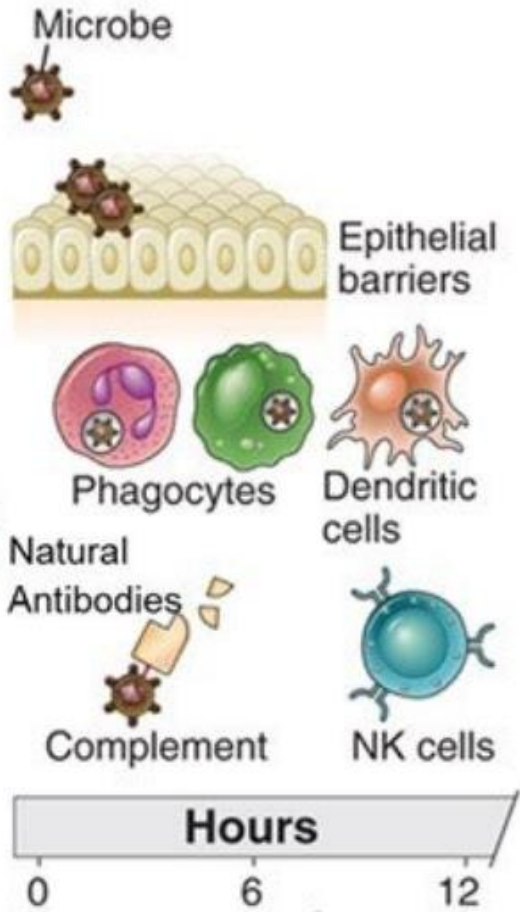


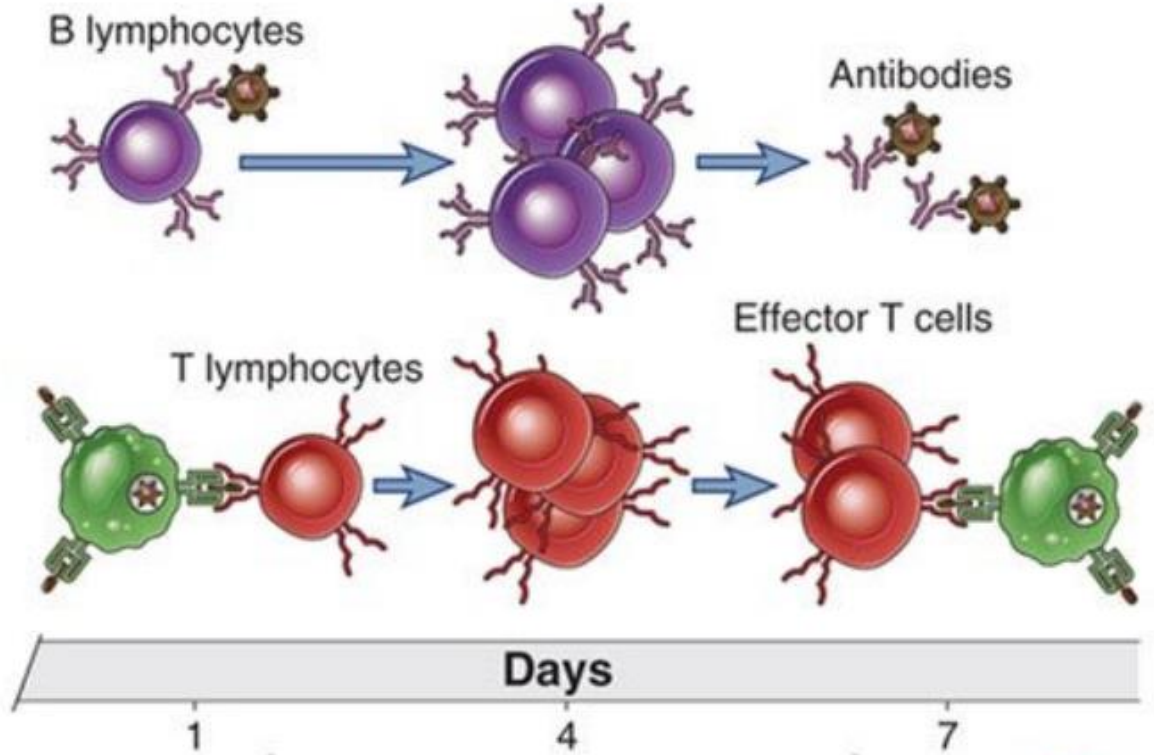
Βιολογία Β - λεμφοκυττάρων

Σωτήρης Τσιόδρας

Innate Immunity

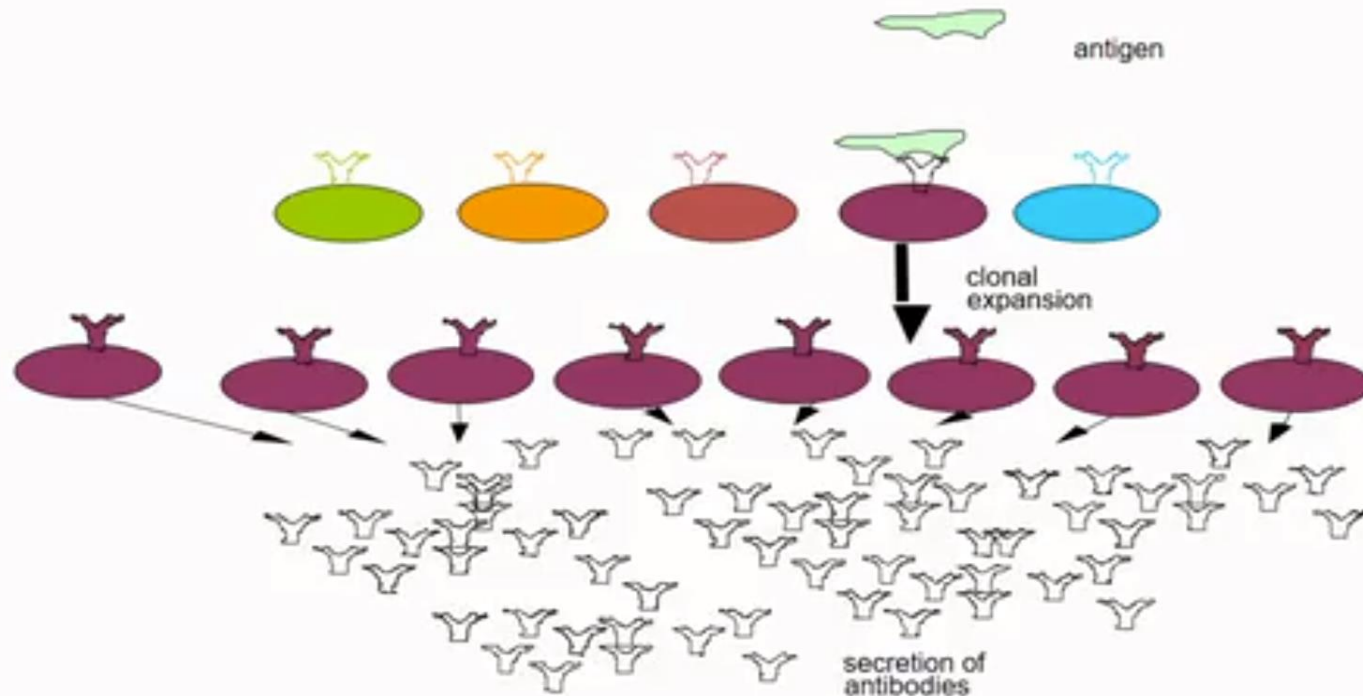


Adaptive Immunity



Time after infection

Main scheme of secretion of antibodies



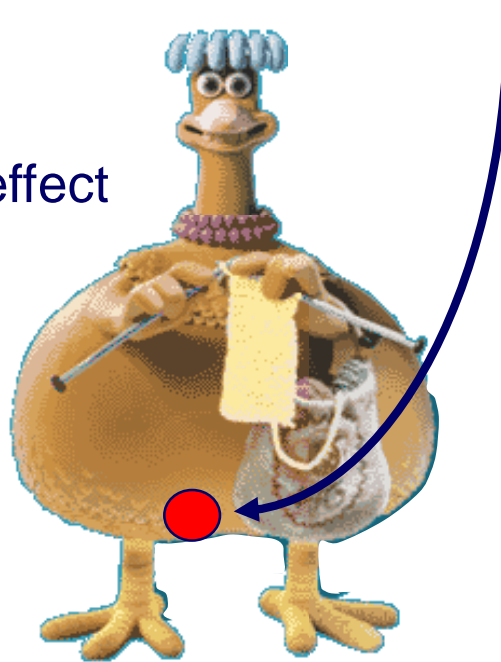
The discovery of B cell immunity

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

Bursectomised chickens were later used in experiments to raise antibodies to *Salmonella* antigens

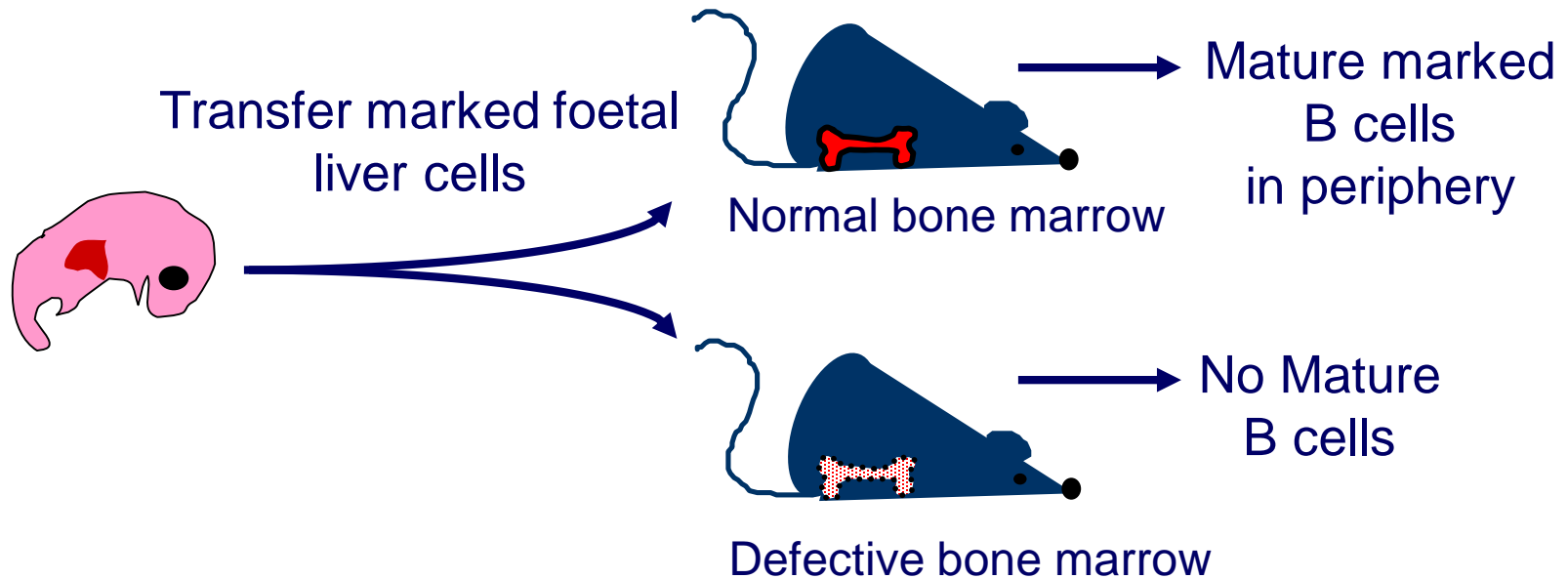


None of the bursectomised chickens made anti-*Salmonella* antibodies

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

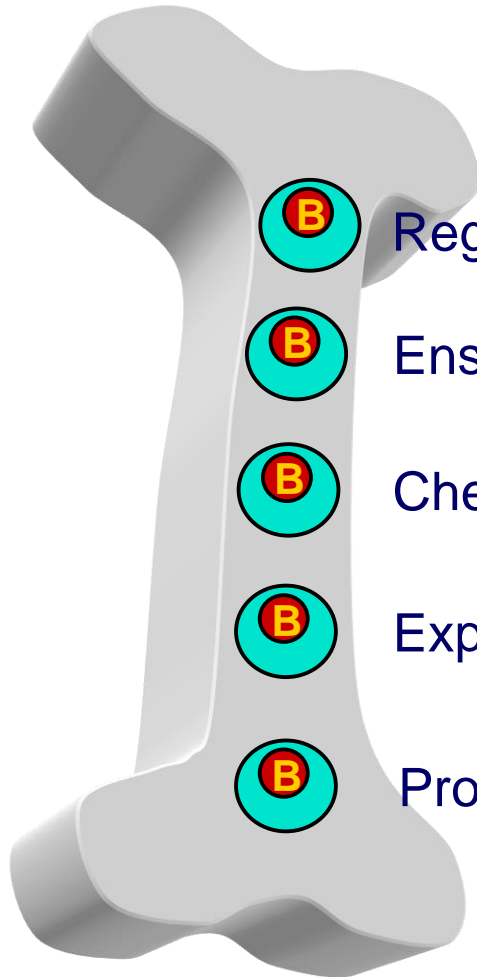
Mammals do not have a bursa of Fabricius

Origin of B cells and organ of B cell maturation



B cell development starts in the foetal liver
After birth, development continues in the bone marrow

B cell development in the bone marrow



Regulates construction of an antigen receptor

Ensures each cell has only one specificity

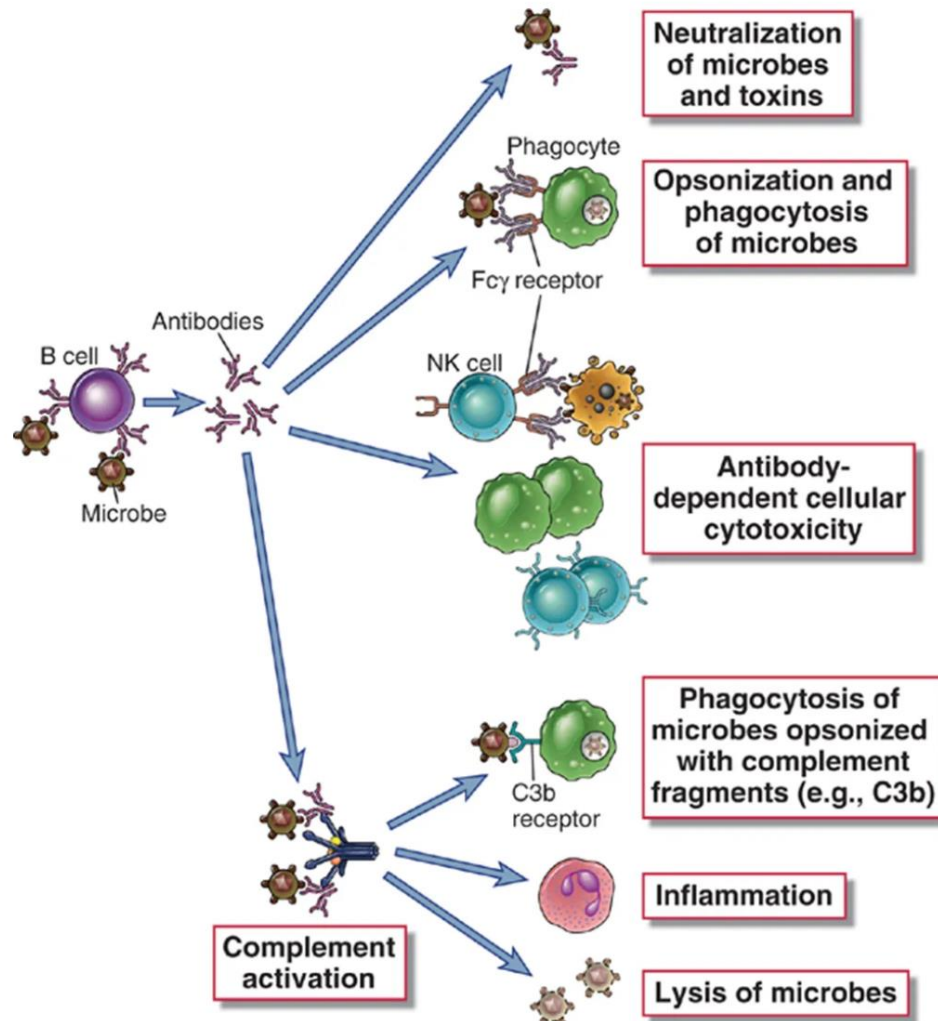
Checks and disposes of self-reactive B cells

Exports useful cells to the periphery

Provides a site for antibody production

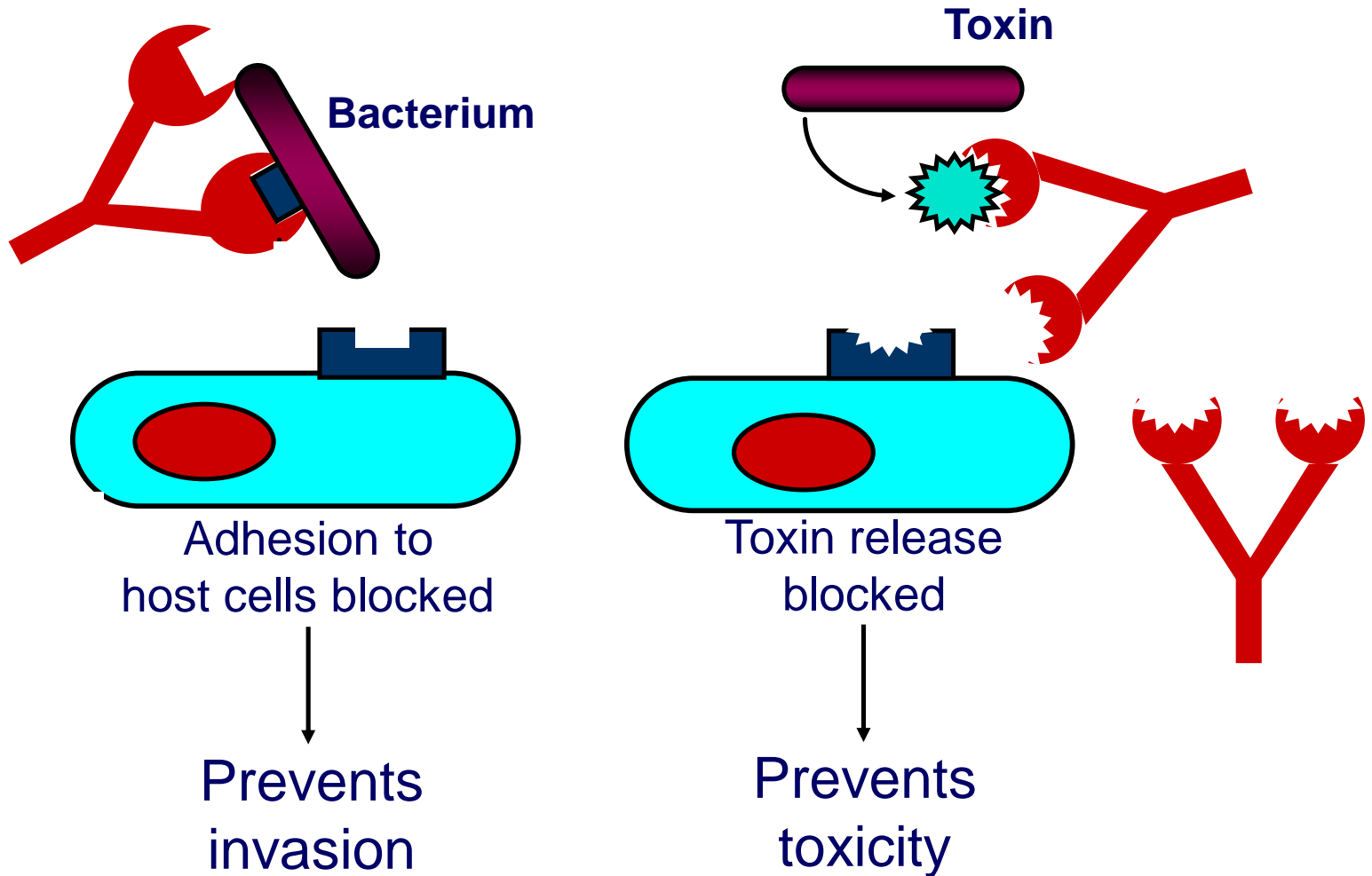
**Bone Marrow provides a
MATURATION & DIFFERENTIATION MICROENVIRONMENT
for B cell development**

The effector functions of Abs



Immune effector mechanisms against extracellular pathogens & toxins

NEUTRALISATION



NEUTRALISING ANTIBODIES

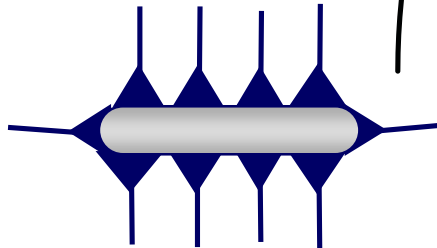
Effector mechanisms against extracellular pathogens

OPSONISATION

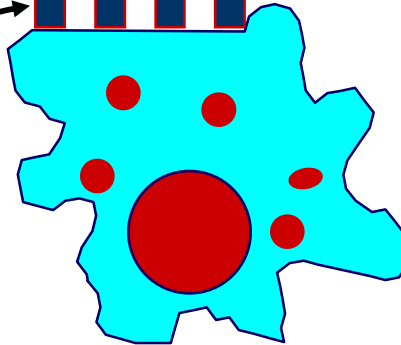
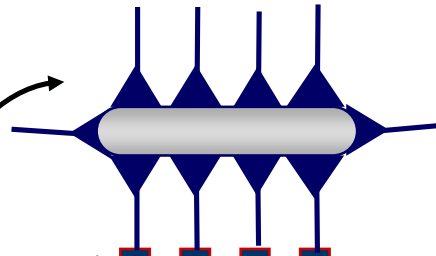
Bacteria in extracellular space



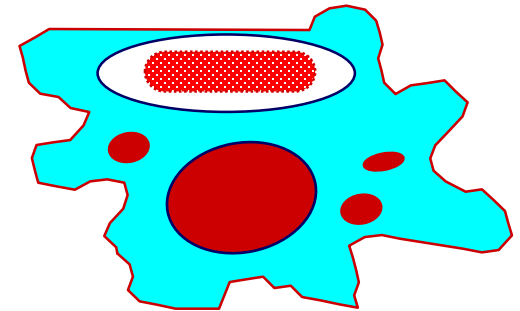
+



OPSONISATION



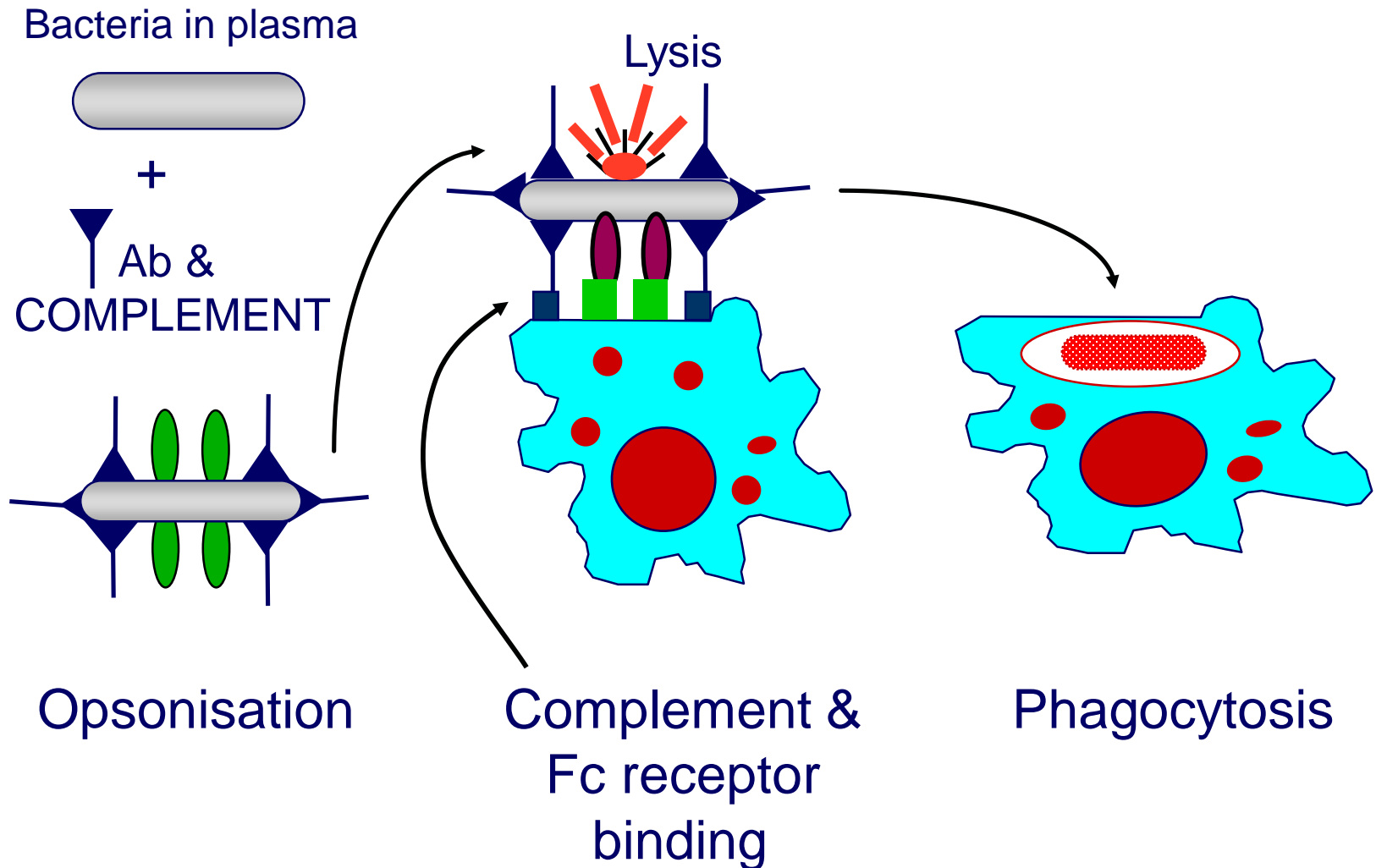
Fc receptor binding



Phagocytosis

Effector mechanisms against extracellular pathogens

COMPLEMENT Activation



Many immune & inflammatory diseases are caused by Antibodies

- **Type 1: Immediate hypersensitivity**

- IgE/Mast cells

- **Type 2: Antibody mediated**

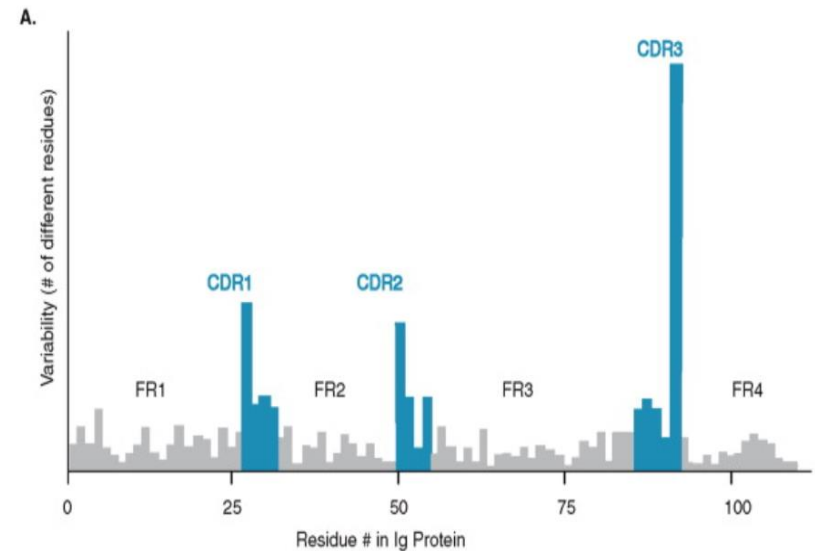
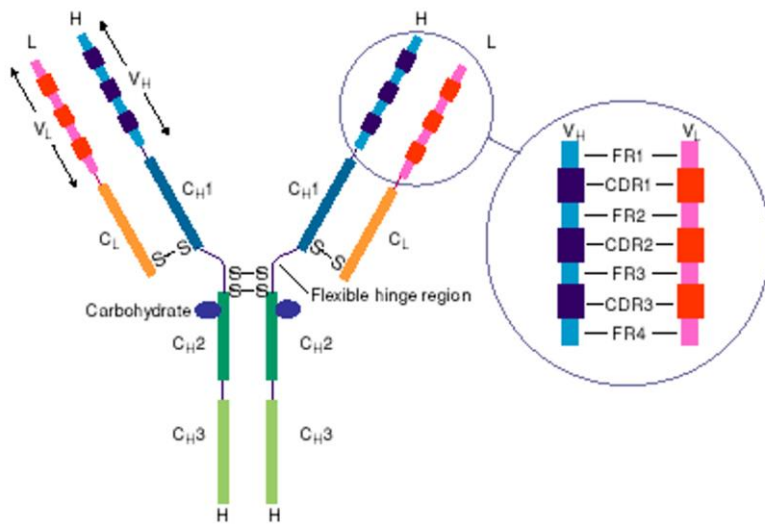
- Antibodies against cell/tissue **antigens**

- **Type 3: Immune complex disease-Common phenomenon in infectious diseases**

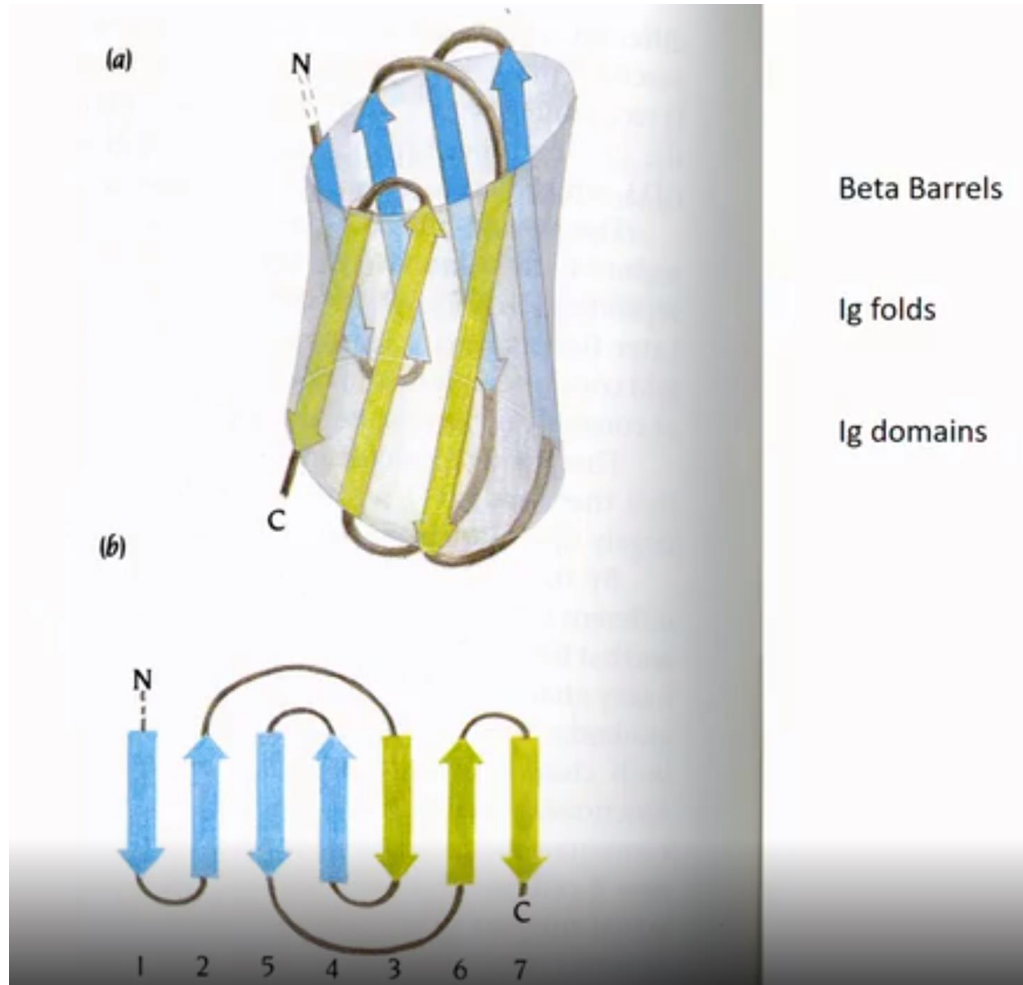
Many immune & inflammatory diseases are caused by Antibodies

- The absence of antibodies is of obvious relevance in Infectious Diseases
 - ✓ XLA, Hyper-IgM syndromes, CVID
- All functional vaccines depend on high affinity antibodies

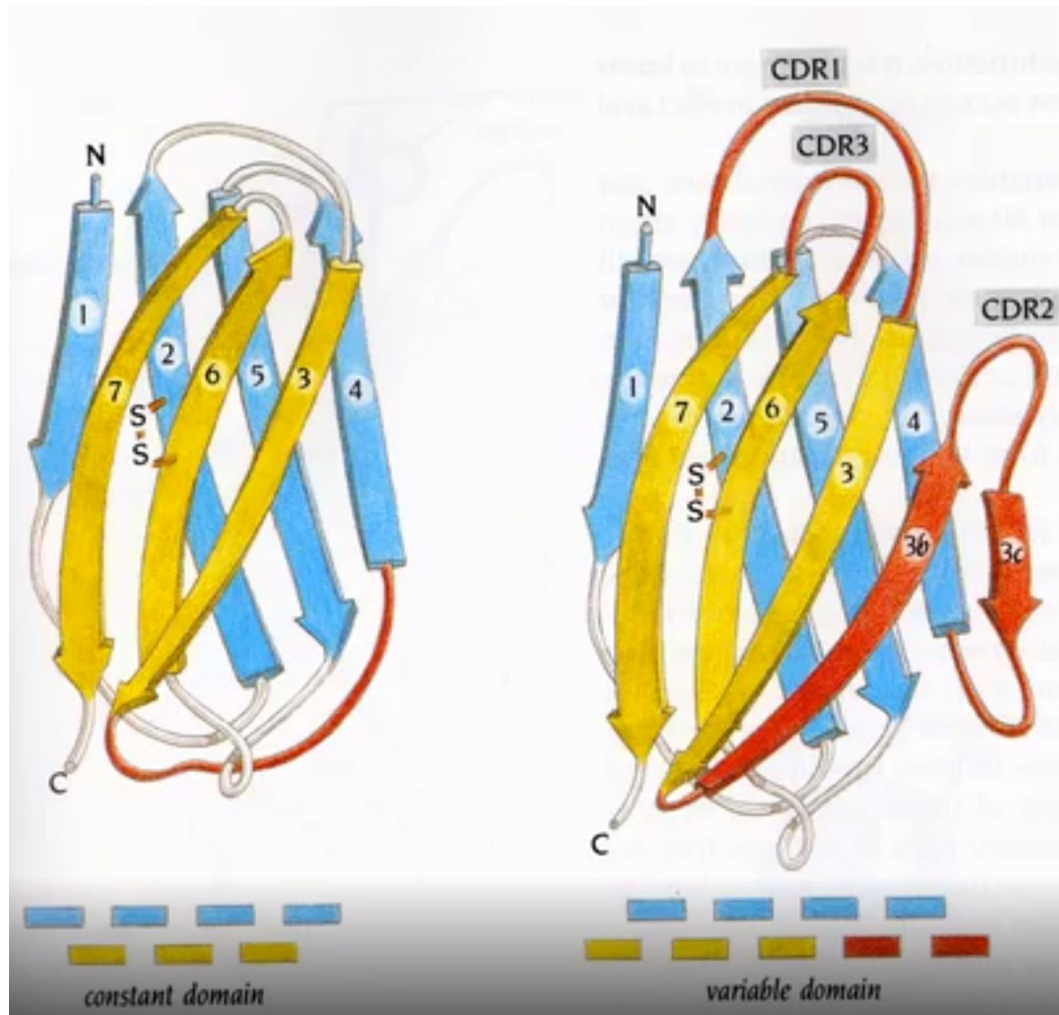
Complementarity determining regions = CDRs = HVRs



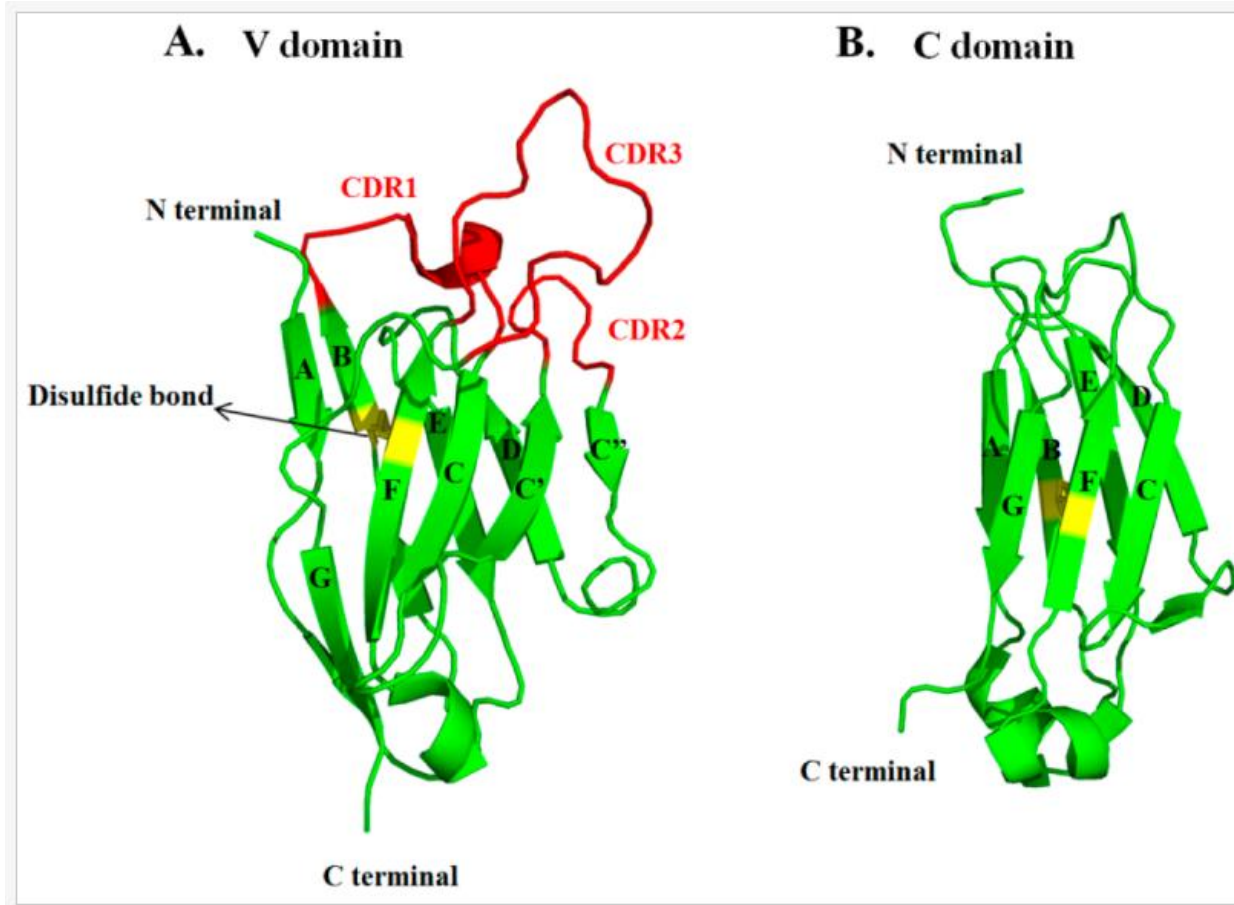
Beta barrels, Ig folds, Ig domains



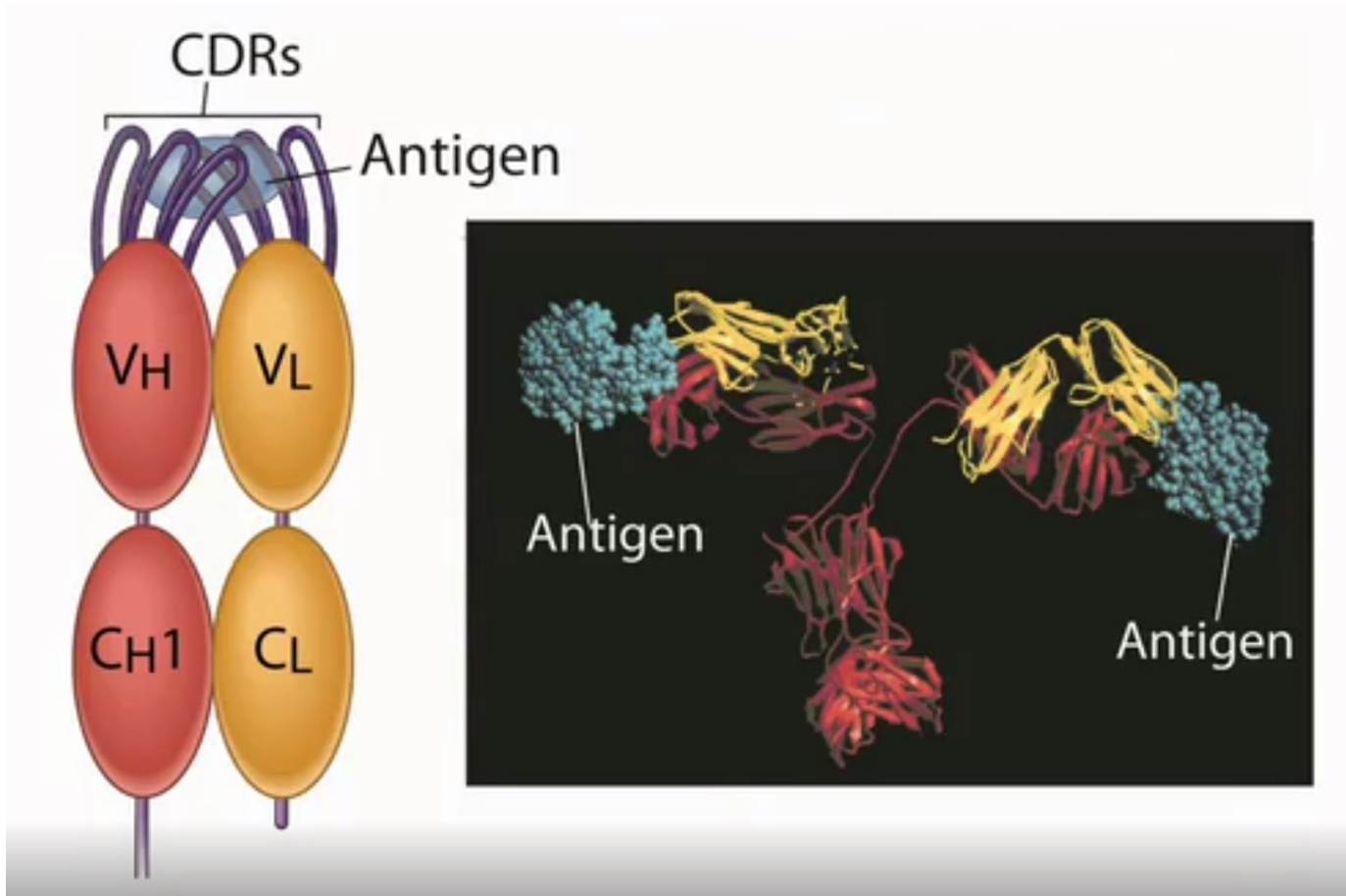
Beta barrels, Ig folds, Ig domains



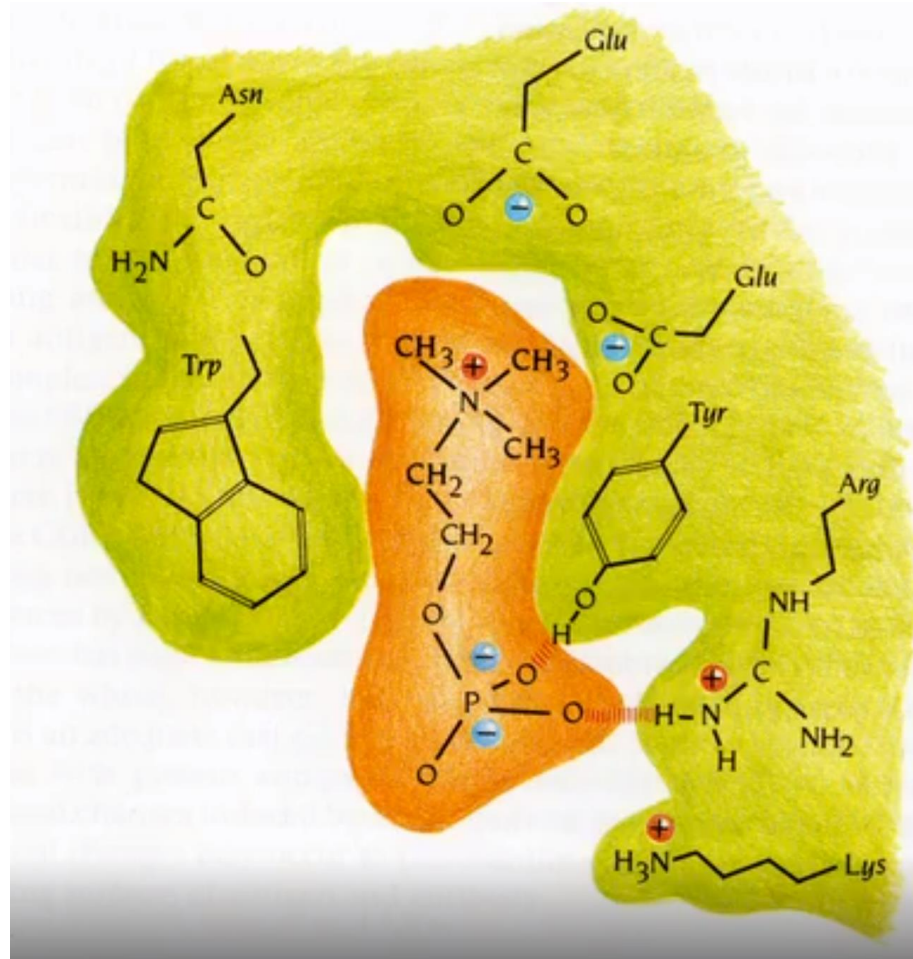
V vs. C domain



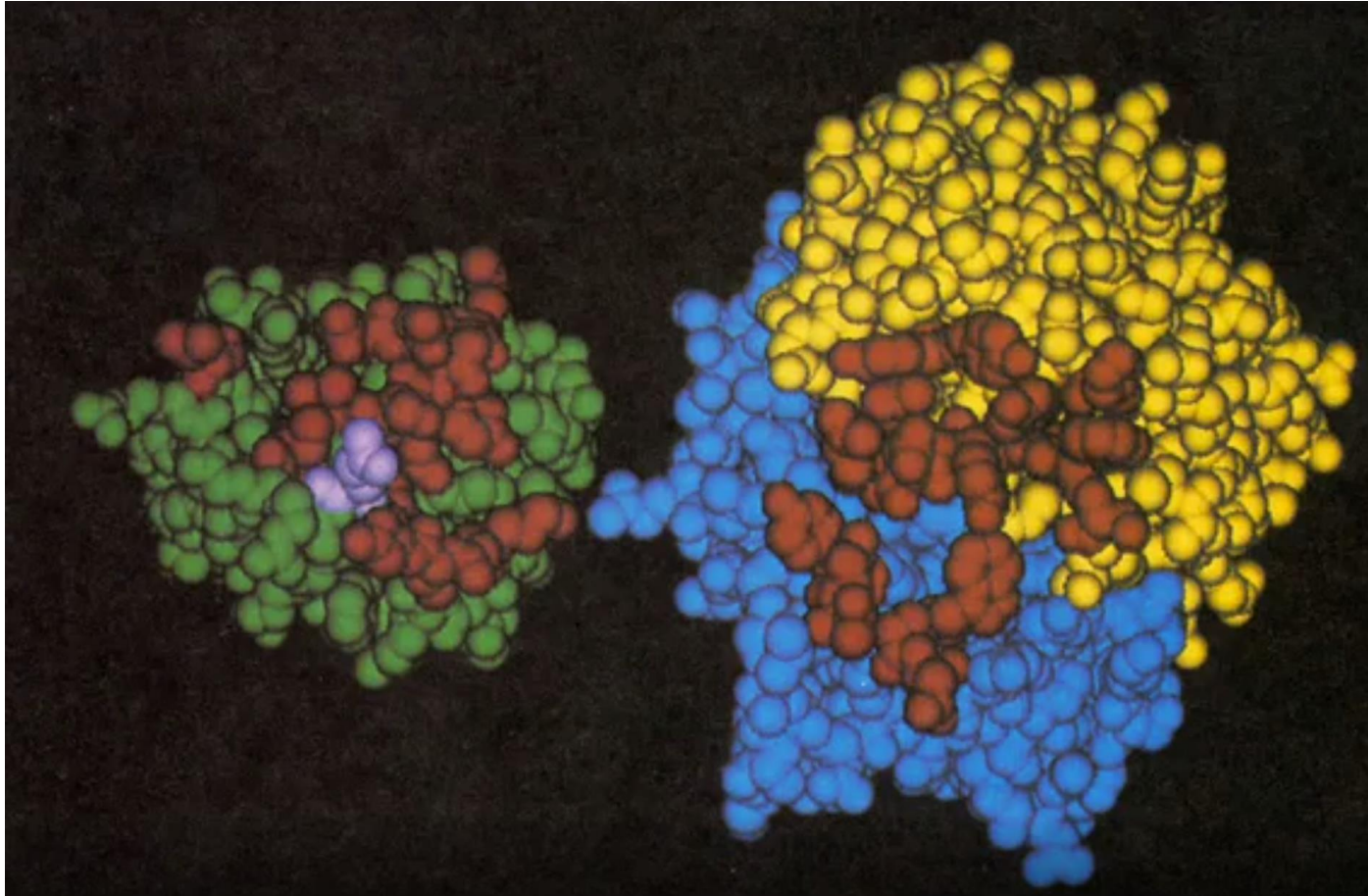
Binding of an Ag by an Ab



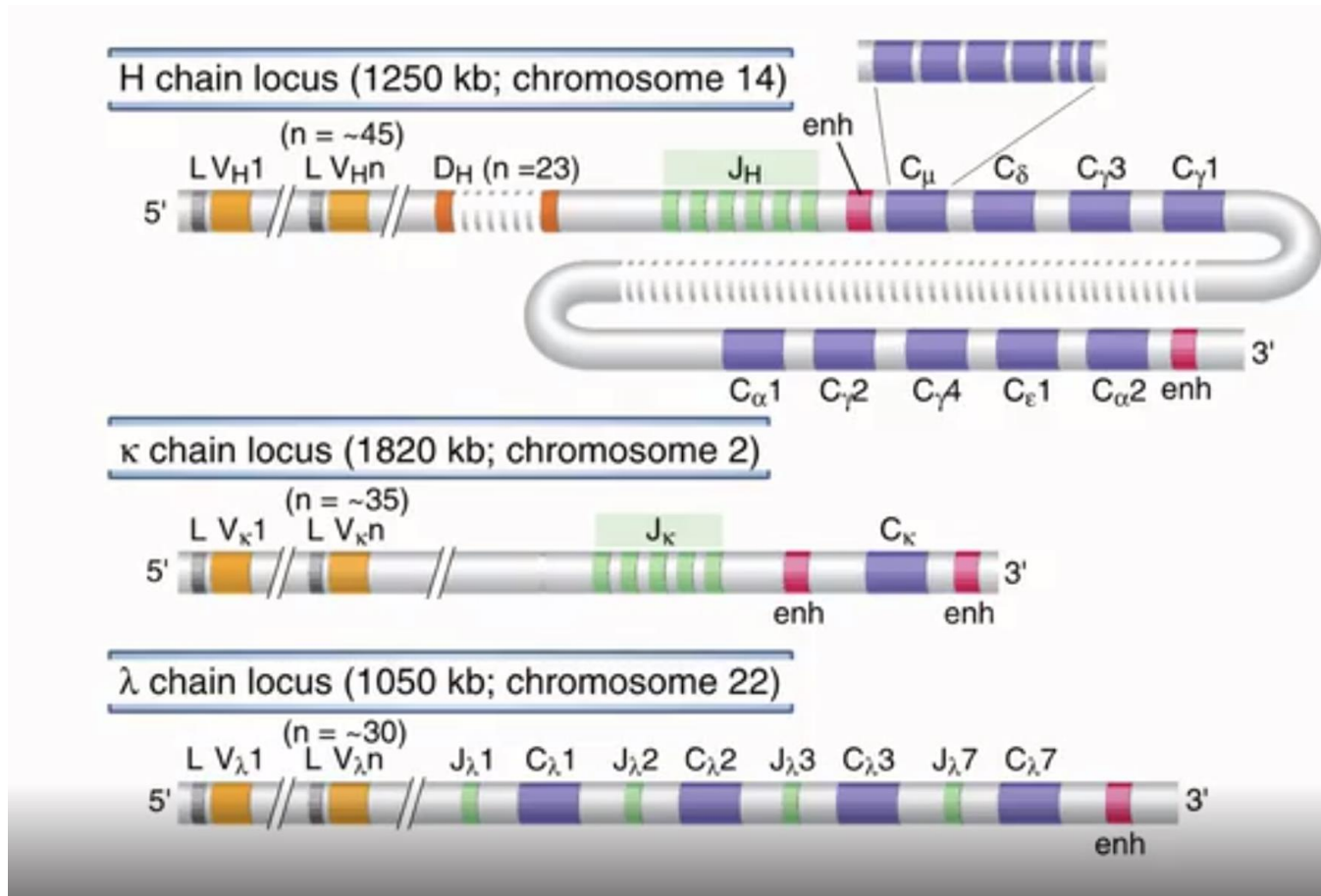
The small molecule concept



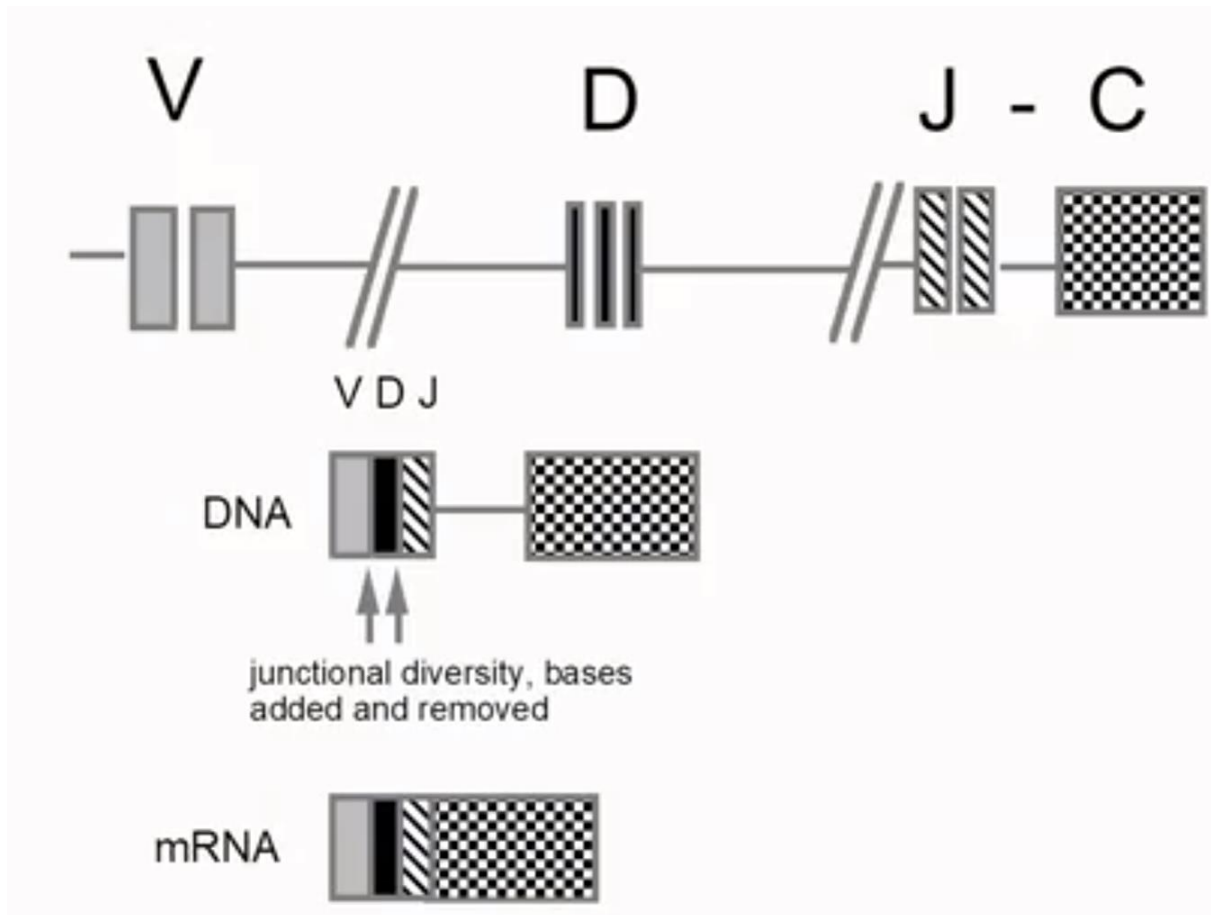
The small molecule concept



Human immunoglobulin genes

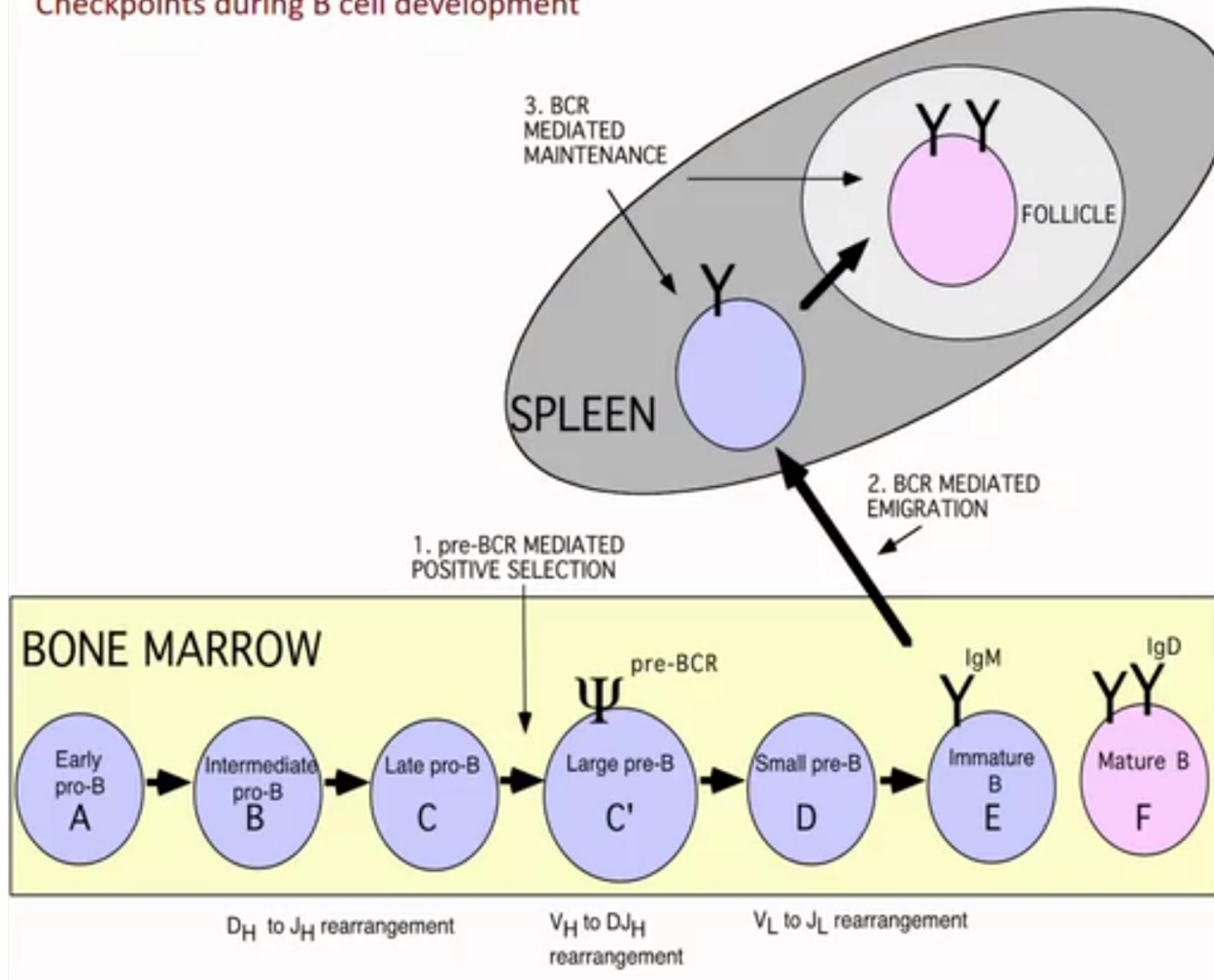


VDJ recombination

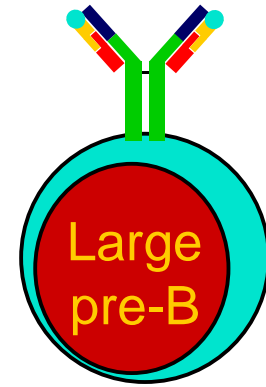
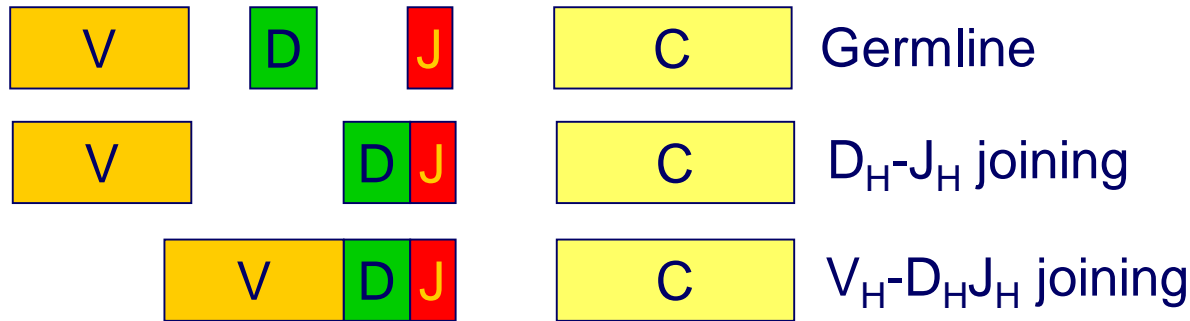


VDJ recombination

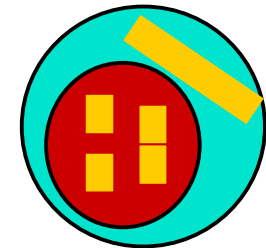
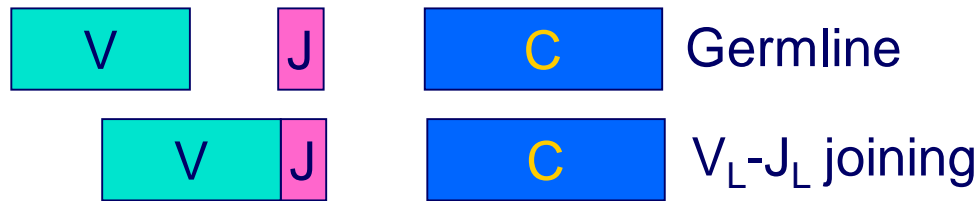
Checkpoints during B cell development



Heavy and light chain rearrangement is potentially wasteful



With two “random” joins to generate a heavy chain there is a 1:9 chance of a rearrangement of being in frame



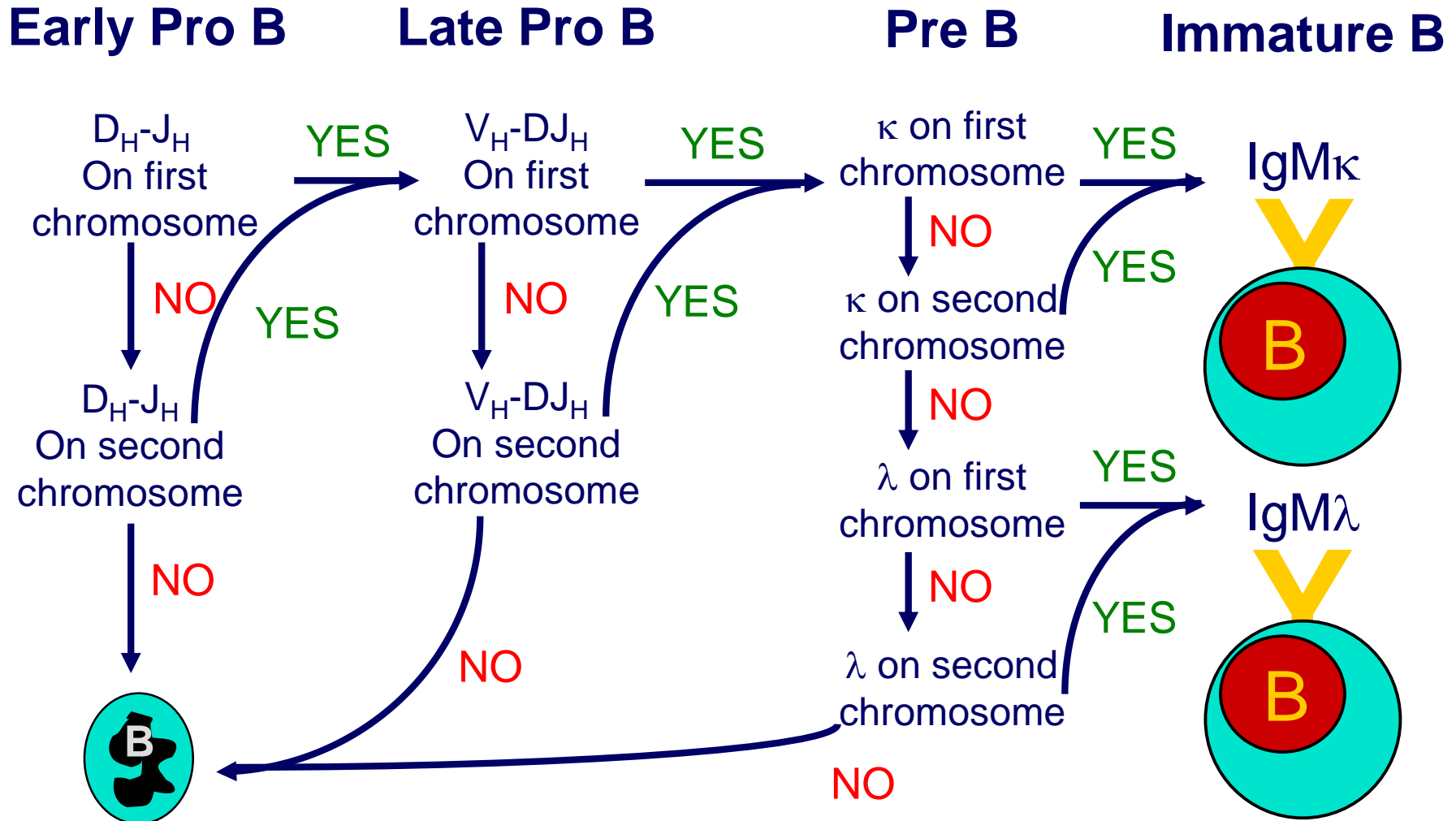
With one “random” join to generate a light chain there is a 1:3 chance of a rearrangement being of frame

Small pre-B

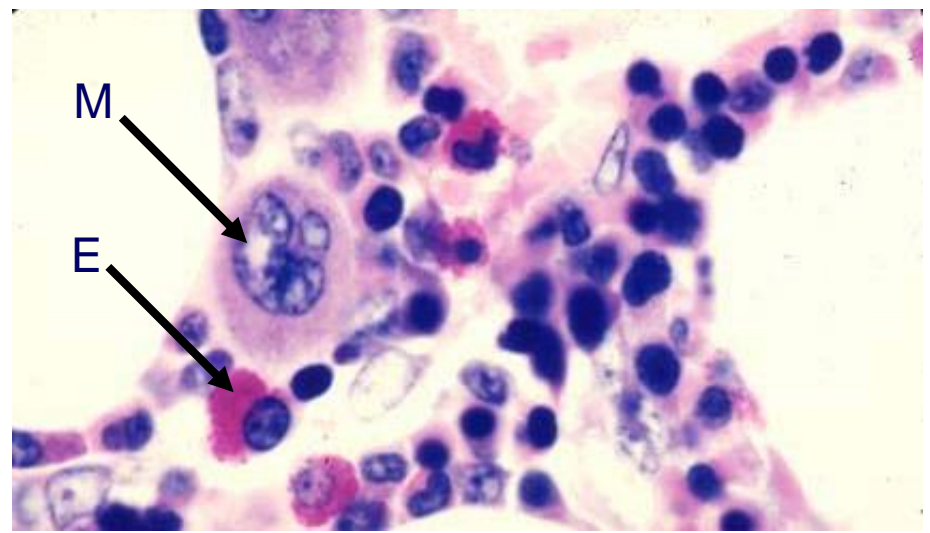
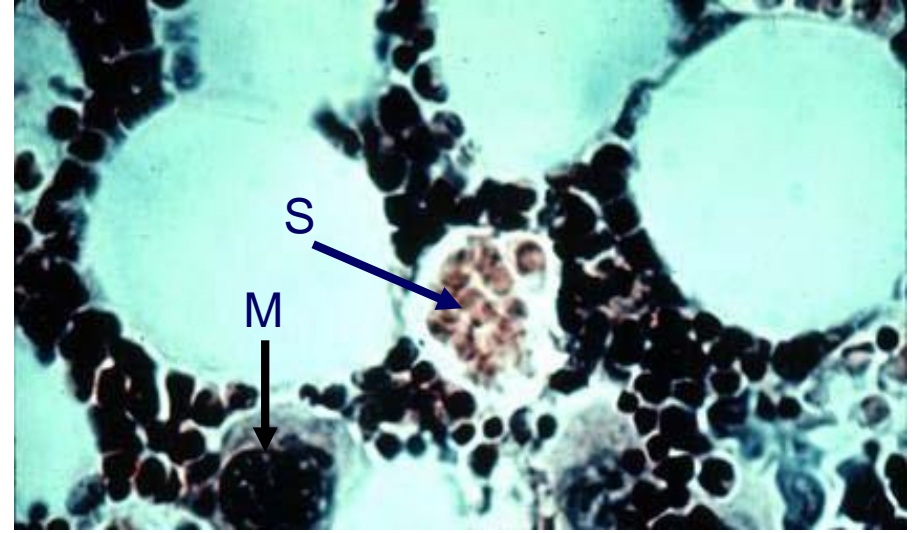
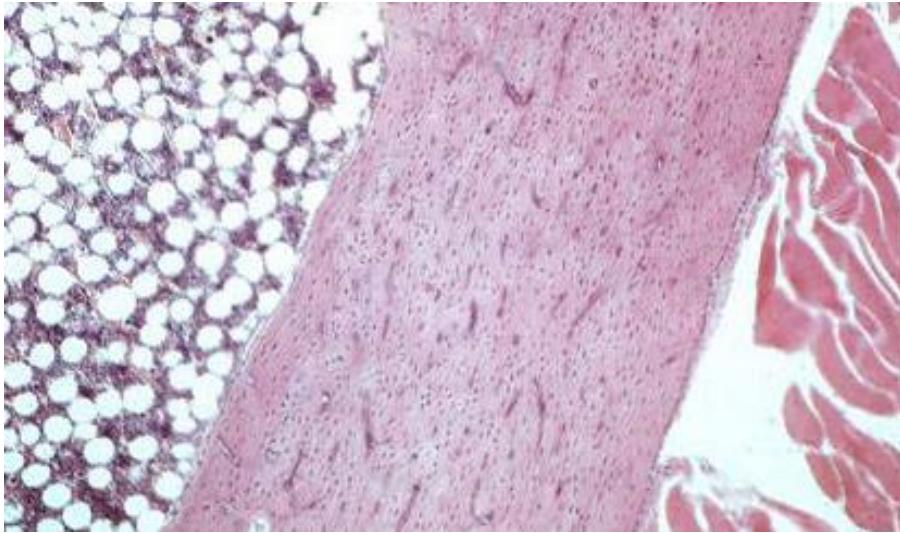
There is, therefore, only a 1:27 chance of an in frame rearrangement

Out of frame rearrangements arrest further B cell maturation

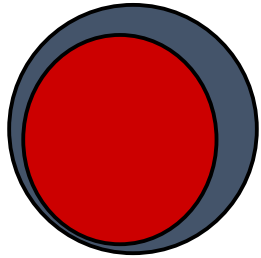
B cells have several chances to successfully rearrange Ig genes



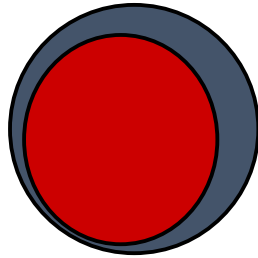
Bone Marrow



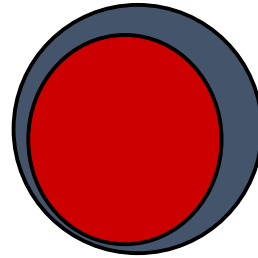
Stages of B cell development



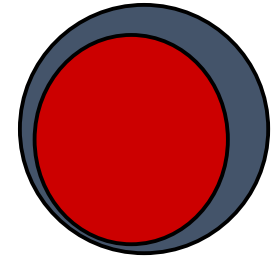
Stem Cell



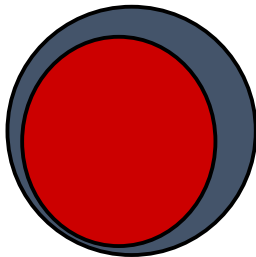
Early pro-B cell



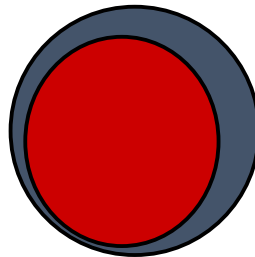
Late pro-B cell



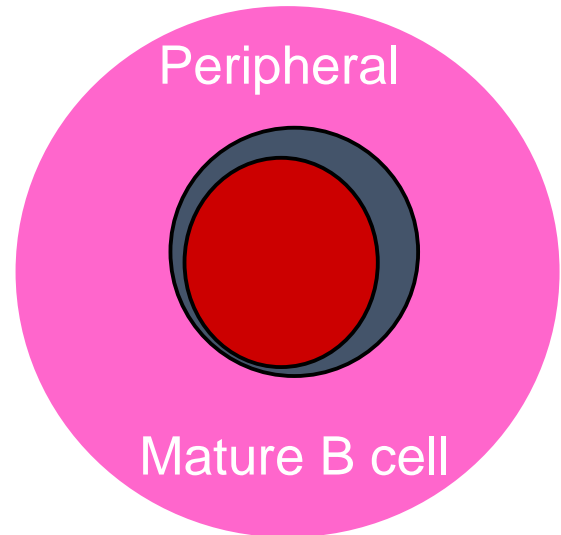
Large pre-B cell



Small pre-B cell

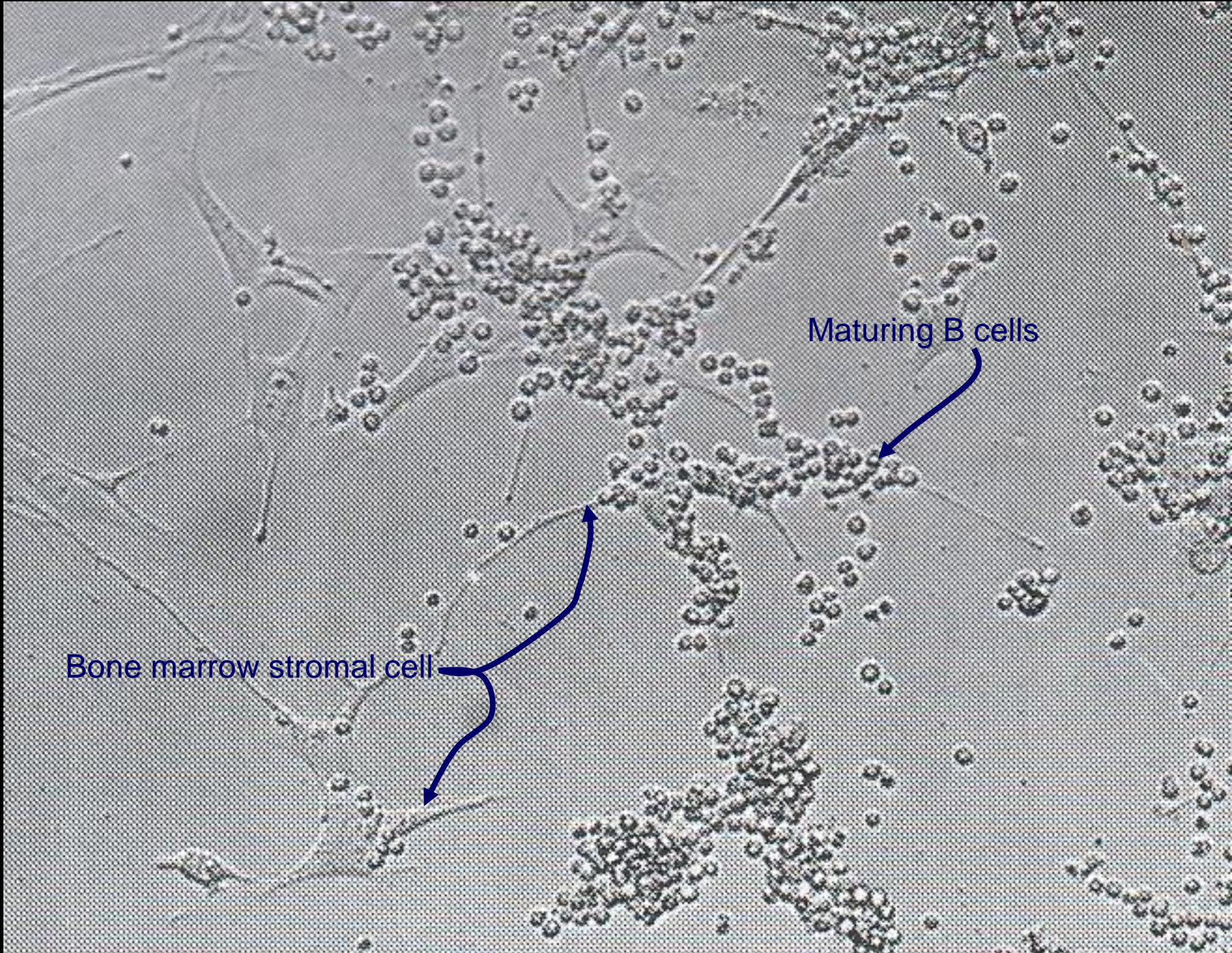


Immature B cell



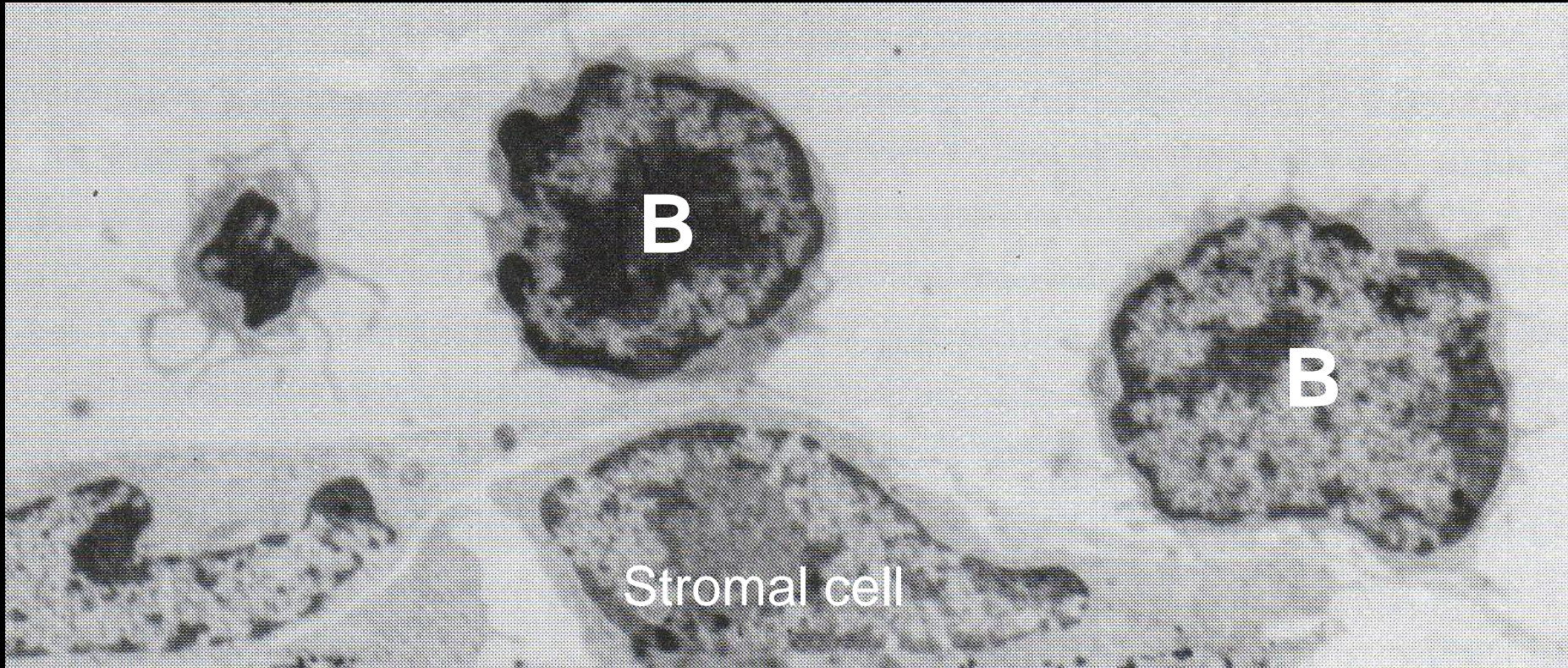
Mature B cell

Each stage of development is defined by rearrangements of IgH chain genes, IgL chain genes, expression of surface Ig, expression of adhesion molecules and cytokine receptors



Maturing B cells

Bone marrow stromal cell



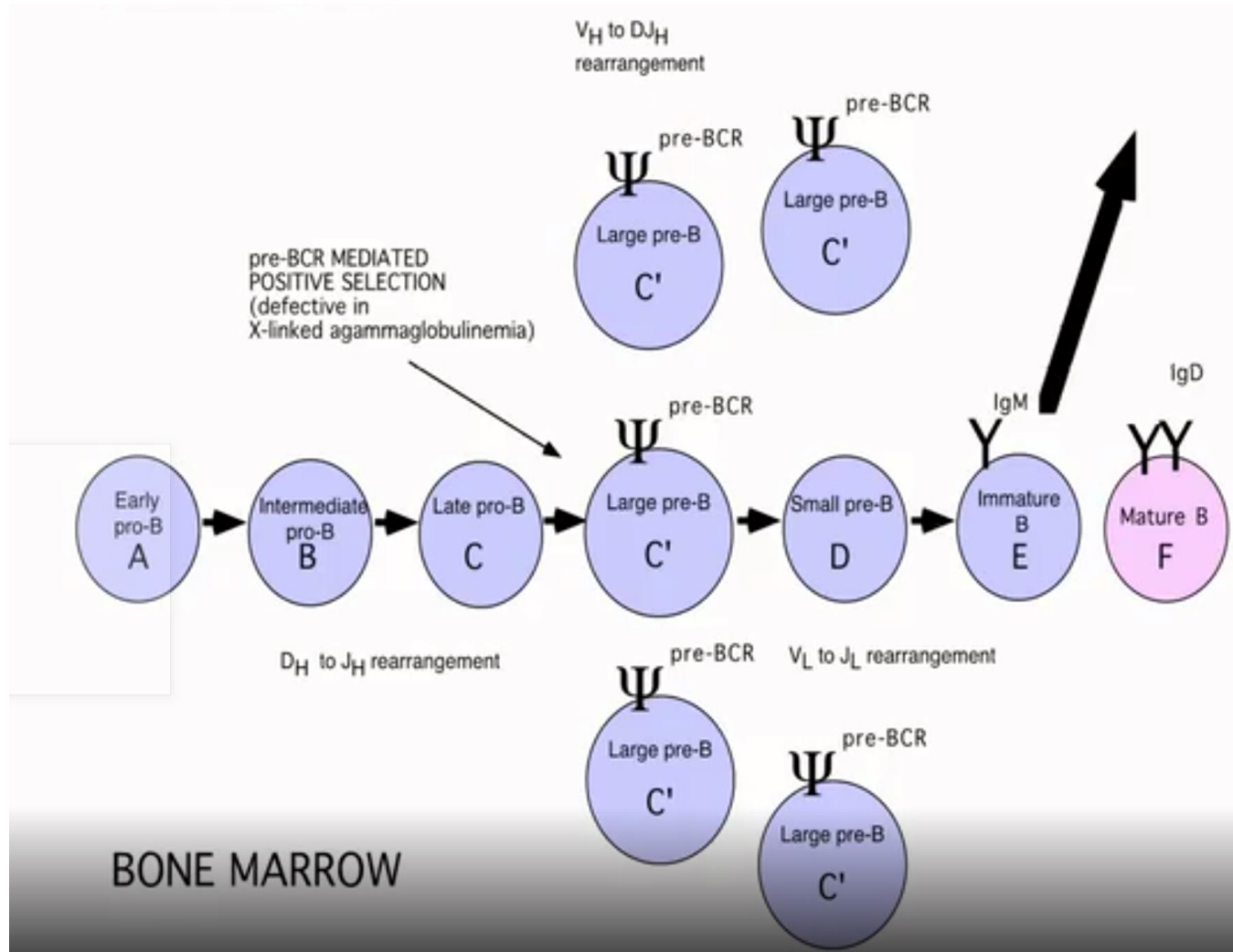
B

B

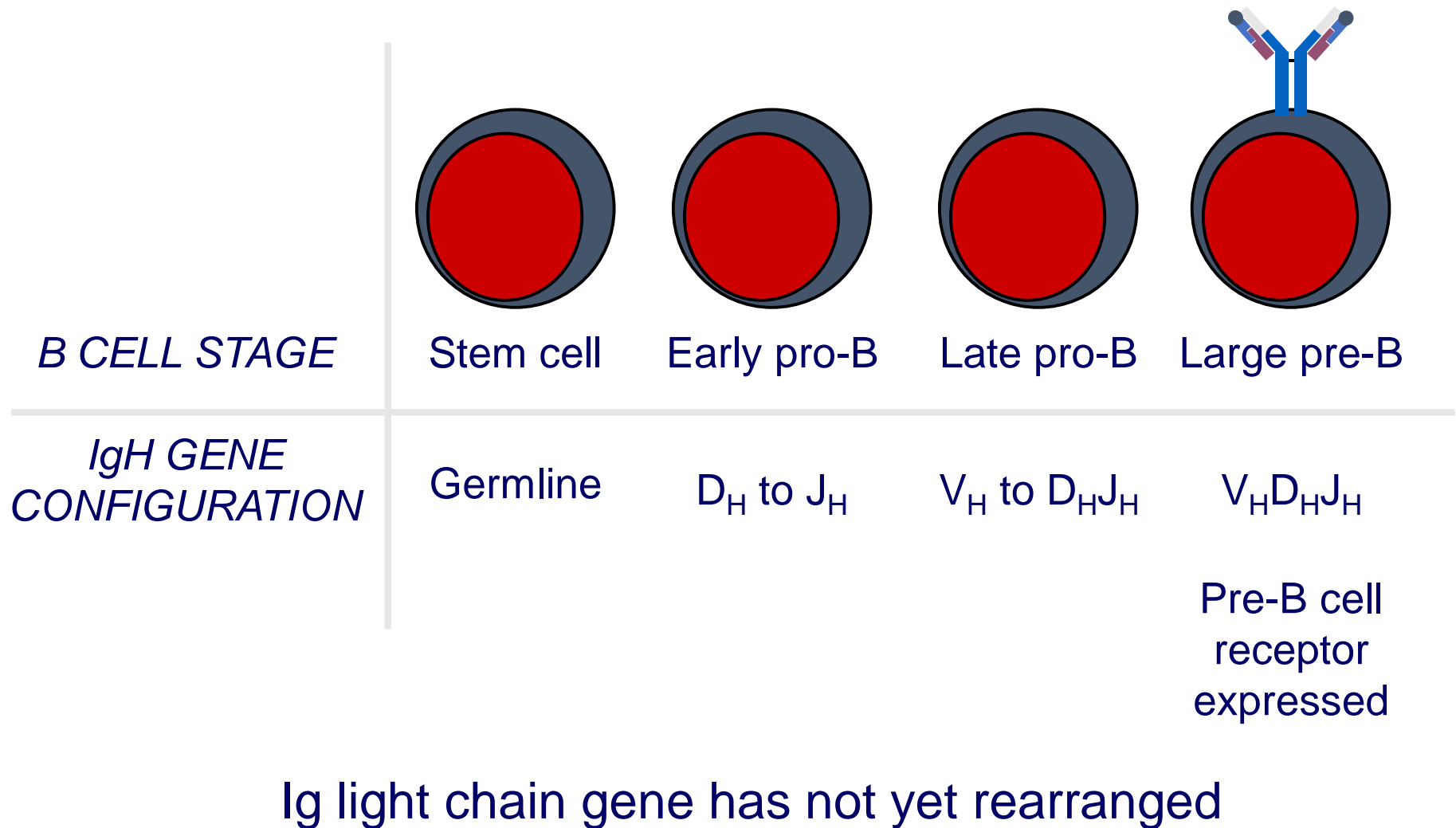
Stromal cell

The pre-BCR checkpoint

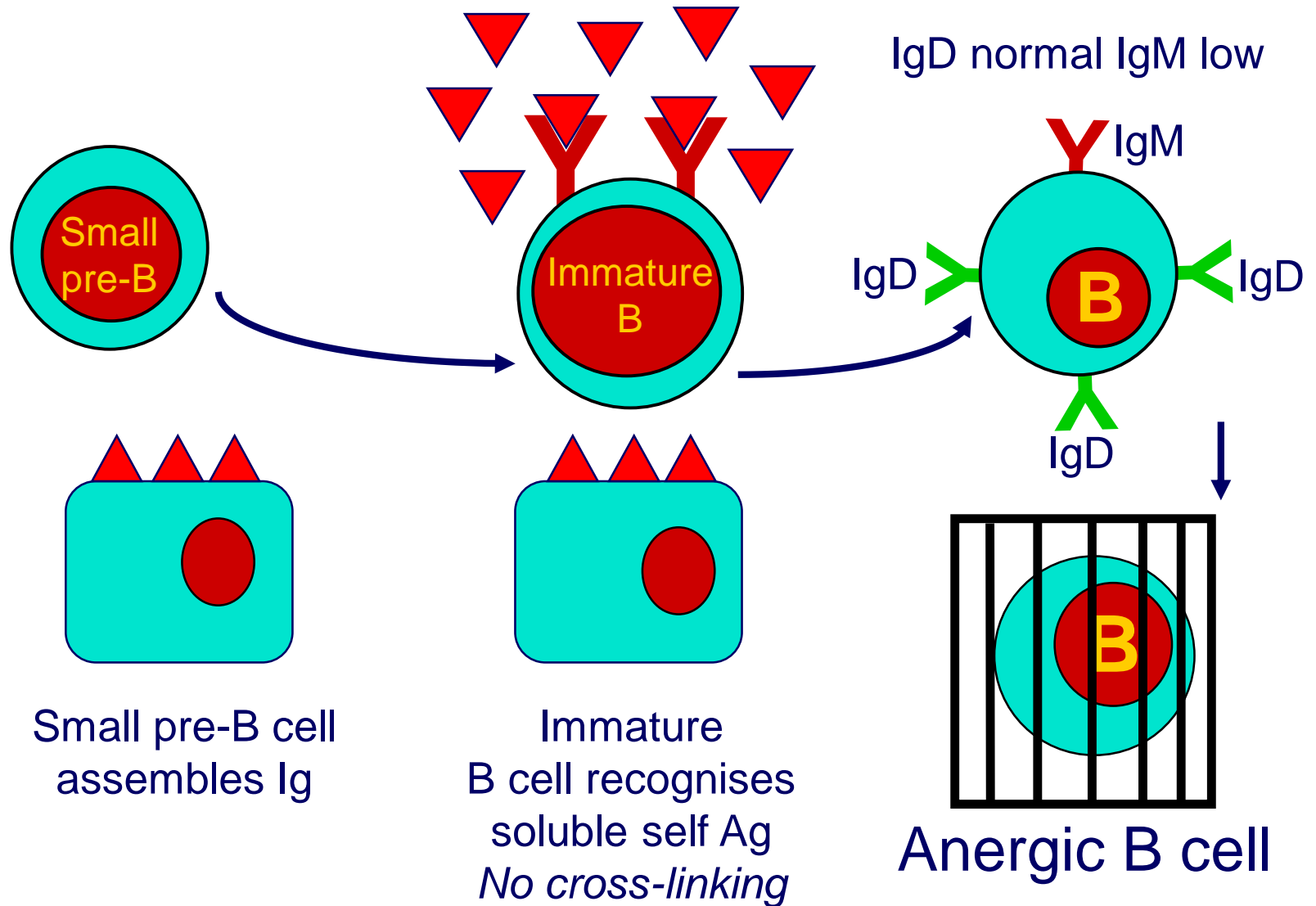
cells not expressing pre-BCR die by apoptosis
cells expressing BCR undergo positive selection



Stages of differentiation in the bone marrow are defined by Ig gene rearrangement

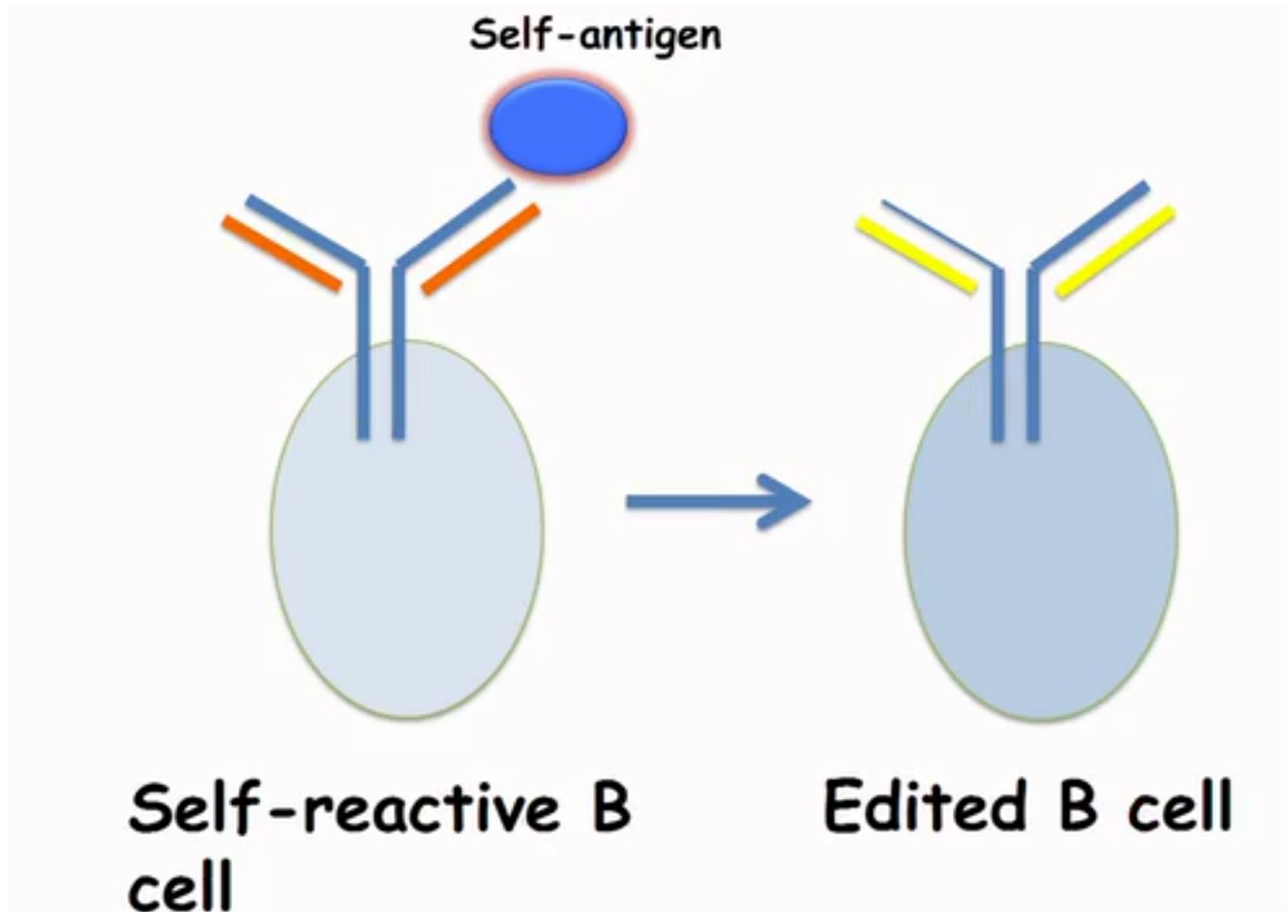


B cell self tolerance: anergy



Receptor editing occurs in the BM

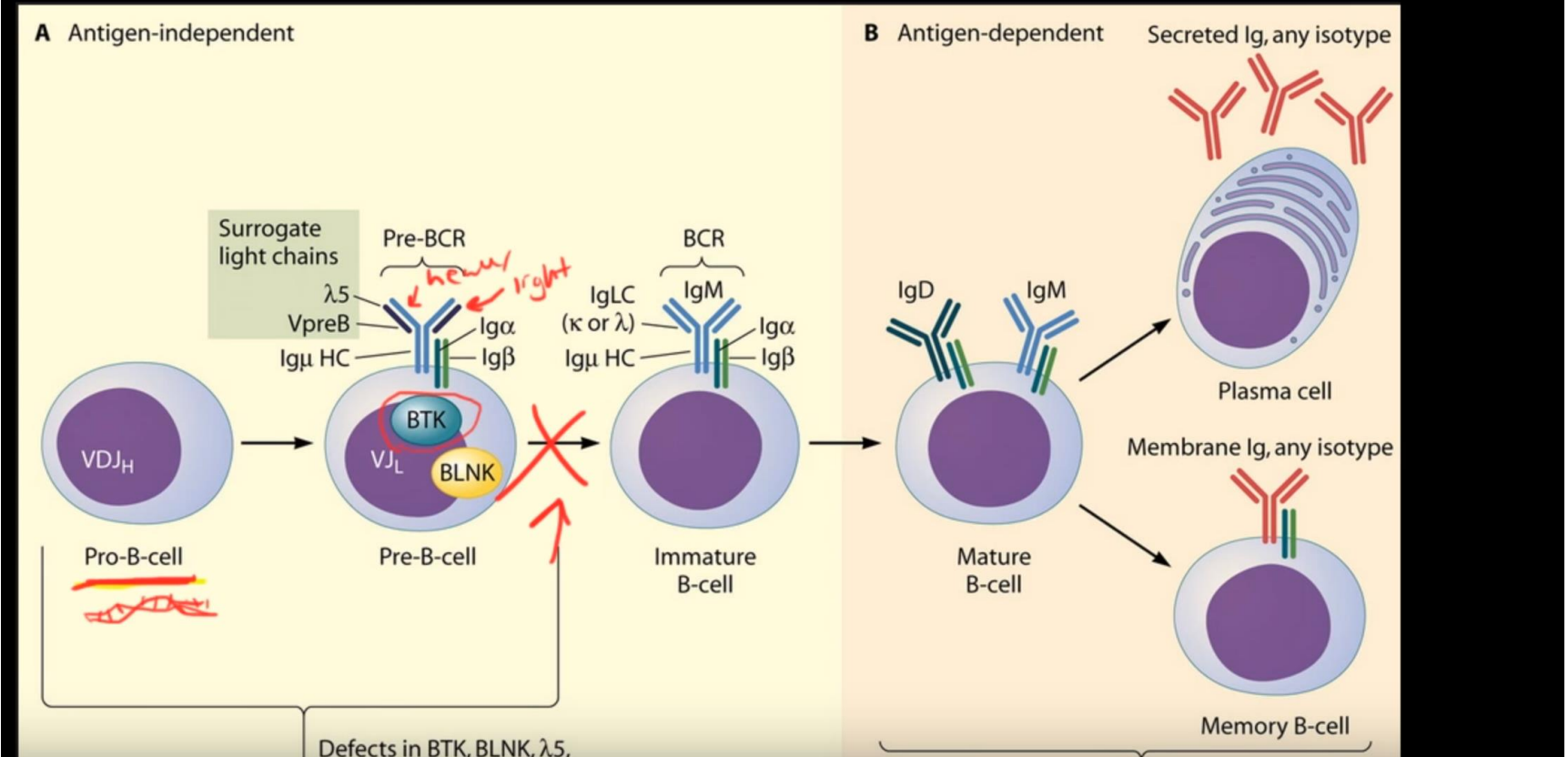
avoid apoptosis -modifying sequence of light chain V & J genes



B-cell tolerance, 20-50% of all naïve B cells!

XLA Brutons disease

B-cell maturation = Ig heavy-chain genes are rearranged first, followed by light-chain rearrangement
In XLA, B-cell maturation stops after the initial heavy-chain gene rearrangement
because of mutations in a tyrosine kinase
The kinase is called Bruton tyrosine kinase or B-cell tyrosine kinase (BTK)

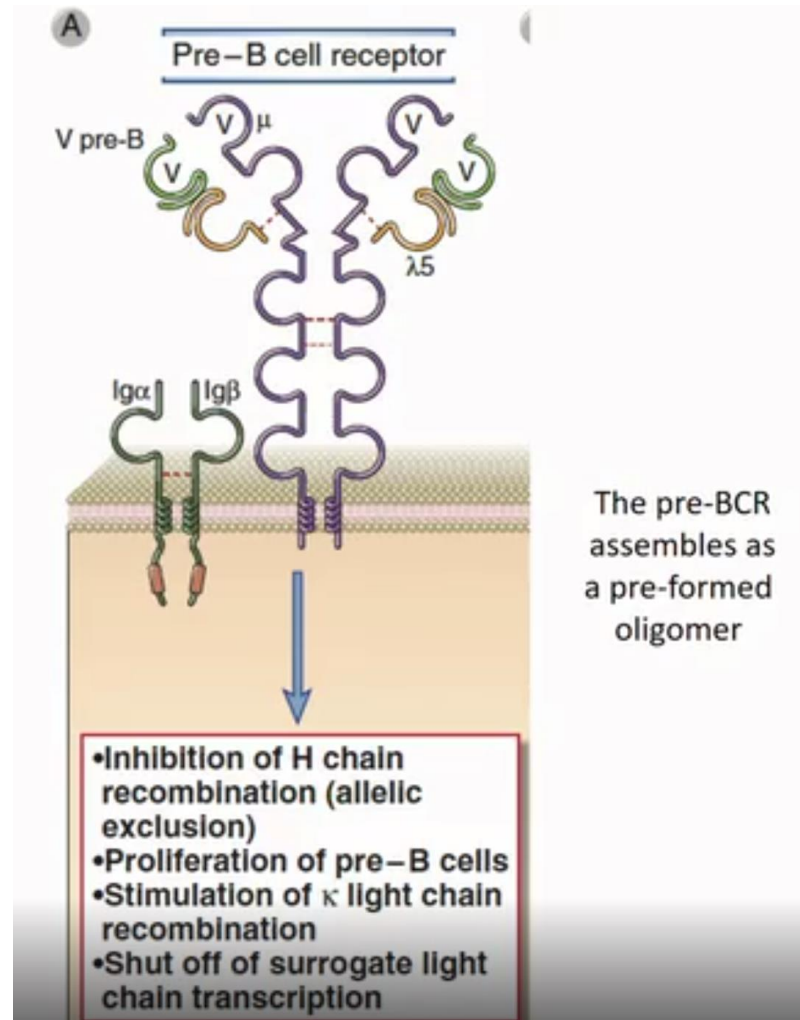


XLA Brutons disease



Diagnosis? Low Igs, Lymphocyte phenotyping->> absent B-cells, genetic testing -> BTK mutation

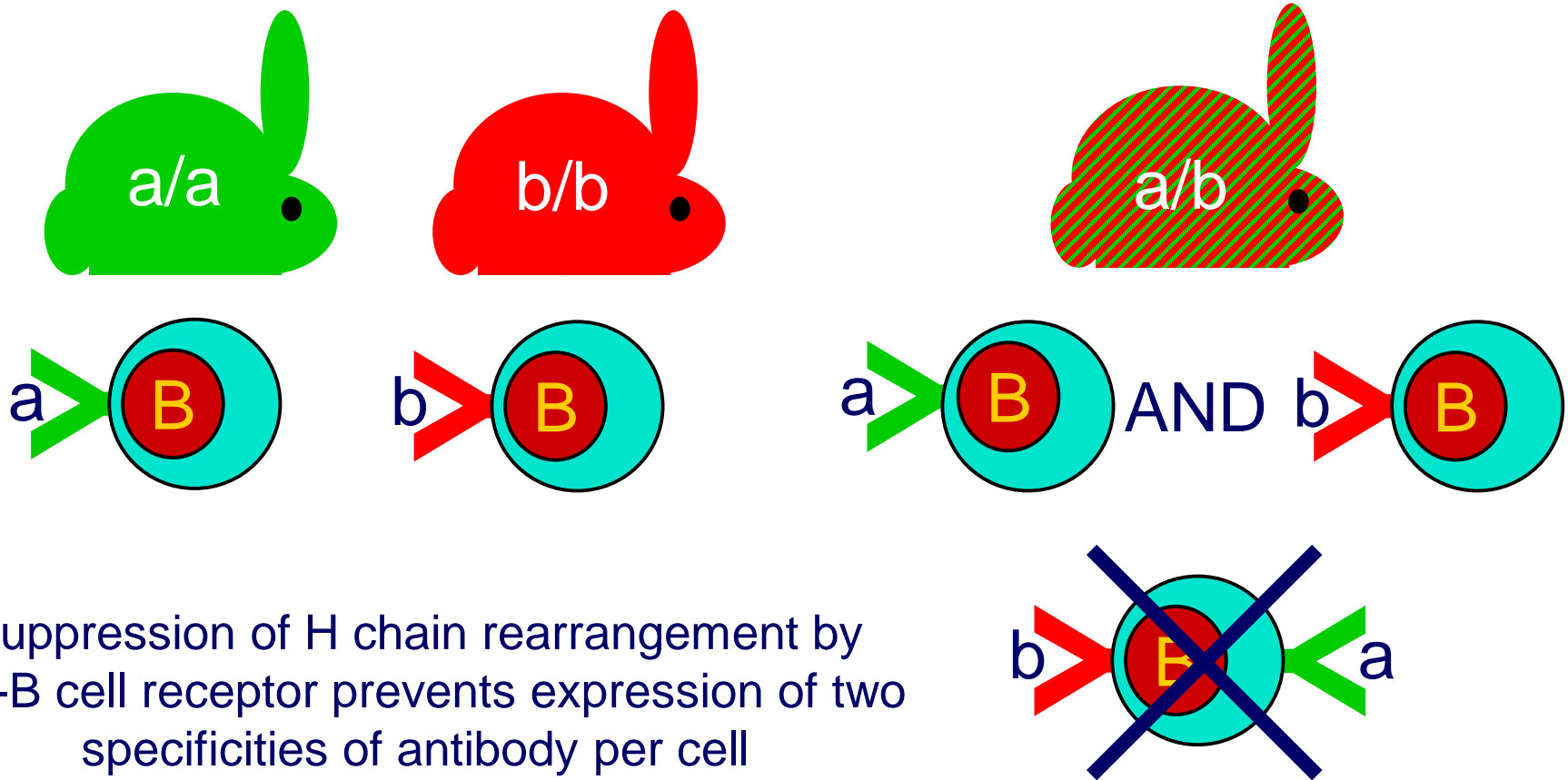
The pre-BCR signals constitutively



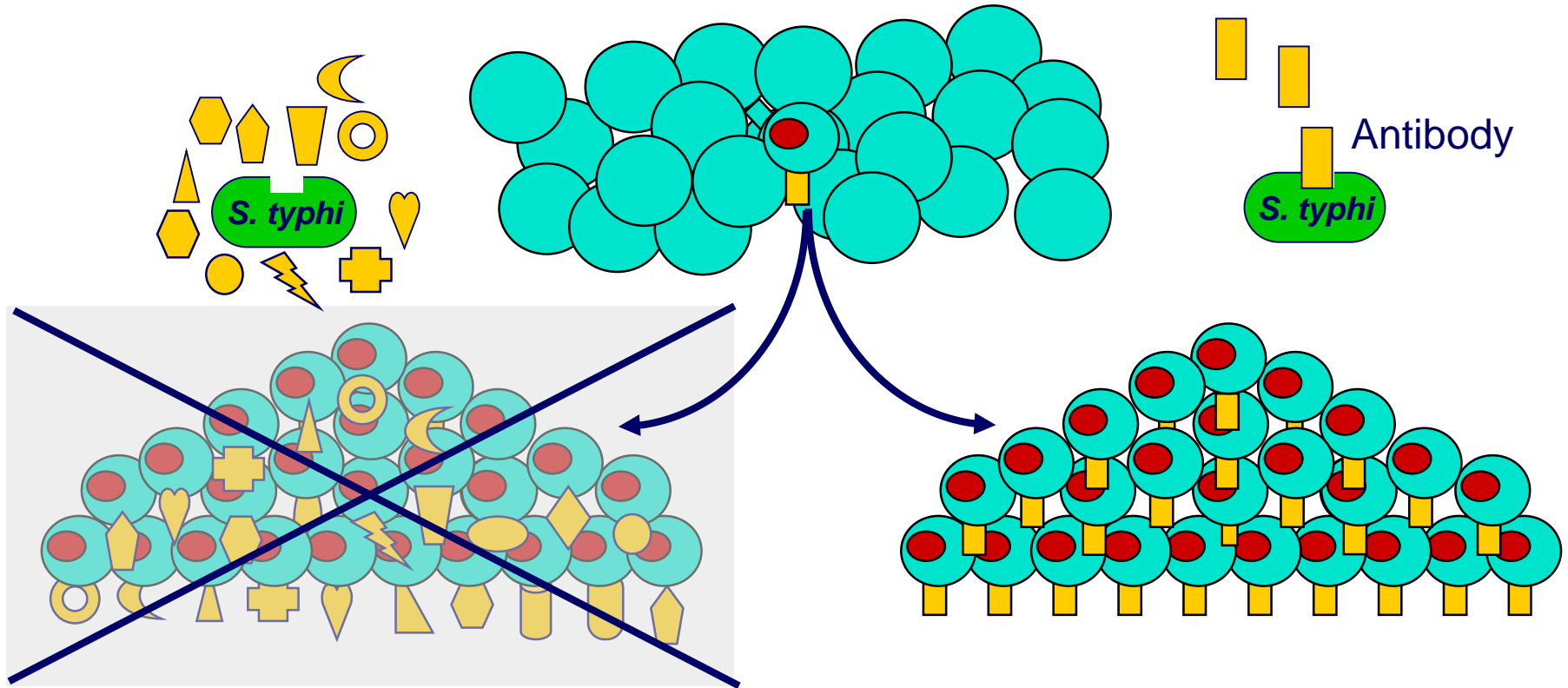
Evidence for allelic exclusion

ALLOTYPE- polymorphism in the C region of Ig – one allotype inherited from each parent

Allotypes can be identified by staining B cell surface Ig with antibodies



Allelic exclusion is needed for efficient clonal selection



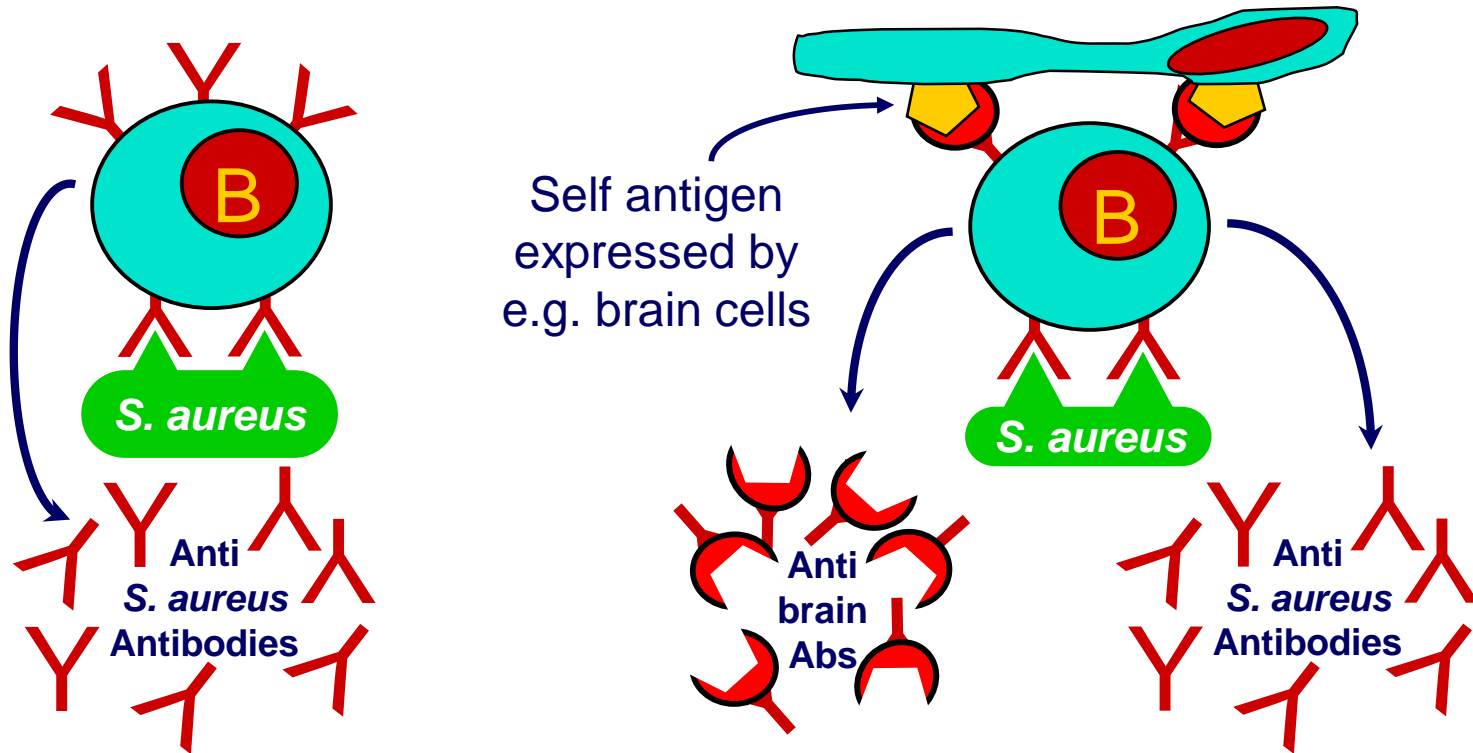
All daughter cells must express the same Ig specificity
otherwise the efficiency of the response would be compromised

Suppression of H chain gene rearrangement helps prevent the emergence of
new daughter specificities during proliferation after clonal selection

Allelic exclusion prevents unwanted responses

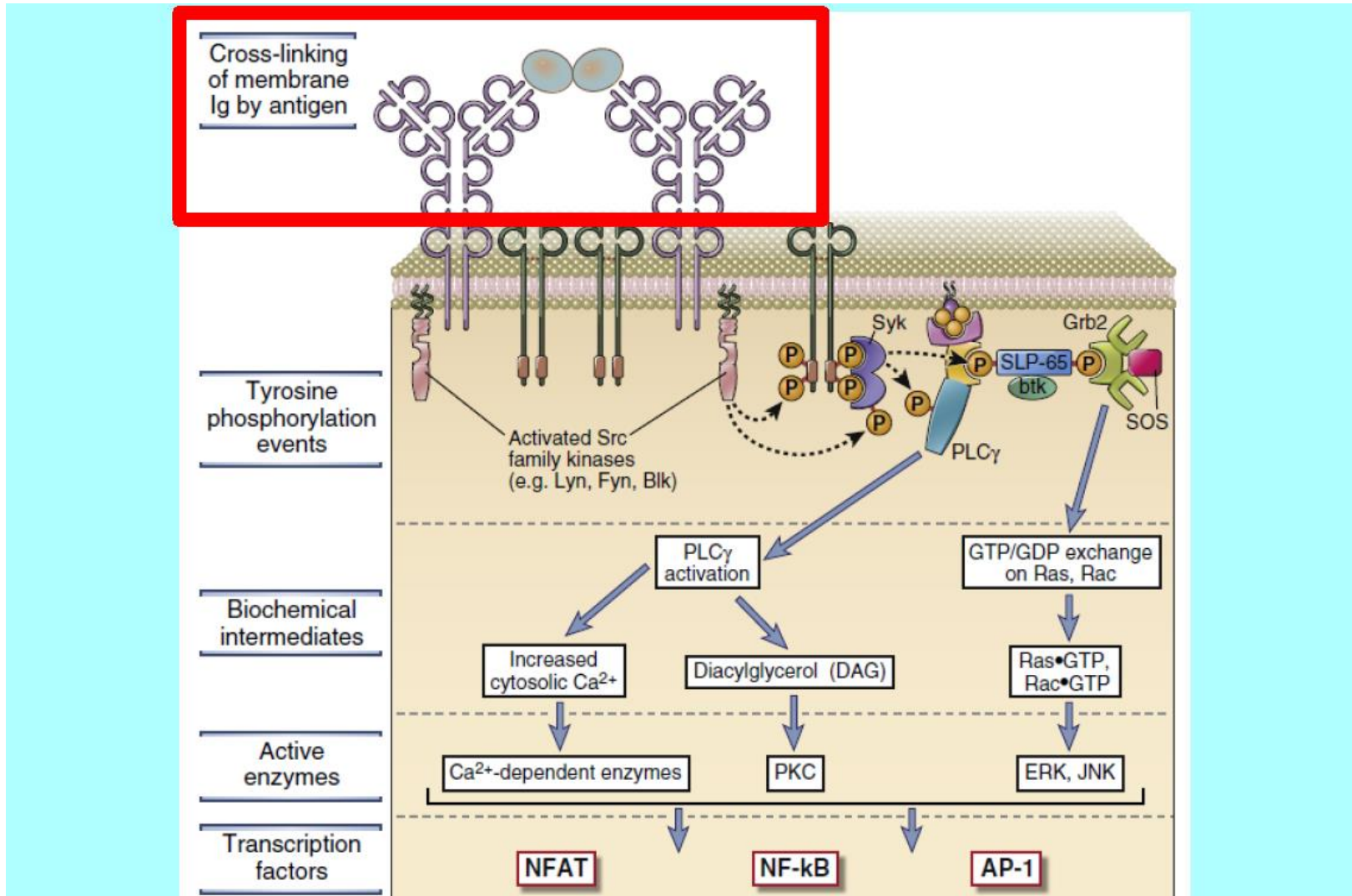
One Ag receptor per cell

IF there were two Ag receptors per cell

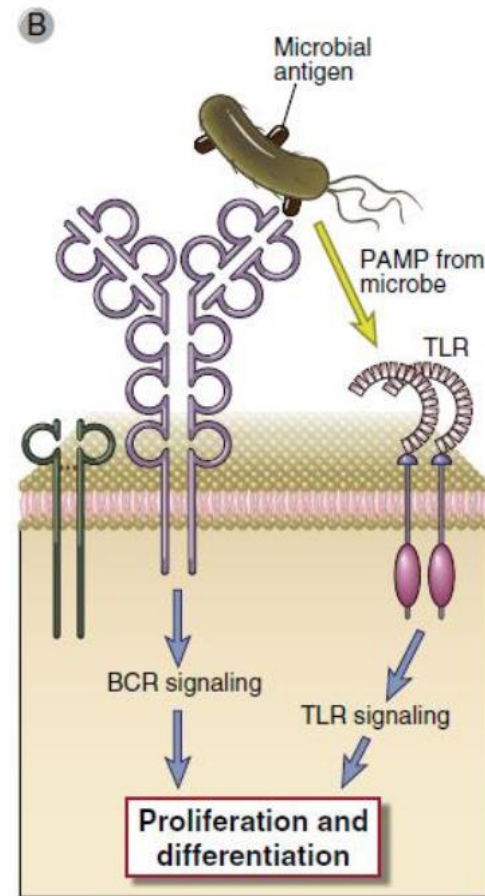
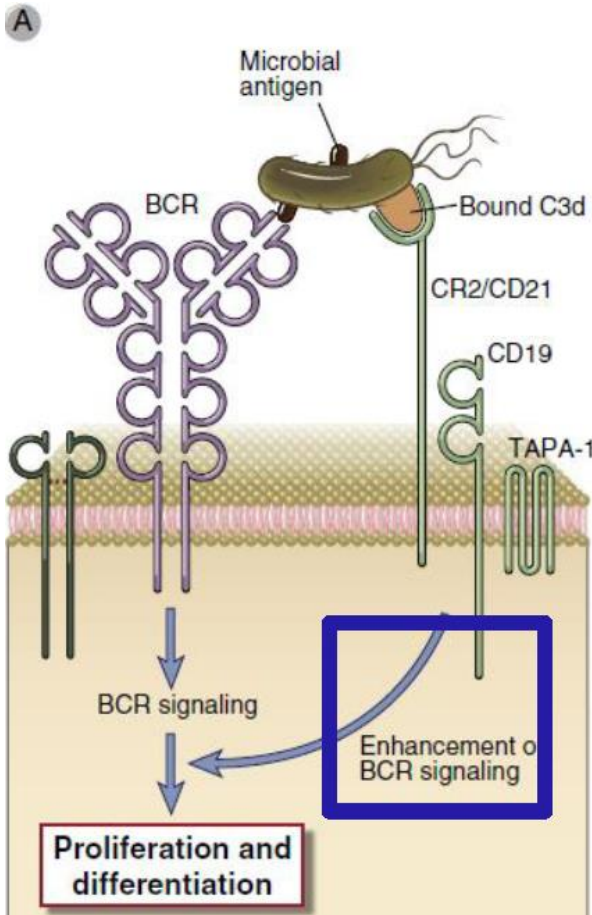


Suppression of H chain gene rearrangement ensures only one specificity of Ab expressed per cell. Prevents induction of unwanted responses by pathogens

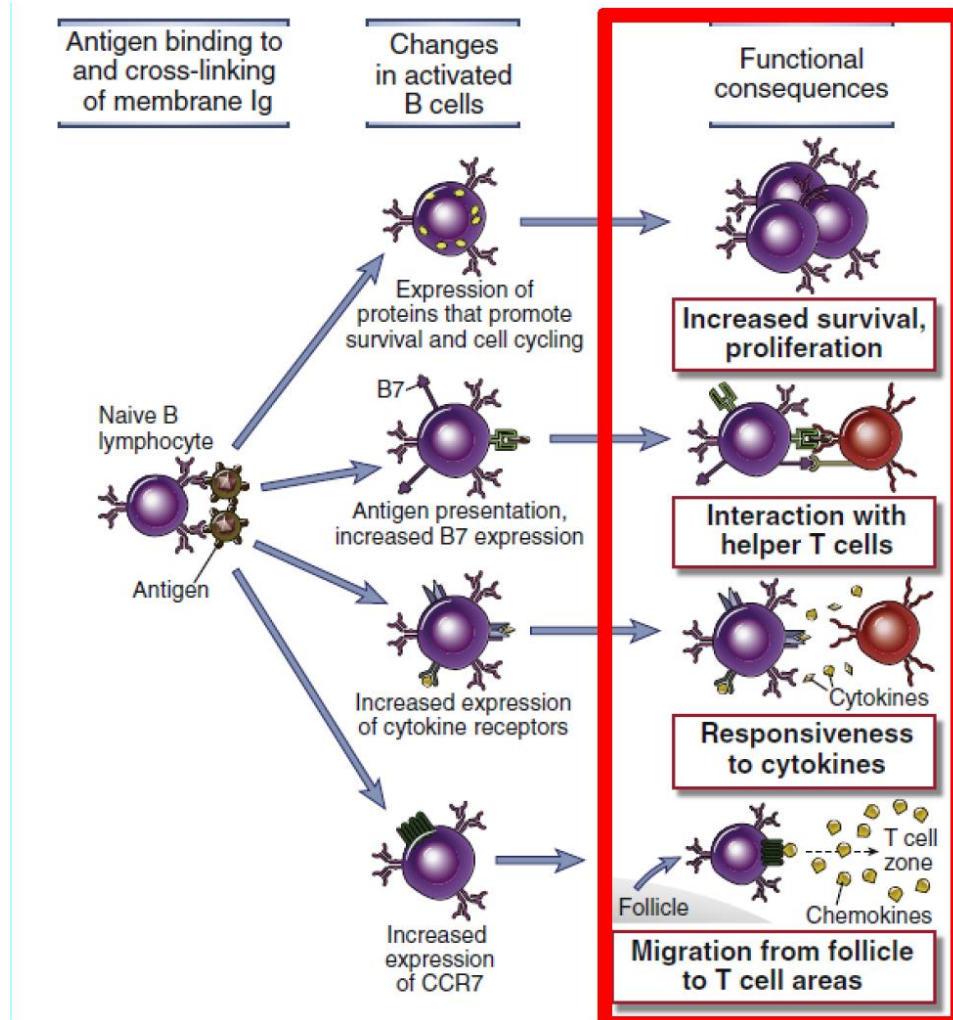
Signal transduction by the BCR complex



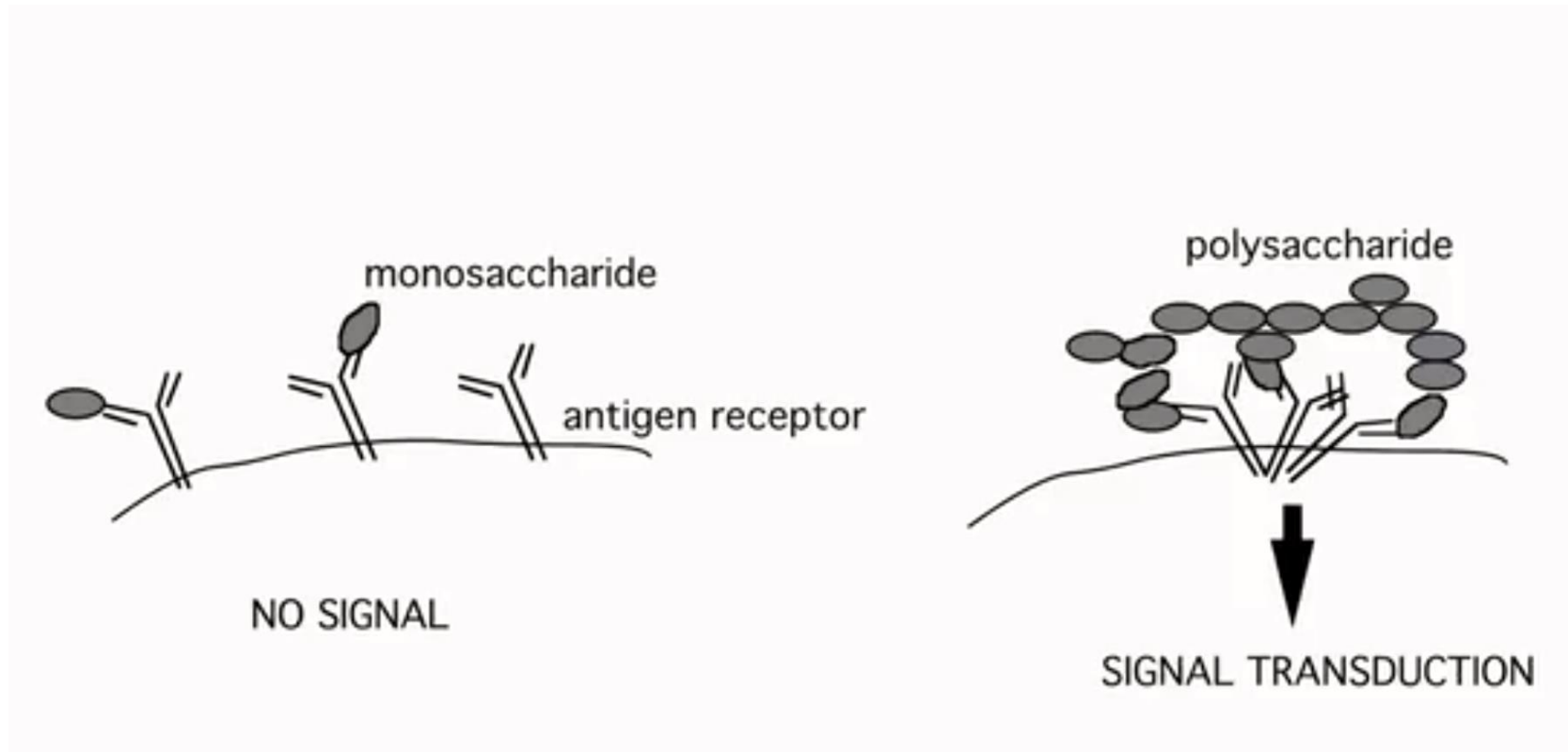
Role of CR2 & TLR in B cell activation



Functional responses induced by Ag-mediated cross-linking of BCR



T independent B cell activation



- Multivalent structures can be T independent antigens
- Responses generally low affinity w limited class switching-short lived plasma cells

Mechanisms of T-Independent Antibody Responses

- T-independent antigens are capable of stimulating B cell proliferation and differentiation in the absence of T cell help
- Most important TI antigens are
 - :Polysaccharides
 - :Glycolipids
 - :Nucleic acids

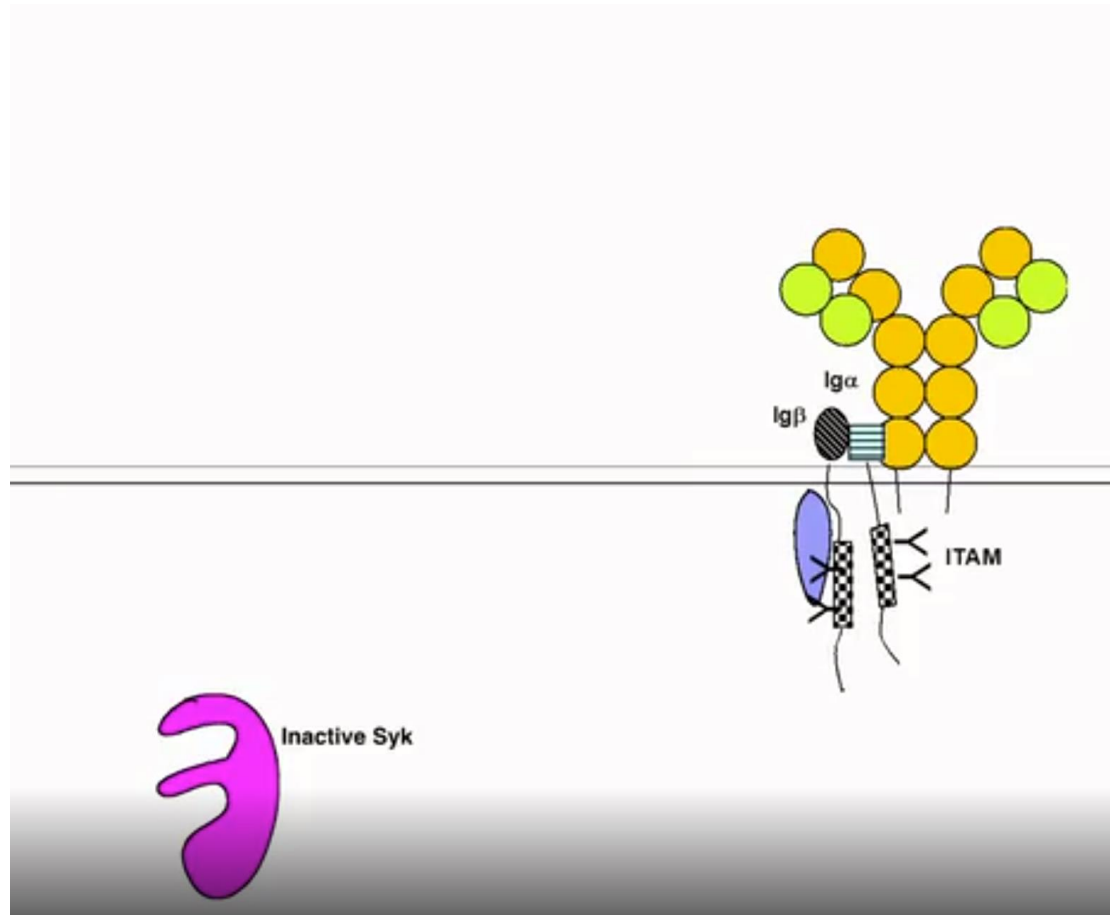
Haptens, antigens, immunogens

- Haptens are small molecules or moieties
 - They are **antigens but not immunogens**
- **All immunogens are antigens**
 - All antigens are not immunogens

Natural antibodies

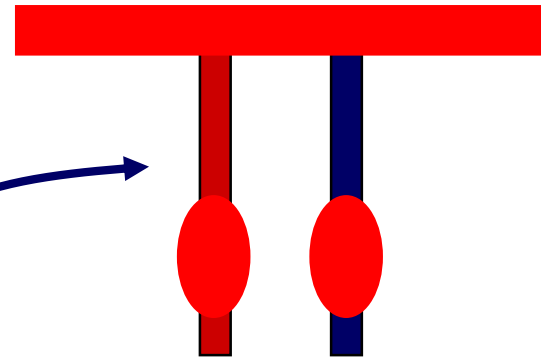
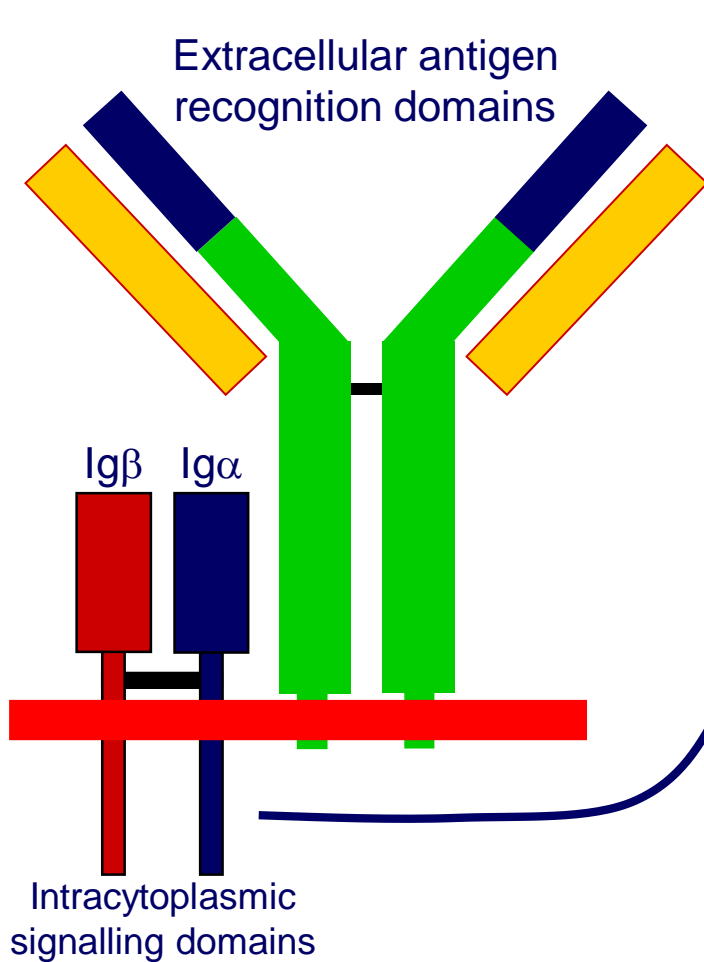
- Low-affinity anti-carbohydrate antibodies
- Postulated to be produced by
 1. Peritoneal B-1 cells
 2. Marginal zone B cells in the spleen
- Protection against bacterial infections & Facilitate phagocytosis of apoptotic cells.
- Anti-ABO blood group antibodies

T independent B cell activation



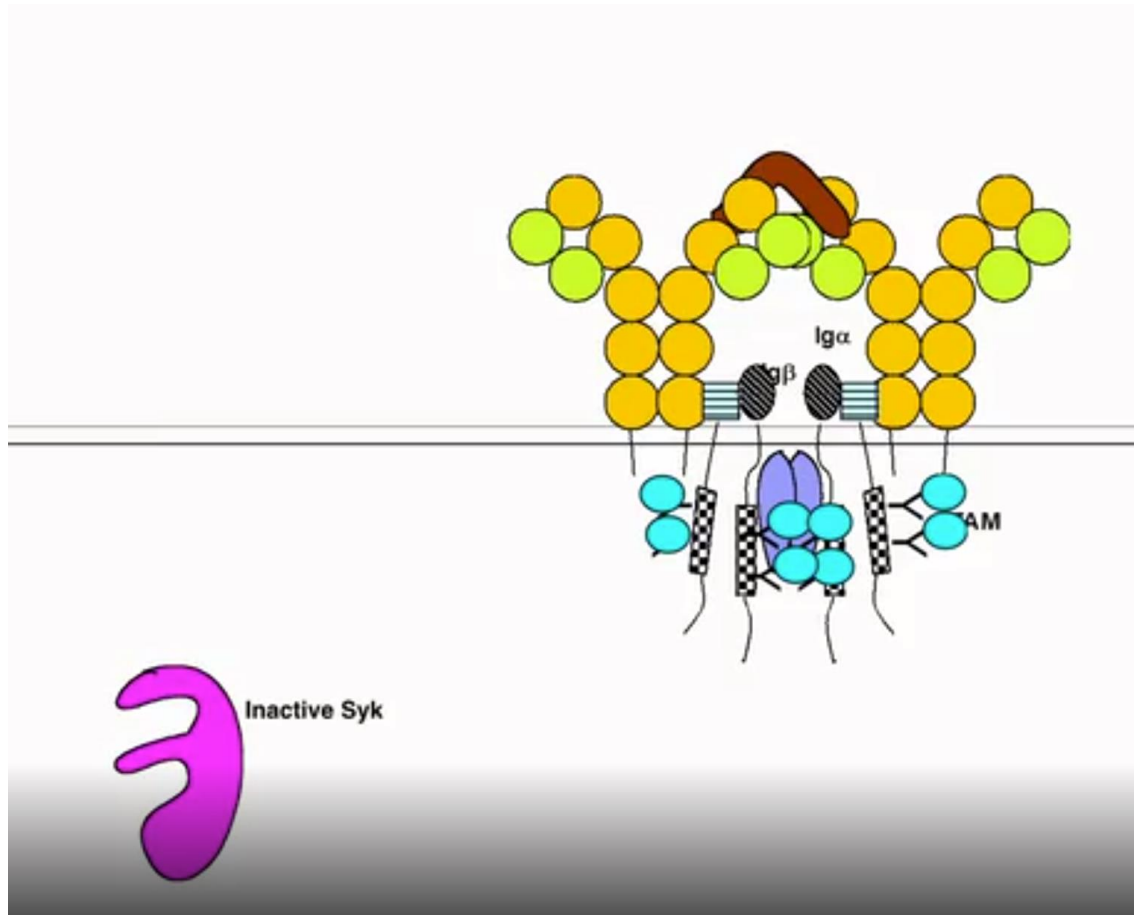
- Cross linking of the receptor

Transduction of signals by the B cell receptor



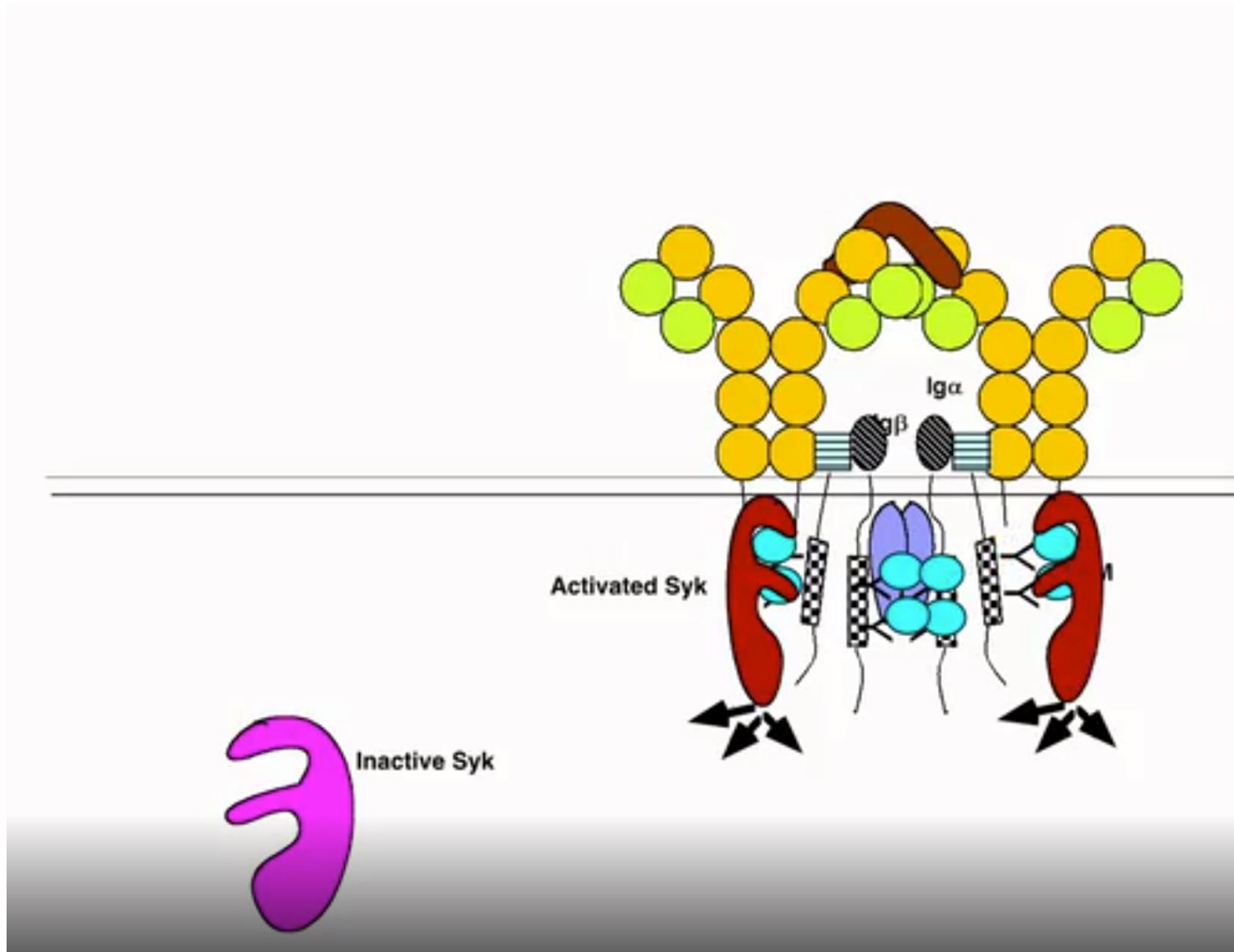
The cytoplasmic domains of the $Ig\alpha$ and $Ig\beta$ contain Immunoreceptor Tyrosine - based Activation Motifs (ITAMS) - 2 tyrosine residues separated by 9-12 amino acids - $YXX[L/V]X_{6-9}YXX[L/V]$

T independent B cell activation



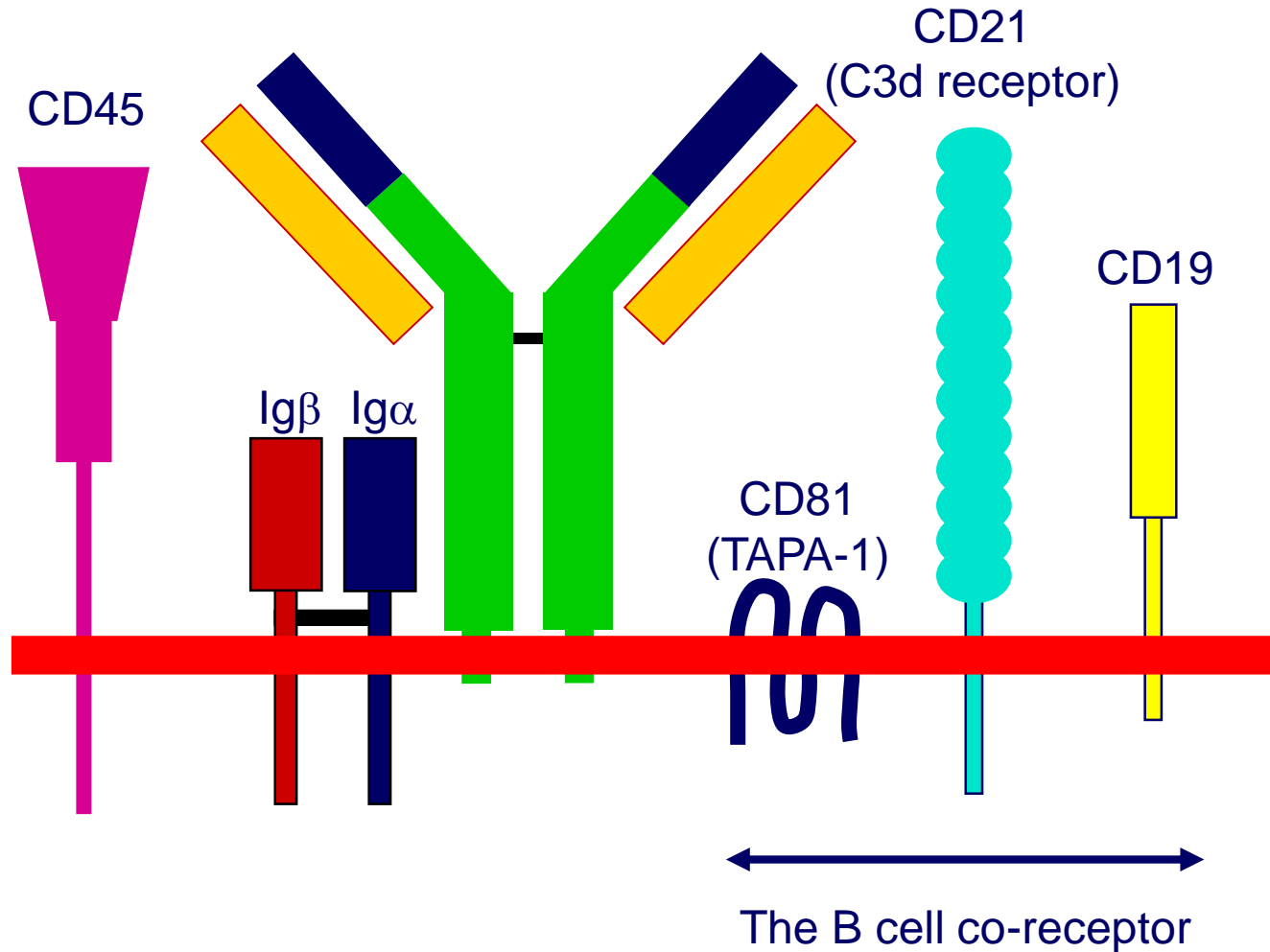
- Cross linking of the receptor

T independent B cell activation

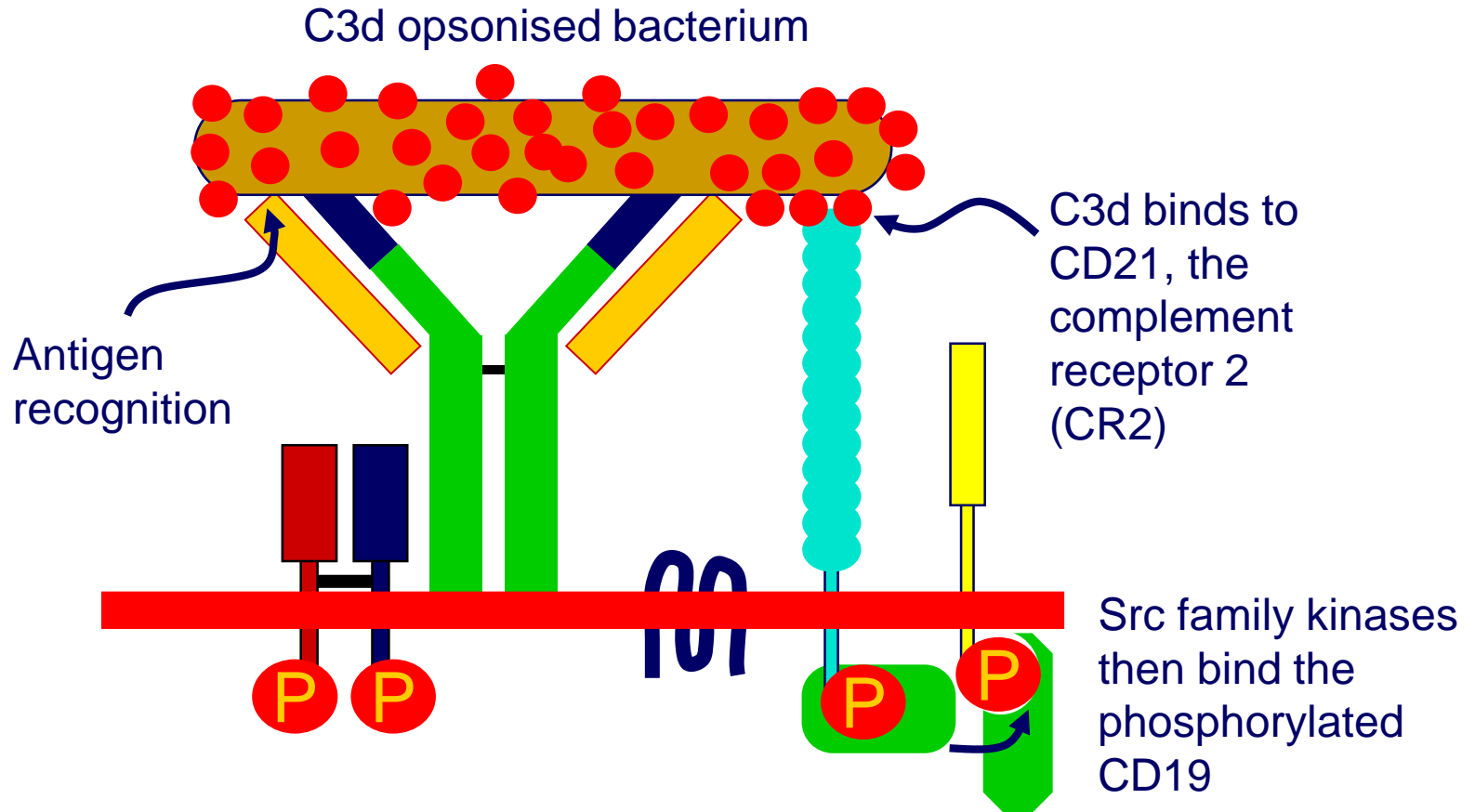


- Cross linking of the receptor

The B cell co-receptor

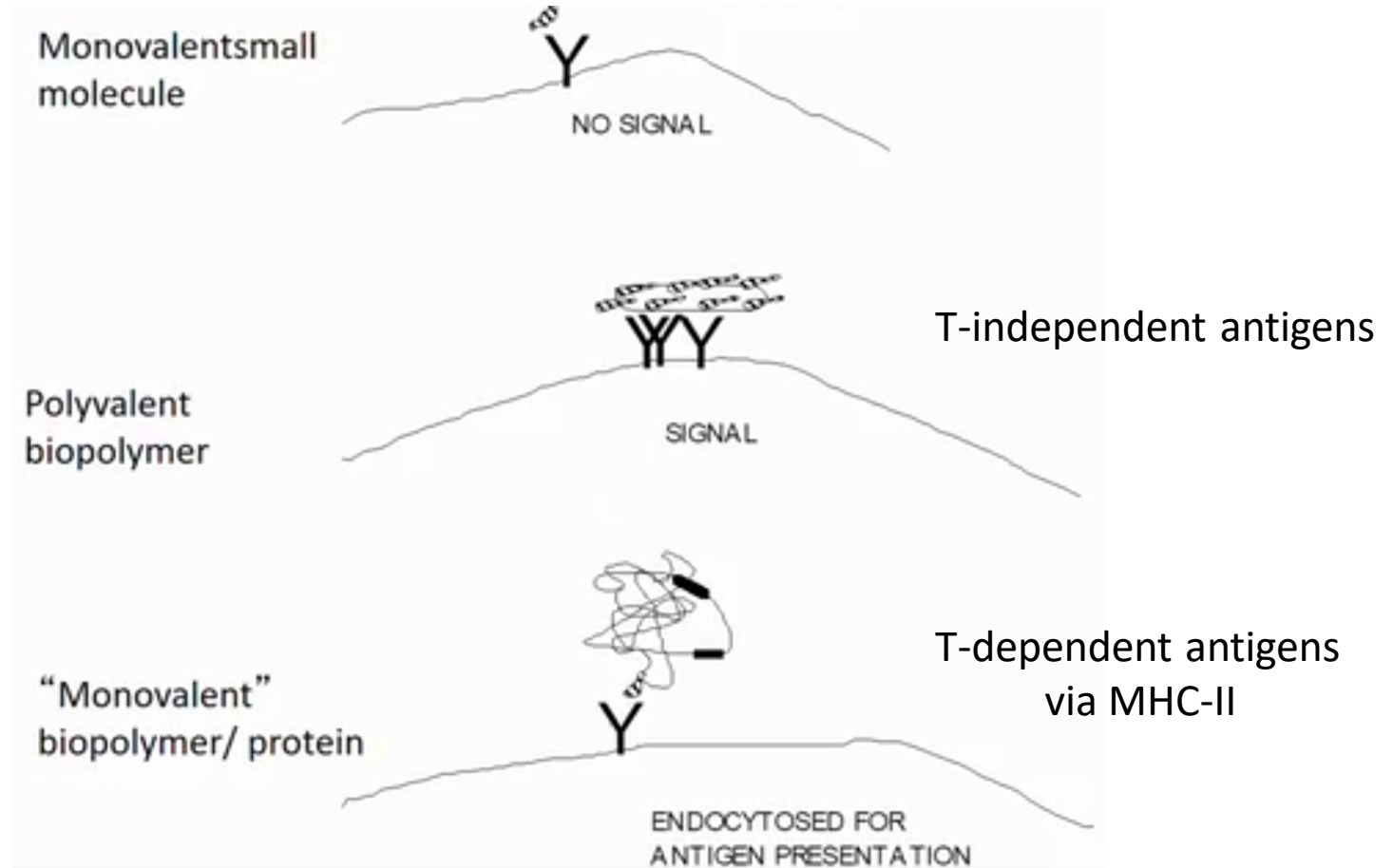


Co-receptor phosphorylation



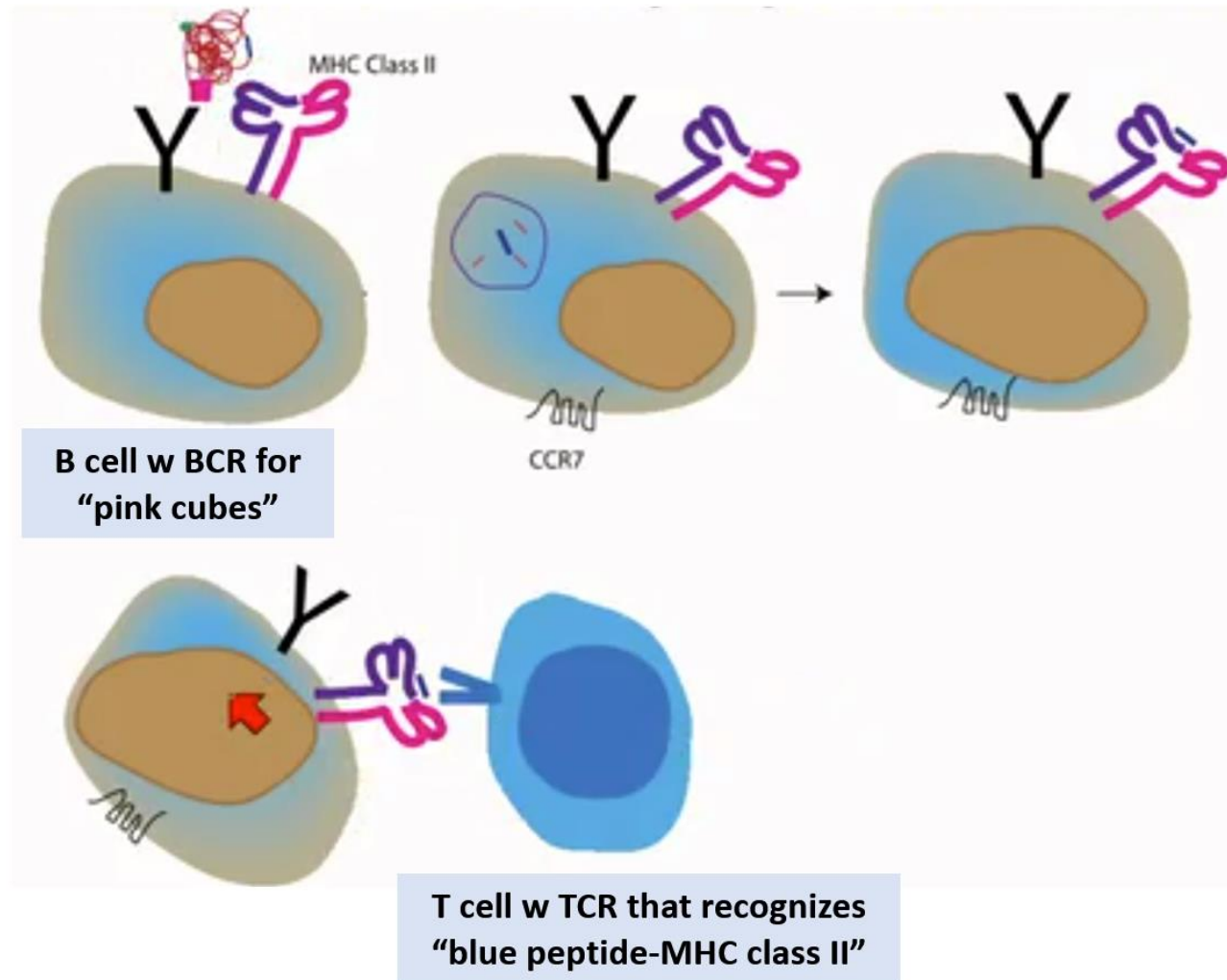
- mlg and CD21 are cross-linked by antigen that has activated complement
- CD21 is phosphorylated and receptor-associated kinases phosphorylate CD19
- Phosphorylated CD19 activates more Src family kinases
- **Ligation of the co-receptor increases B cell receptor signalling 1000 -10,000 fold**

T-independent antigens

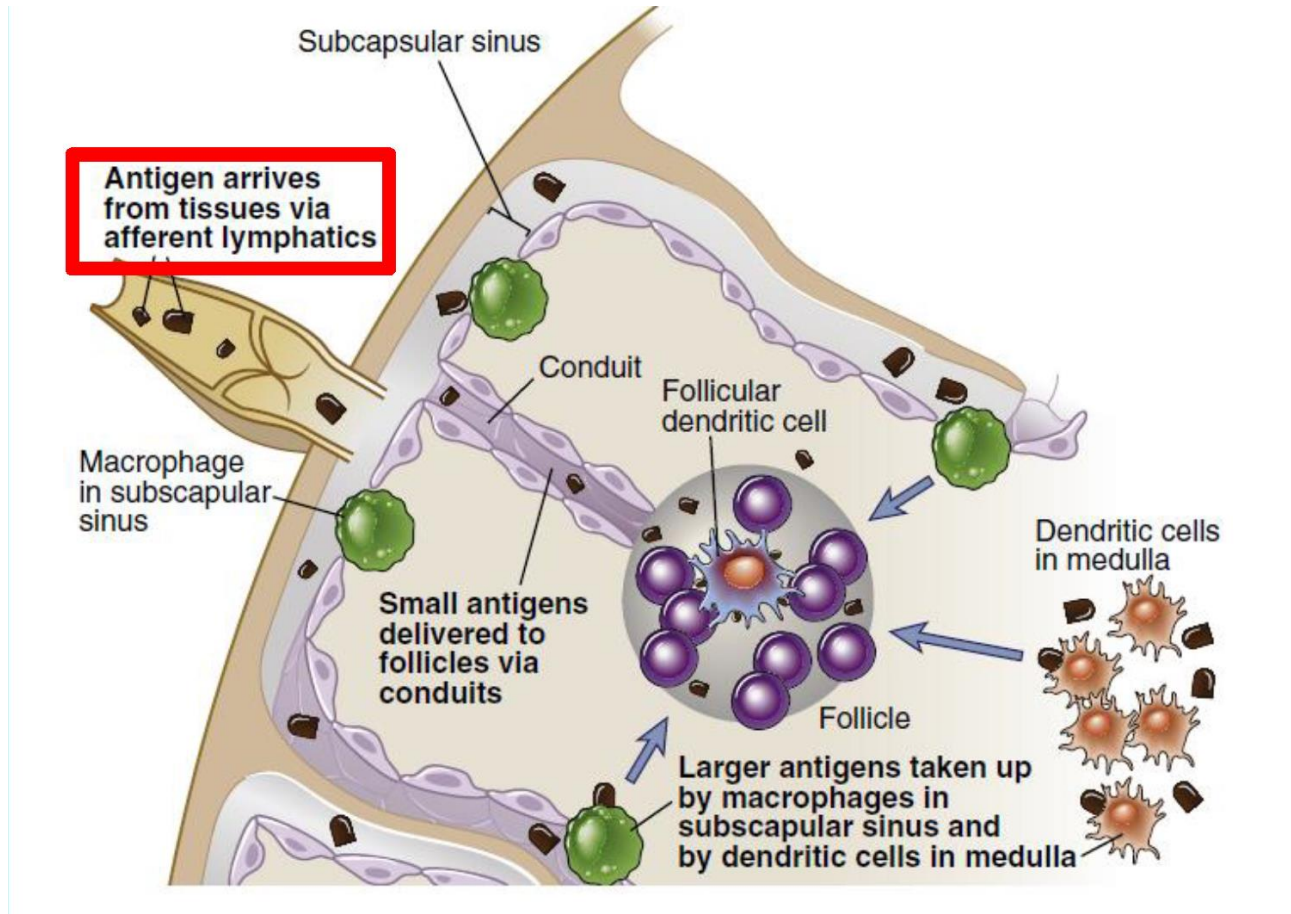


Proteins are endocytosed after they are seen by the BCR

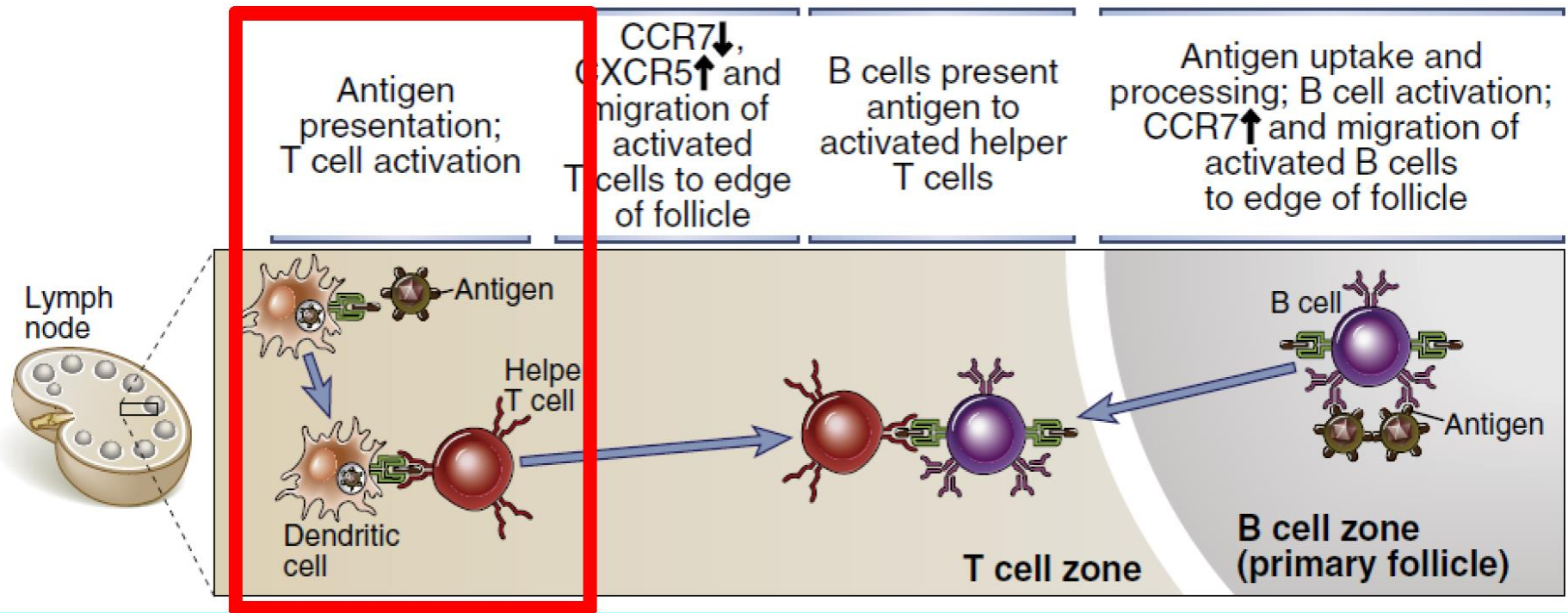
Distinct B & T cell epitopes must be linked



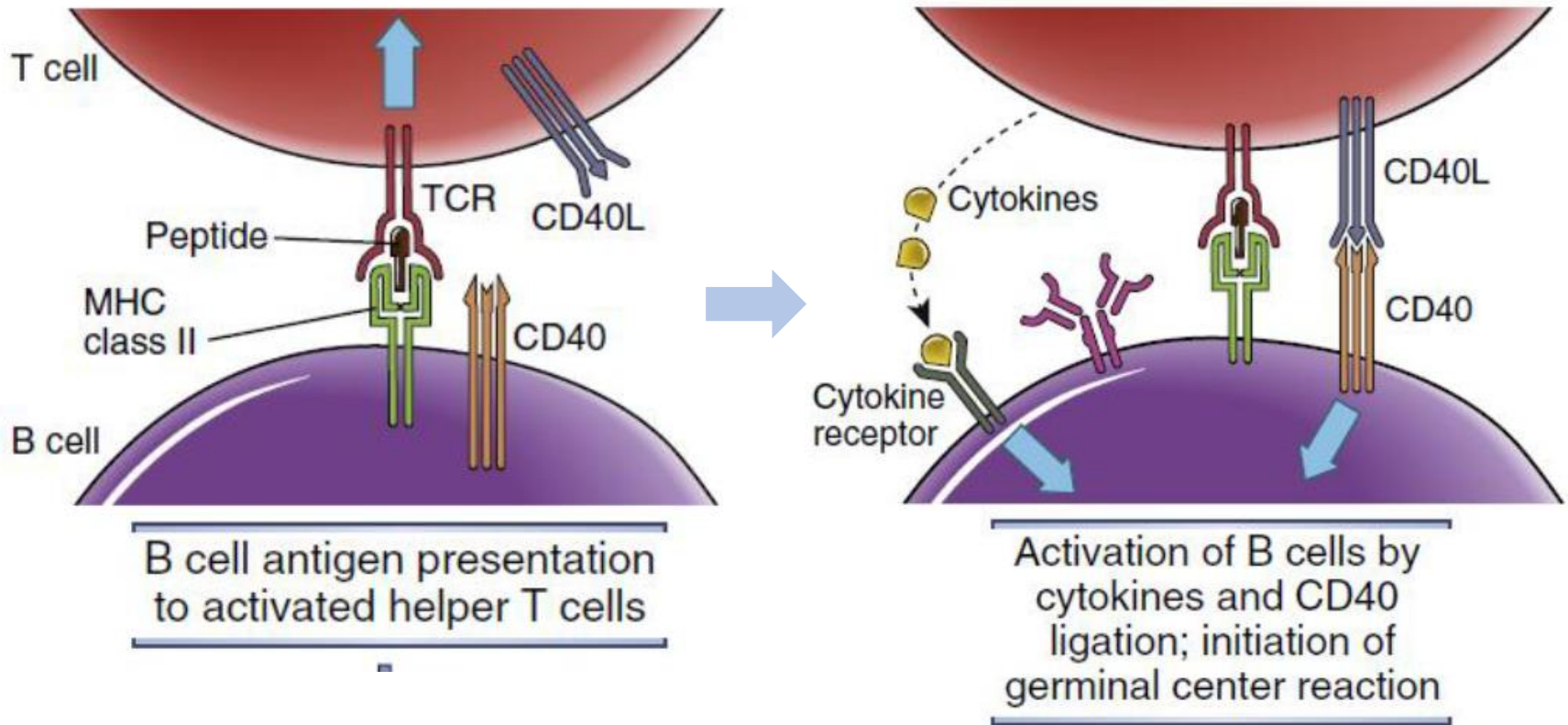
Ag delivery to follicular B cells



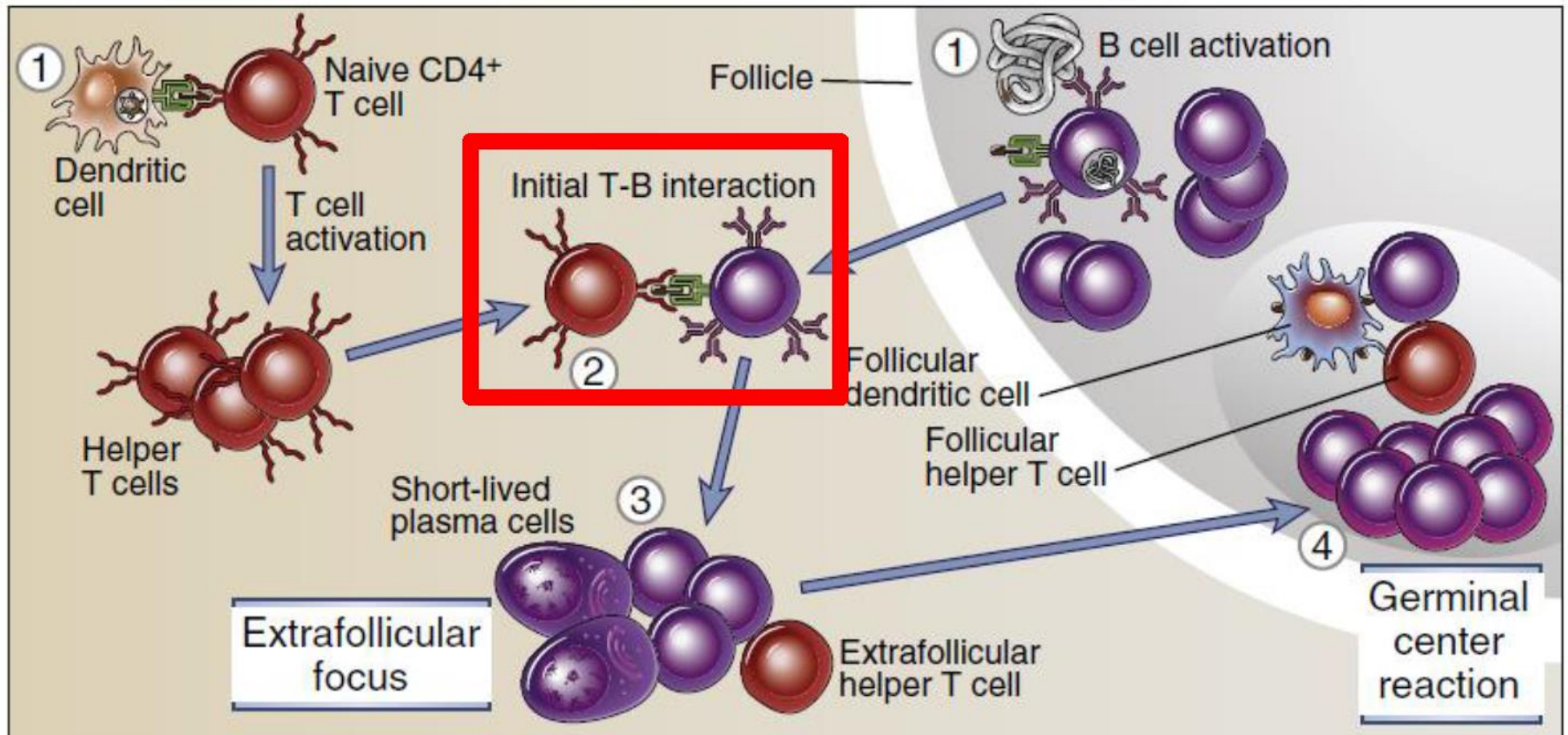
T-B cell migration and interactions



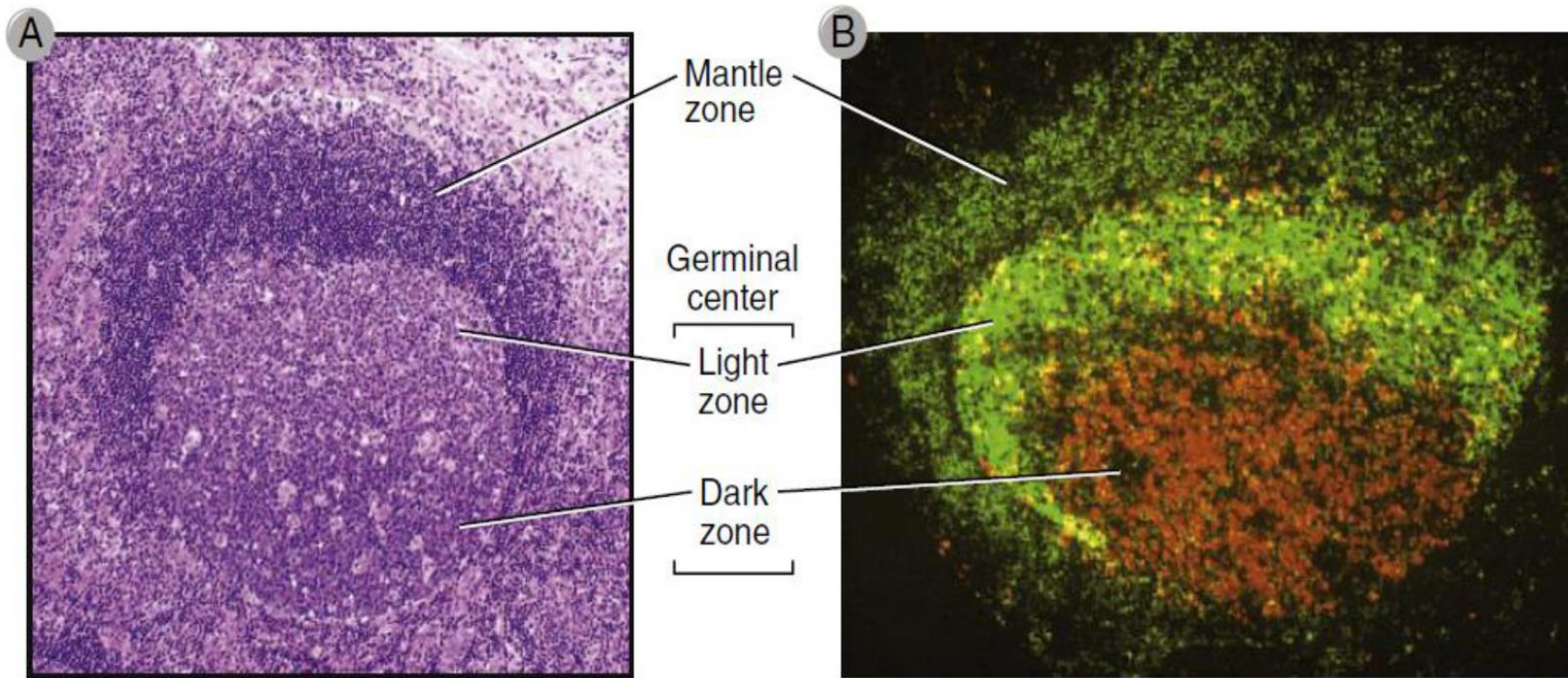
Helper T cell activation of B cells



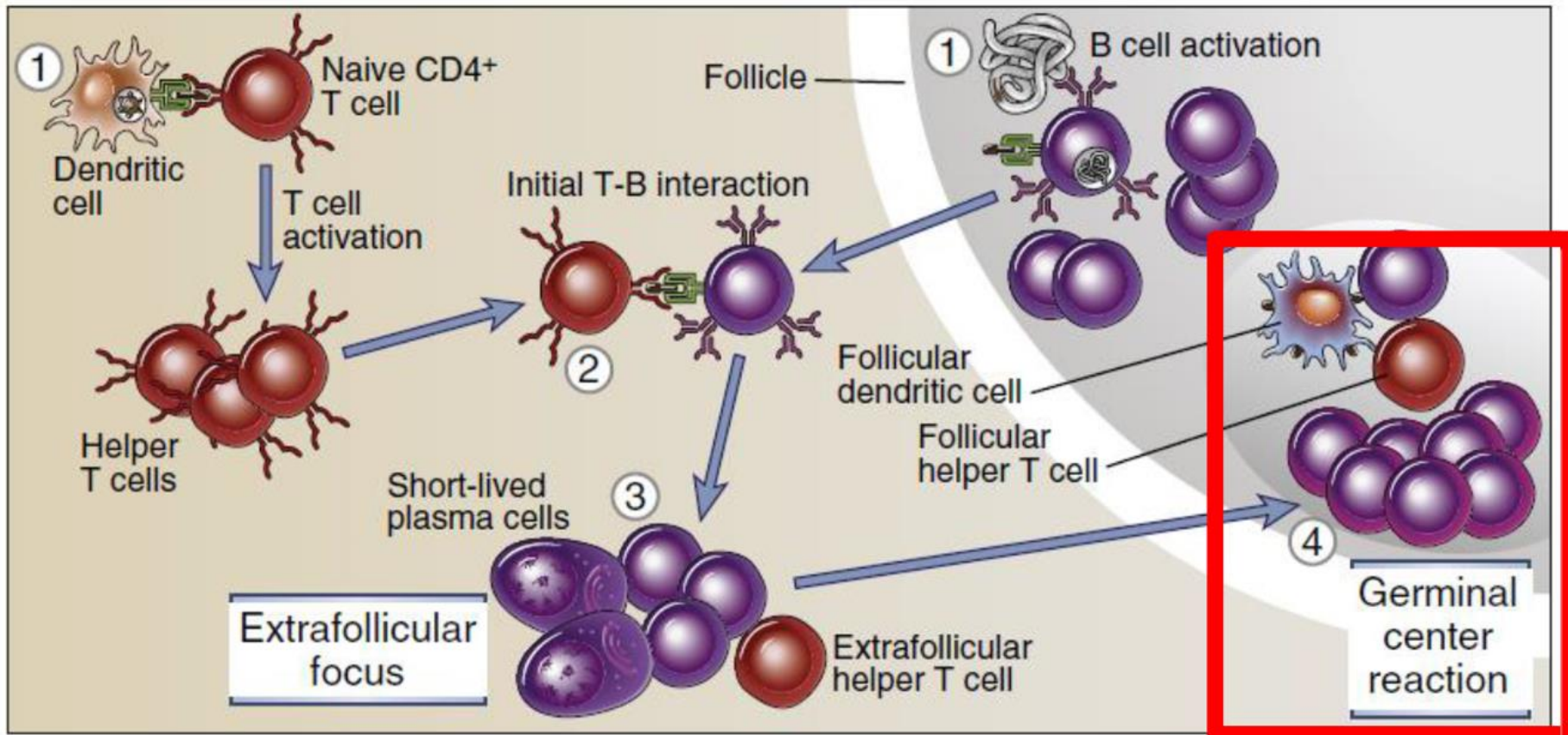
Events in T dependent humoral response



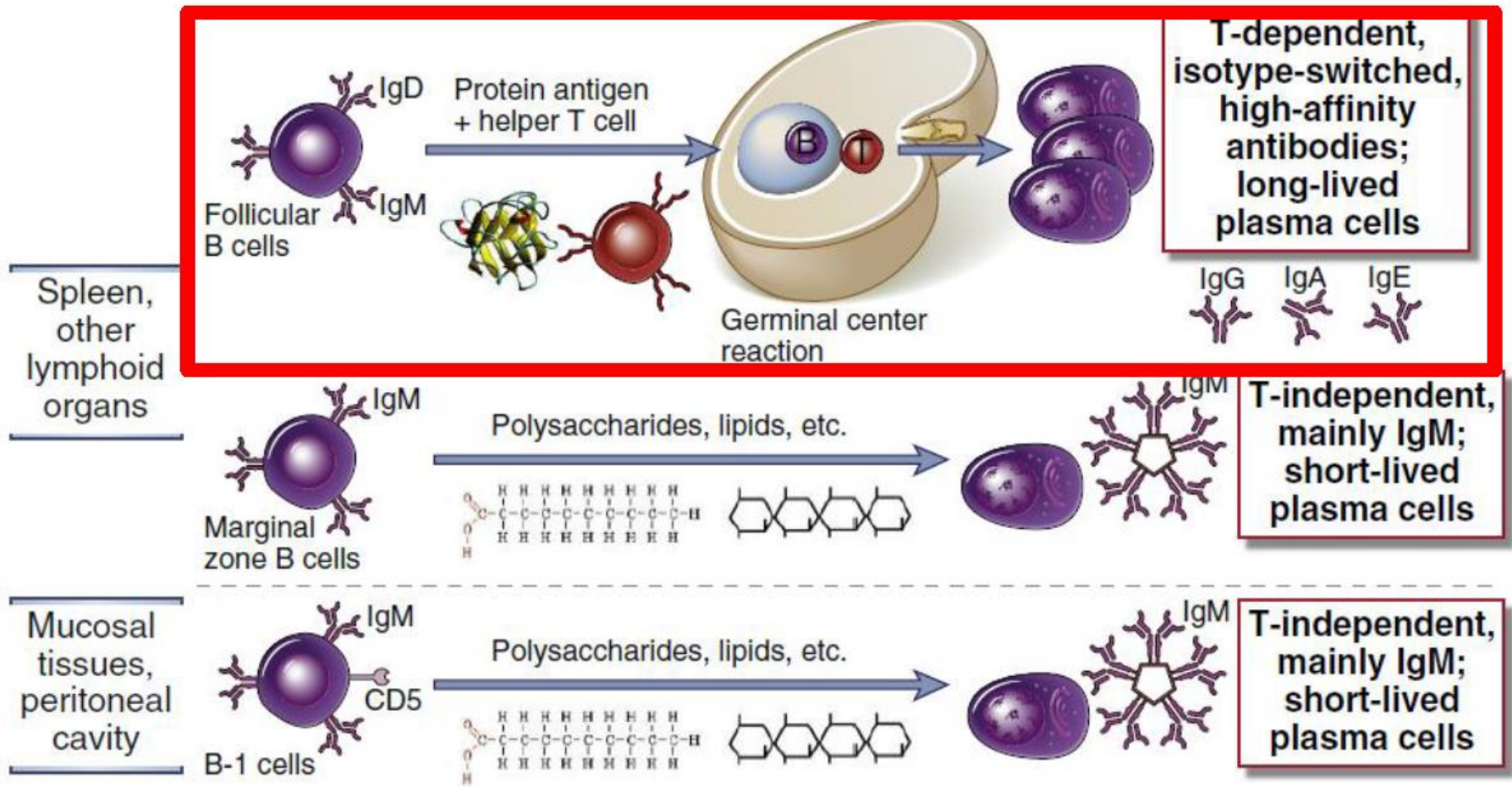
The germinal center in lymphoid organs



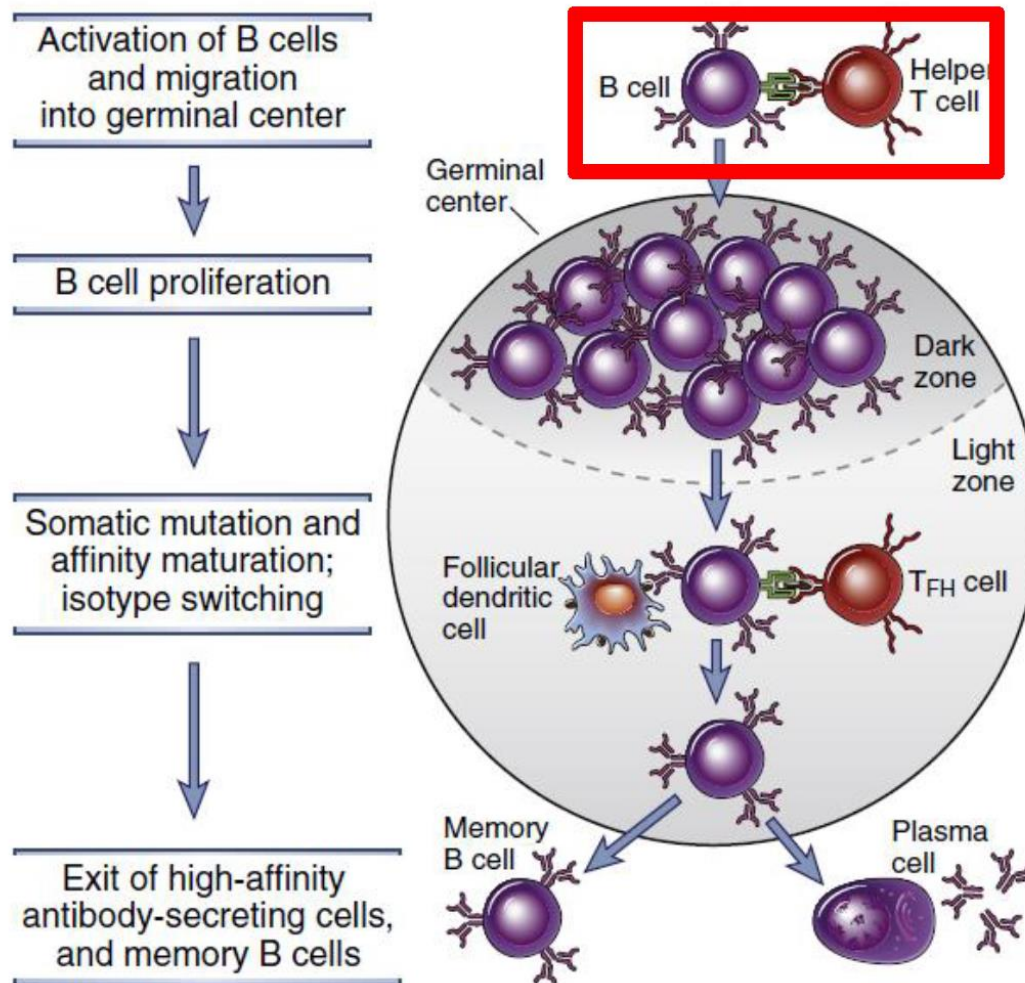
Events in T dependent humoral response



Distinct B cell subtypes mediate different types of Ab responses



B cell antigen presentation to helper T cells

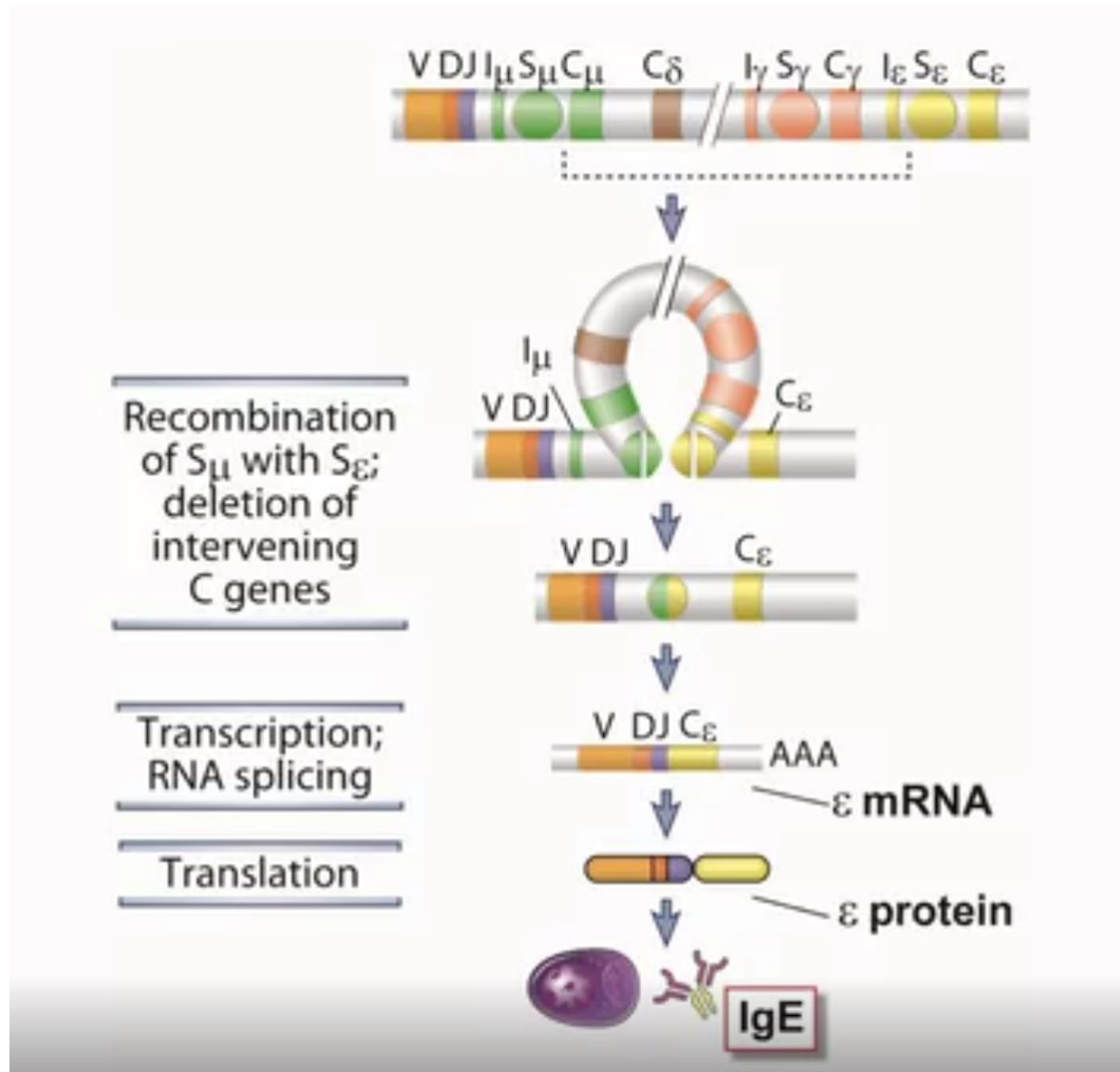


Sequential T-B interactions in immunity

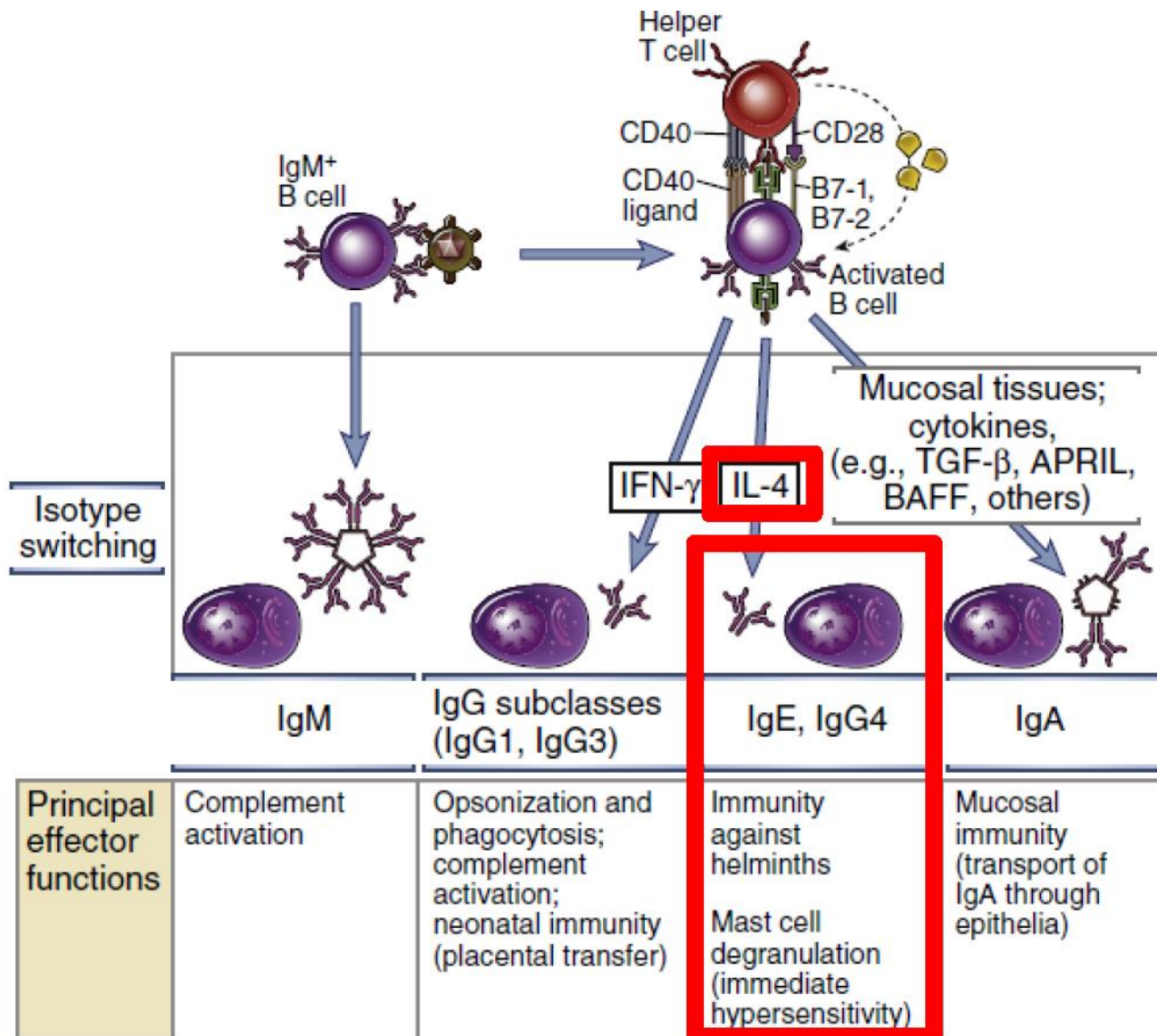
- **T-> B – Forms extrafollicular B cell focus**
- **B-> T – Generates T follicular helper cells**
- **T-> B – Selection of high affinity B cells**

Heavy chain isotype switching, memory cells

constant region of HC changes IgM-IgG, affinity remains

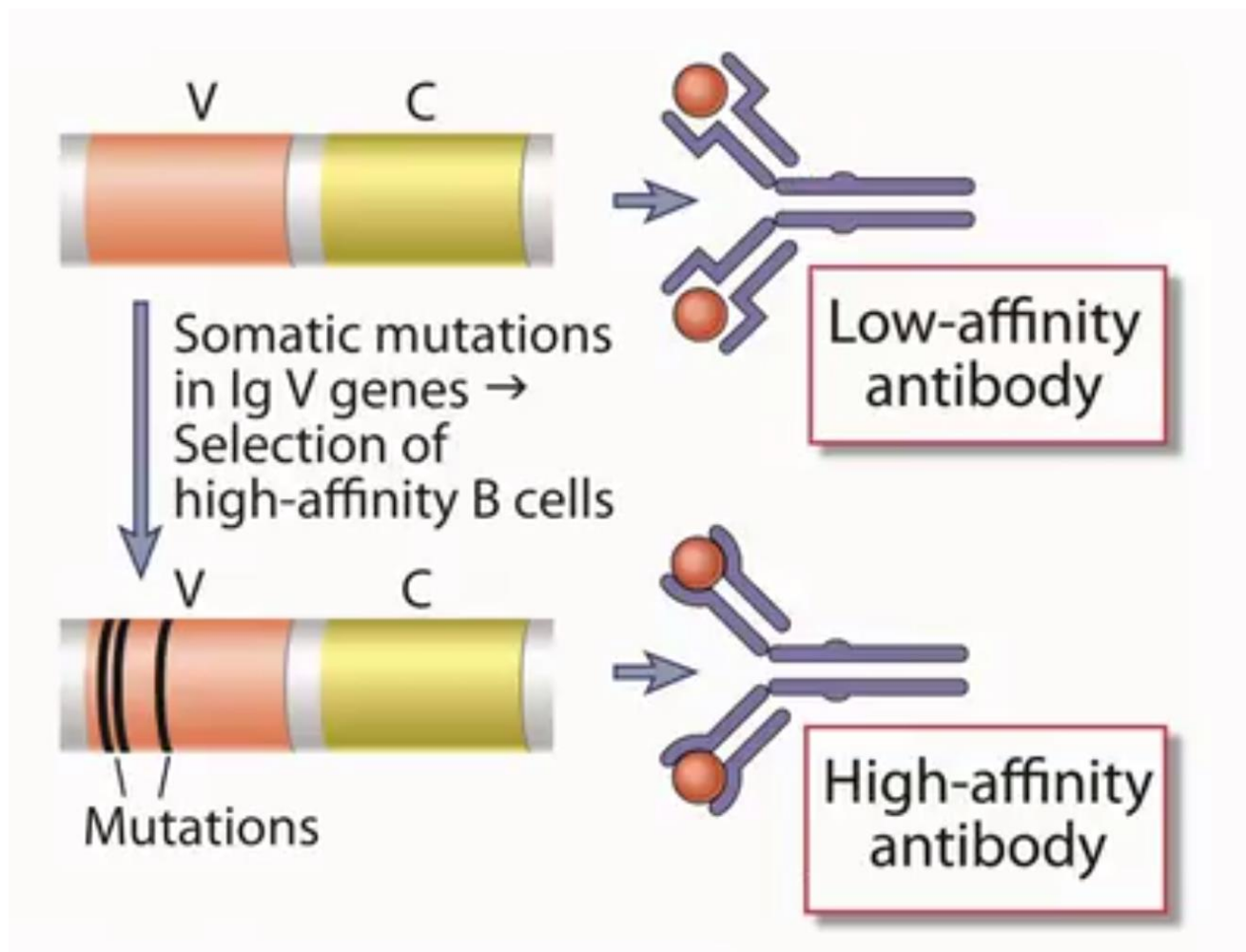


Heavy chain isotype switching, memory cells

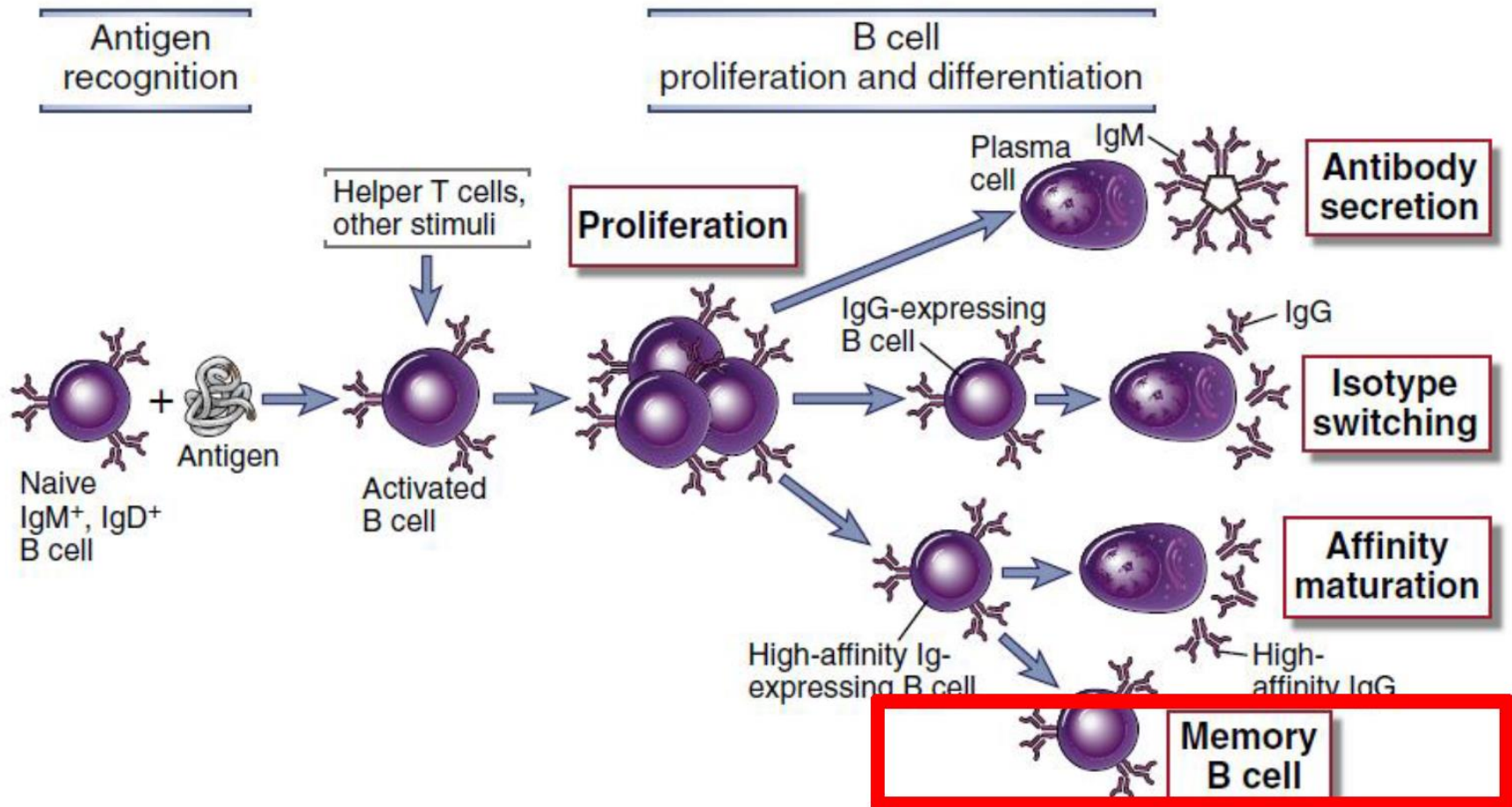


Affinity maturation, memory cells

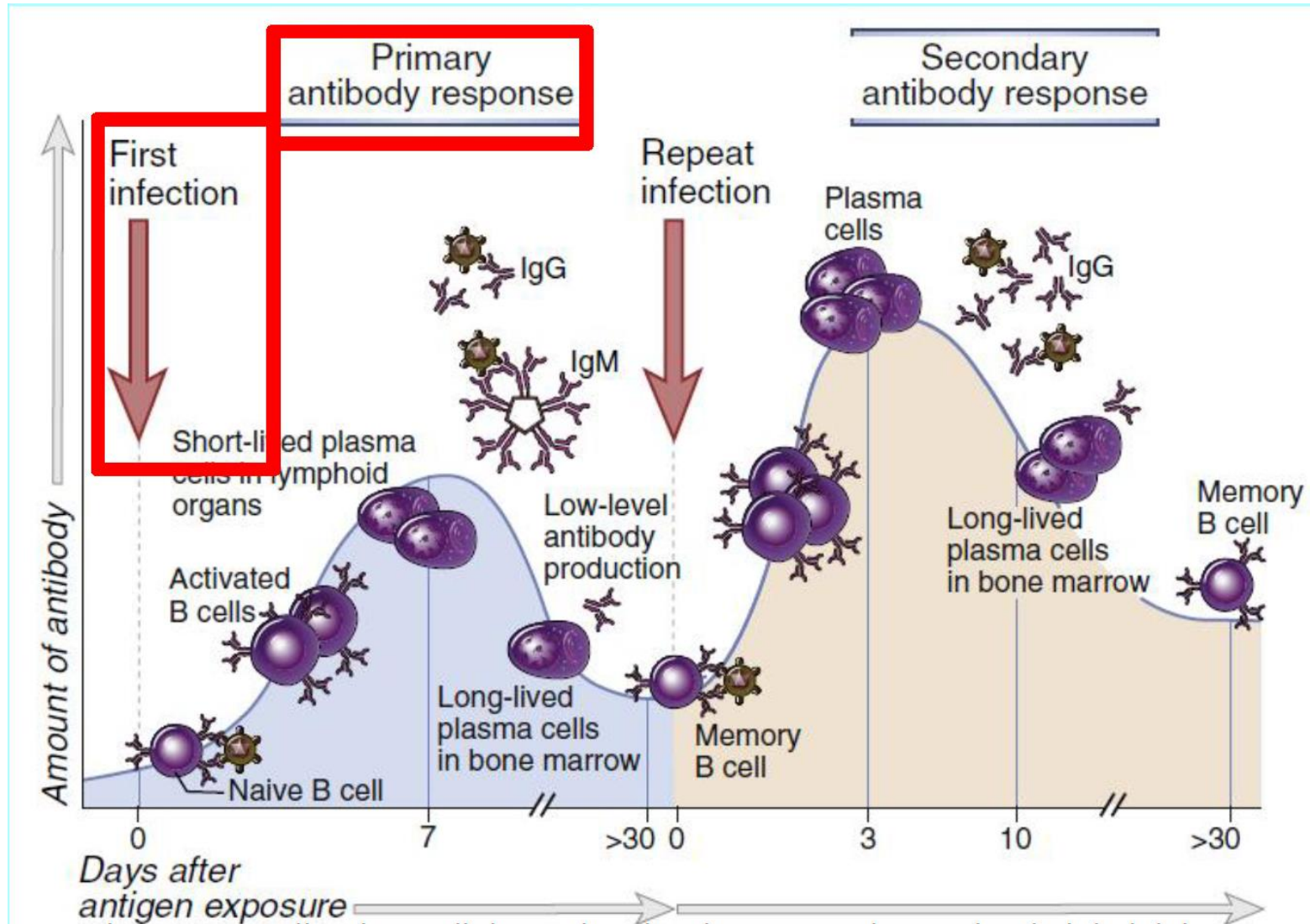
somatic hypermutation of CDR, 1-2/cell generation
positive/clonal selection via Tfh



Overview of B-cell activation



Overview of B-cell activation

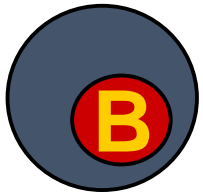


1ry vs 2ry Response

Feature	Primary response	Secondary response
Peak response	Smaller	Larger
Antibody isotype	Usually IgM > IgG	Relative increase in IgG and, under certain situations, in IgA or IgE
Antibody affinity	Lower average affinity, more variable	Higher average affinity (affinity maturation)
Induced by	All immunogens	Mainly protein antigens

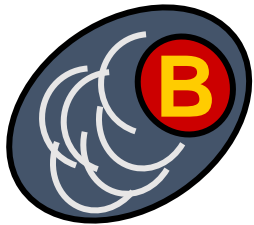
Plasma cells

	Surface Ig	Surface MHC II	High rate Ig secretion	Growth	Somatic hypermut'n	Isotype switch
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Mature B cell

High	Yes	No	Yes	Yes	Yes
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Plasma cell

Low	No	Yes	No	No	No
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T dependent vs T independent

TABLE 12-2 Properties of Thymus-Dependent and Thymus-Independent Antigens

	Thymus-Dependent Antigen	Thymus-Independent Antigen
Chemical nature	Proteins	Polymeric antigens, especially polysaccharides; also glycolipids, nucleic acids
Features of Antibody Response		
Isotype switching	Yes; IgG, IgE, and IgA	Little or no; may be some IgG and IgA
Affinity maturation	Yes	No
Secondary response (memory B cells)	Yes	Only seen with some antigens (e.g., polysaccharides)

B-cells

- Important WBC-small lymphocytes
- Humoral immunity of adaptive immune system
- Function in the bone marrow
- Express BCR on membrane-bind Ag – initiate Ab response
- Can present Ag/APC & collaborate w Tfh cells