B Cell Activation and Differentiation

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Lecture 11 Kuby (Chapter 11)

March 23, 2009

Goals of Lecture

- Mechanism of BCR signaling via ITAM sequences

- Distinguish Primary vs Secondary Immune Response
- TI vs TD

- Distinguish between membrane and secreted form of Ig

- T and B cell interactions:

  Germinal Center Maturation and role of AID.

  Class Switching (CSR)

  Somatic Hypermutation (SHM)

  Memory B cells and Plasma Cells

B Cell Activation

Ab Responses to most Ags require thymus (TD)

Ag must be a protein

Humoral response leads to:

- **Affinity maturation**
- **Isotype Switching**
- **Memory B cells**

TD antigen

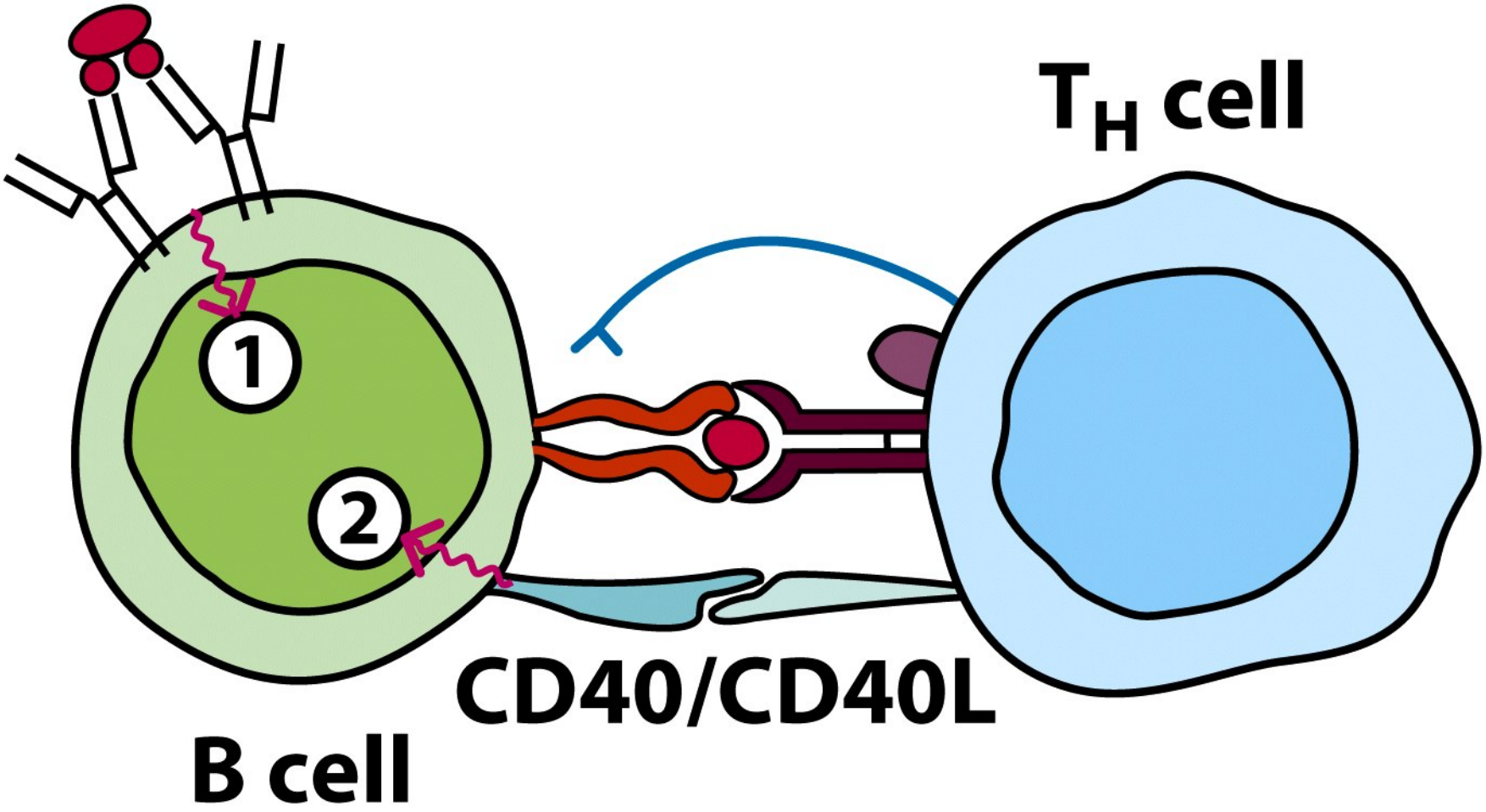

Figure 11-7b
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B Cell Activation

Ab Responses to few Ags does not require thymus (TI)

Response is mainly IgM with no memory

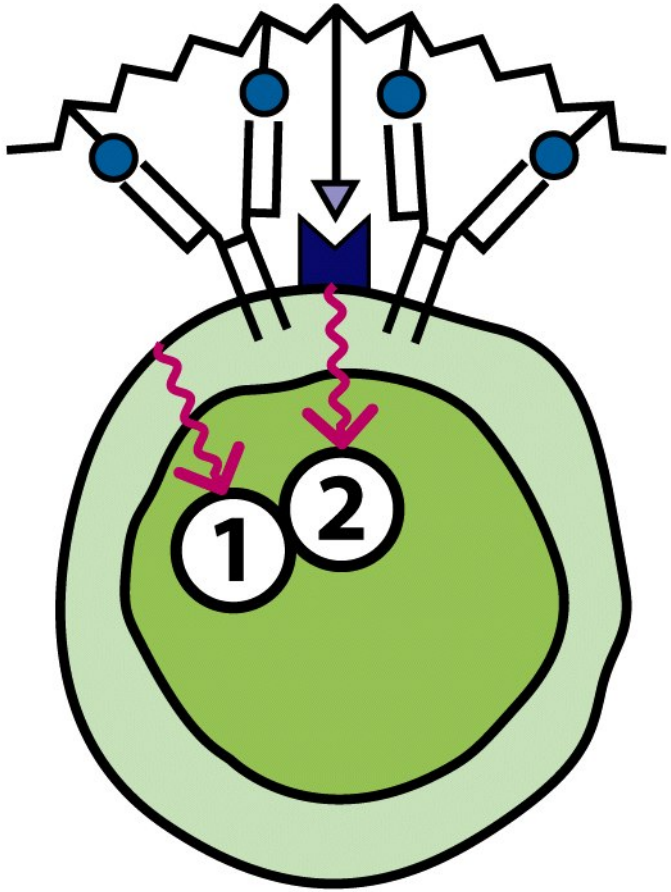
TI-1 Ags Bacterial cell wall components, LPS

act as polyclonal B cell activators or B cell mitogens

LPS can also bind to TLR4 to activate most B cells

TI-2 Ags Repeating eptiopes that induce cross-linking

TI-1 antigen



B cell

Figure 11-7a
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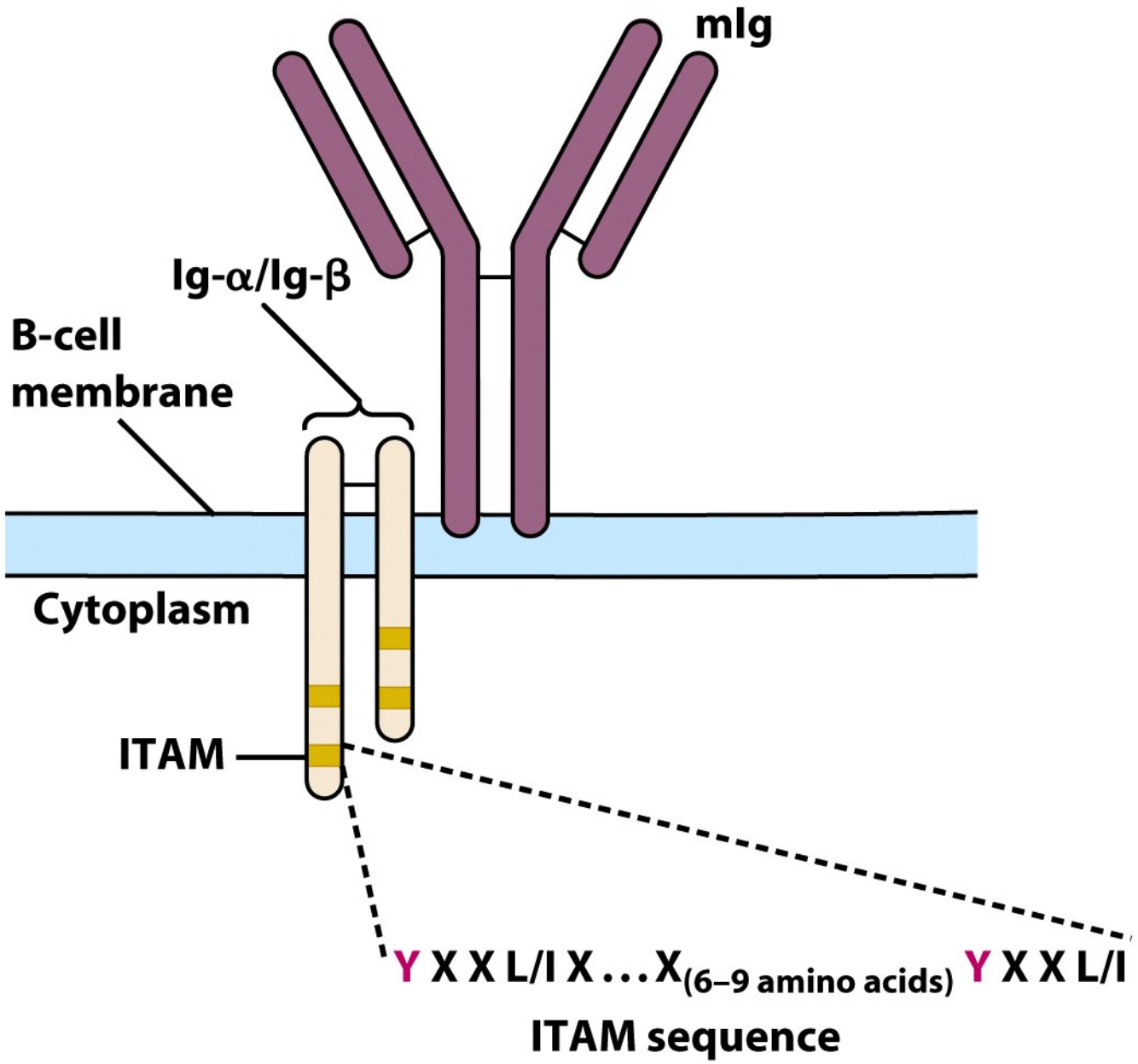

Figure 11-8
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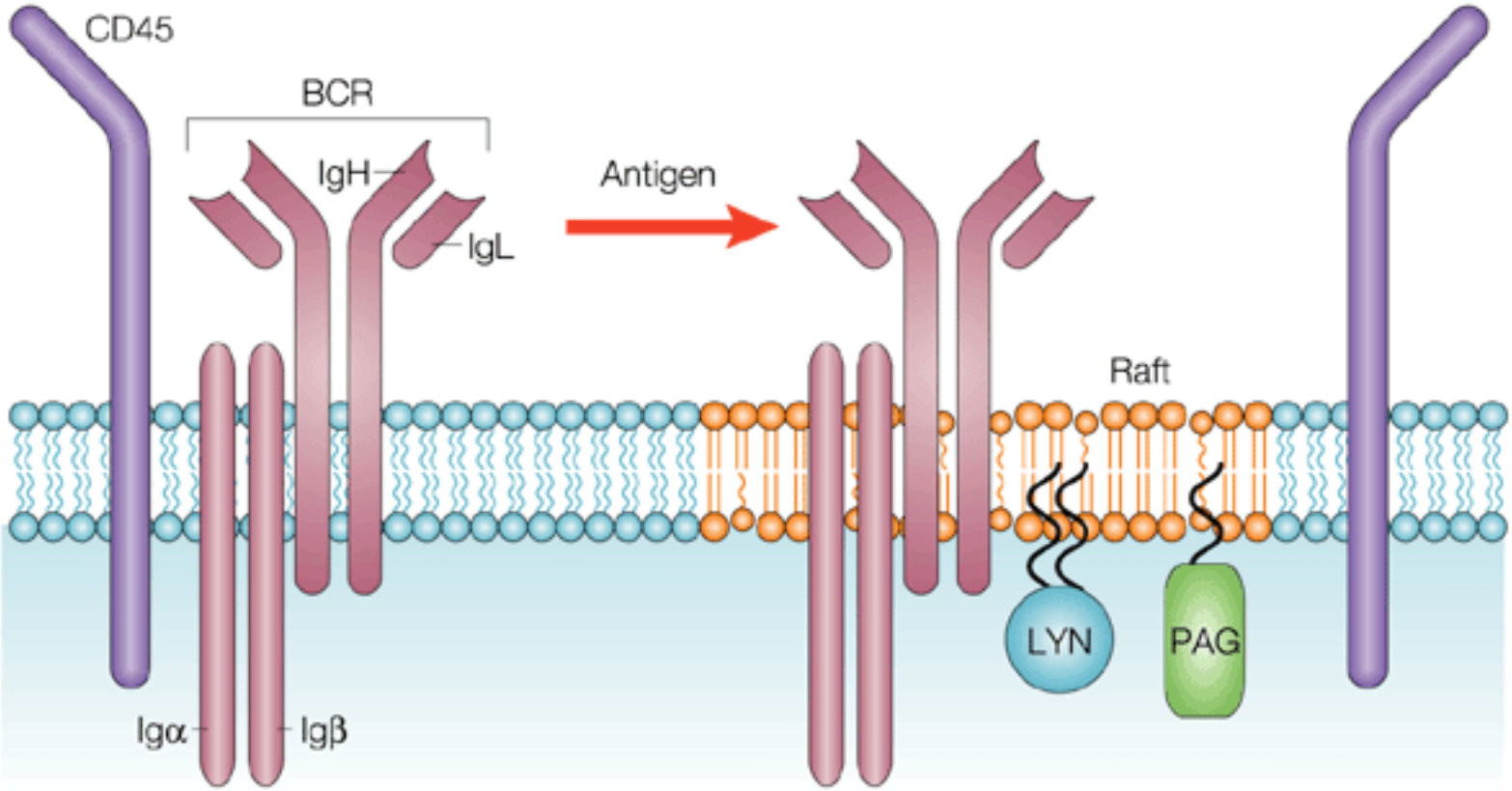
Immunoreceptor tyrosine-based activation motif



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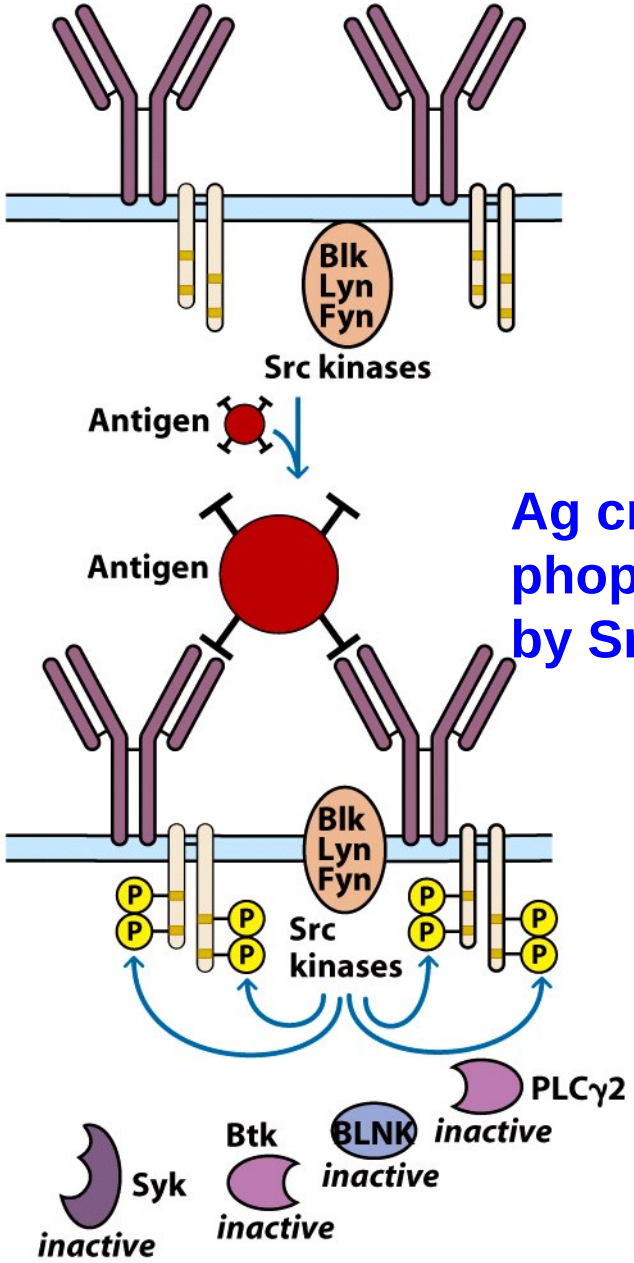
Raft translocation of the BCR by ligand binding.
In resting B cells, the B-cell receptor (BCR) is excluded from lipid rafts, as are most plasma-membrane proteins, including CD45. The rafts concentrate glycosylphosphatidylinositol (GPI)-linked proteins and myristylated proteins, such as LYN and phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG). After antigen engagement, the BCR relocates within rafts. IgH, immunoglobulin heavy chain; IgL, immunoglobulin light chain.



Ag cross links mIg triggers phophorylation of ITAMs by Src kinases

Figure 11-9 part 1
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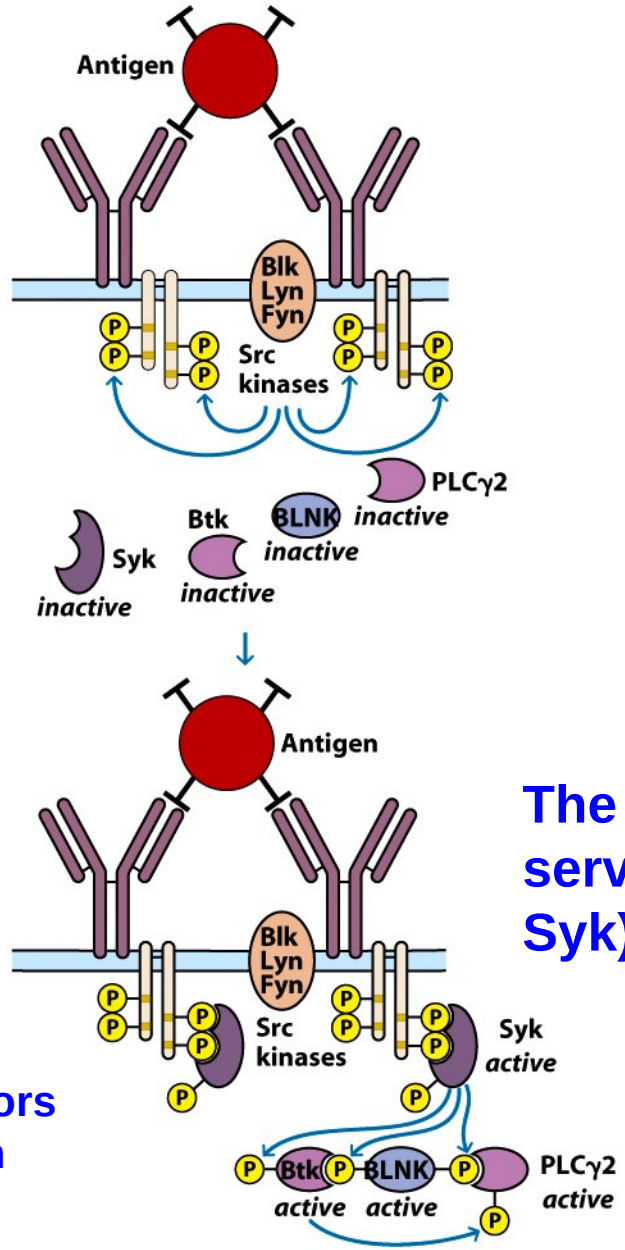

Figure 11-9 part 2
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The activated ITAMS serve as docking site of Syk)

Activation of transcription factors and change in gene expression

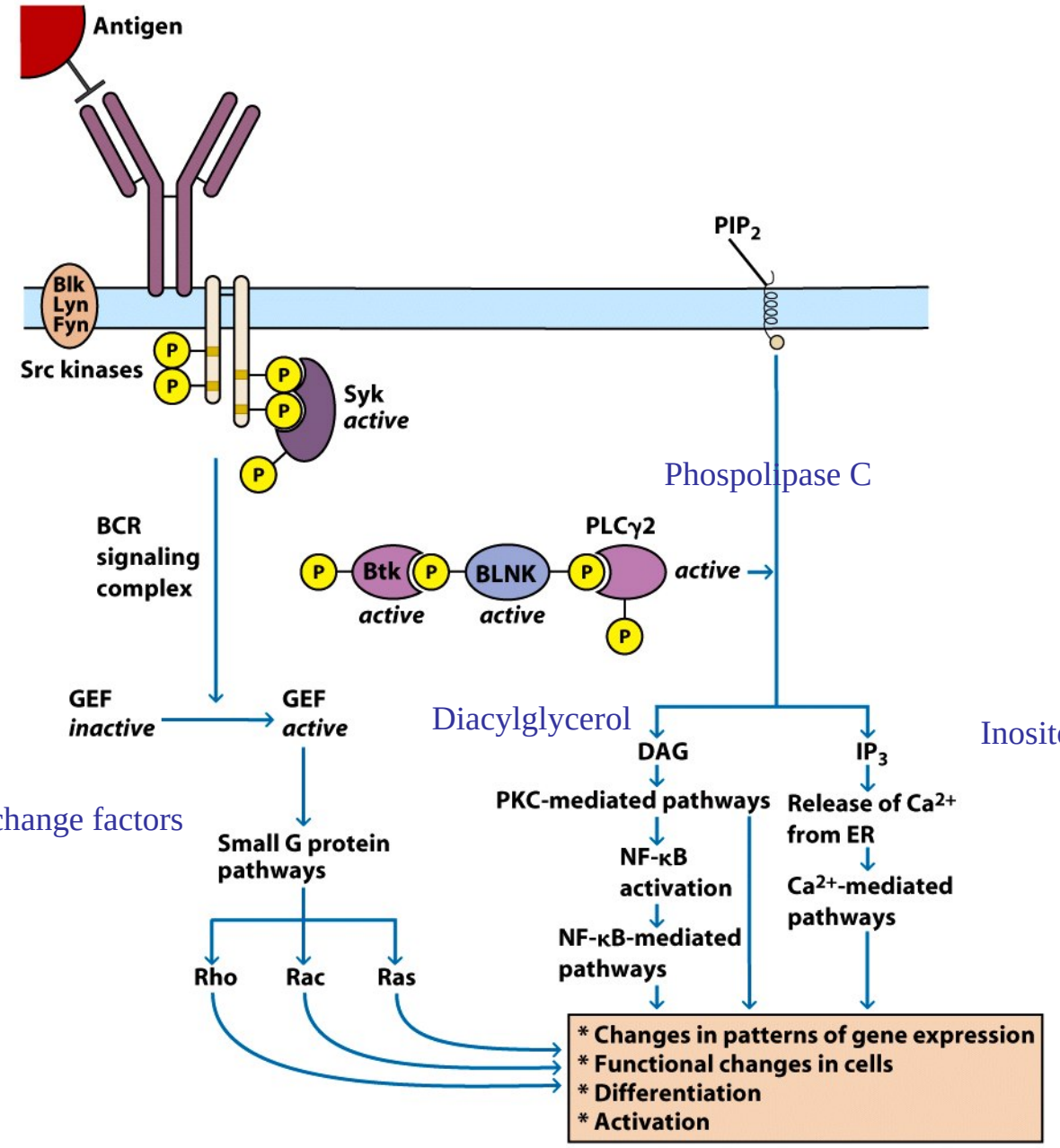

Figure 11-10
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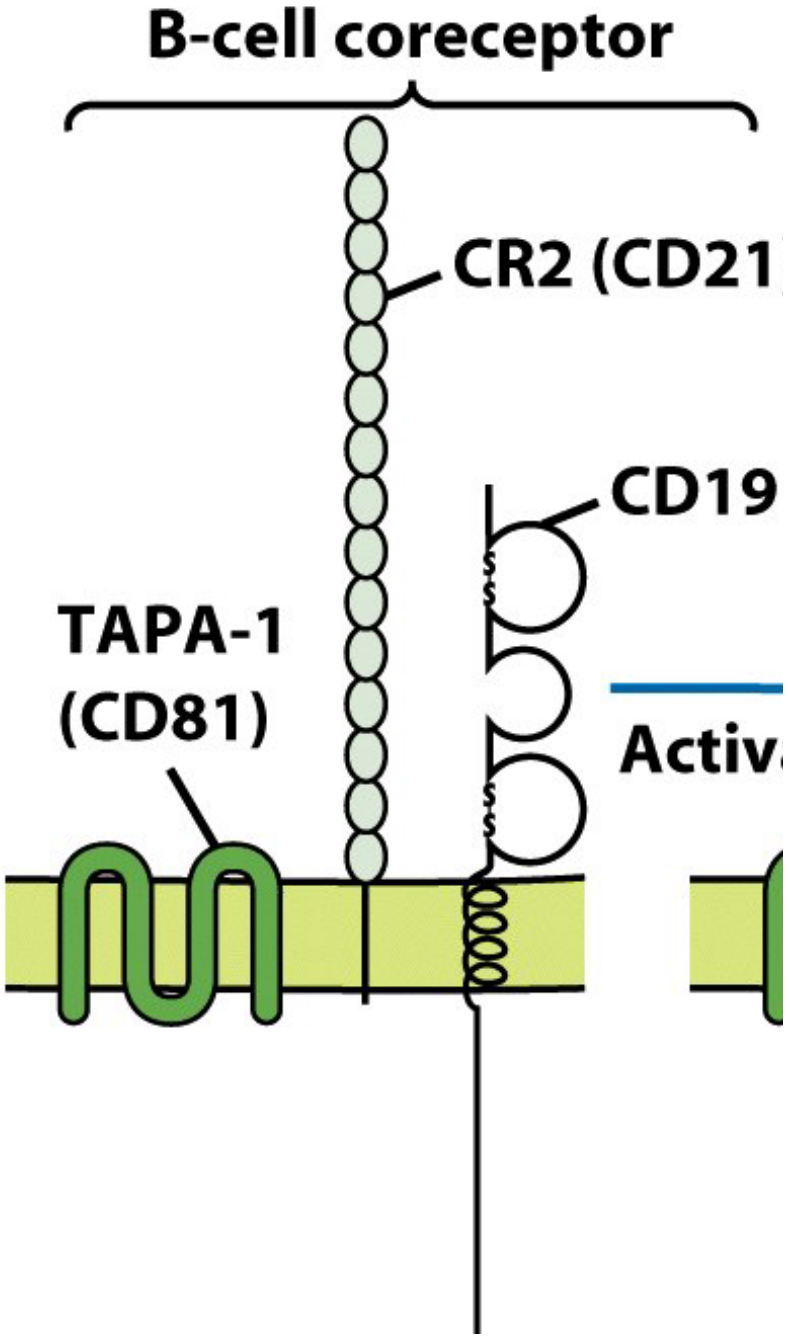
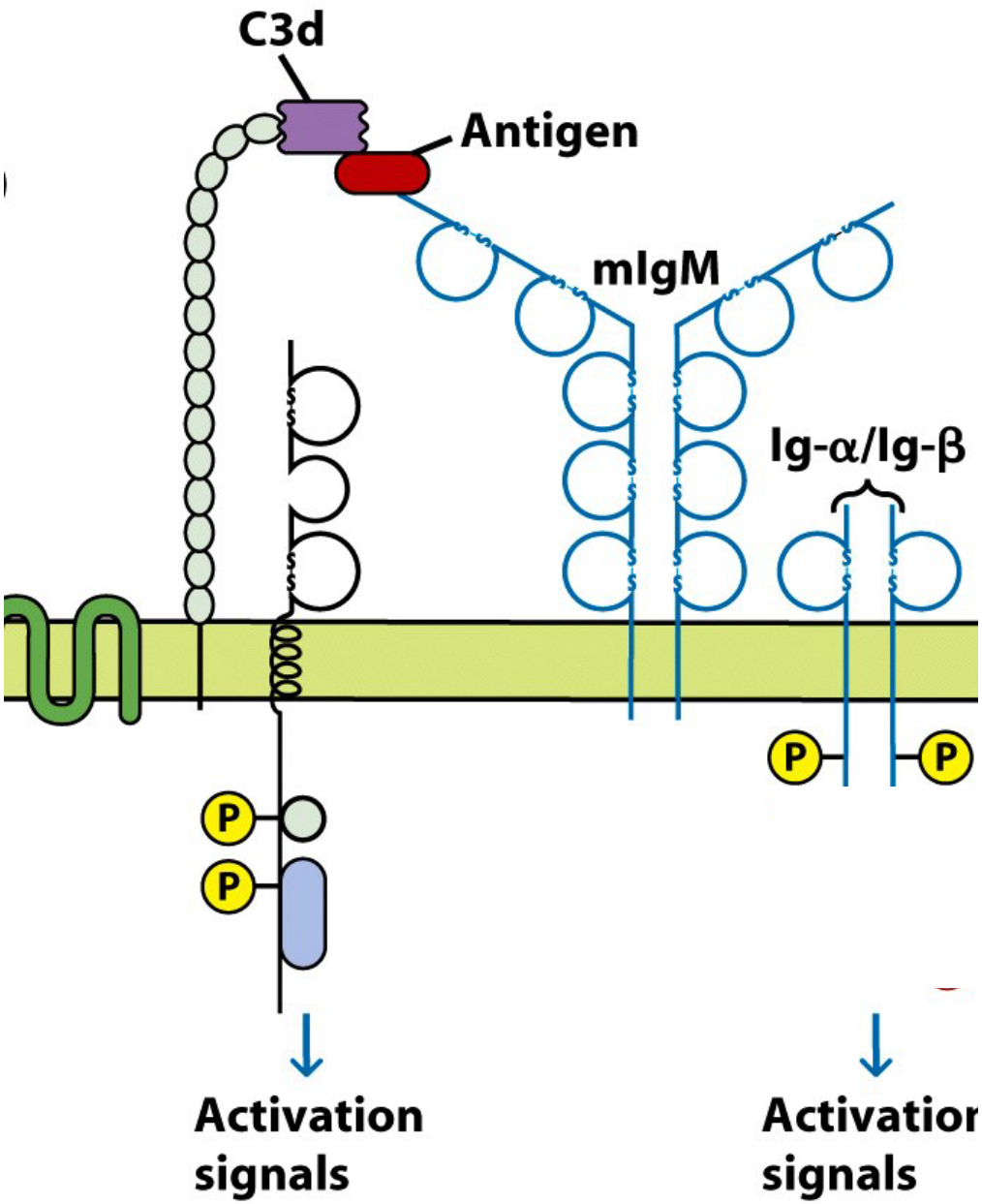


Receptor for complement


C3d
Antigen
mIgM
Ig-α/Ig-β
P
P
P
P
Activation signals
Activation signals

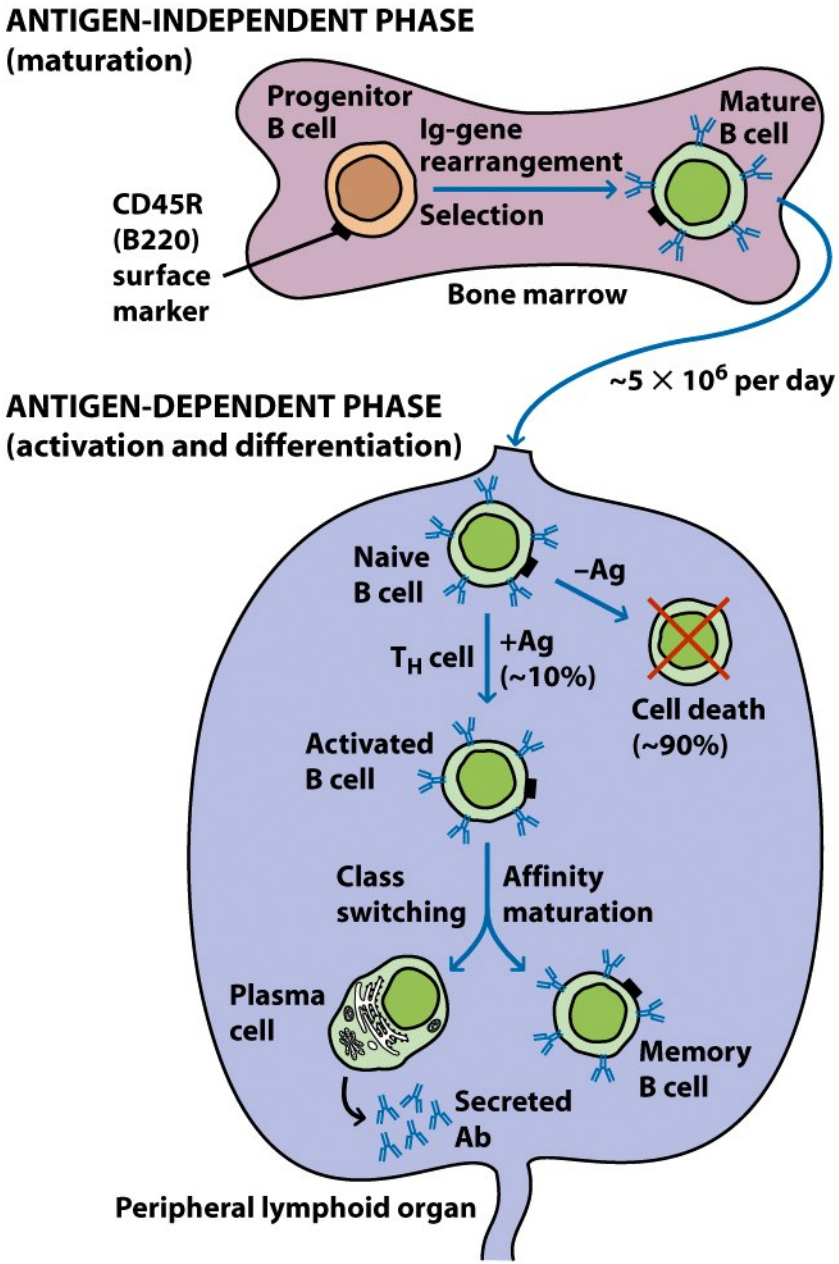


Figure 11-1
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Antigen cross-links mIg, generating signal ①, which leads to increased expression of class II MHC and co-stimulatory B7. Antigen-antibody complexes are internalized by receptor-mediated endocytosis and degraded to peptides, some of which are bound by class II MHC and presented on the membrane as peptide–MHC complexes.

T_H cell recognizes antigen–class II MHC on B-cell membrane. This plus costimulatory signal activates T_H cell.

1. T_H cell begins to express CD40L.
2. Interaction of CD40 and CD40L provides signal ②.
3. B7-CD28 interactions provide costimulation to the T_H cell.

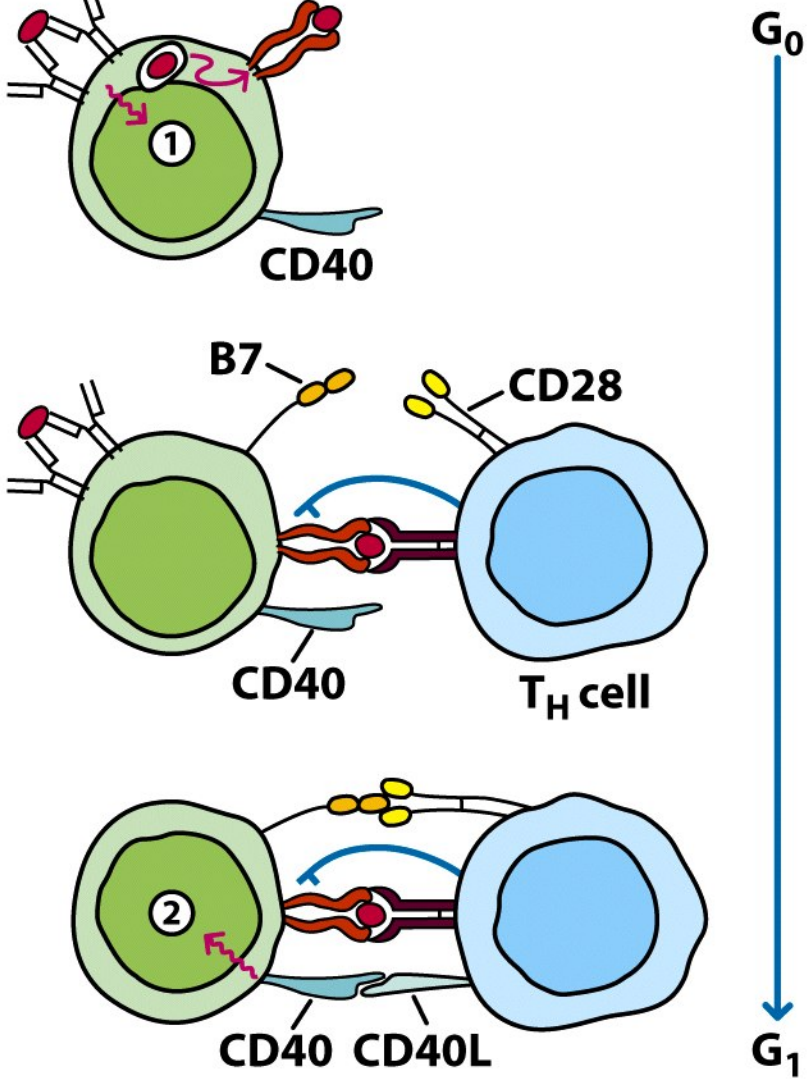

Figure 11-12 part 1
Kuby IMMUNOLOGY, Sixth Edition

1. T_H cell begins to express CD40L.

2. Interaction of CD40 and CD40L provides signal ②.

3. B7-CD28 interactions provide costimulation to the T_H cell.

1. B cell begins to express receptors for various cytokines.

2. Binding of cytokines released from T_H cell in a directed fashion sends signals that support the progression of the B cell to DNA synthesis and to differentiation.

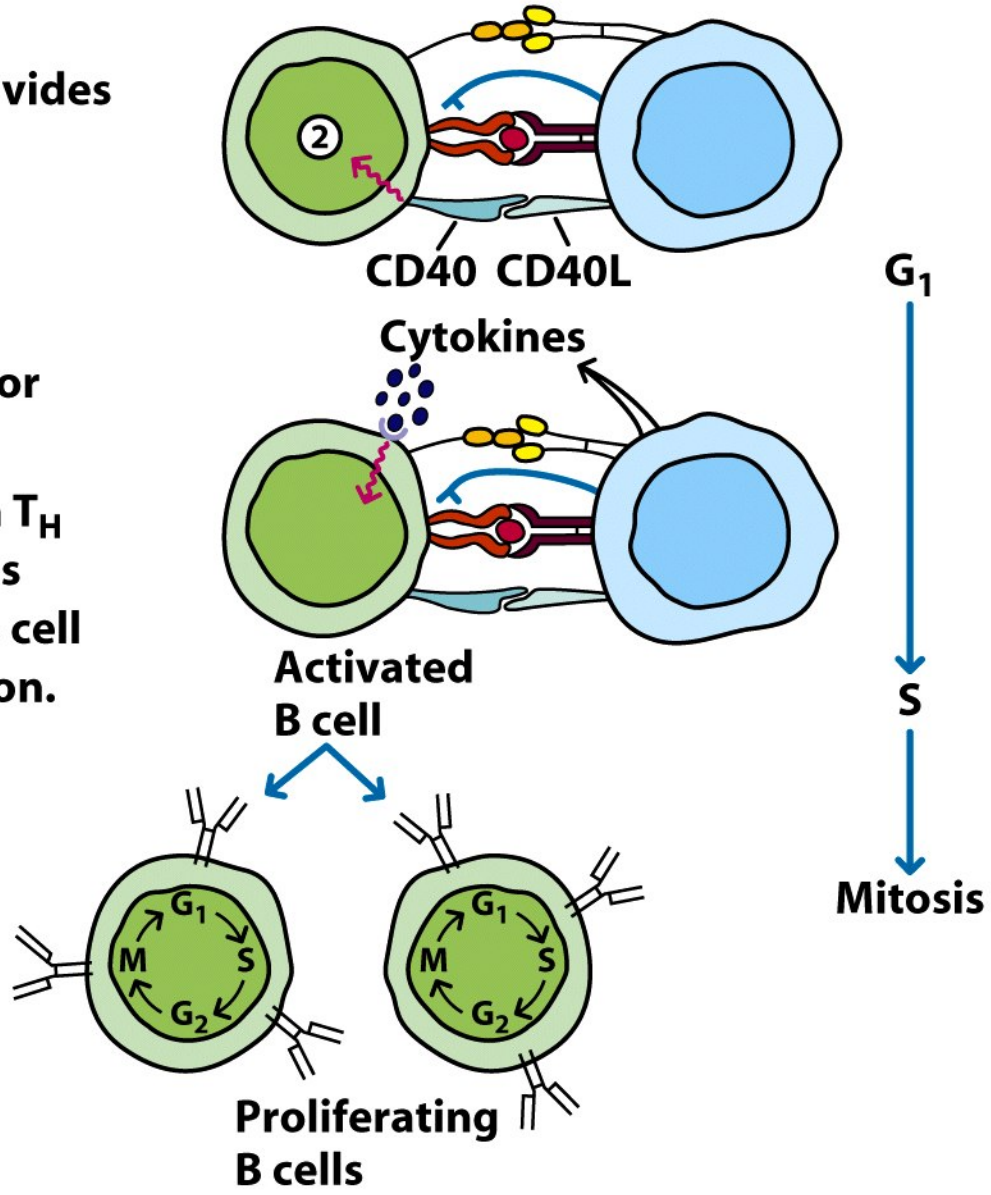

Figure 11-12 part 2
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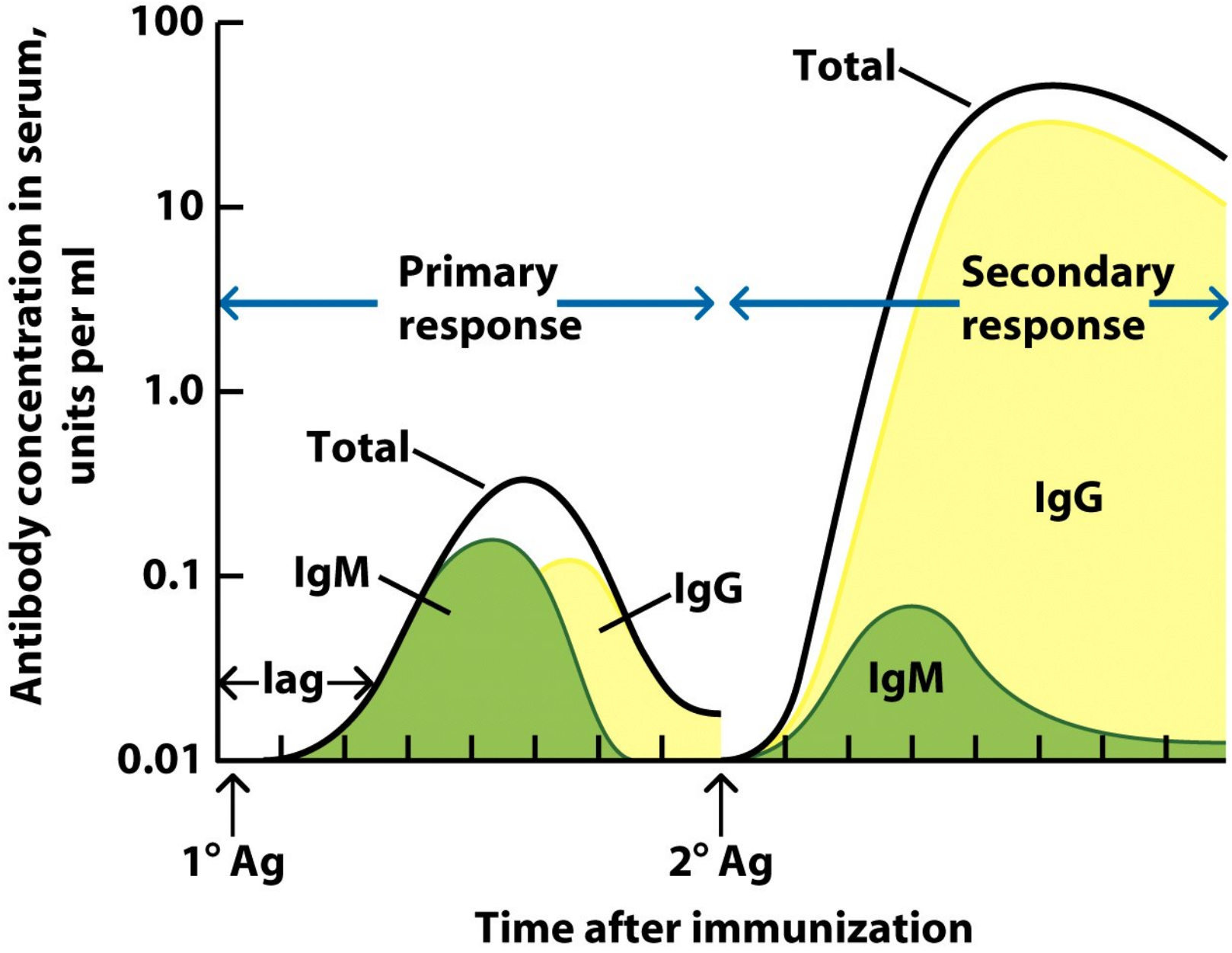

Figure 11-16
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TABLE 11-4 Comparison of primary and secondary antibody responses

Property	Primary response	Secondary response
Responding B cell	Naive B cell	Memory B cell
Lag period following antigen administration	Generally 4–7 days	Generally 1–3 days
Time of peak response	7–10 days	3–5 days
Magnitude of peak antibody response	Varies depending on antigen	Generally 100–1000 times higher than primary response
Isotype produced	IgM predominates early in the response	IgG predominates
Antigens	Thymus dependent and thymus independent	Thymus dependent
Antibody affinity	Lower	Higher

Table 11-4
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T cells recognize different epitope from B cell epitope: Artificial system hapten (B cell epitope: DNP), carrier (T cell epitope BSA or BGG). Need T help to get secondary Ab response

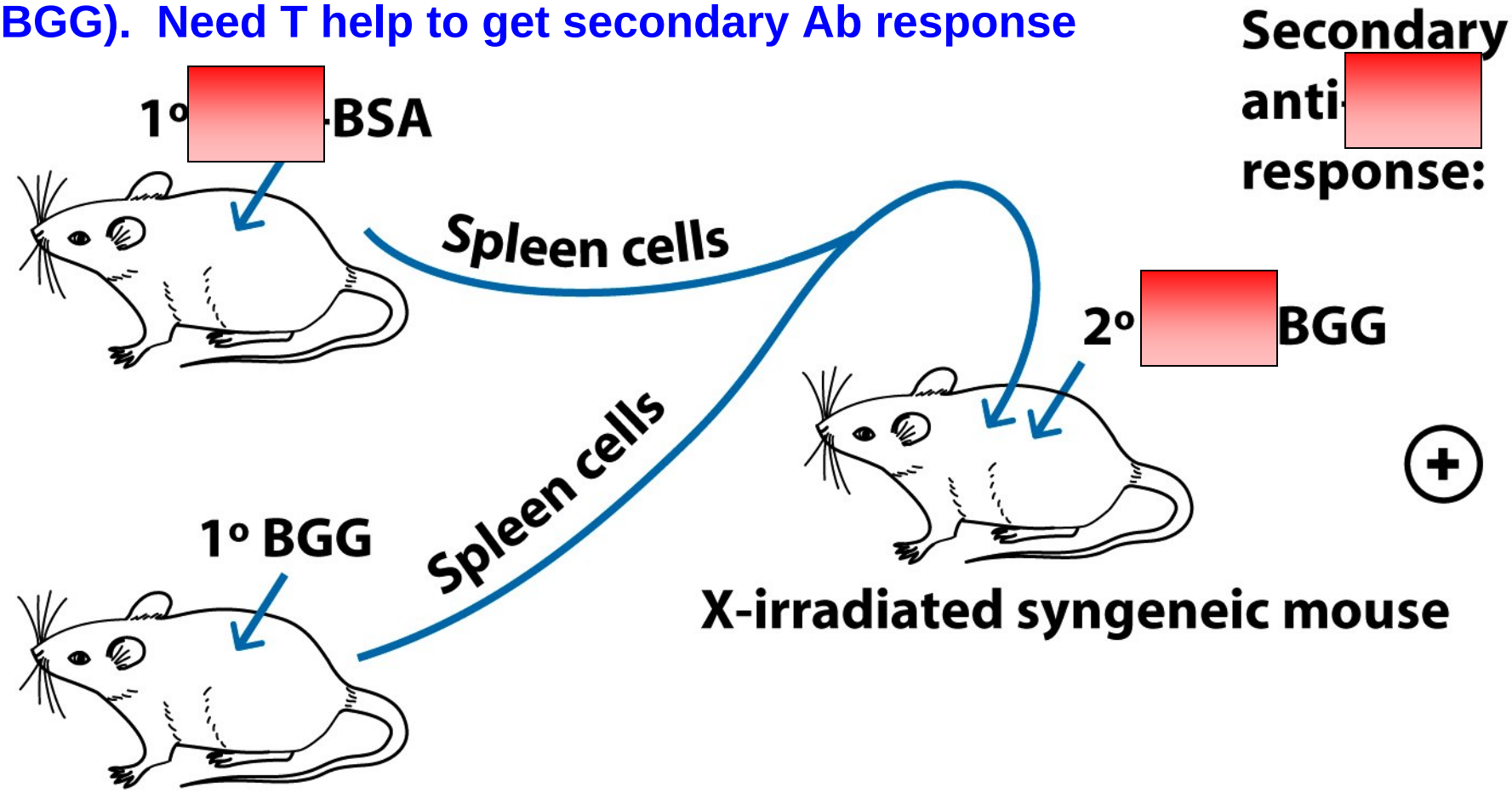

Figure 11-17a
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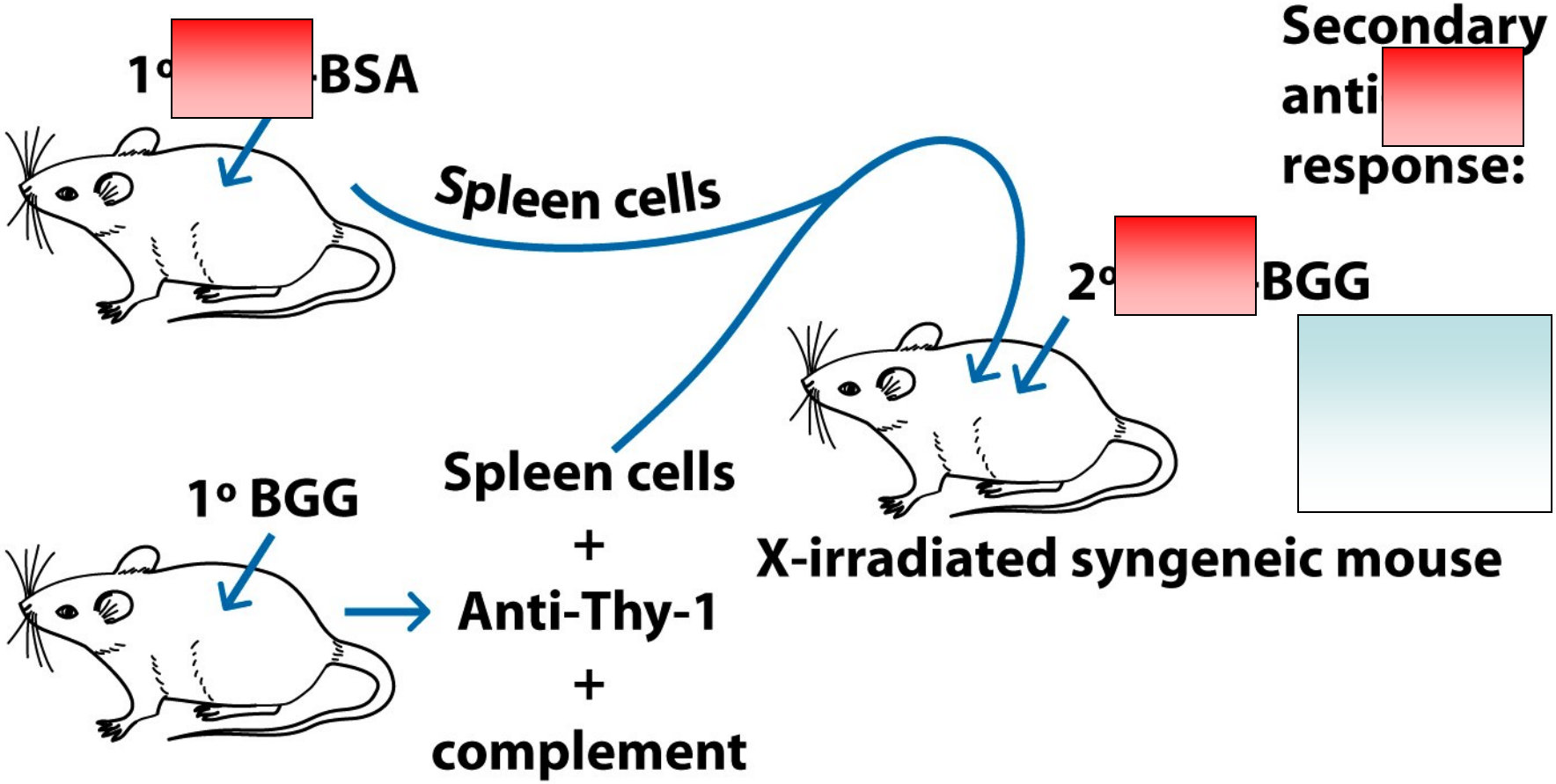

Figure 11-17b
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T cells recognizing carrier required --BUT not T Cells recognizing B cell epitope (next slide)

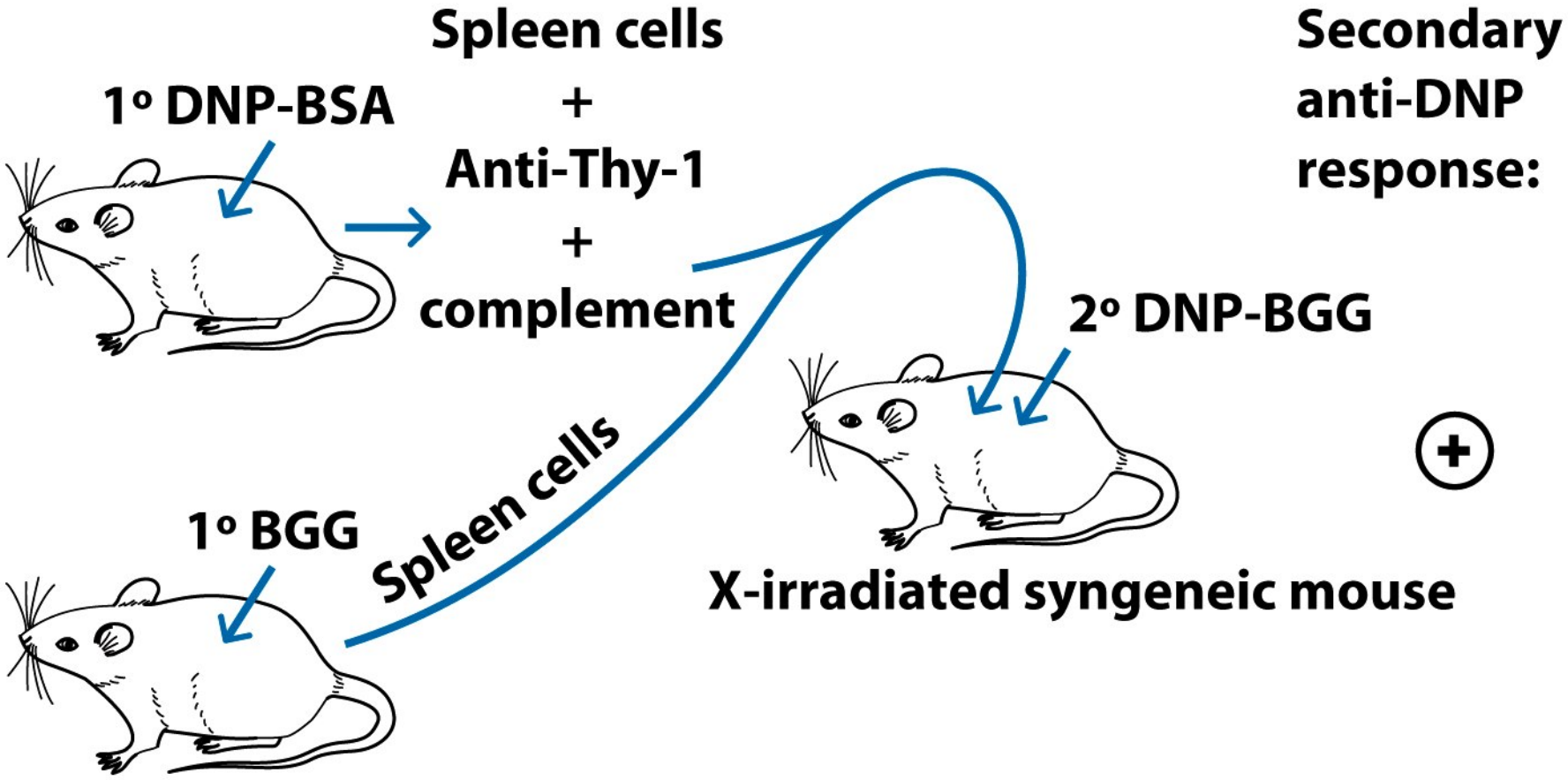

Figure 11-17c
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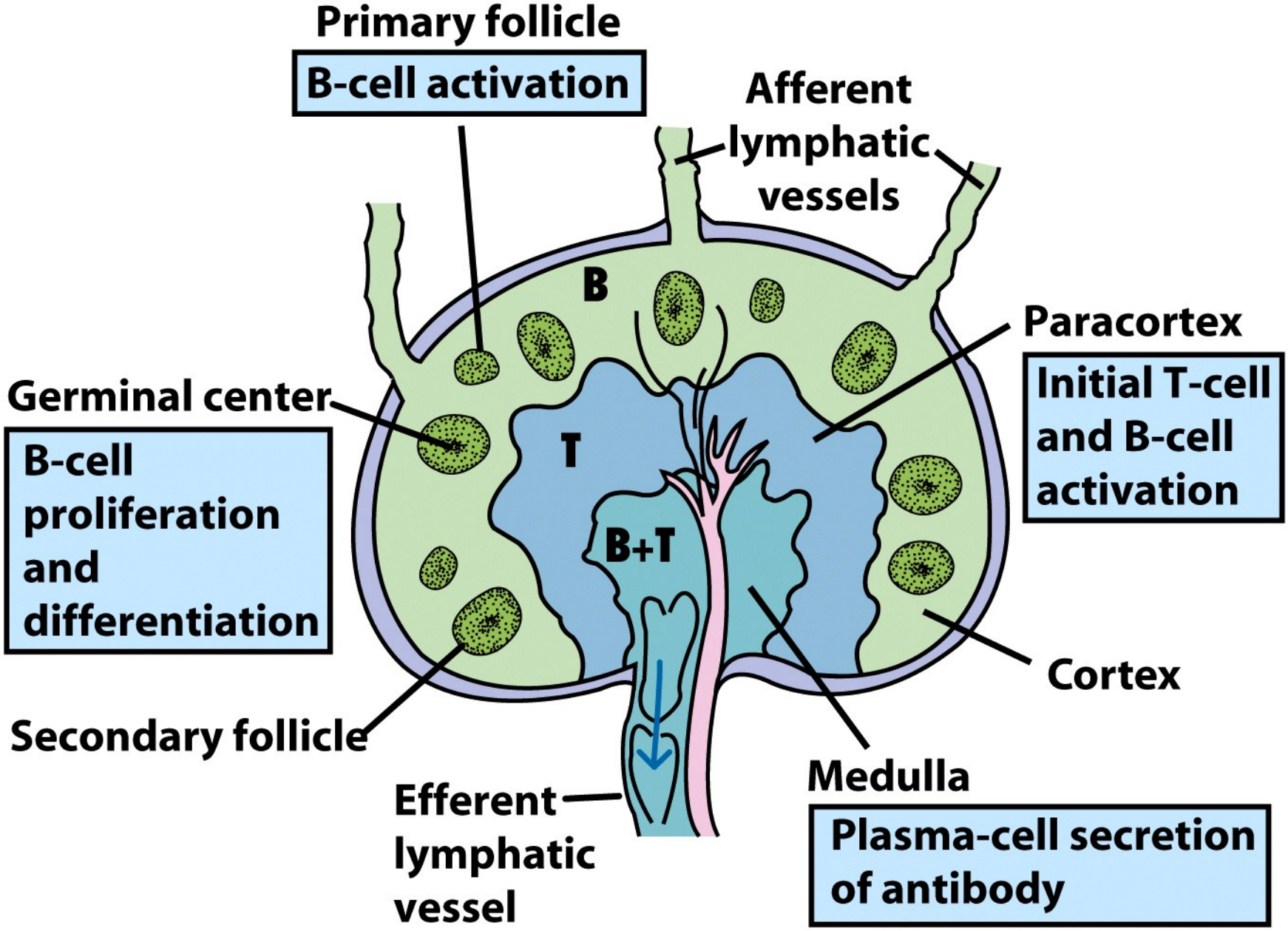

Figure 11-18
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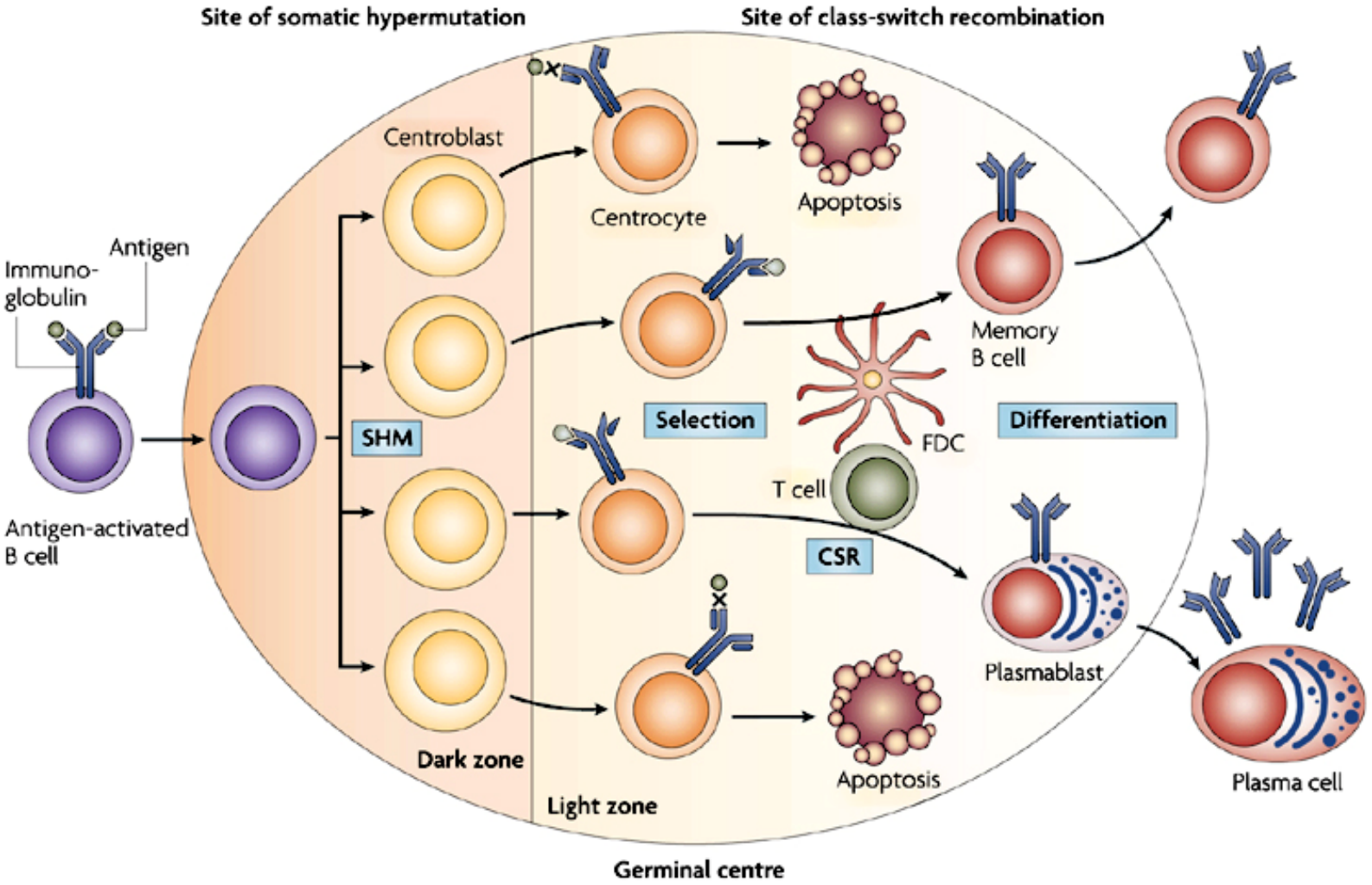

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- Combinatorial and Junctional diversity creates repertoire of naïve, mature B cells and T cells
- B cells undergo further diversification after antigen stimulation
  - Class switching of constant regions
  - Affinity maturation of variable regions (somatic hypermutation)

Mechanisms of Somatic Hypermutation and Isotype Switching

- Both require active transcription of genes
- Both require AID (Activation Induced Cytidine Deaminase)

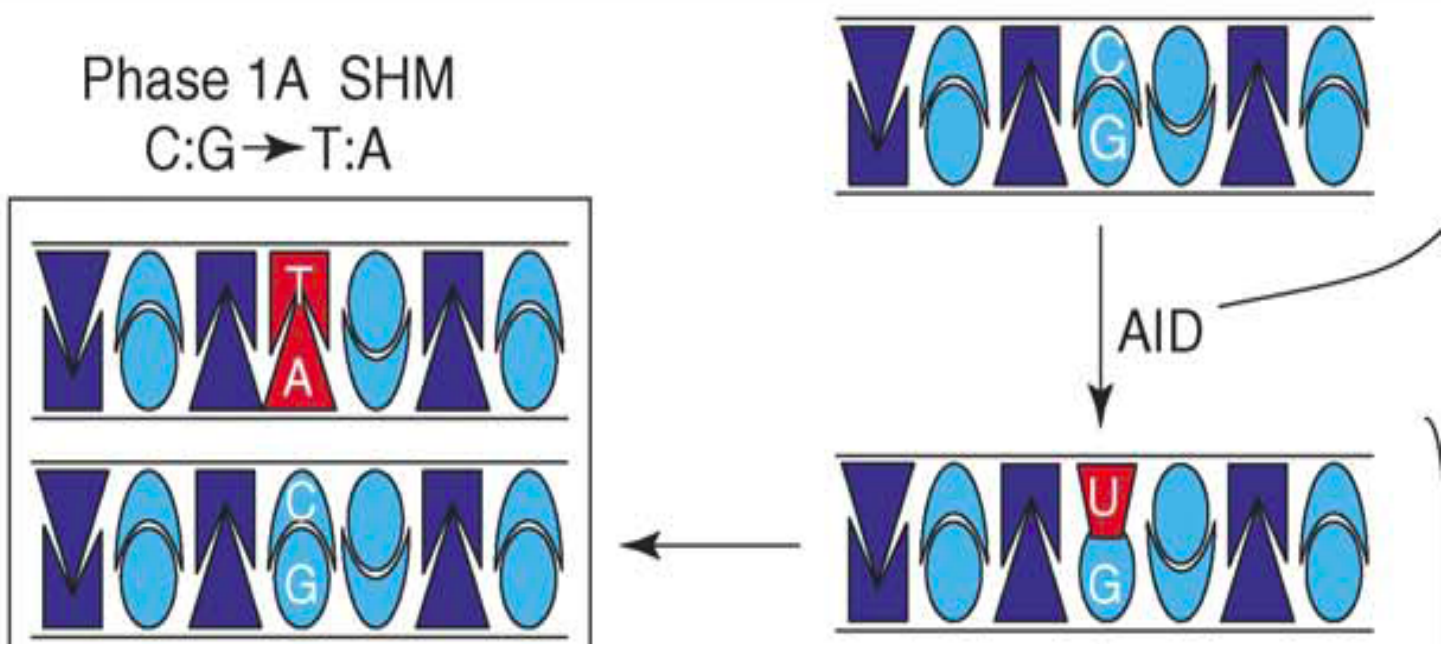
- **Affinity maturation** by somatic hypermutation increases antibody affinity from 10^{-7} - 10^{-9} nM to 10^{-9} - 10^{-11} nM (100-fold increase in affinity)

Somatic hypermutation (SHM)

- SHM targets immunoglobulin genes (but not T cell receptor genes)
- SHM requires active transcription
- SHM involves DNA single-strand breaks

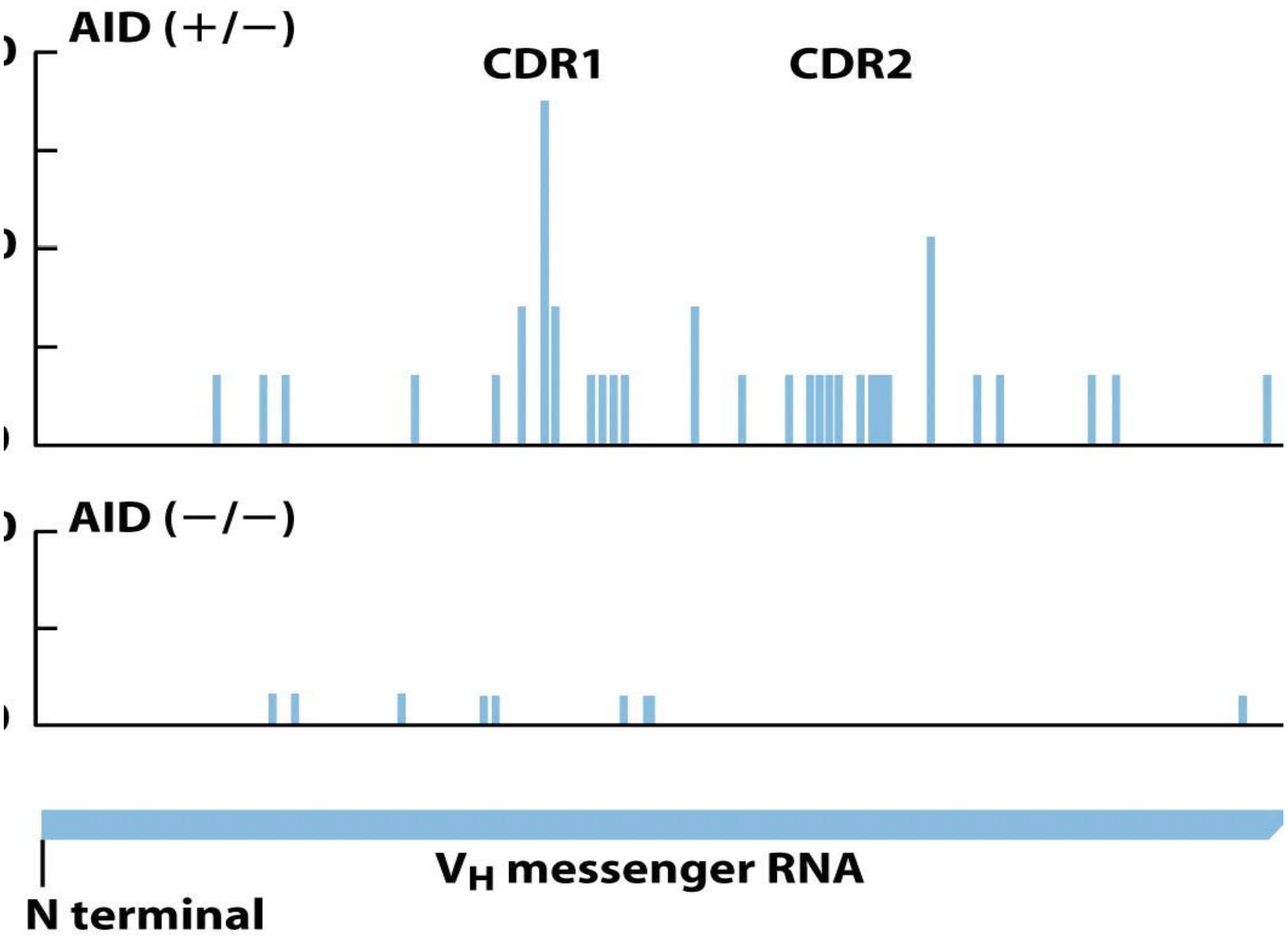
Model for somatic hypermutation

- Activation-induced deaminase (AID)
  - Expressed only in activated B cells
  - Converts C to U in single-stranded DNA
- Other proteins insert mutations
  - Uracil DNA glycosylase converts U to an apurinic site
  - AP endonuclease nicks the DNA adjacent to the AP site
  - Exonuclease removes the AP ribose
  - An error-prone polymerase fills in the gap


Phase 1A SHM
C:G→T:A
C
G
AID
U
G
T
A
C
G


NH2
O

Mutations in variable-region mRNA



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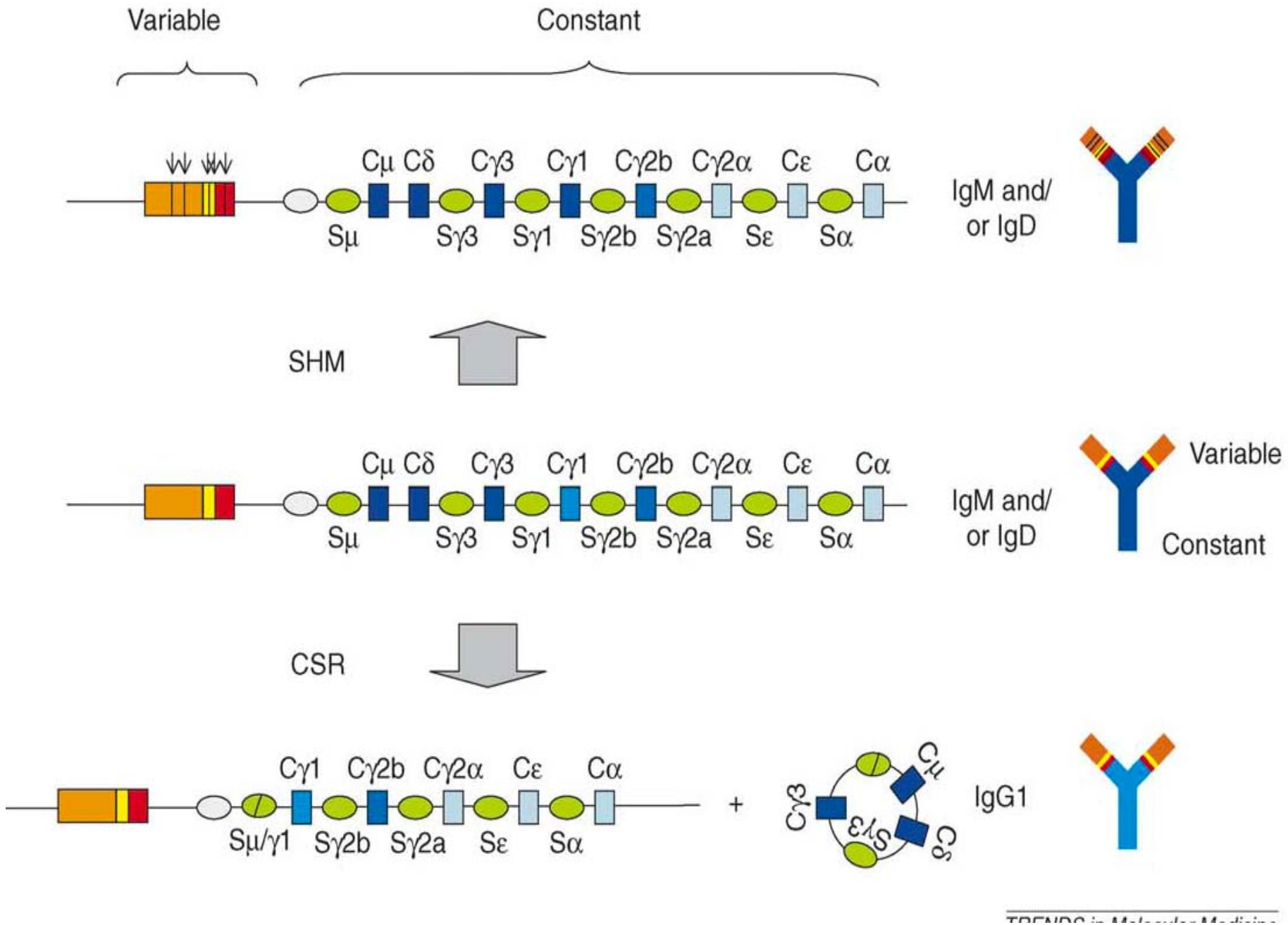
Variable
Constant
Cμ Cδ Cγ3 Cγ1 Cγ2b Cγ2α Cε Cα
Sμ Sγ3 Sγ1 Sγ2b Sγ2a Sε Sα
IgM and/
or IgD
SHM
Cμ Cδ Cγ3 Cγ1 Cγ2b Cγ2α Cε Cα
Sμ Sγ3 Sγ1 Sγ2b Sγ2a Sε Sα
IgM and/
or IgD
Variable
Constant
CSR
Cγ1 Cγ2b Cγ2α Cε Cα
Sμ/γ1 Sγ2b Sγ2a Sε Sα
+
Cμ
Cγ3
Sγ3
Cδ
IgG1

Isotype Switching involves recombination at **switch signals**

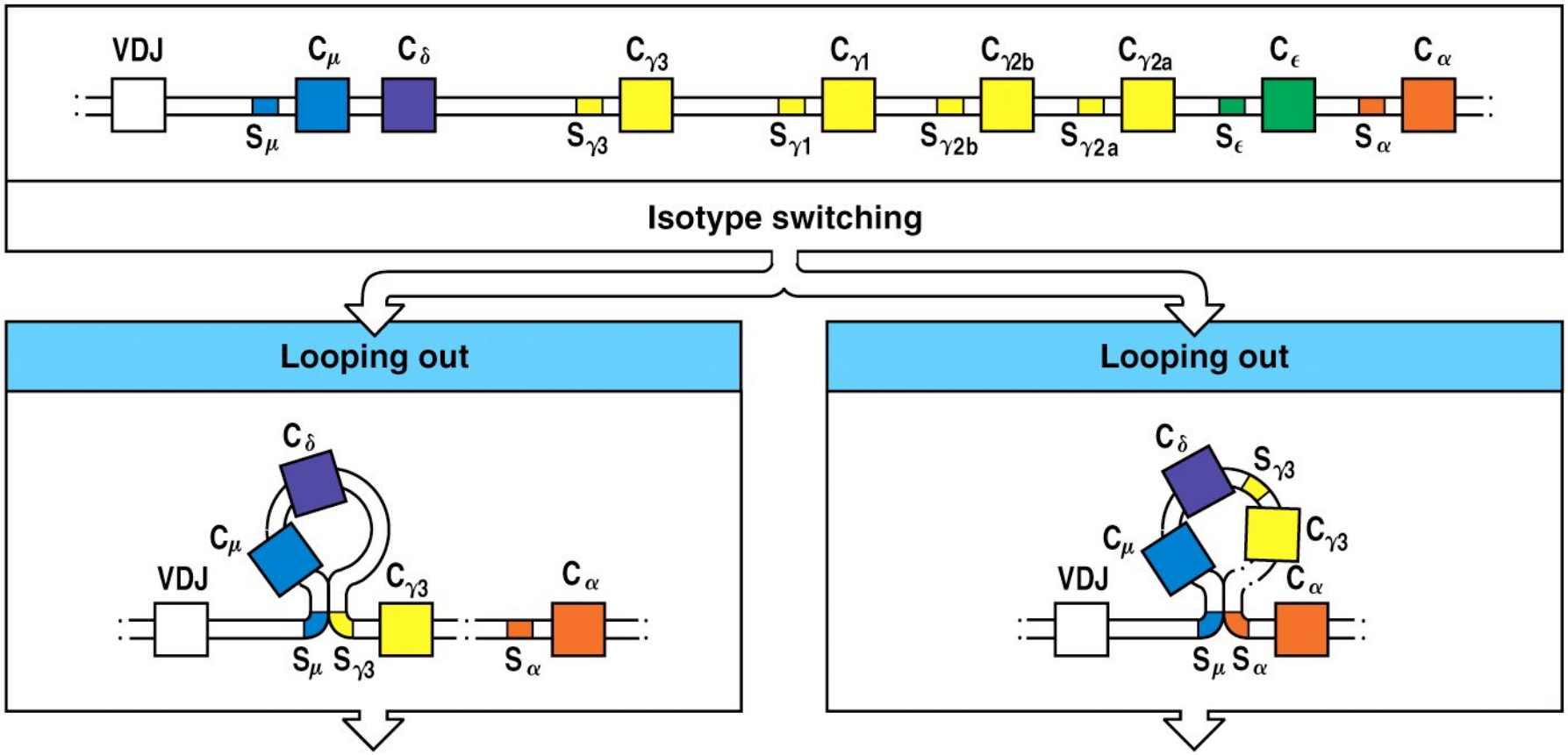

Figure 4-21 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Isotype Switching

- Other (than IgM/IgD) isotypes are produced by class-switch recombination (CSR), a process that exchanges the constant region of the heavy chain (CH) with a set of downstream constant-region genes.

- This deletional-recombination reaction, which requires the enzyme activation-induced cytidine deaminase (AID), involves the generation of DNA breaks at switch (**S**) regions, which precede the constant-region genes, followed by the repair of DNA.

- This leads to a rearranged CH locus and deletion of the intervening sequence as an episomal circle.

- Cytokines stimulate transcription through the CH gene and determine the immunoglobulin isotype that the B cell will switch to.

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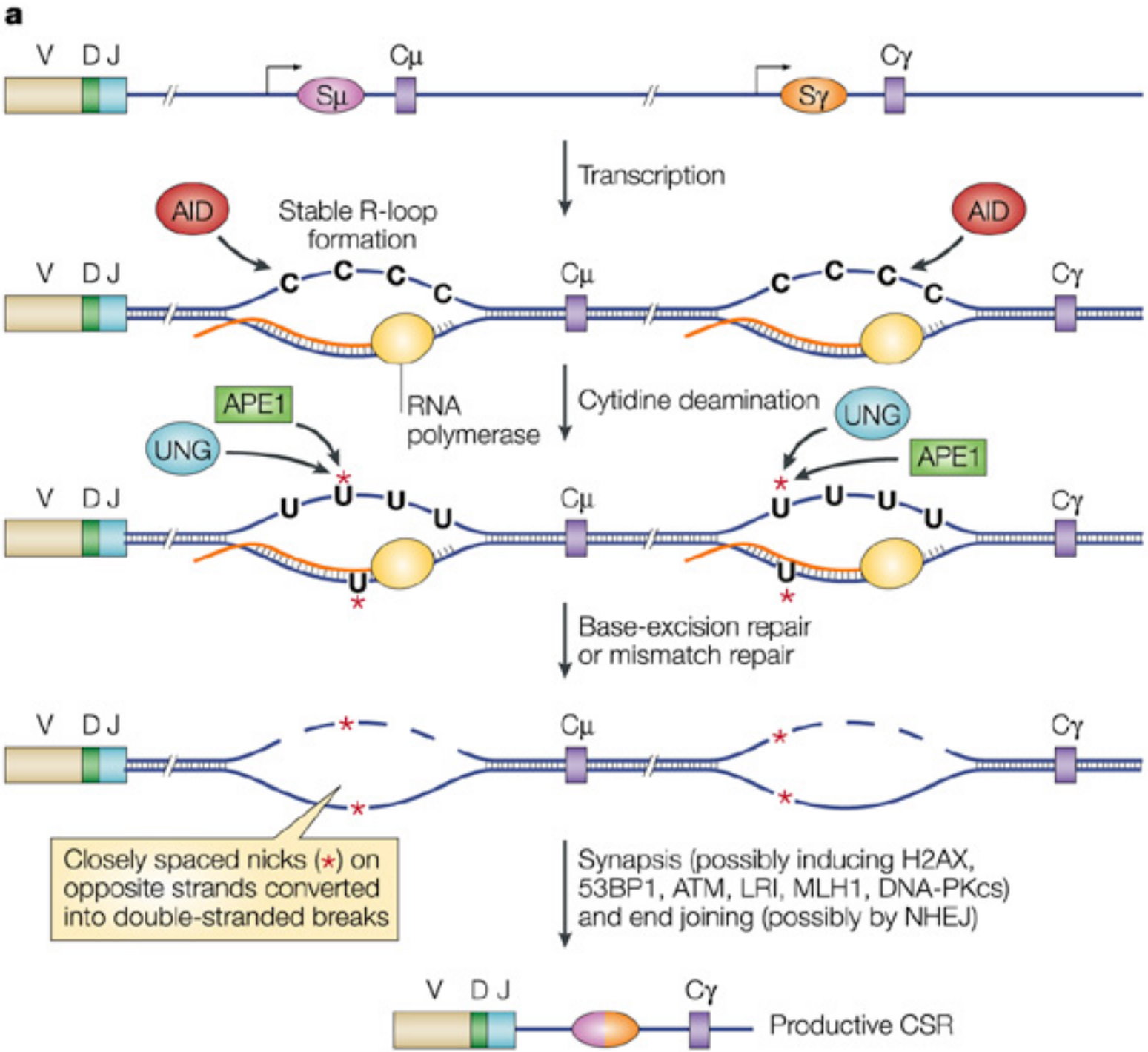
a
V D J
Sμ
Cμ
Sγ
Cγ
Transcription
AID
Stable R-loop formation
RNA polymerase
Cytidine deamination
APE1
UNG
Base-excision repair or mismatch repair
Closely spaced nicks (*) on opposite strands converted into double-stranded breaks
Synapsis (possibly inducing H2AX, 53BP1, ATM, LRI, MLH1, DNA-PKcs) and end joining (possibly by NHEJ)
Productive CSR

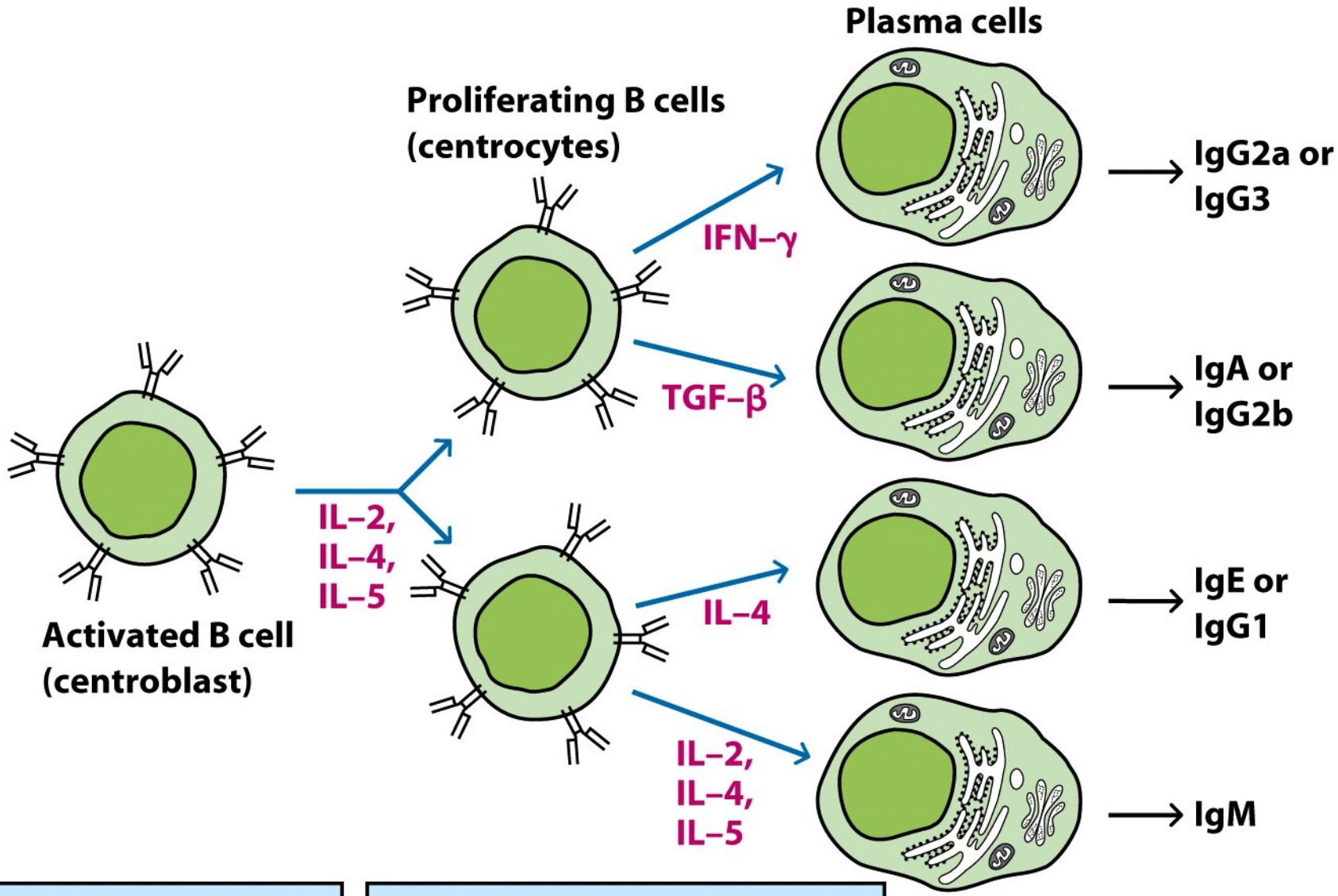

Figure 11-22
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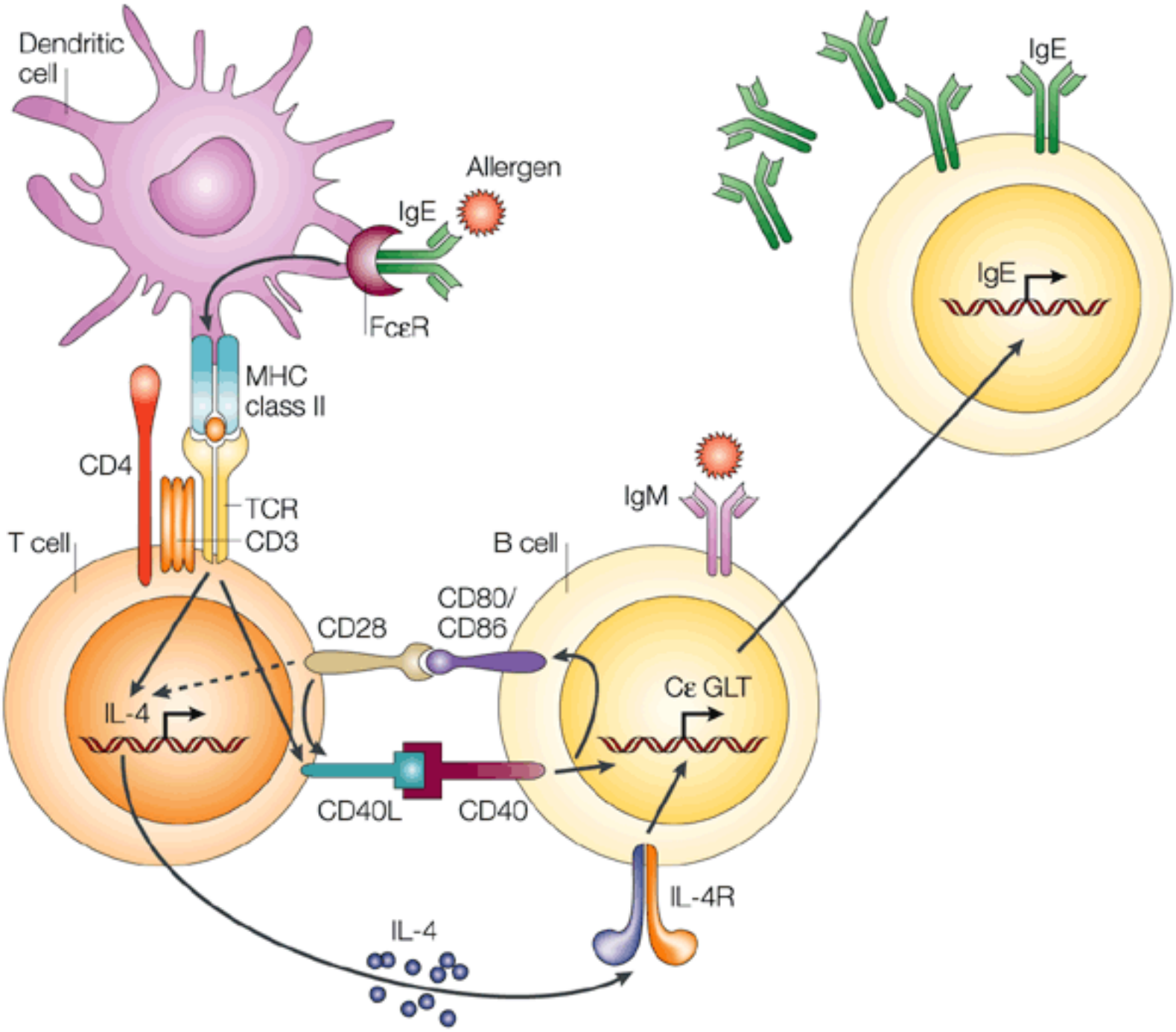
Dendritic cell
Allergen
IgE
FcεR
MHC class II
CD4
TCR
CD3
T cell
IgM
B cell
CD80/ CD86
CD28
IL-4
Cε GLT
CD40L
CD40
IL-4R
IL-4
IgE
IgE

Cellular interactions important for IgE class-switch recombination.

- Uptake of allergens by dendritic cells allows for the presentation of antigenic determinants to T cells.

- Stimulation of specific CD4+ T cells leads to the production of interleukin-4 (IL-4) and the upregulation of expression of CD40 ligand (CD40L) by T cells.

- CD40 stimulation of allergen-specific B cells upregulates the expression of the co-stimulatory molecules CD80 and CD86, which allows for more efficient T-cell expression of CD40L and enhanced stimulation of B cells through the induction of IL-4.

- CD40-mediated stimulation of B cells also synergizes with IL-4-receptor (IL-4R) signals to enhance the transcription of C germline transcripts (C GLTs) and activation-induced cytidine deaminase (*AID*), rearrangement of the IgE genomic locus and production of IgE antibodies.

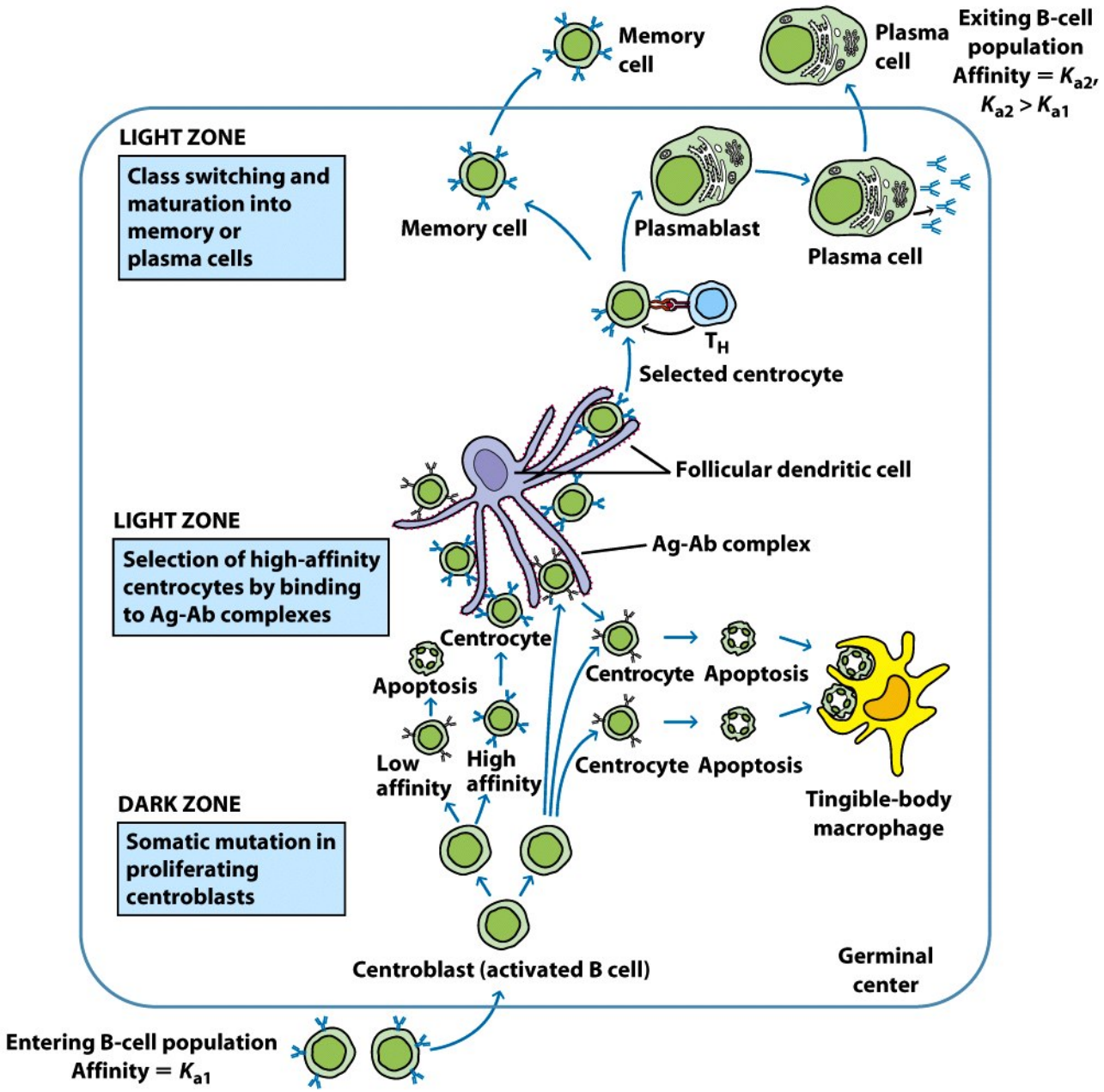

Figure 11-20
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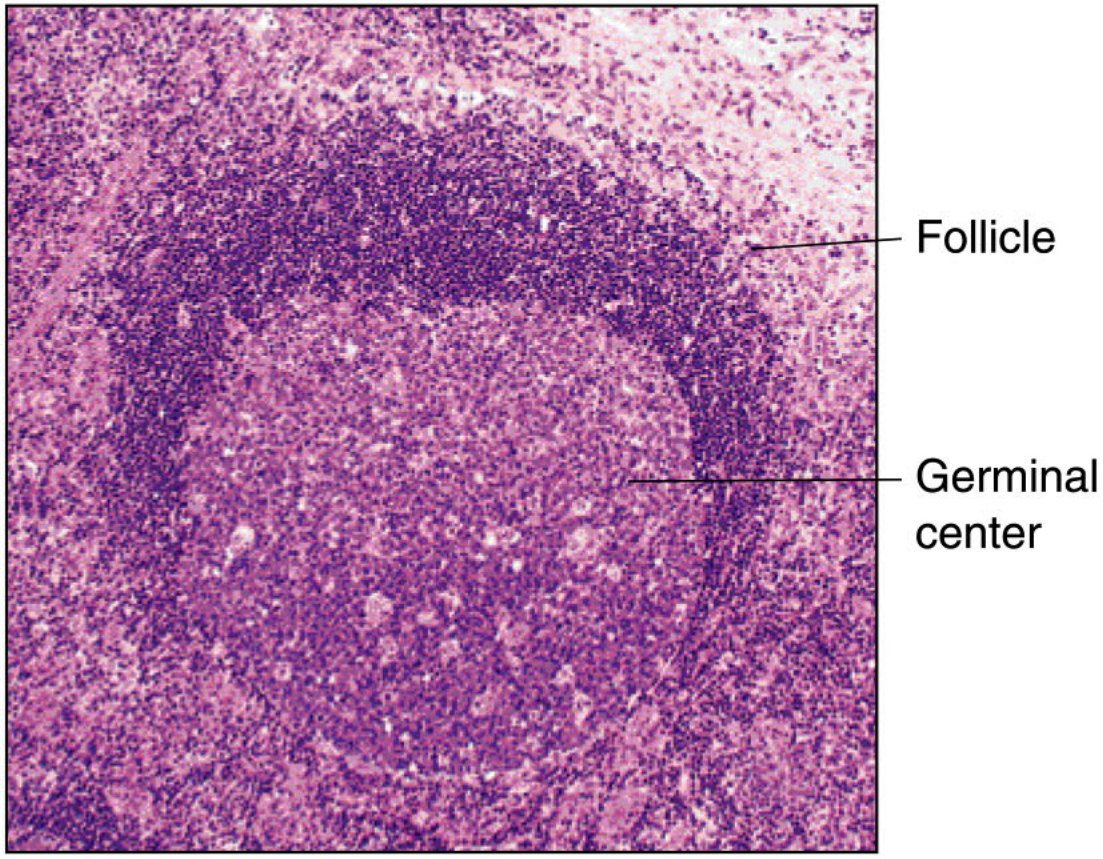
Secondary follicle in a lymph node



From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 9-14b

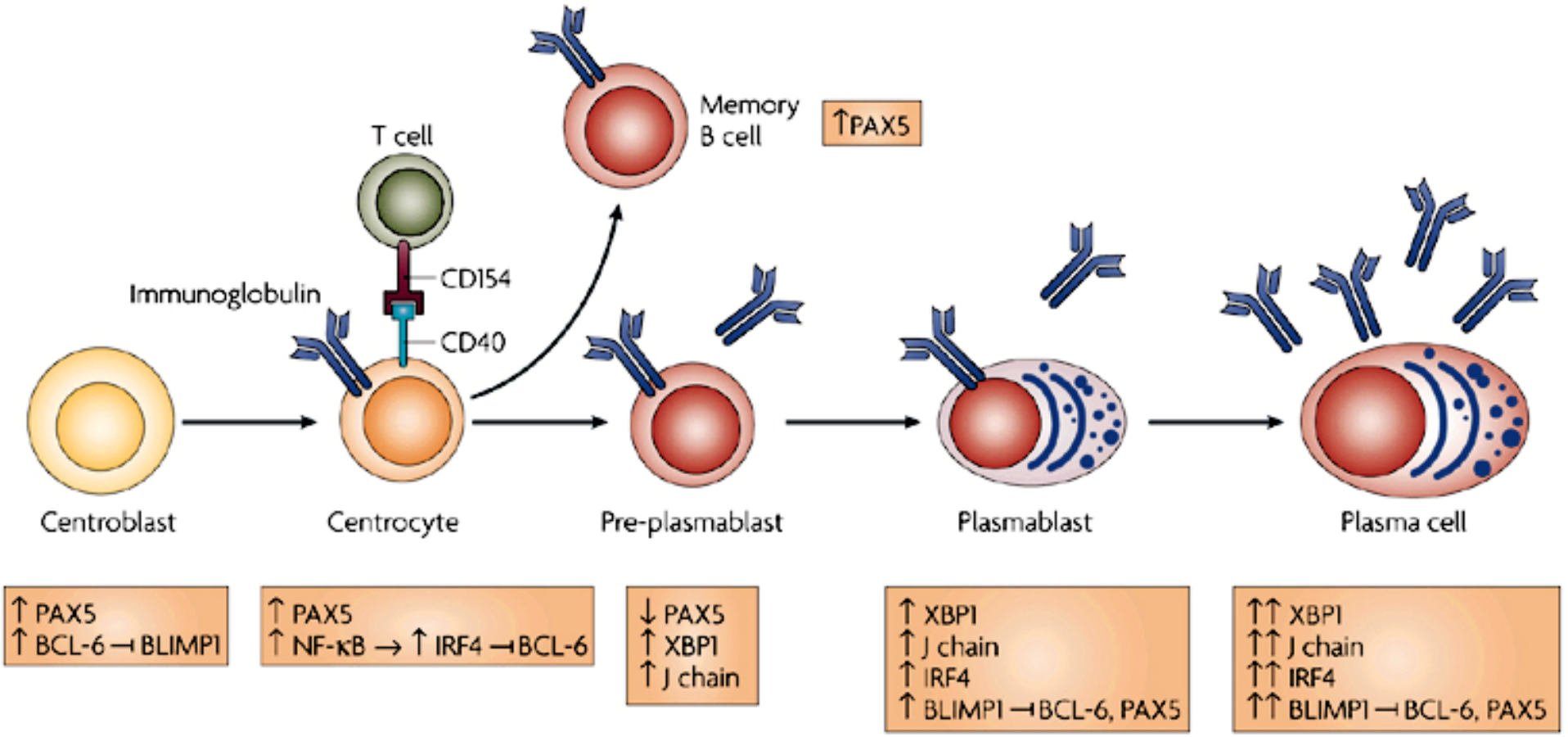


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TABLE 11-6 Comparison of naive and memory B cells

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 mIg.

Table 11-6
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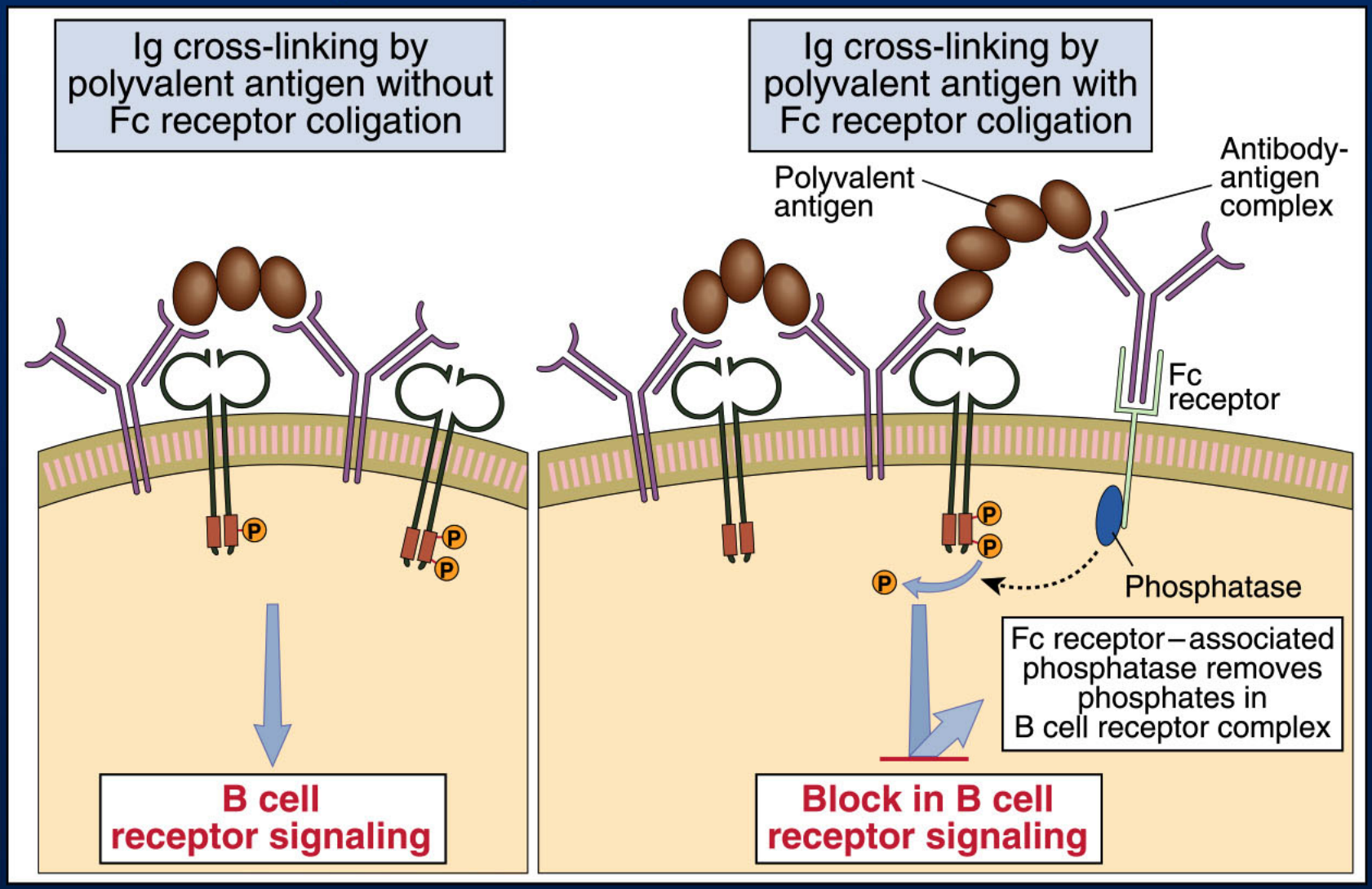
Slide 9-20

Fc receptor regulation of B cell activation



From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 9-17