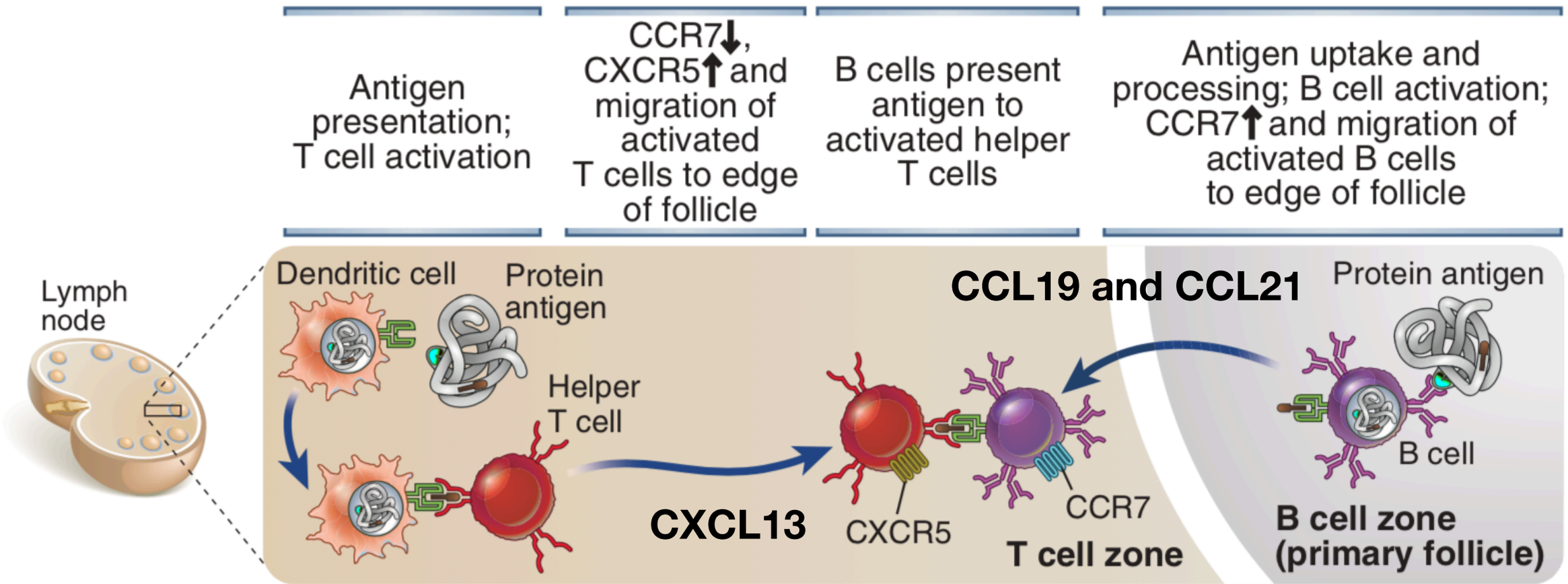
T-dependent B cell activation

Follicular B cells (lymphoid organs and blood) - protein antigens

T-B border interaction

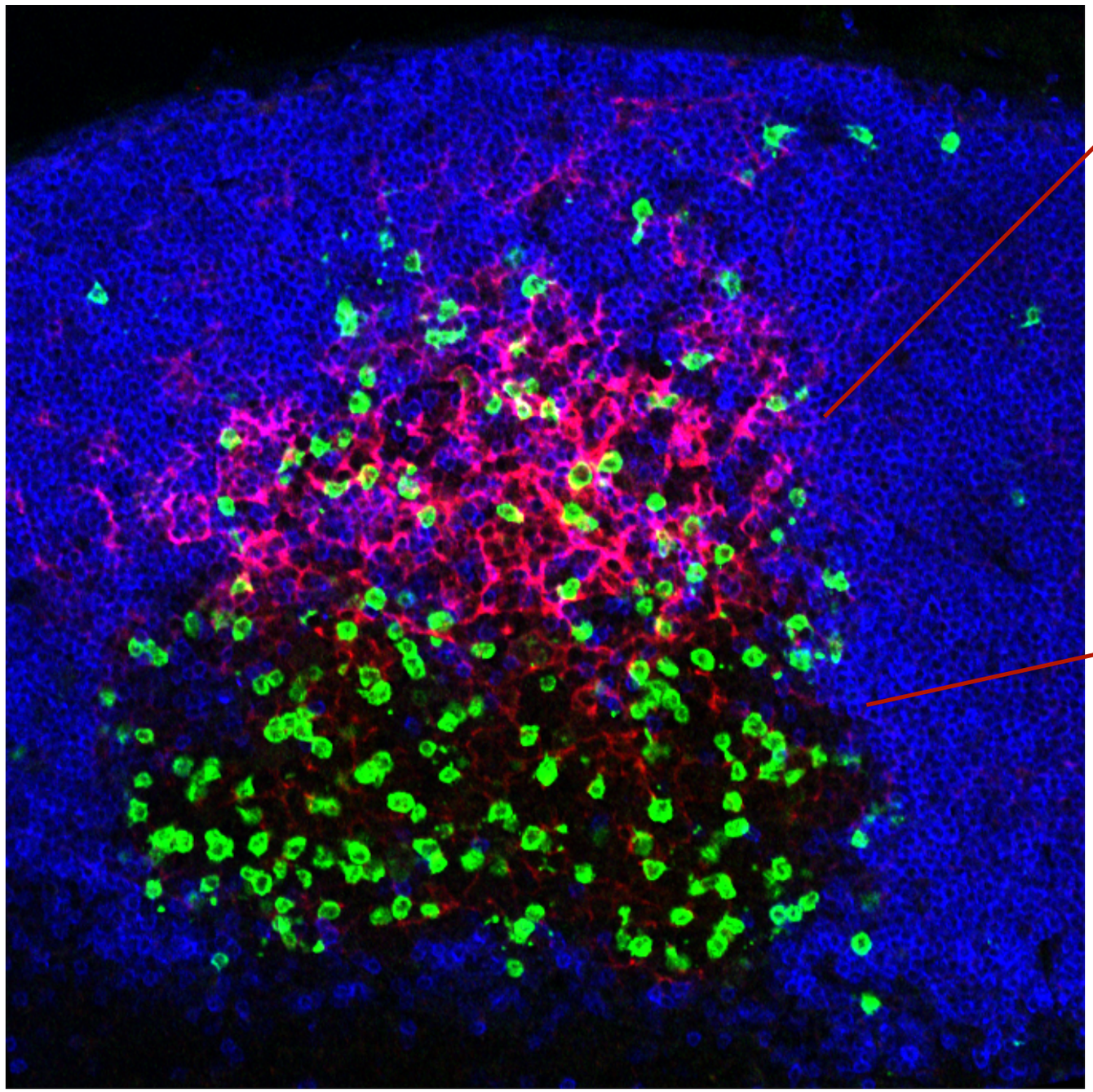


Presentation in MHCII

CD40 activated on B

TRAF and ↑ NFK, AP-1

Germinal center reaction



Light zone (selection)

Tfh FDCs B

Dark zone
(proliferation and SHM)

Responding B cells

Naive B cells

**FDCs (CR1,CR2,CR3,FCR
no BM derived No MHCII)**

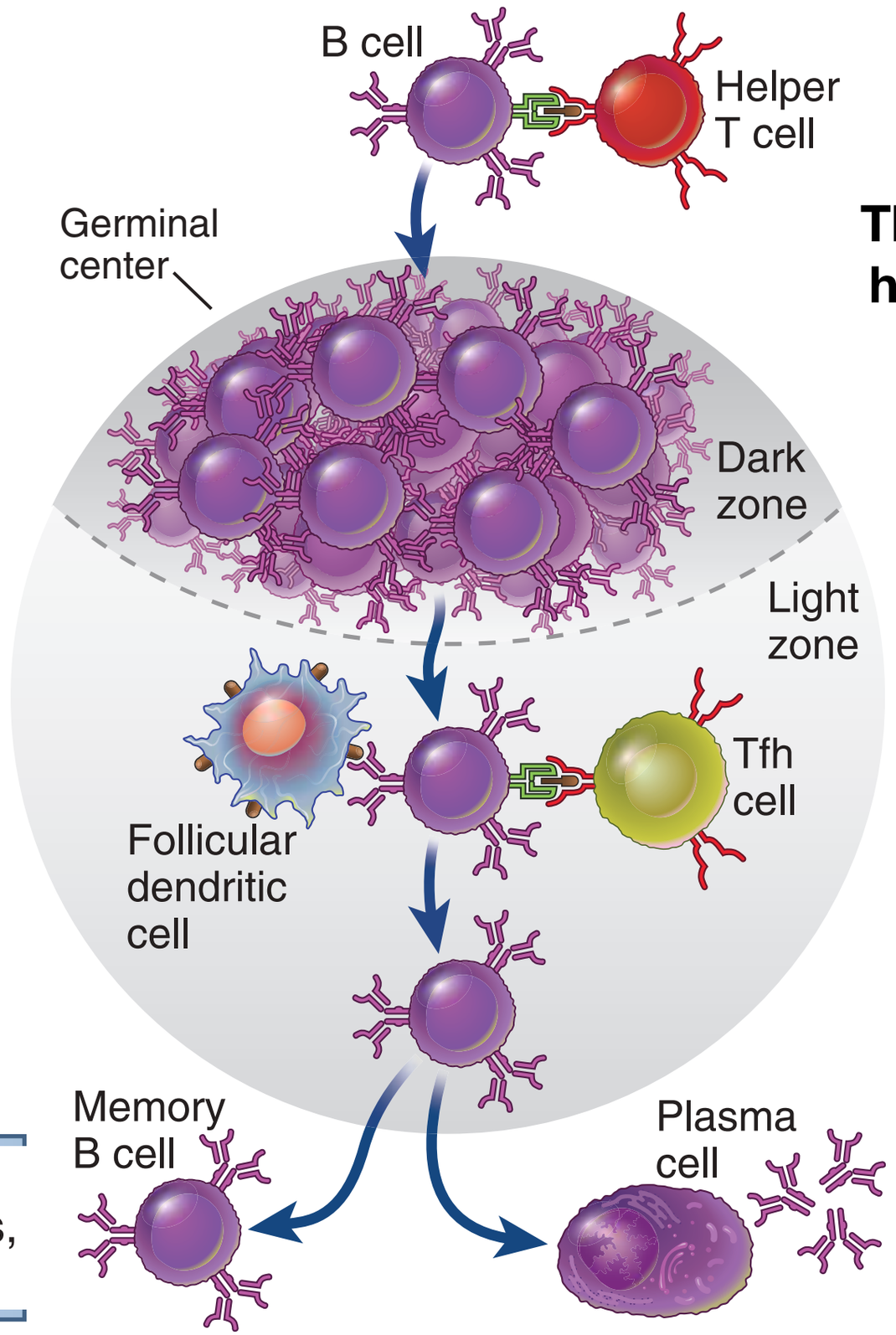
The germinal center reaction in a lymph node

Activation of B cells and migration into germinal center

B cell proliferation and somatic hypermutation

Selection of high affinity B cells; isotype switching

Exit of high-affinity antibody-secreting cells, and memory B cells



ThF differentiated after high activation of TCR

BCl6 repressor

Alpha chain of ILR

ICOS and IL21

Heavy chain class switching

B cells initially express **IgM** and **IgD (alternative splicing)**

After class switch B cells will express other isotypes: **IgG, IgA** and **IgE**

During the process of class switch the constant region in the heavy chains is modified, but antigen specificity does not change

SAME SPECIFICITY - DIFFERENT EFFECTOR FUNCTIONS

5-14 IgM and IgD are derived from the same pre-mRNA transcript and are both expressed on the surface of mature B cells

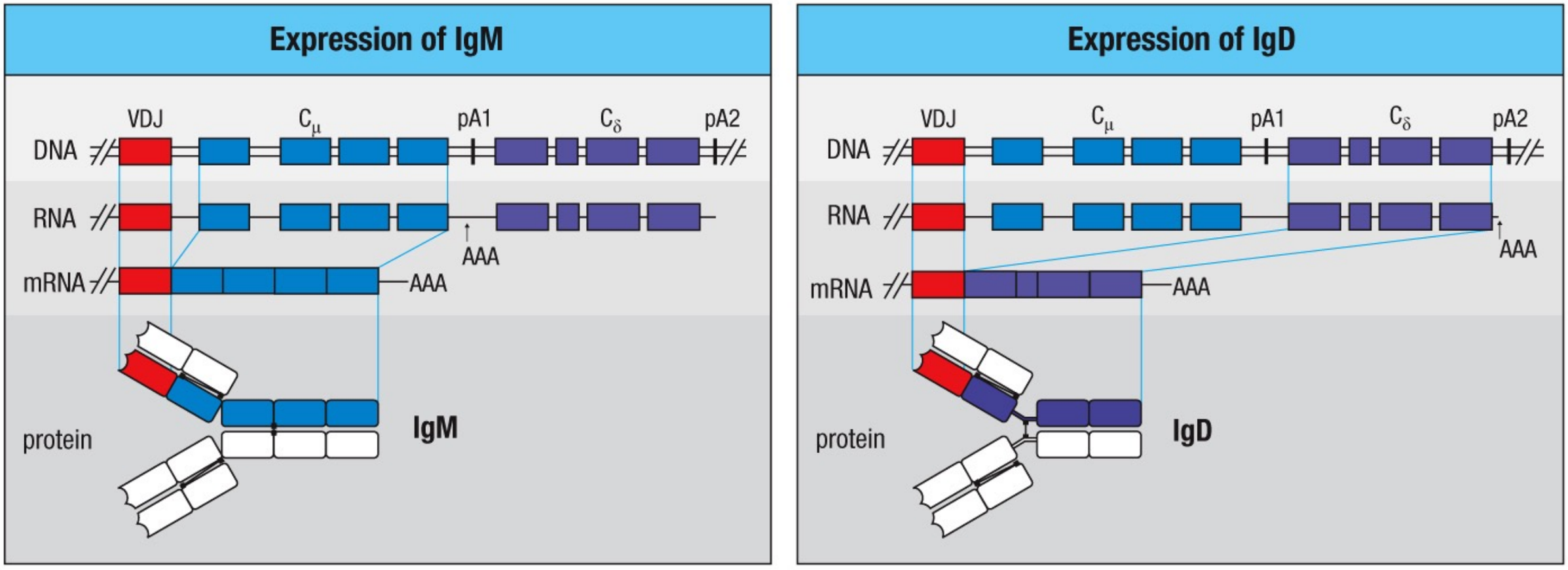


Figure 5.21 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Class switching is directed by cytokines

Cytokines are produced by Th cells and, in some cases (mucosal sites) by other cells in the environment

INFγ

IL-4

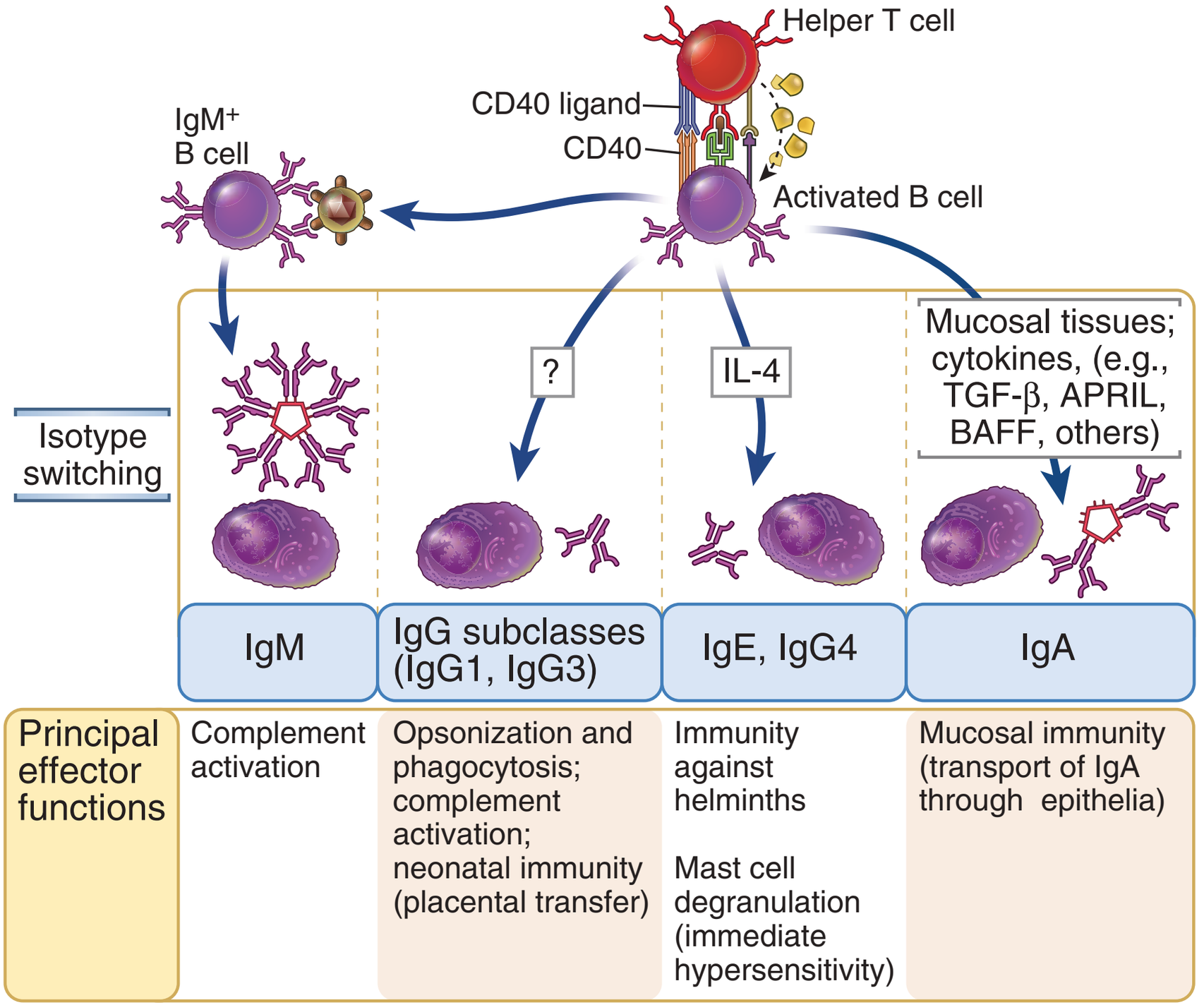
TGF-β, BAFF and APRIL

Class switching is directed by cytokines

Cytokines are produced by Th cells and, in some cases (mucosal sites) by other cells in the environment

INFγ	IgG1, IgG3
IL-4	IgE, IgG4
TGF-β, BAFF and APRIL	IgA

Ig heavy chain isotype switching

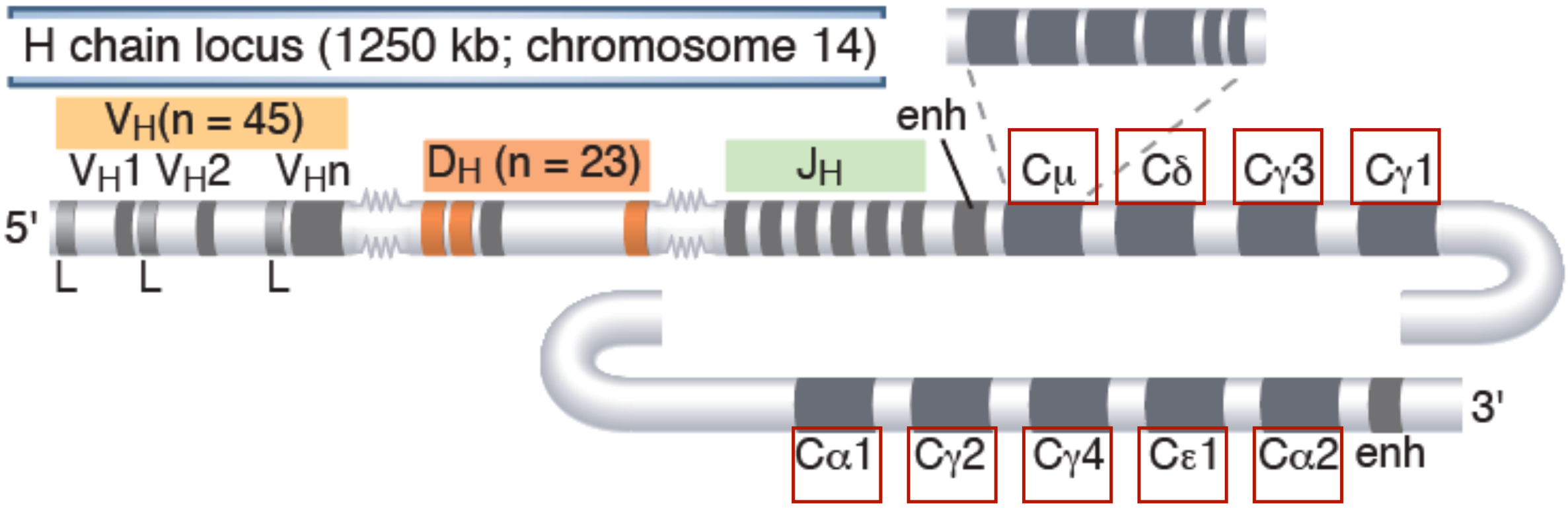


Class switching requires CD40 stimulation and AID expression

CD40-CD40L interaction between B cells and T cells leads to **AID** expression in B cells

AID enzyme is required for class switch recombination, its expression is tightly regulated

Heavy chain locus contains nine different constant regions

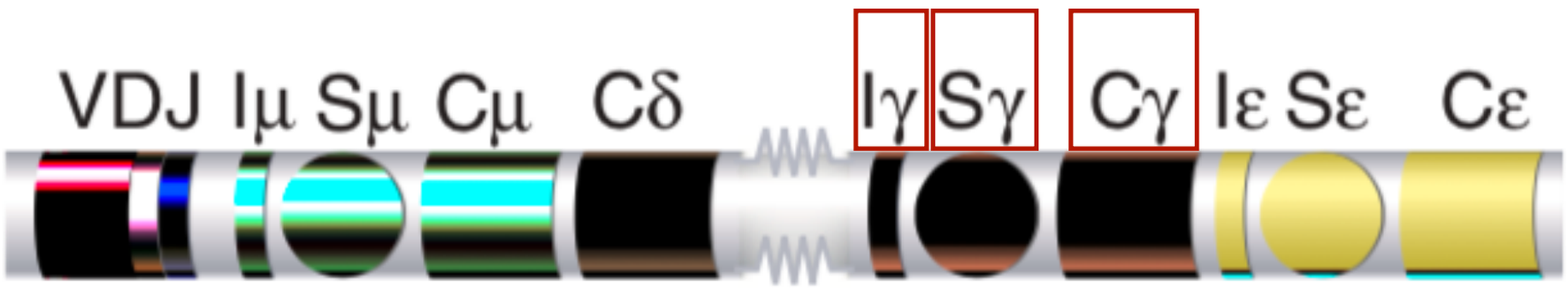


IgM, IgD, IgG3, IgG1, IgA1, IgG2, IgG4, IgE and IgA2

Every C is associated with a switch region S

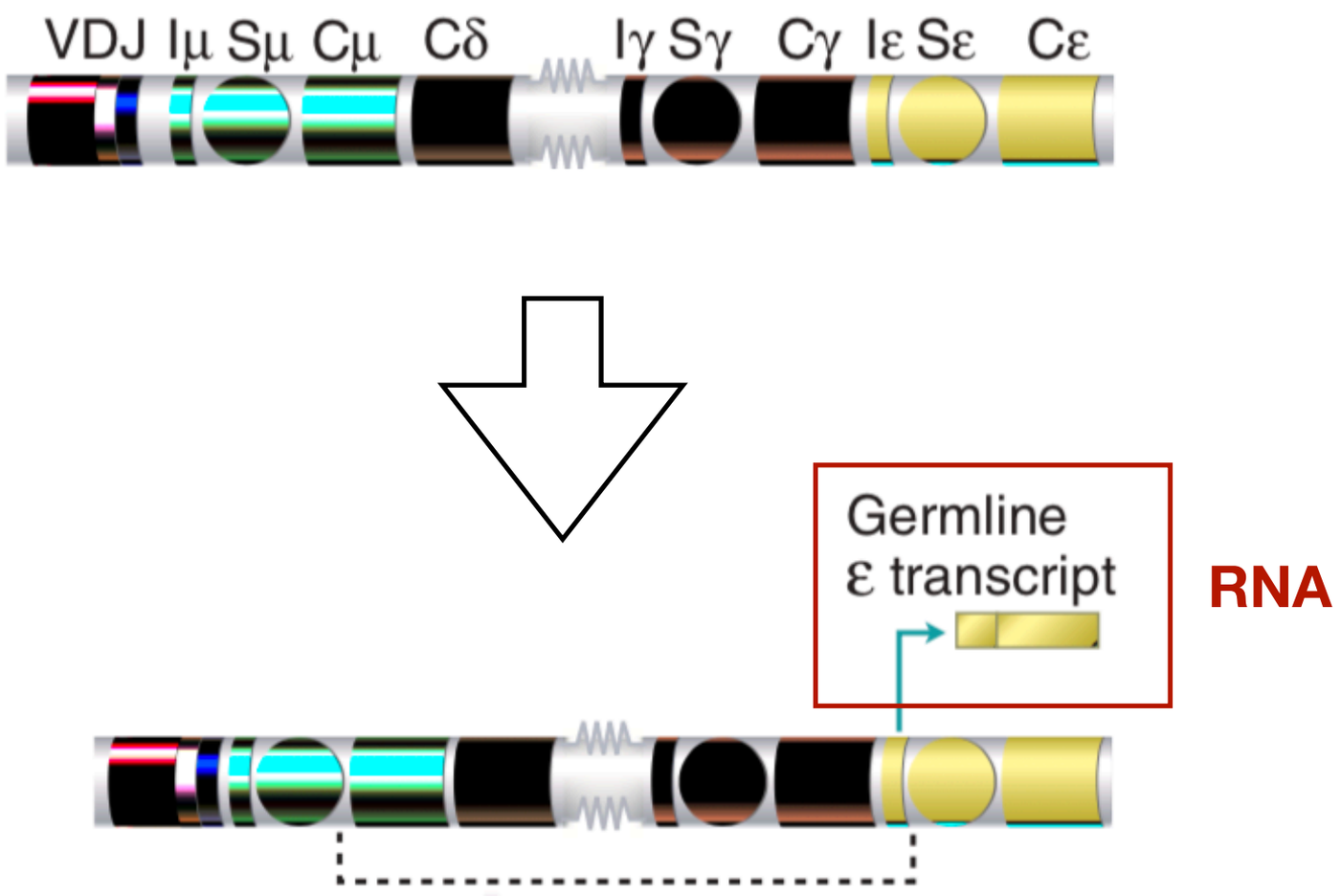
At the 5’ of every C (except for Cδ) there are:

- small exon **(I)**, downstream of a promote region, where transcription initiates
- GC rich DNA sequences called **switch regions (S)**

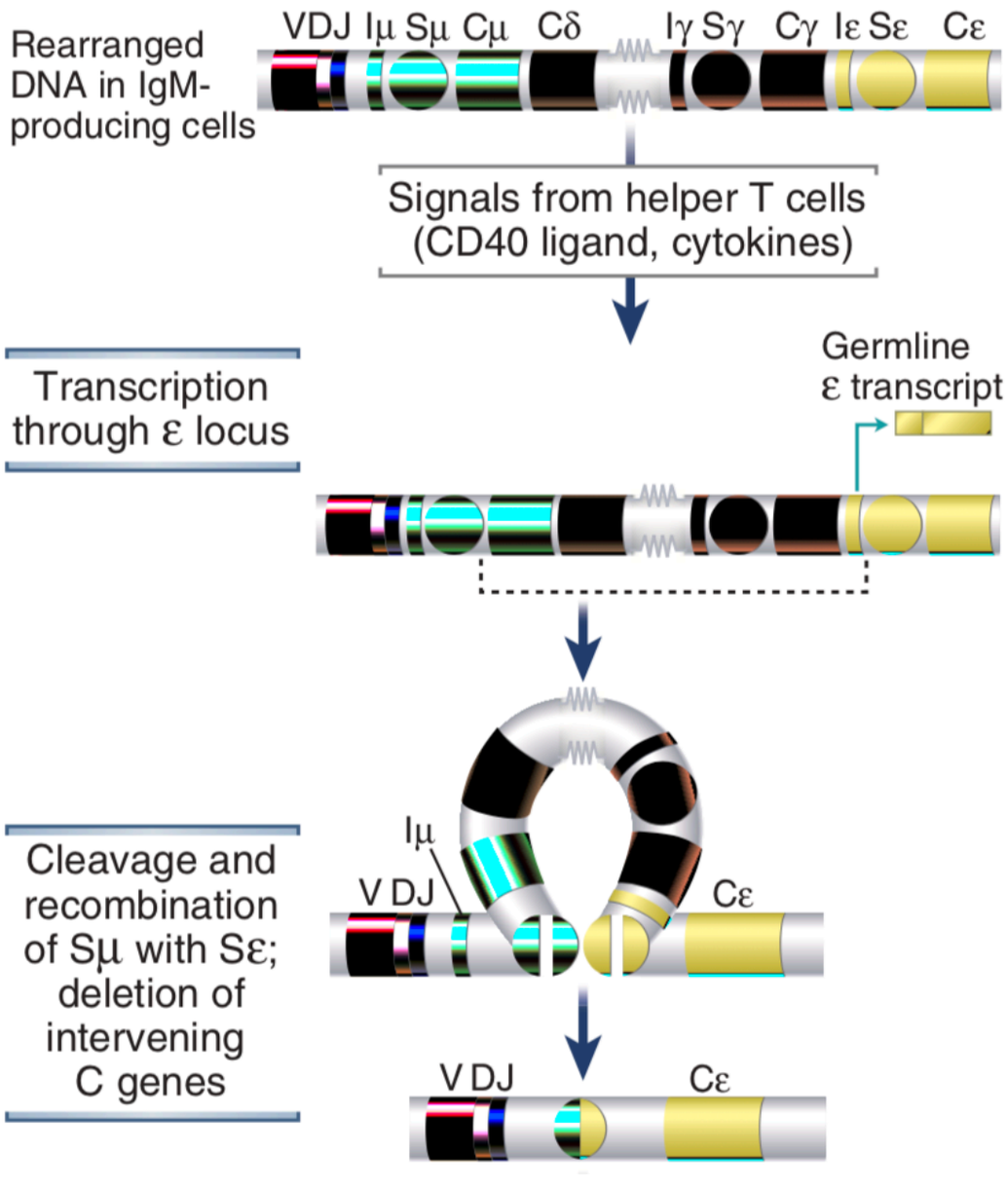


Germline transcripts are transcribed

Various signaling leads to the transcription at different promoter sites. These mRNA are not translated and are called **germline transcripts**



Transcription is associated to chromatin and DNA opening



Accessible switch regions in the chromosome recombine. The sequence in between is lost.

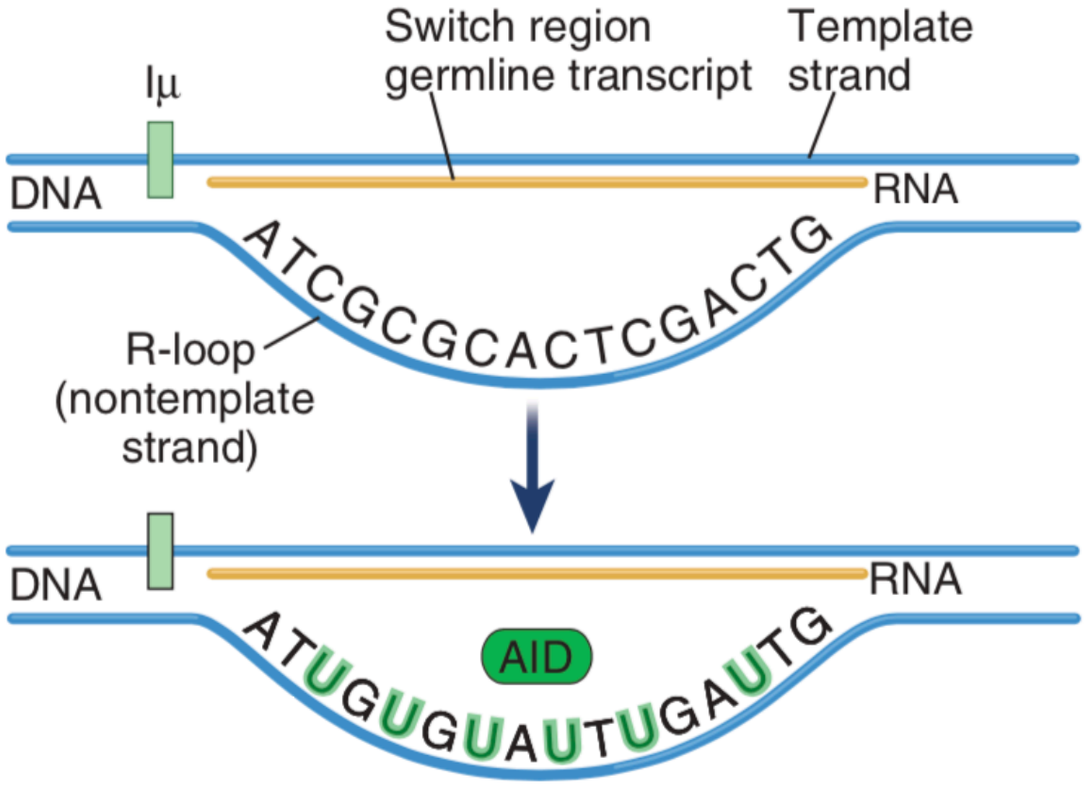
..but how exactly DNA recombine??

In general DNA recombination requires:

1 - Induction of a double strand break (DSB)

1 - Repair of a double strand break (DSB)

AID, UNG and APE1 cooperate to induce DSB in the switch regions

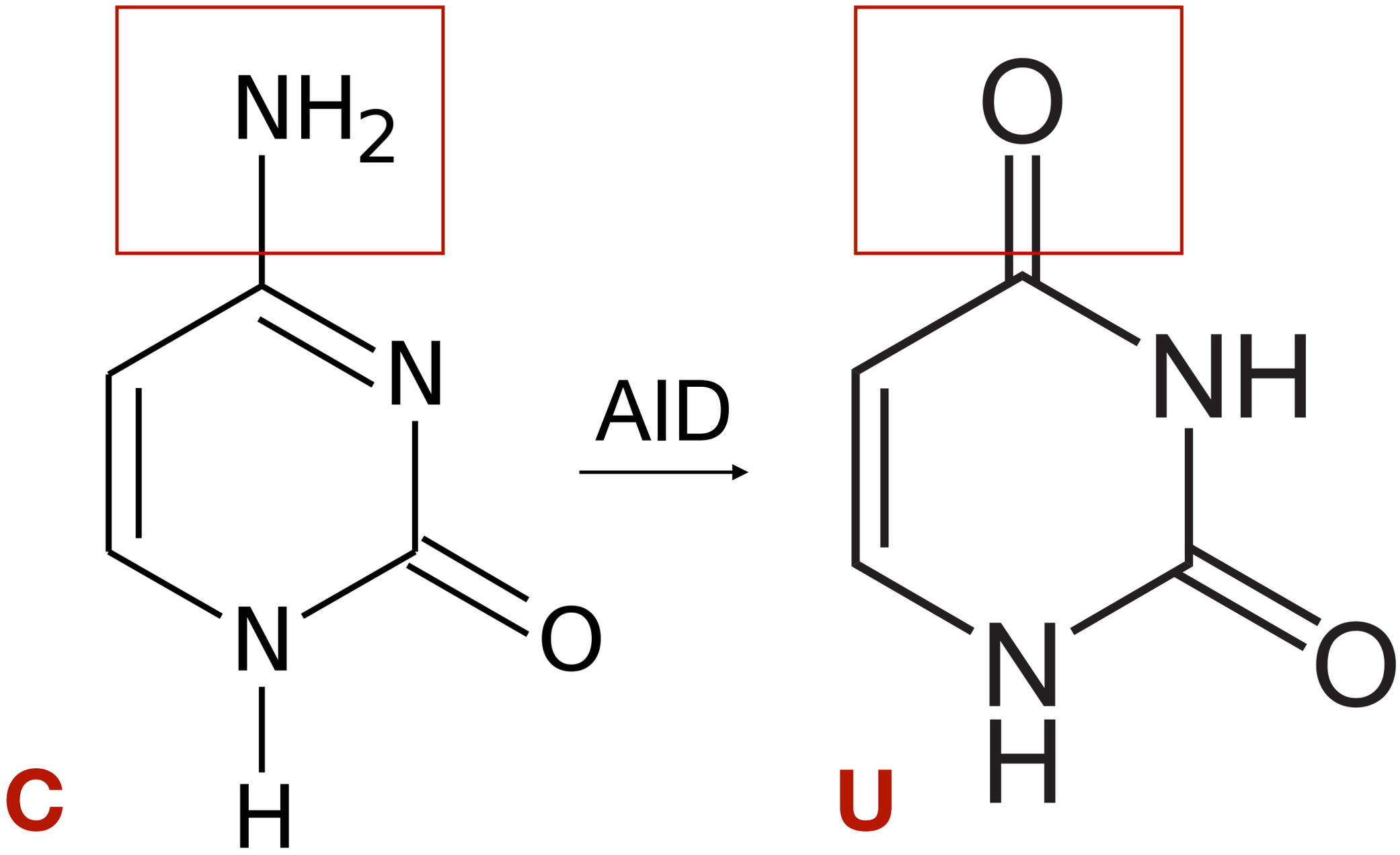


The germline transcript binds firmly to the template strand (GC rich)

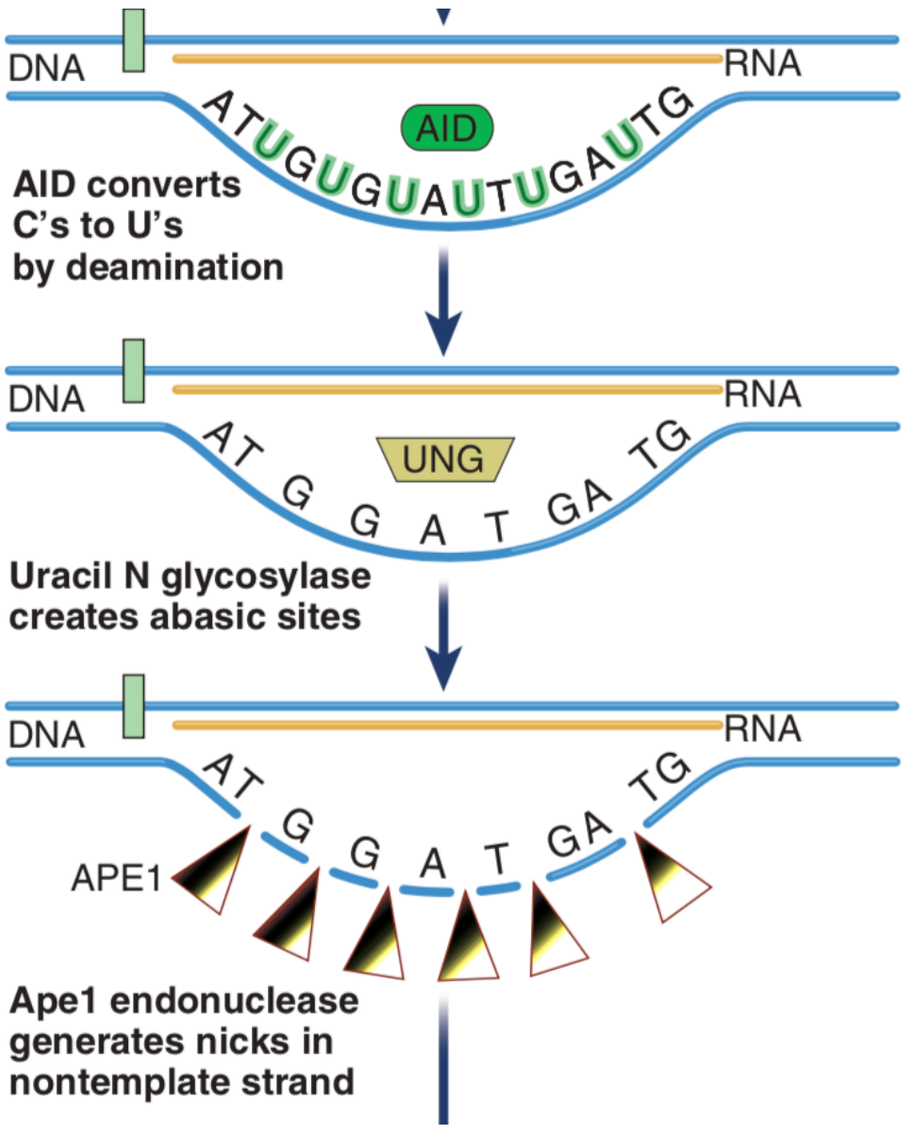
A single strand DNA loop (R loop) is available for AID (AID only bind ssDNA)

AID converts C to U by deamination

AID =Activation-Induced cytidine Deaminase


NH2
N
N
H
O
C
AID
O
NH
N
H
O
U

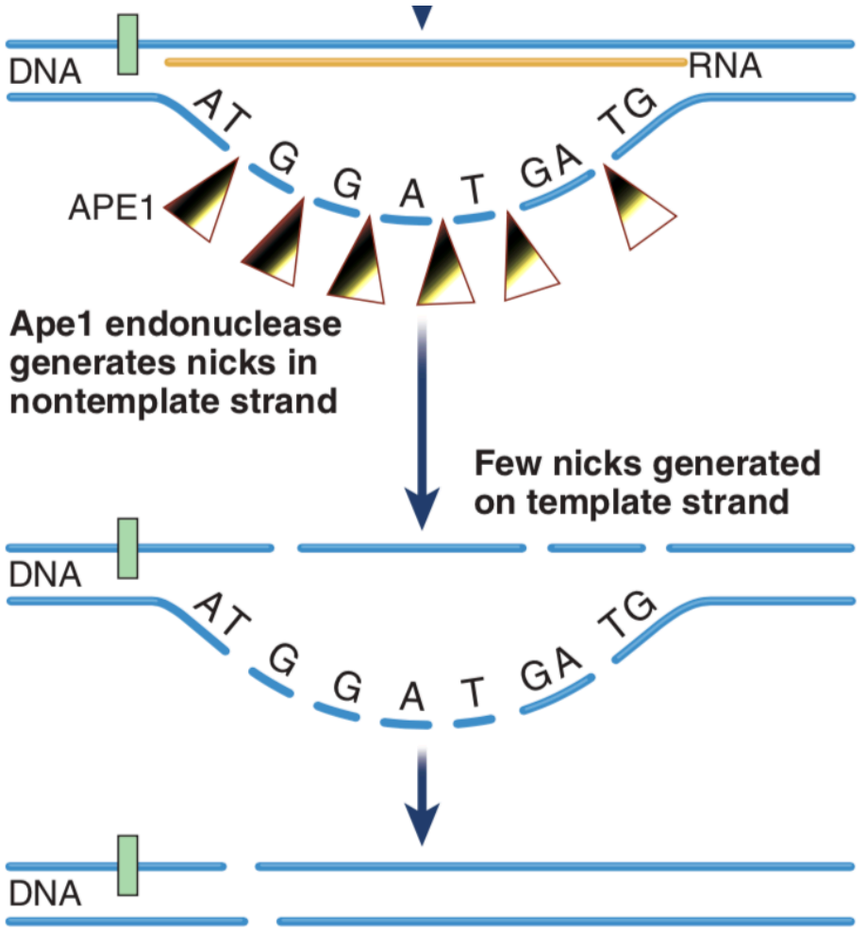
AID, UNG and APE1 cooperate to induce DSB in the switch regions



UNG (= Uracil N glycosylase) removes U base from the DNA stand and creates **abasic sites** which are cleaved by the endonuclease Ape1

Single strand DNA breaks are formed

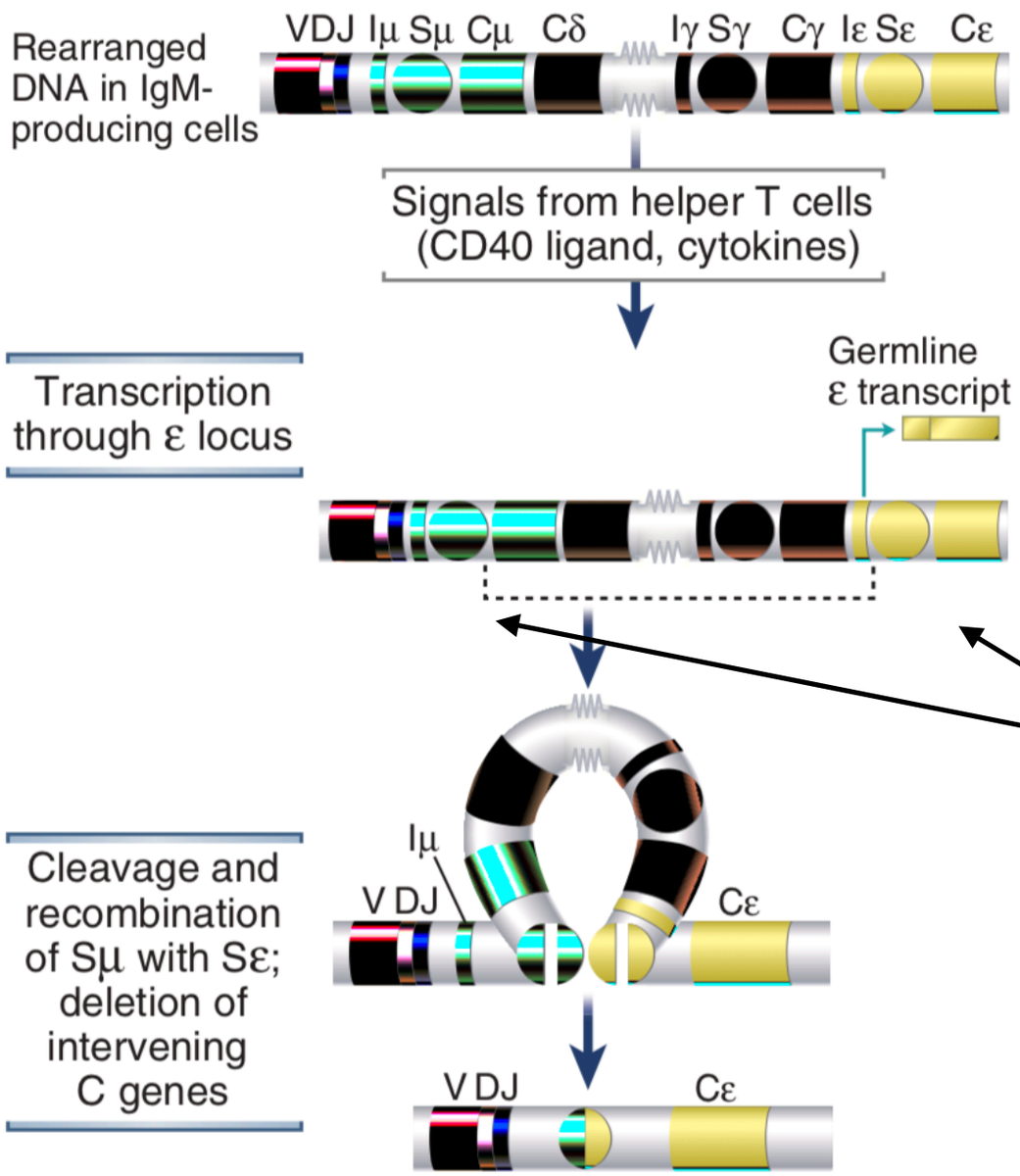
AID, UNG and APE1 cooperate to induce DSB in the switch regions



RNA associated to the DNA in the switch region is degraded by a complex called RNA exosome

AID, UNG and APE1 create breaks also on the second strand

Double strand DNA breaks are formed



Double strand breaks are induced here

DNA repair mechanism lead to DNA ligation

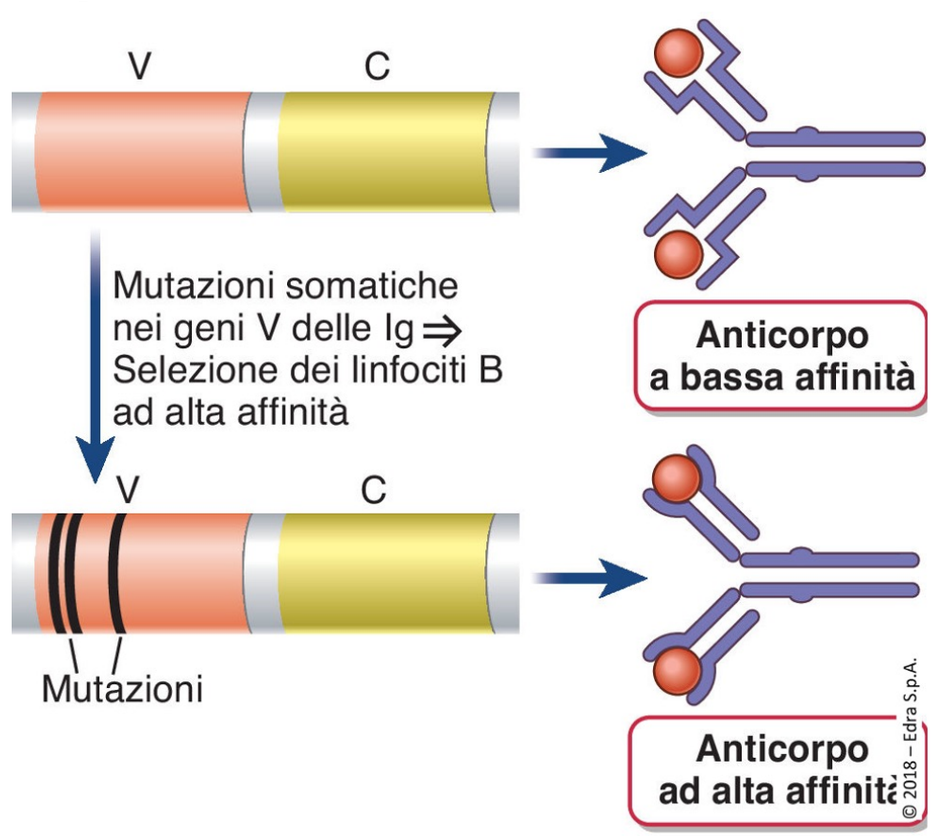
Affinity maturation

Production of antibody with increased affinity for the T dependent antigens

Affinity maturation relies on two processes:

1 Somatic hypermutation of the immunoglobulin genes

2 Affinity-based selection



Somatic hypermutation

B cells in the dark zone of germinal centers proliferate at high speed and express AID. AID deaminates C in U at specific hotspots contains the sequence **AGCT**.

When DNA replicates, Us are converted in T > insertion of point mutations

Us can also be removed by UNG, with the formation of abasic sites. During replication, abasic sites are repaired by addition of random nucleotides > insertion of point mutations

MSH2 and MSH6 involved in process of DNA mismatch repair
engage error-prone DNA polymerase

Both heavy and light chains are targeted by AID and incorporate mutations

10-10 Mismatch and base-excision repair pathways contribute to somatic hypermutation following initiation by AID

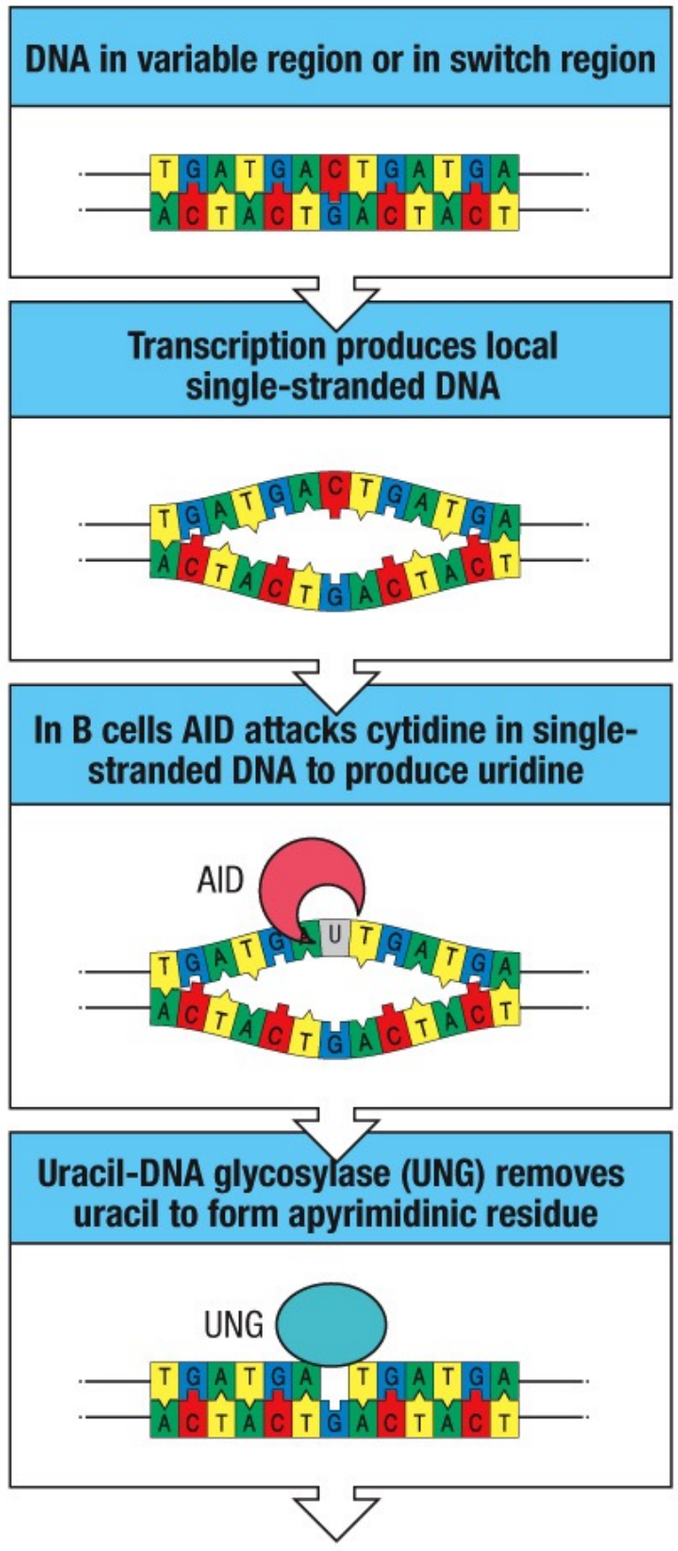
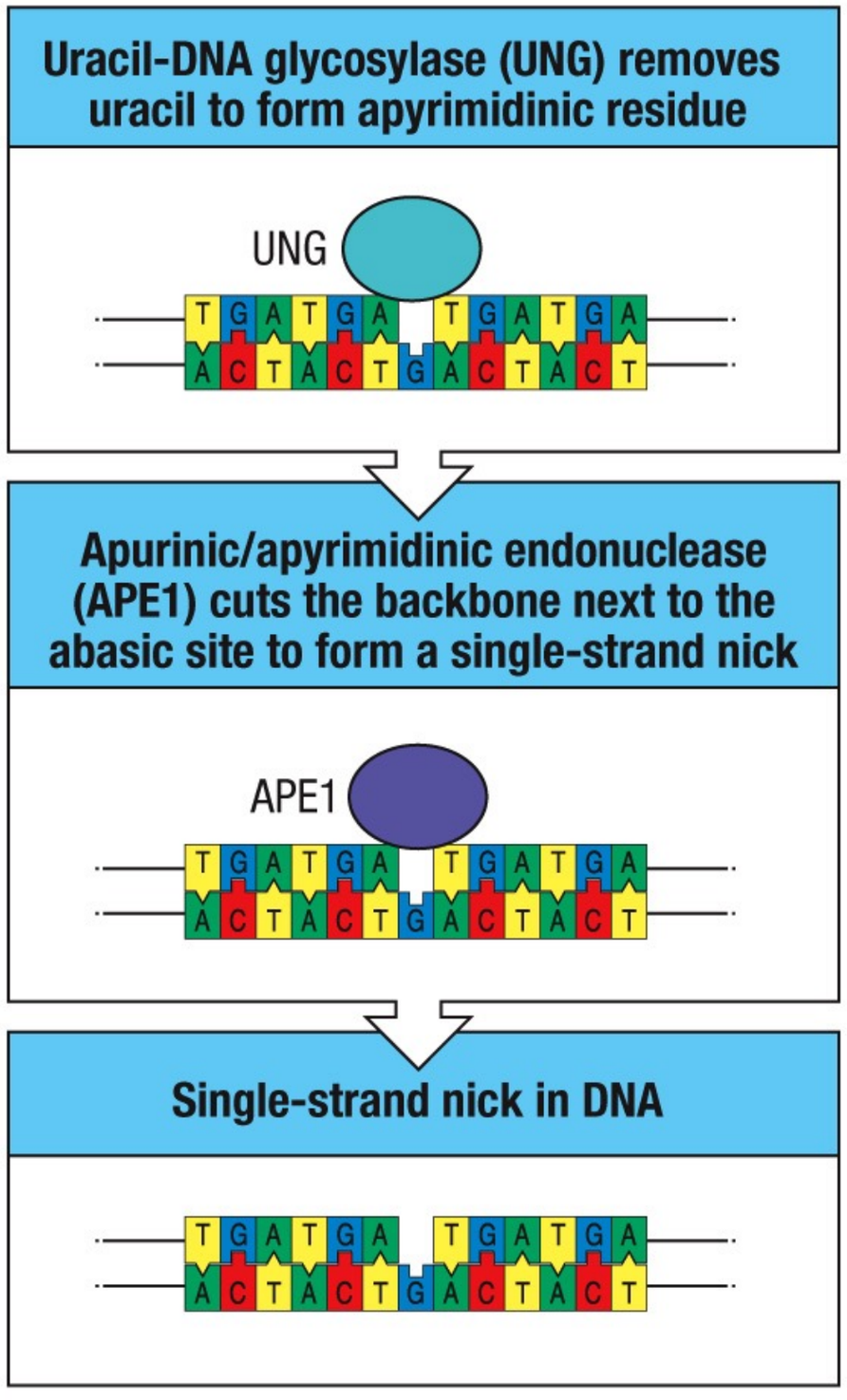


Figure 10.20 (part 1 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

10-10 Mismatch and base-excision repair pathways contribute to somatic hypermutation following initiation by AID



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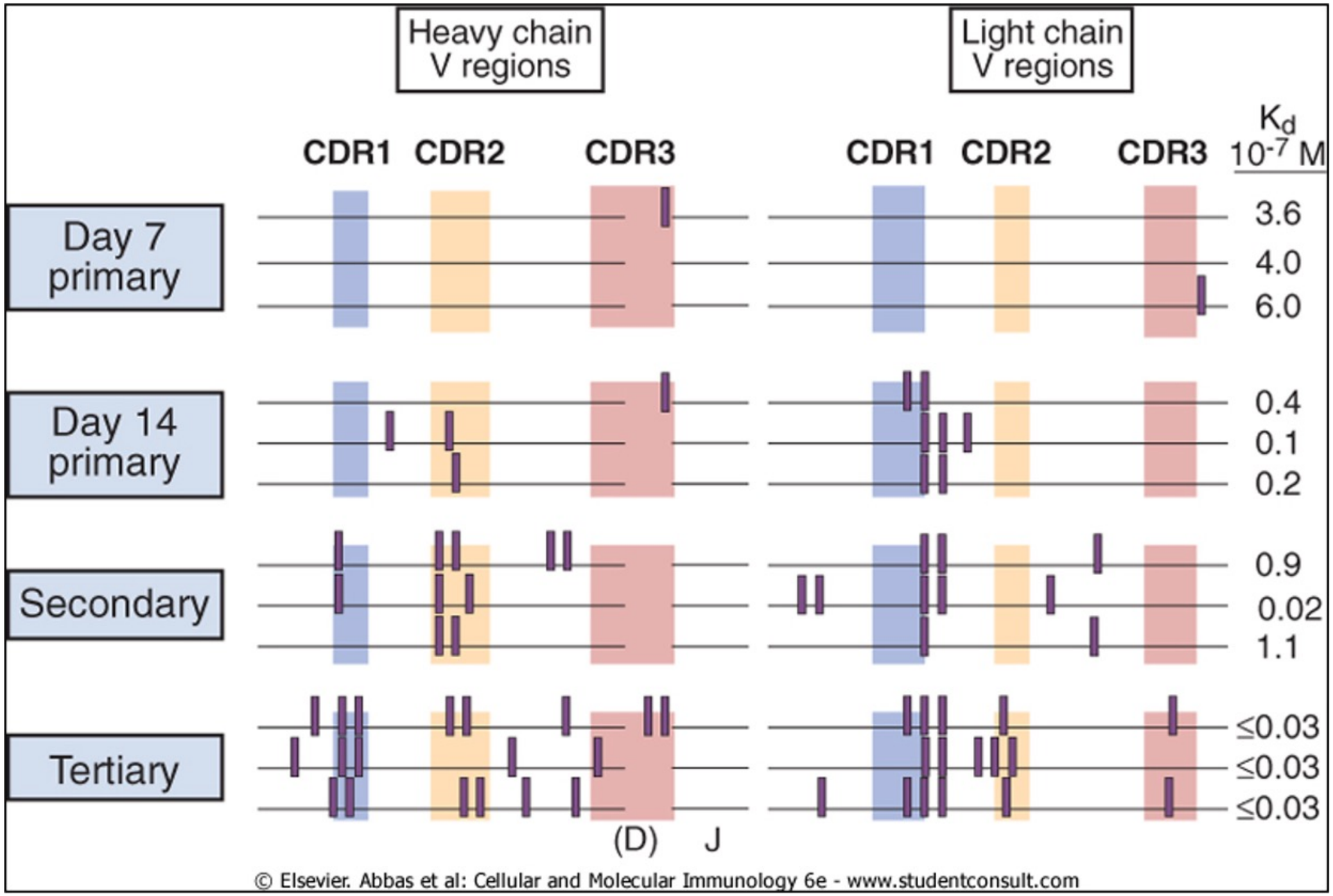
Mutations preferentially accumulate in the CRDs



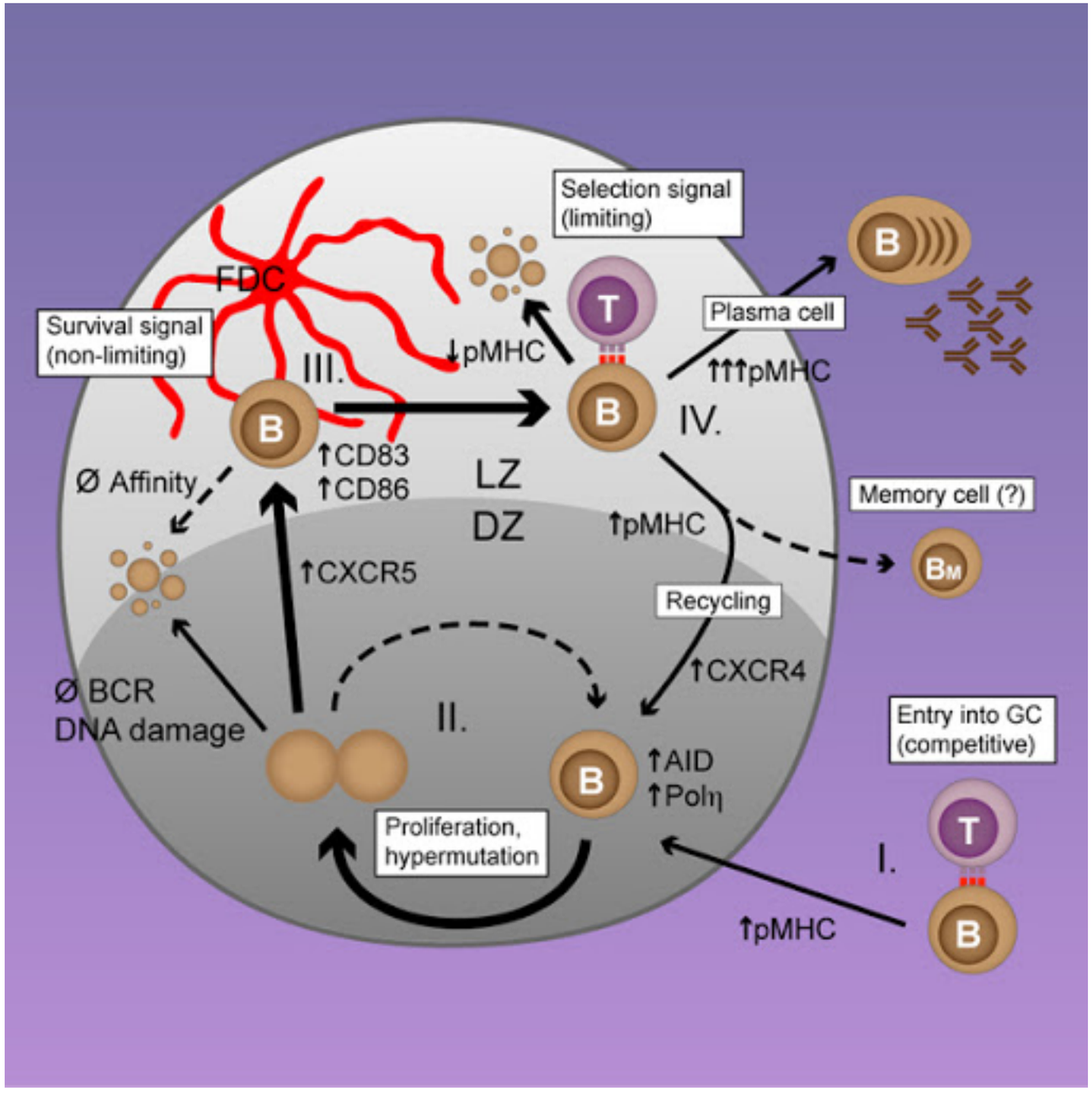
Side effects of somatic hypermutation

- Linfoma (B cell tumors)

  The DNA breaks associated with somatic hypermutation and isotype switching predispose to chromosomal translocations of various oncogenes into Ig gene loci

- Generation of self-reactive clones

Affinity based selection



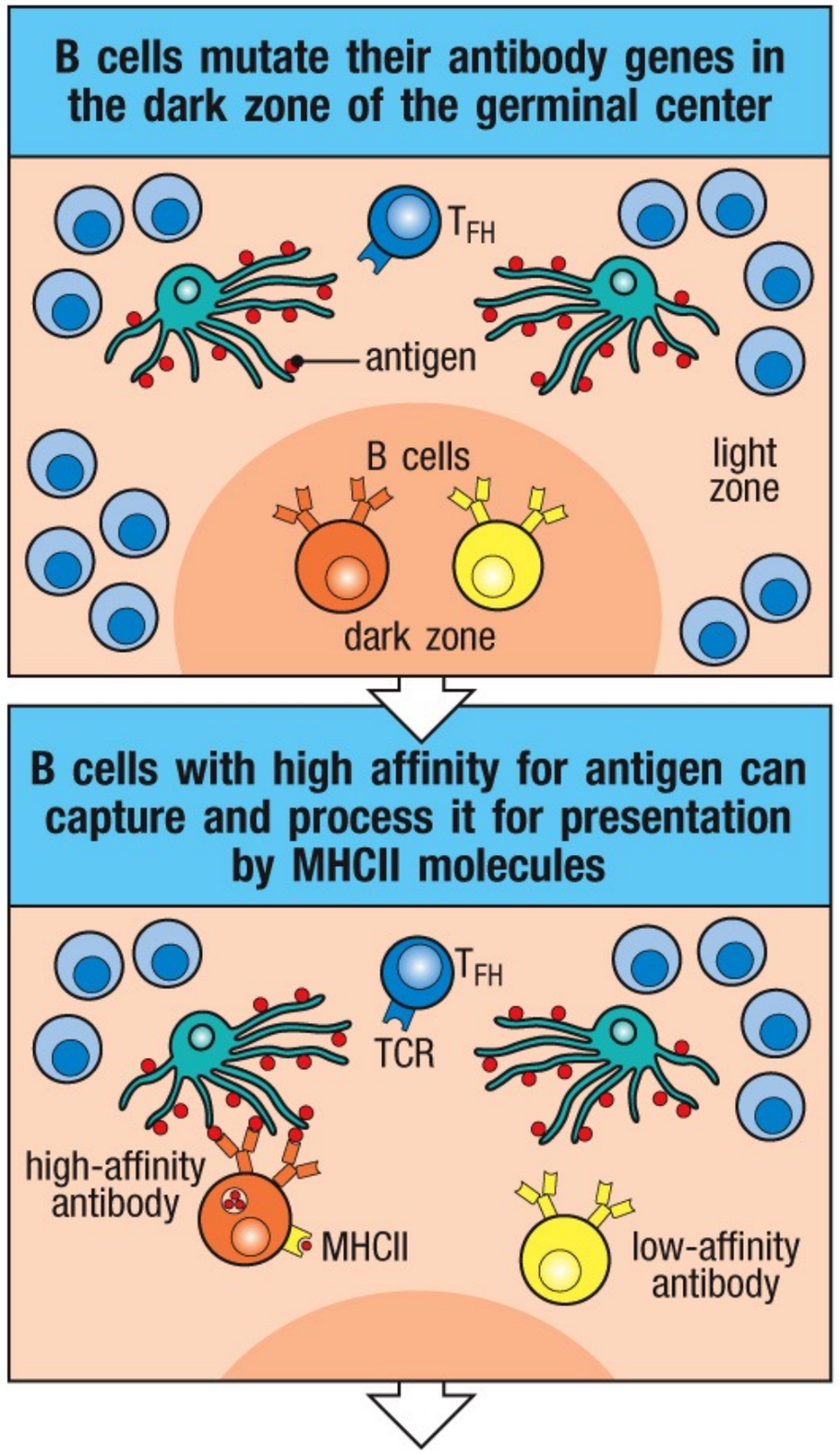
In the light zone, antigen and T cell help are limited

Survival signal are due to the recognition of the soluble antigen or antigen linked by FDCs (complement and Antibody)

B cells compete for antigen uptake and survival signals delivered by Tfh (CD40L)

Only the B cells that can access T cell help survive

10-7 Germinal center B cells undergo V-region somatic hypermutation, and cells with mutations that improve affinity for antigen are selected



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10-7 Germinal center B cells undergo V-region somatic hypermutation, and cells with mutations that improve affinity for antigen are selected

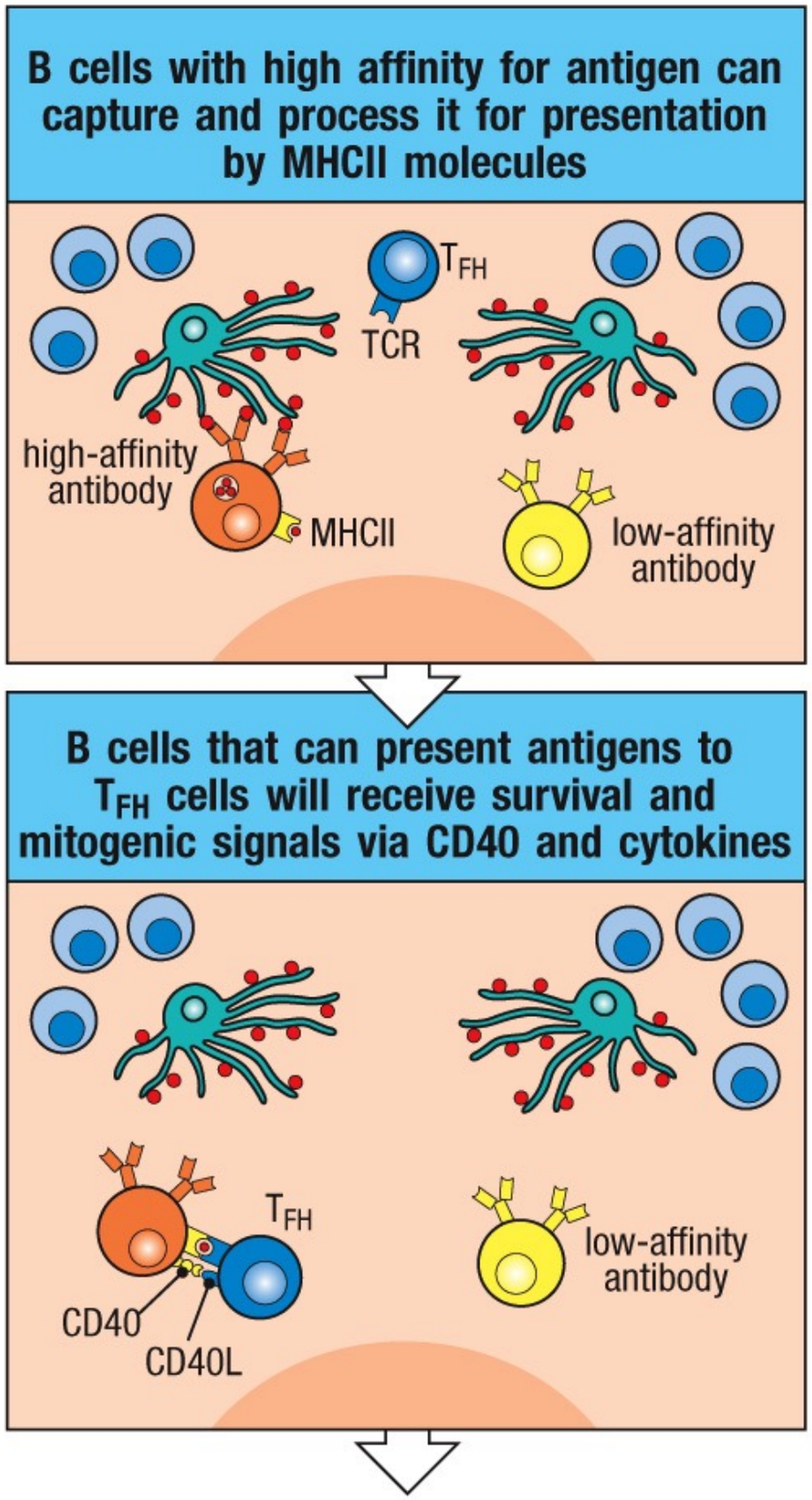


Figure 10.15 (part 2 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

10-7 Germinal center B cells undergo V-region somatic hypermutation, and cells with mutations that improve affinity for antigen are selected

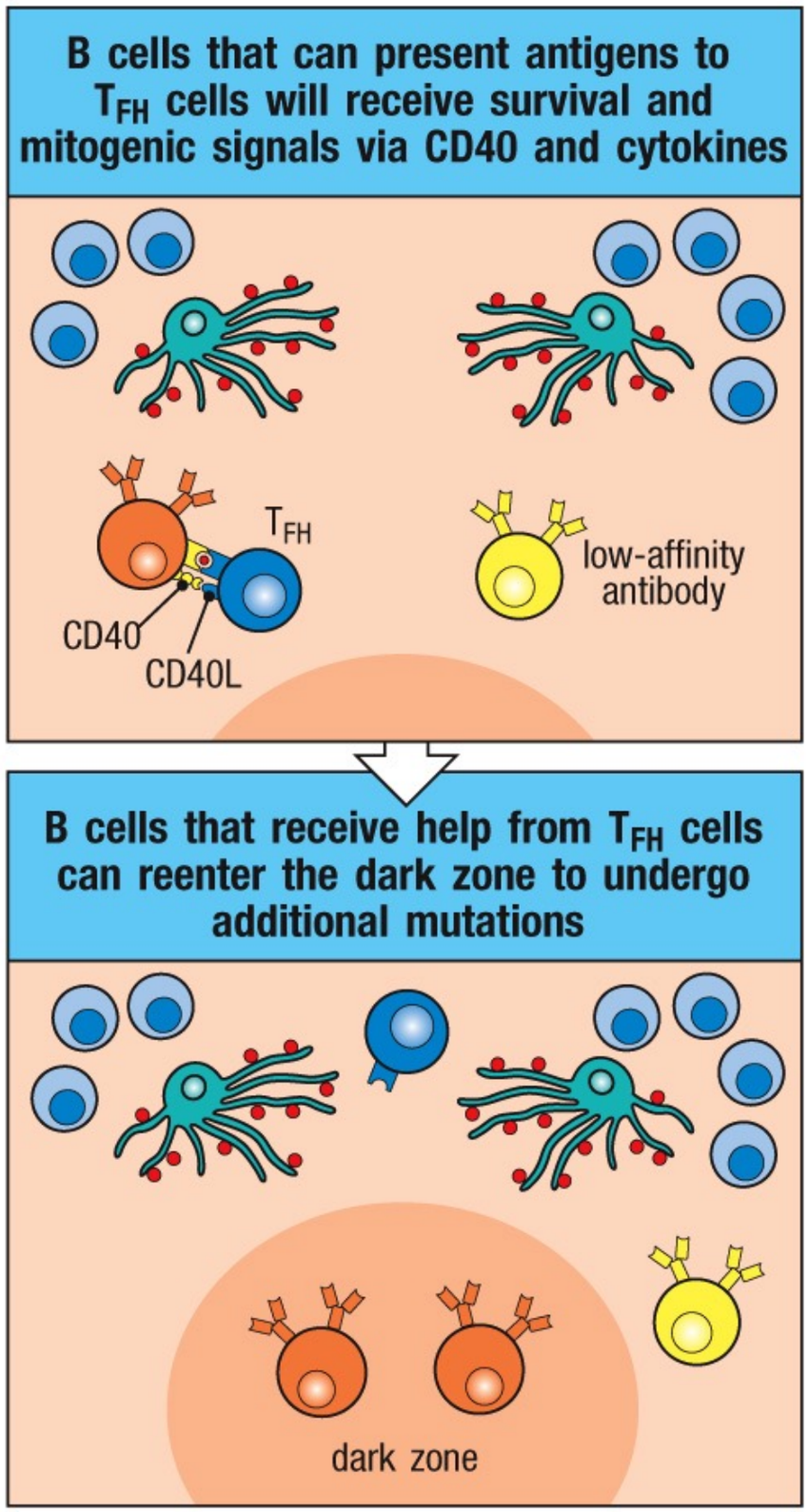


Figure 10.15 (part 3 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

B cells exit the GC and differentiate into PC or Memory

Plasma cells (PC)

Effector B cells, their main function is the production of large amounts of antibodies. CD20 is lost. They have high amount of cytoplasm and are very big.

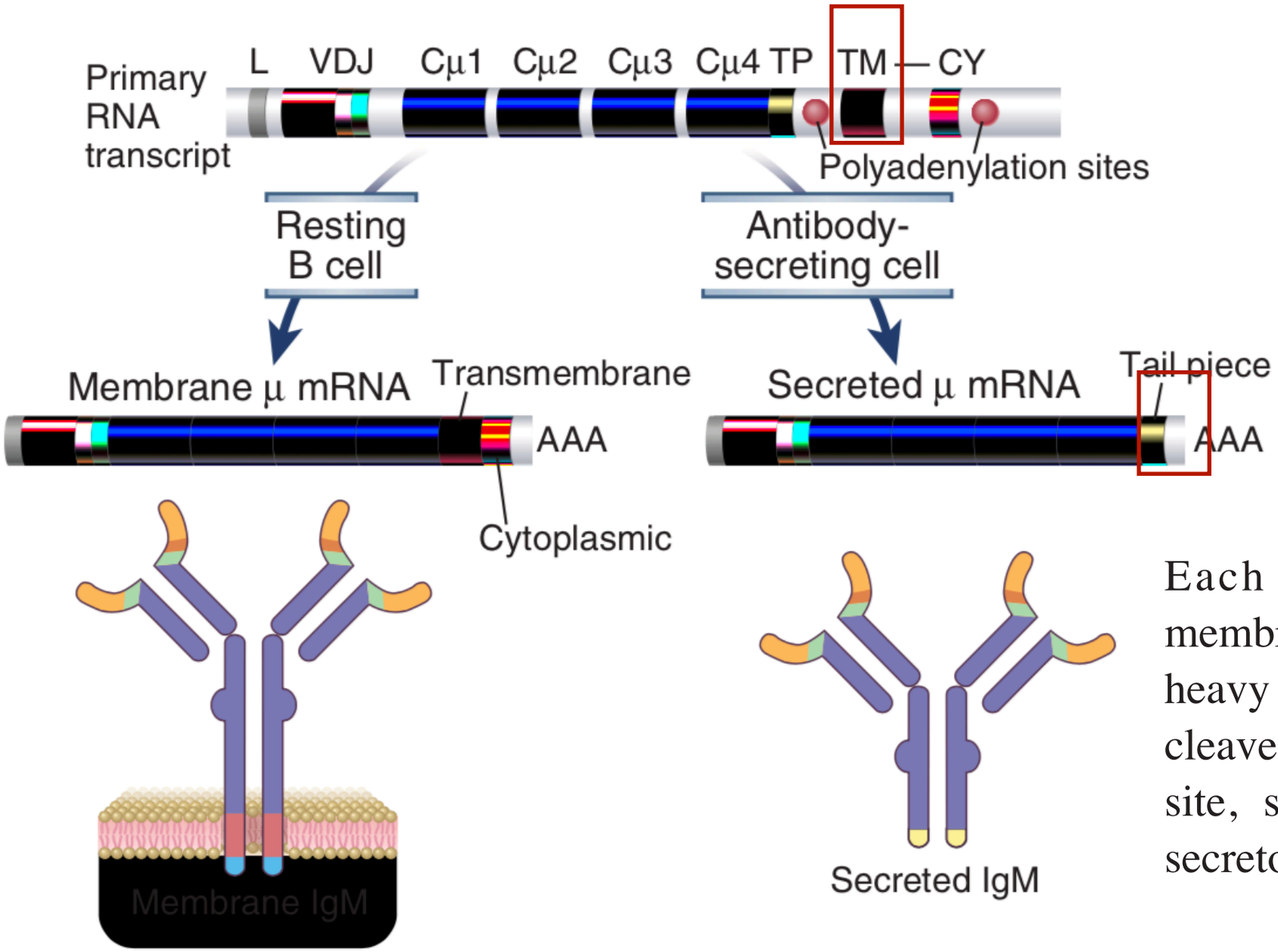
Short lived (T-independent, early T-dependent, home in secondary lymphoid organs and in the periphery)

Long lived (GC reaction, home in the bone marrow)

Plasma cells produce the soluble form of the Ig and can survive for many years.

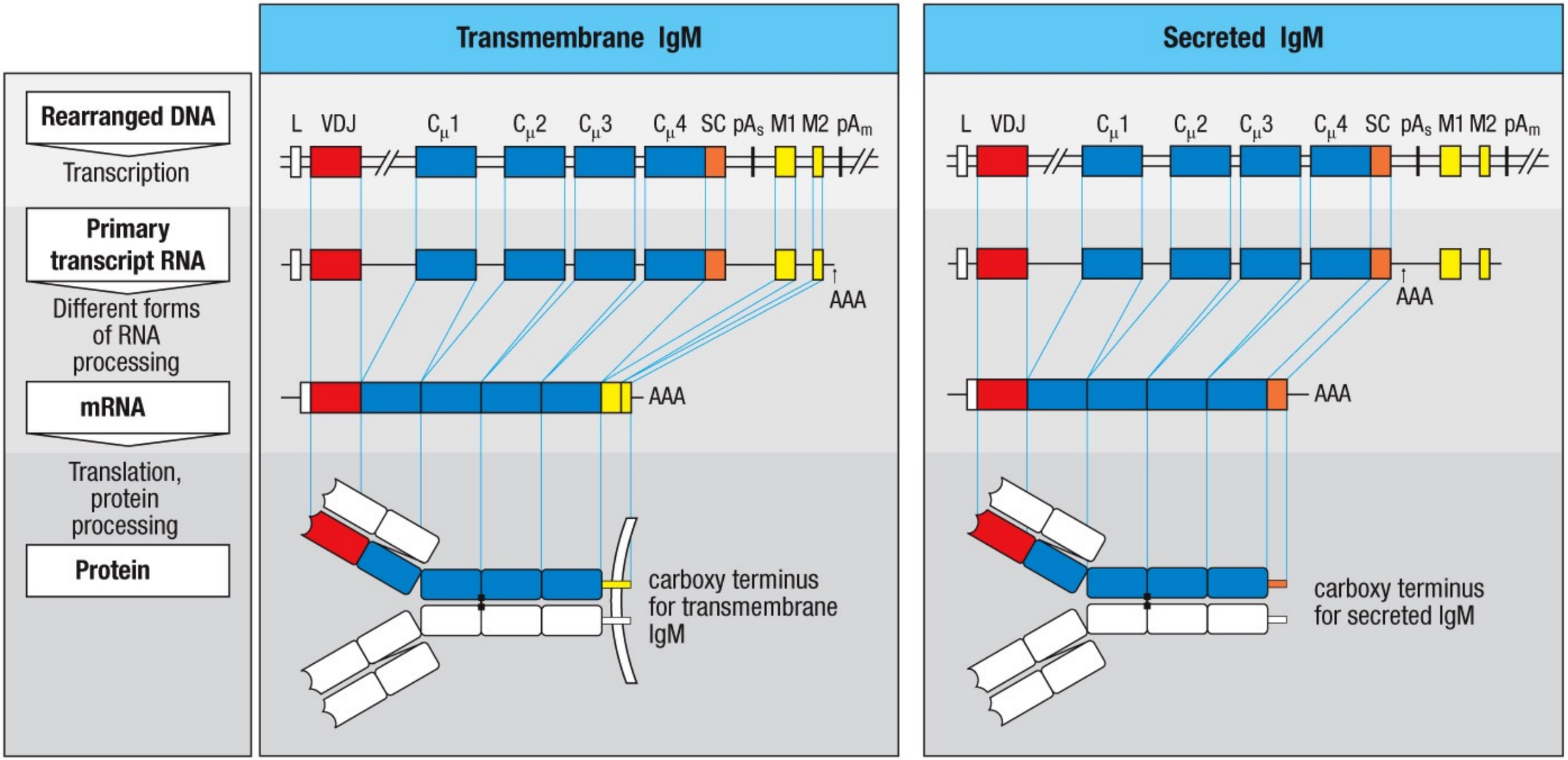
Plasma cells produce the soluble form of the Ig

Alternative splicing of the transcript



Each B cell can synthesize both membrane and secreted Ig. Most of the Ig heavy chain mRNA in a plasma cell is cleaved at the upstream polyadenylation site, so most of this mRNA is of the secretory form.

5-15 Transmembrane and secreted forms of immunoglobulin are generated from alternative heavy-chain mRNA transcripts



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Memory B cells

Generated by germinal center reactions, will rapidly reactivate to plasma cells in case of secondary antigen exposure
Express anti-apoptotic Bcl-2 molecule, generated by T dependent antigens

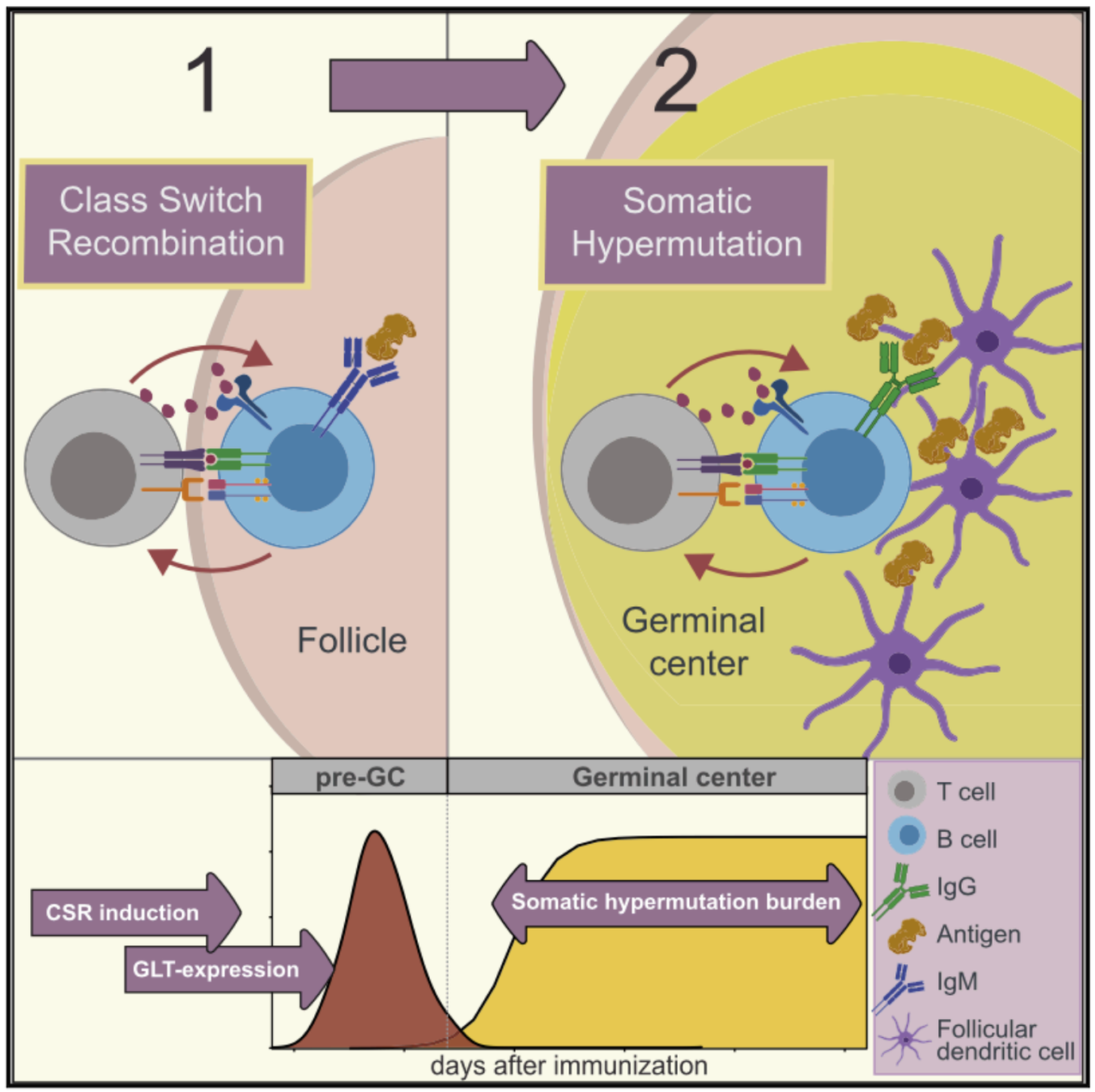
Recirculate through the blood and lymphoid organs

Article

Immunity

Class-Switch Recombination Occurs Infrequently in Germinal Centers

Graphical Abstract



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In Brief

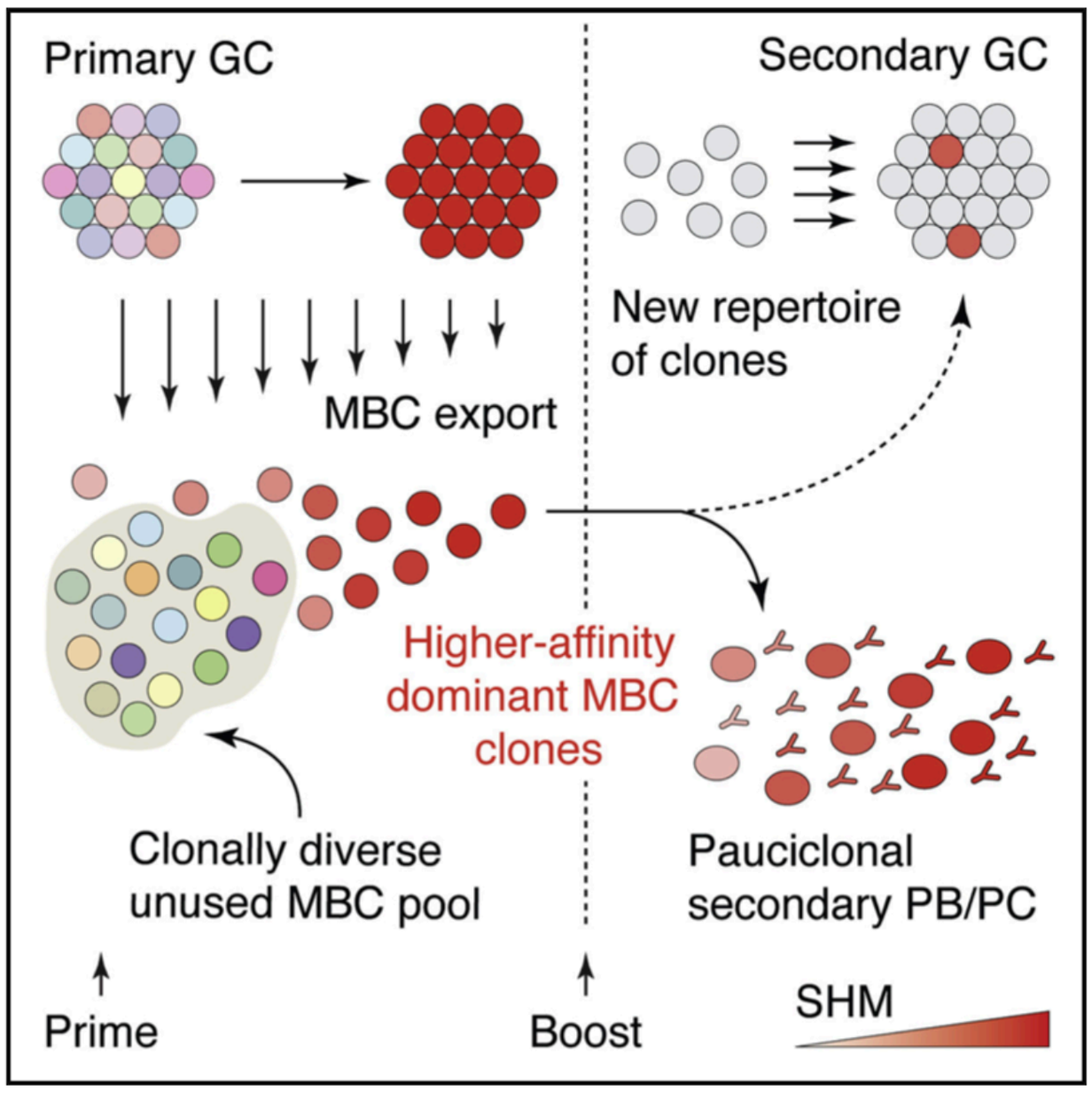
Germinal centers (GCs) have long been considered sites in which Ig class-switch recombination (CSR) is favored. Roco et al. show that CSR occurs during the initial T cell:B cell interaction prior to GC formation and rapidly declines as B cells differentiate into GC cells and somatic hypermutation commences.

Article

Cell

Restricted Clonality and Limited Germinal Center Reentry Characterize Memory B Cell Reactivation by Boosting

Graphical Abstract



Authors

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In Brief

A clonal dynamics analysis of the transition between primary and recall B cell responses in mice reveals a clonality bottleneck that constricts the breadth of the secondary antibody response and limits reentry of previously matured B cells into secondary germinal centers.