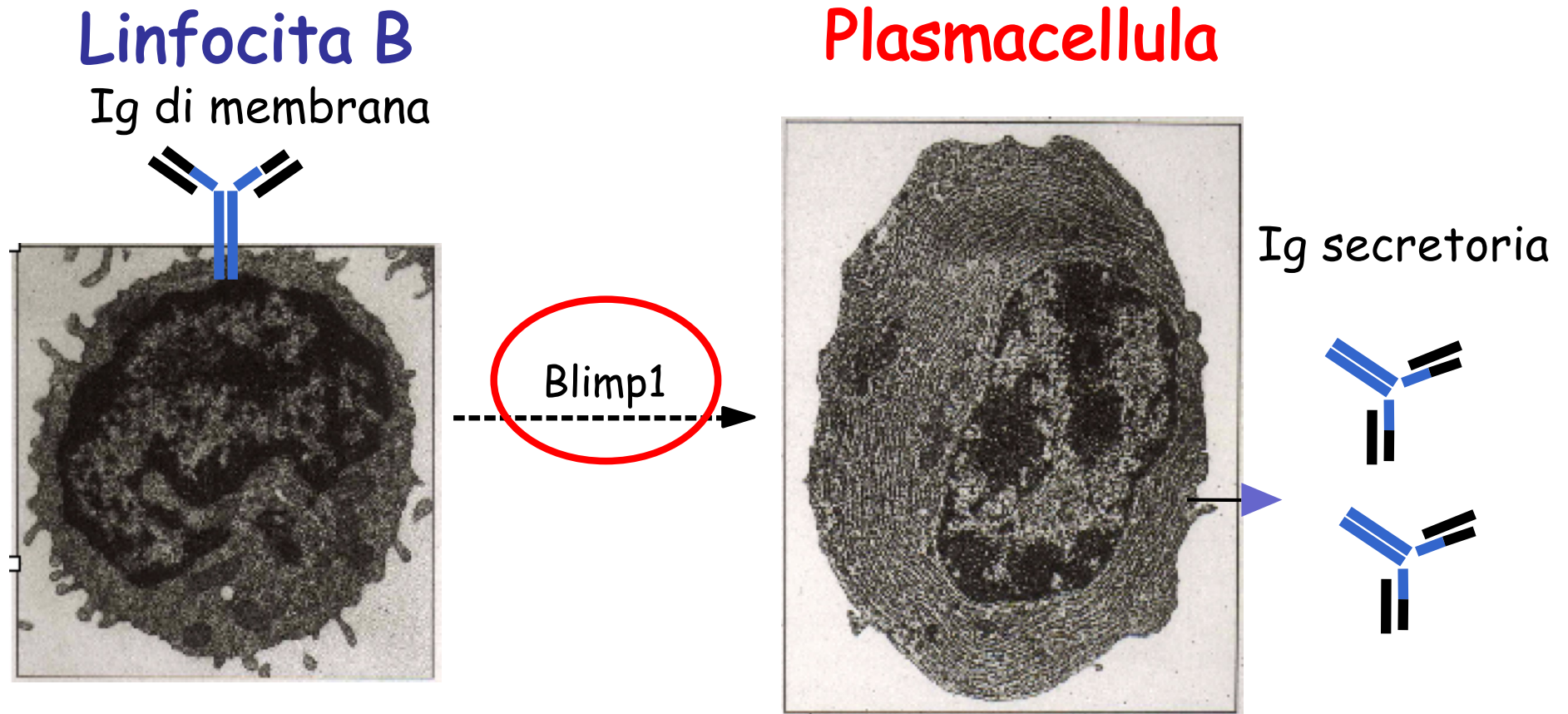


Long-lived plasma cells in bone marrow





Plasma cells lose *CXCR5* expression and acquire *CXCR4* expression. Guided by chemokines such as *CXCL12*, they reach the bone marrow, where they can live for months or years

Il fattore trascrizionale Blimp1 promuove il differenziamento in plasmacellule

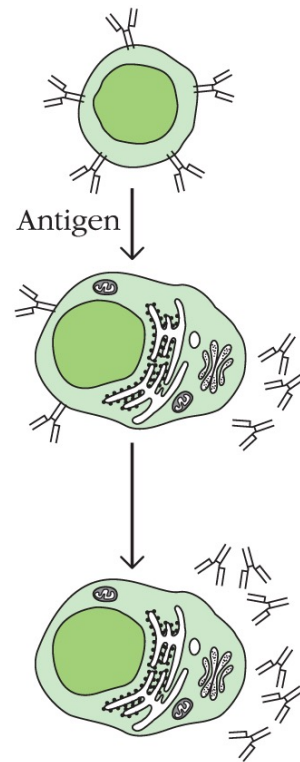


BLIMP1 = B-lymphocyte-induced maturation protein 1

Caratteristiche delle plasmacellule

Proprietà						
Intrinseche				Inducibili		
Cellula della linea B	Ig di superficie	MHC di classe II di superficie	Secrezioni ad alta velocità di Ig	Crescita	Ipermutazione somatica	Switch isotipico
 Cellula B a riposo	Sì	Sì	No	Sì	Sì	Sì
 Plasmacellula	No	No	Sì	No	No	No

T-Dependent B-Cell Responses: Types of Antibody-Secreting Cells



Naïve B cells. Bear cell surface IgM. Do not secrete antibody.

Plasmablasts. Differentiated B cells that have begun to secrete antibodies, have not yet lost the capacity to proliferate, and still bear cell surface BCRs.

Plasma cells. Differentiated B cells that can no longer divide, bear little to no cell surface immunoglobulin, and rapidly secrete large numbers of antibody molecules.

Also referred to as antibody forming cells or AFCs

© Macmillan Learning



Match the B-cell type with the correct description.

A: naïve B cell	1: secrete large numbers of antibodies, can no longer proliferate, little to no surface BCR expression
B: plasmablast	2: bear cell surface IgM; do not secrete antibody
C: plasma cell	3: can secrete antibodies, can proliferate, still have surface BCRs



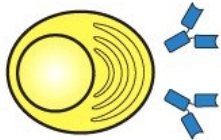
	Intrinsic properties			Inducible by antigen stimulation		
B-lineage cell	Surface Ig	Surface MHC class II	High-rate Ig secretion	Growth	Somatic hyper-mutation	Class switch
 Resting B cell	High	Yes	No	Yes	Yes	Yes
 Plasmablast	High	Yes	Yes	Yes	Unknown	Yes
 Plasma cell	Low	No	Yes	No	No	No

Figure 10.9 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Characteristics of memory B lymphocytes

TABLE 11-6 Comparison of naive and memory B cells		
Property	Naive B cell	Memory B cell
Membrane markers		
Immunoglobulin	IgM, IgD	IgM, IgD(?), IgG, IgA, IgE
Complement receptor	Low	High
Anatomic location	Spleen	Bone marrow, lymph node, spleen
Life span	Short-lived	May be long-lived
Recirculation	Yes	Yes
Receptor affinity	Lower average affinity	Higher average affinity due to affinity maturation*
Adhesion molecules	Low ICAM-1	High ICAM-1
* Affinity maturation results from somatic mutation during proliferation of centroblasts and subsequent antigen selection of centrocytes bearing high-affinity mlg.		

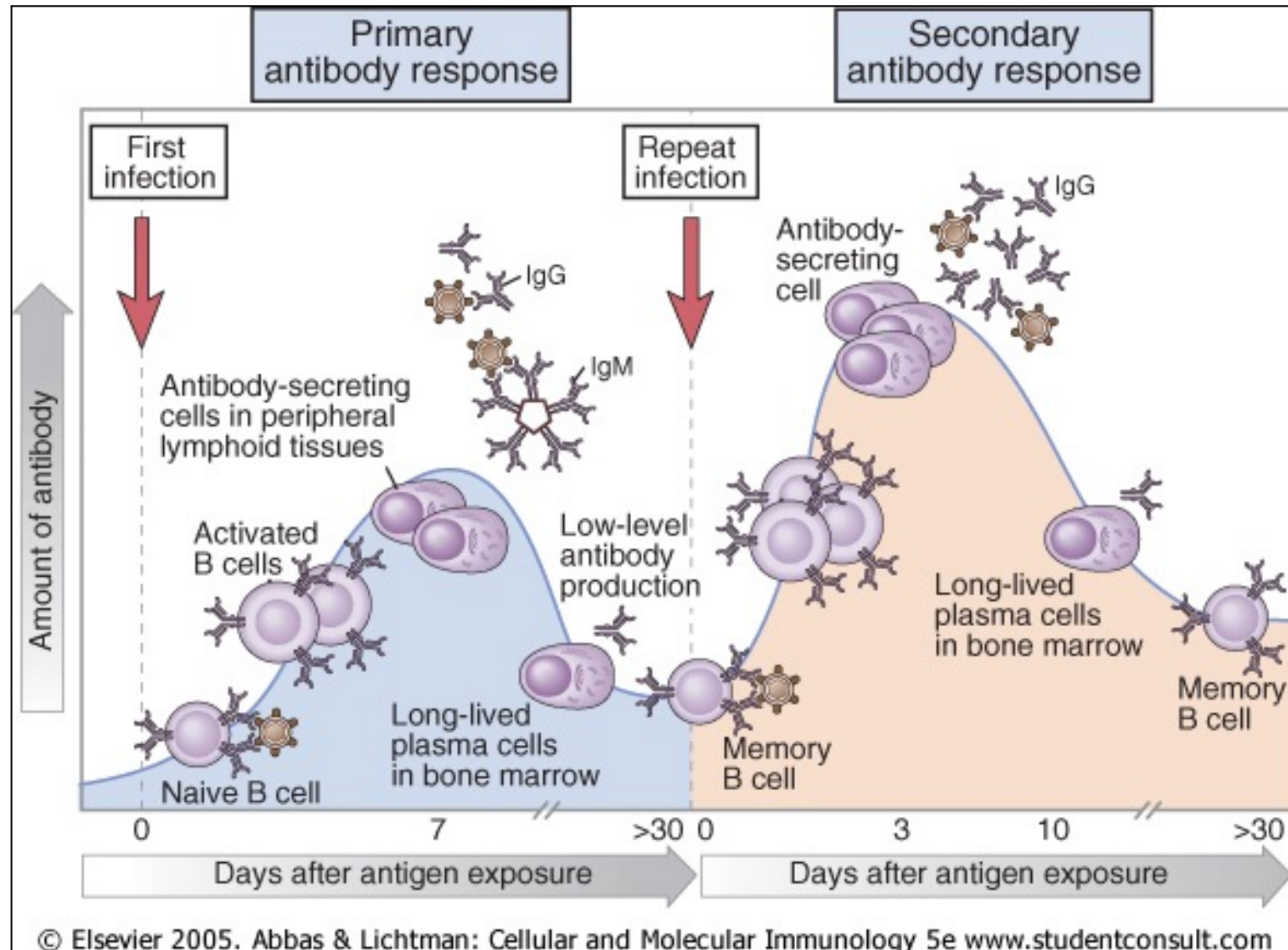
Table 11-6
Kuby IMMUNOLOGY, Sixth Edition
 © 2007 W.H. Freeman and Company

**Threshold for
activation**

HIGH

LOW

Features of primary and secondary antibody responses



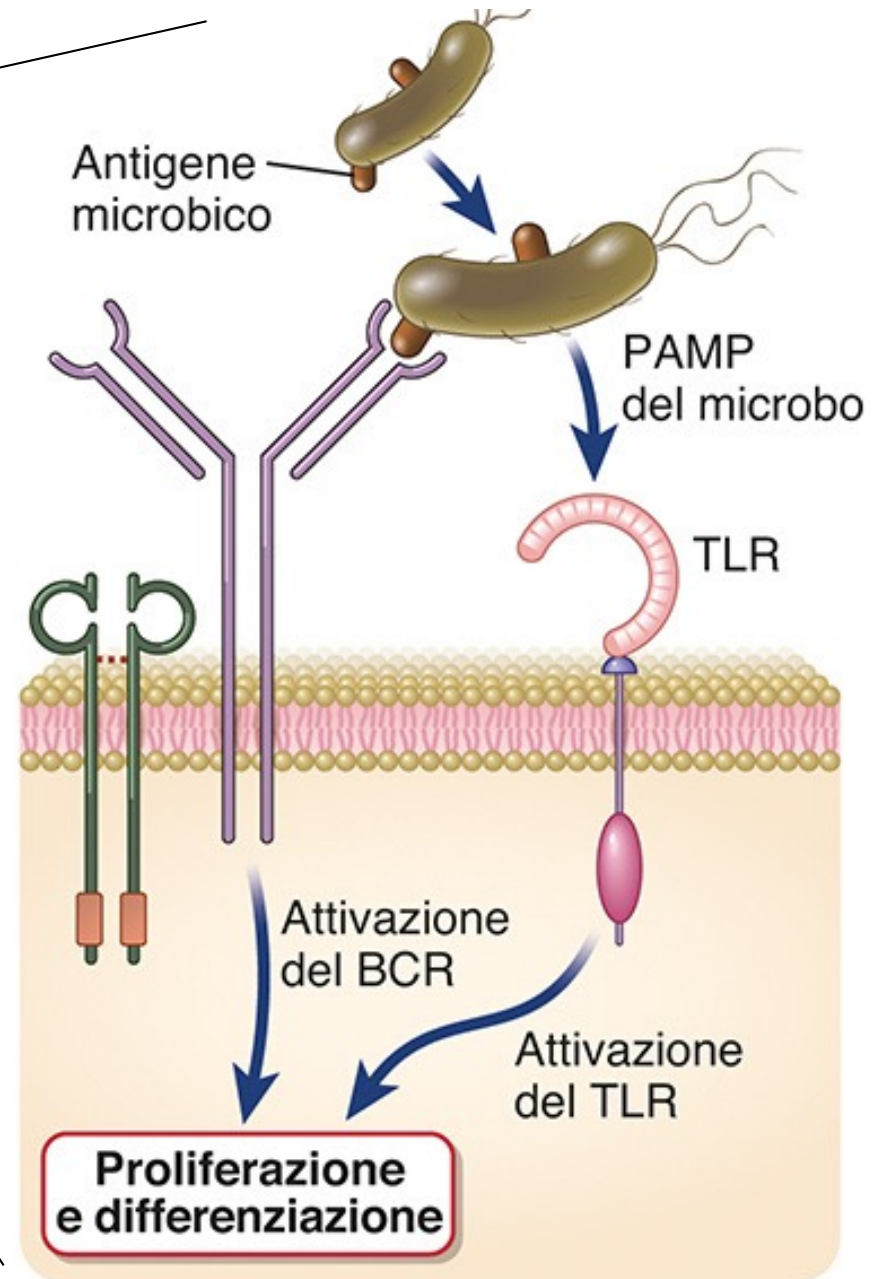
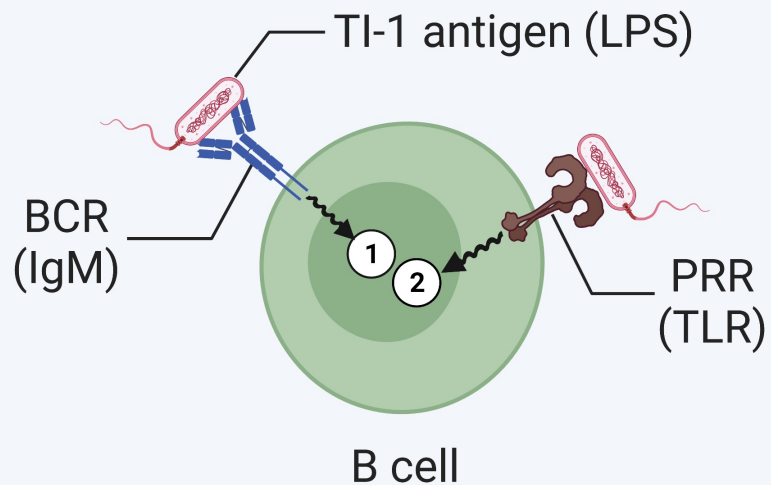
T-Independent B-Cell Responses

- T-independent Ag can stimulate antibody production without the need for T-cell help.
 - Nude mice have a *Foxn1* mutation that results in athymia.
 - They can still respond to T-independent antigens with antibodies.
 - **TI-1 Ag**
 - typically bacterial cell-wall components
 - bind to ty PRRs and BCR on B cells
 - **TI-2 Ag**
 - polymeric protein Ag and capsular polysaccharides
 - crosslink large number of BCRs

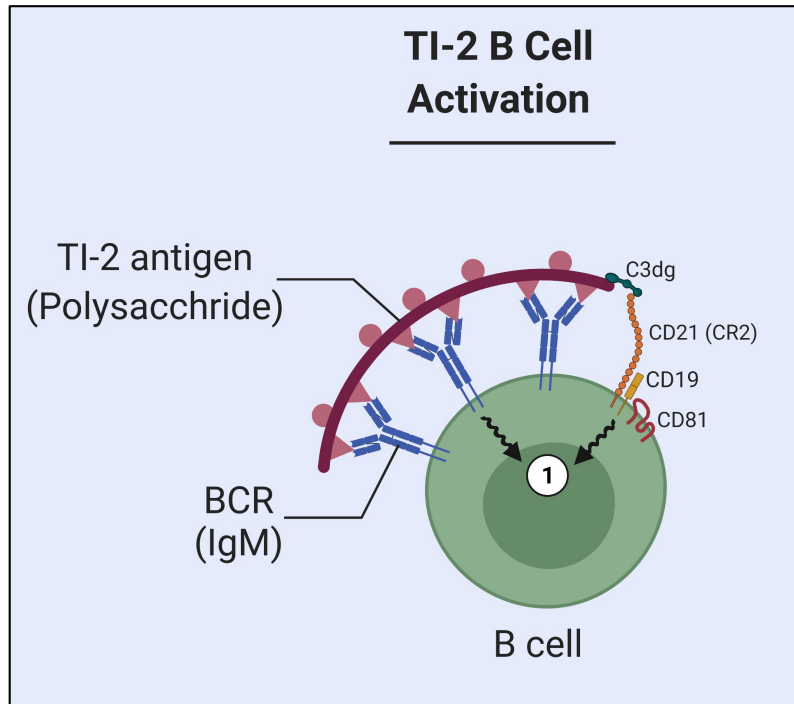


TI-1 activation = bind to TLRs and BCR

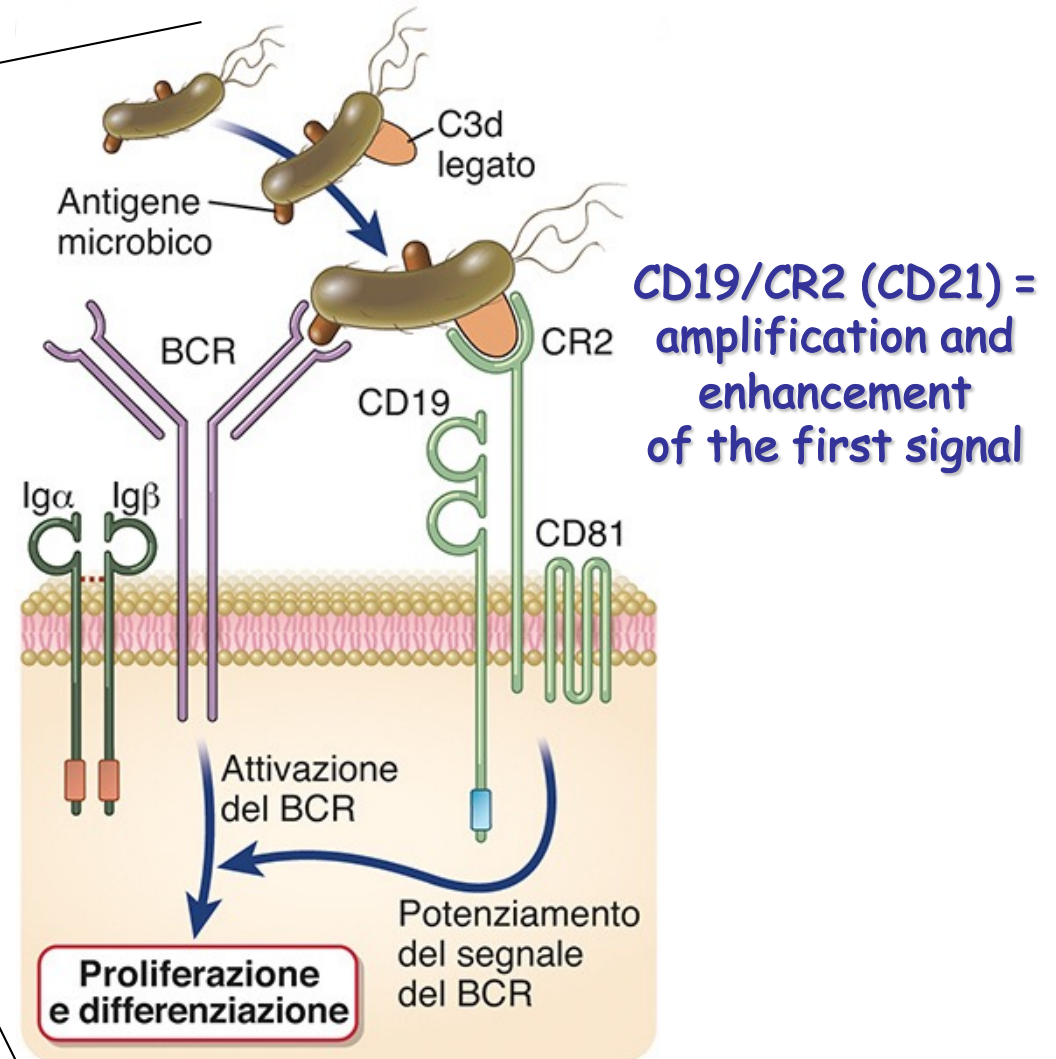
TI-1 B Cell Activation



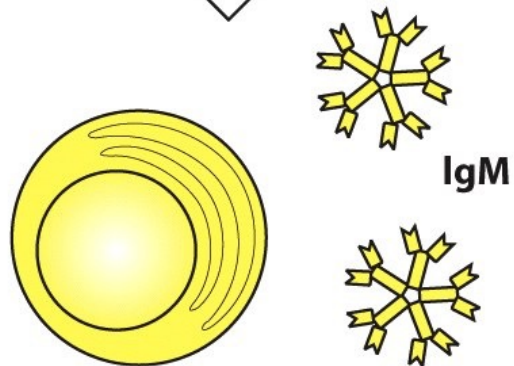
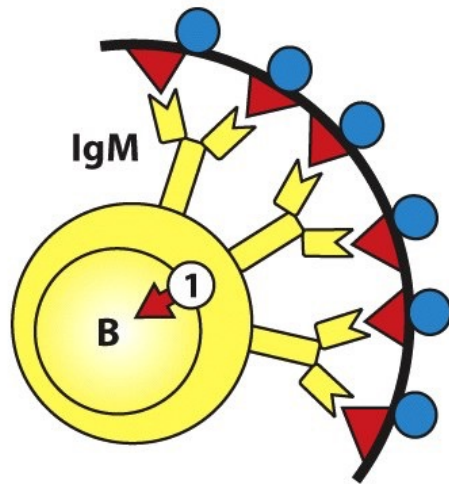
TI-2 activation = crosslink large number of BCRs



The co-receptor complex amplifies the signal transduced by the BCR



TI-2 antigens alone can signal B cells to produce IgM antibody



Activated dendritic cells release a cytokine, BAFF, that augments production of antibody against TI-2 antigens and induces class switching

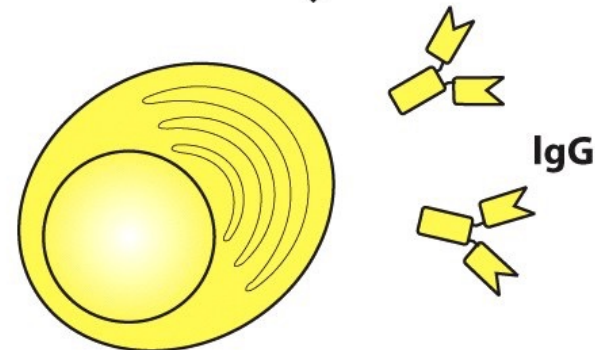
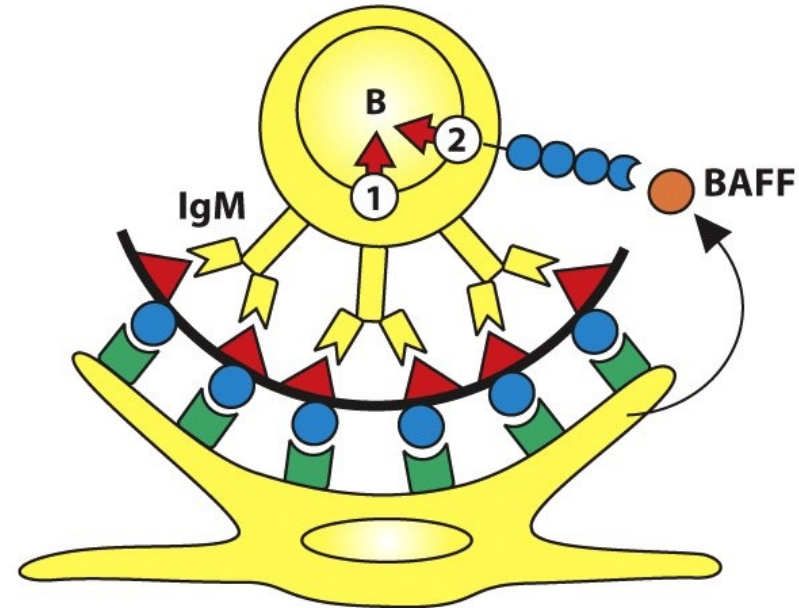


Figure 10.19 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Proprietà degli Ag timo-dipendenti e timo-indipendenti

	TD antigen	TI-1 antigen	TI-2 antigen
Antibody response in infants	Yes	Yes	No
Antibody production in congenitally athymic individual	No	Yes	Yes
Antibody response in absence of all T cells	No	Yes	No
Primes T cells	Yes	No	No
Polyclonal B-cell activation	No	Yes	No
Requires repeating epitopes	No	No	Yes
Examples of antigen	Diphtheria toxin Viral hemagglutinin Purified protein derivative (PPD) of <i>Mycobacterium tuberculosis</i>	Bacterial lipopolysaccharide <i>Brucella abortus</i>	Pneumococcal polysaccharide <i>Salmonella</i> polymerized flagellin Dextran Hapten-conjugated Ficoll (polysucrose)

Figure 9-18 Immunobiology, 6/e. (© Garland Science 2005)

Summary

- B-cell activation is well characterized and highly intricate.
 - Somatic hypermutation, affinity selection, and class switching recombination are elegant mechanisms used to increase antibody response efficiency.
- Antibody responses can be generated with and without T-cell assistance, depending on the antigen type and structure.
- Memory B-cell formation is unclear at present but is an area of active research.