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B cell activation

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Immunity and Infection BSc course 2011-12
October 21, 2011

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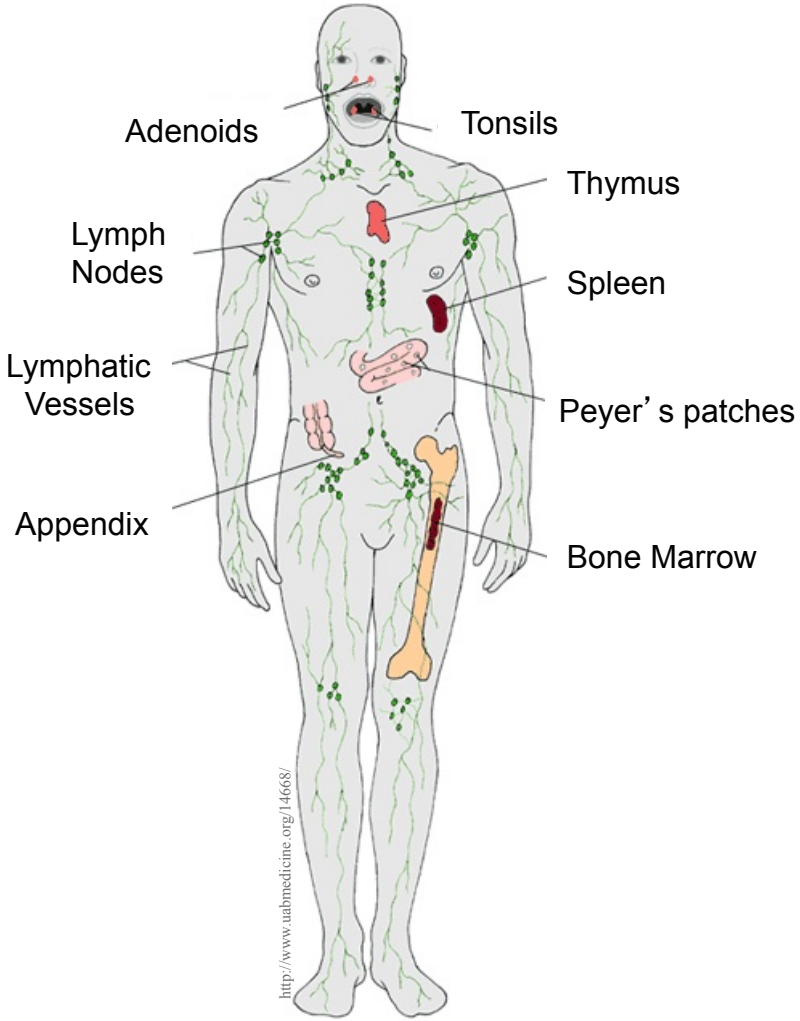
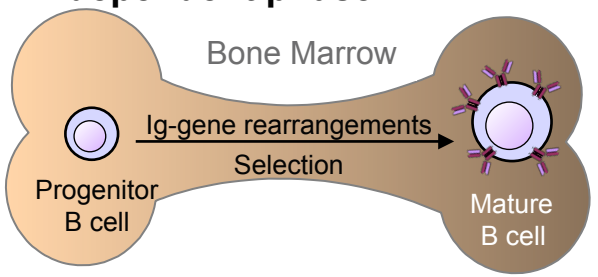
Overview of B cell development and activation

Focus: Events in a B cell's life in the secondary lymphoid organs

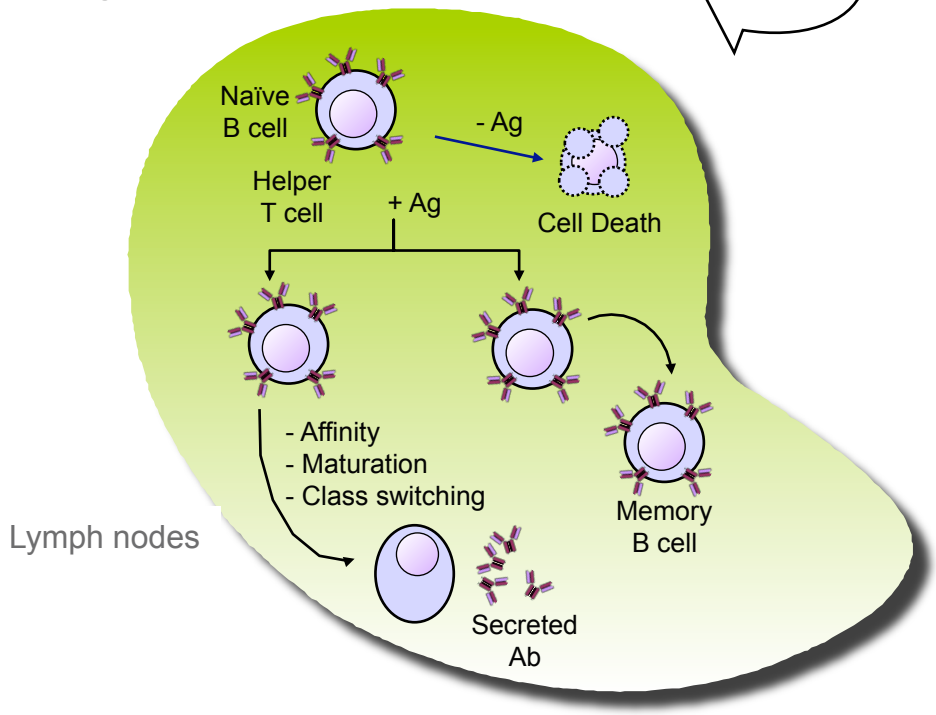
The Immune System



Antigen-independent phase



Antigen-dependent phase



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We will...

- **Overview of signalling through B cell receptor (BCR), including role of co-receptors**
  - Structure of BCR
  - Tyrosine kinases involved
  - Second messengers
  - BCR co-receptors
  - Transcription factors
- **Describe the T-independent B cell activation**
- **Describe the T-dependent B cell activation**
- **Relate the B cell activation to the microanatomy**

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Learning objectives

To be able to:

- **Describe the key stages of the signalling through the BCR from the antigen recognition to the early response of B cell**:
  - *Define the B cell receptor*
  - *Identify the key events at each phosphorylation step*
  - *List the transcription factors and the second messenger pathways involved in the signal transduction*
  - *Outline the changes in B cell phenotype and function*
- **Give at least one example of B cell co-receptor and describe how this modulate the BCR signalling**
- **Describe the sequence of events in T-dependent B cell activation and relate them to the microanatomy within the peripheral lymphoid organs**

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Why activate B cells ?

- **To generate high affinity, soluble & surface expressed antibody**
  - •effector functions of Ab
  - •efficient antigen presentation to T cells

This requires

- •B cell proliferation
- •Somatic hypermutation of Ig V regions
- •Differentiation into Ab secreting cells (plasma cells)

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B cell activation

Requires 2 signals:

1. Signal 1 occurs through the B-cell receptor

- Ag engagement of the BCR initiates signal cascades that result in the transcription of variety of genes associated with B-cell activation

2. Signal 2 is derived from cell-cell interactions, cytokine stimulation (T-dependent) or from Ag directly

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B cell activation

Requires 2 signals:

1. Signal 1 occurs through the B-cell receptor

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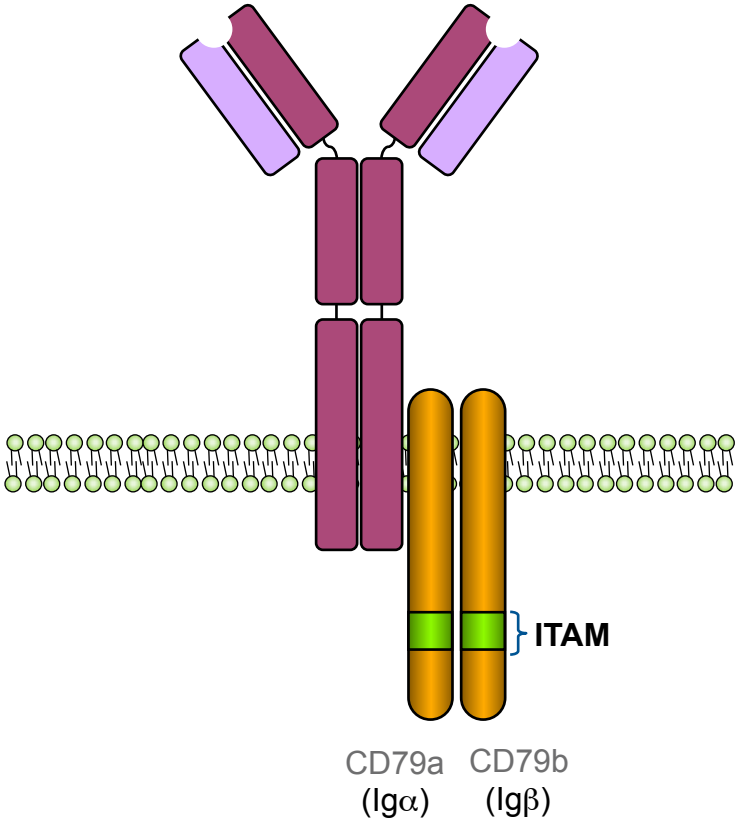
B Cell Receptor (BCR)

- Naïve B cell express membrane (m)IgM, and soon acquire mIgD.

- mIg assocates with CD79a (Igα) and CD79b (Igβ) to form the B cell receptor (BCR).

- Igα and Igβ each have a conserved sequence motif named ITAM (Immunoreceptor Tyrosine-based Activation Motif), containing two tyrosines separated by 9-12 aminoacids:

$$[D/E]\text{-}X_{(7)}\text{-}[D\backslash E]\text{-}X_{(2)}\text{-}Y\text{-}X_{(2)}\text{-}L\text{-}X_{(7)}\text{-}Y\text{-}X_{(2)}\text{-}L$$



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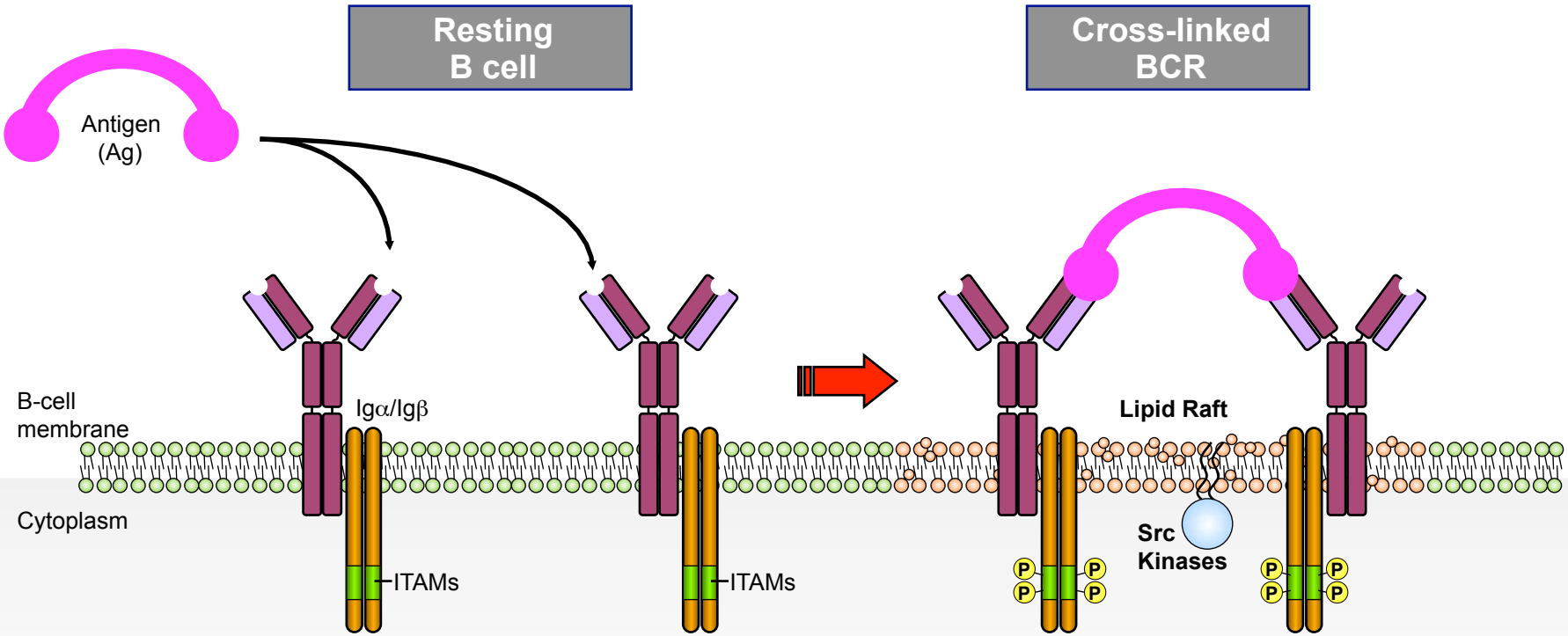
Clustering of receptors



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Protein kinase: Definition

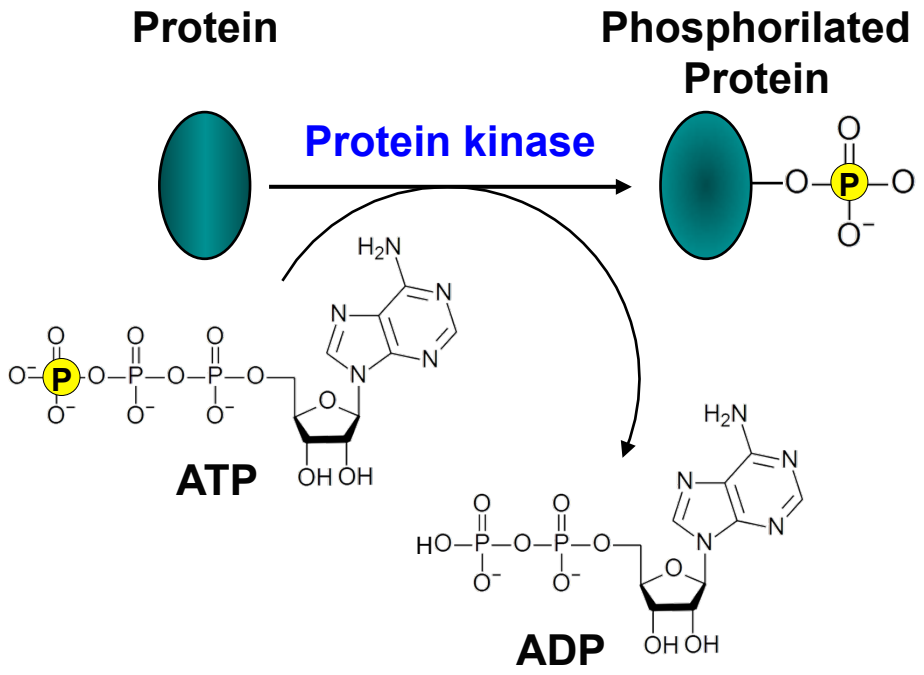
A kinase is an enzyme that modifies other proteins by adding phosphate groups (phosphorylation).

Phosphorylation usually results in a functional change of the target protein (substrate).

The activity of a kinase involves removing a phosphate group from ATP and covalently attaching it to an amino acids that have a free hydroxyl group.

Most kinases act on both serine and threonine, others act on tyrosine.

By contrast, phosphatase is an enzyme that removes a phosphate group from its substrate.



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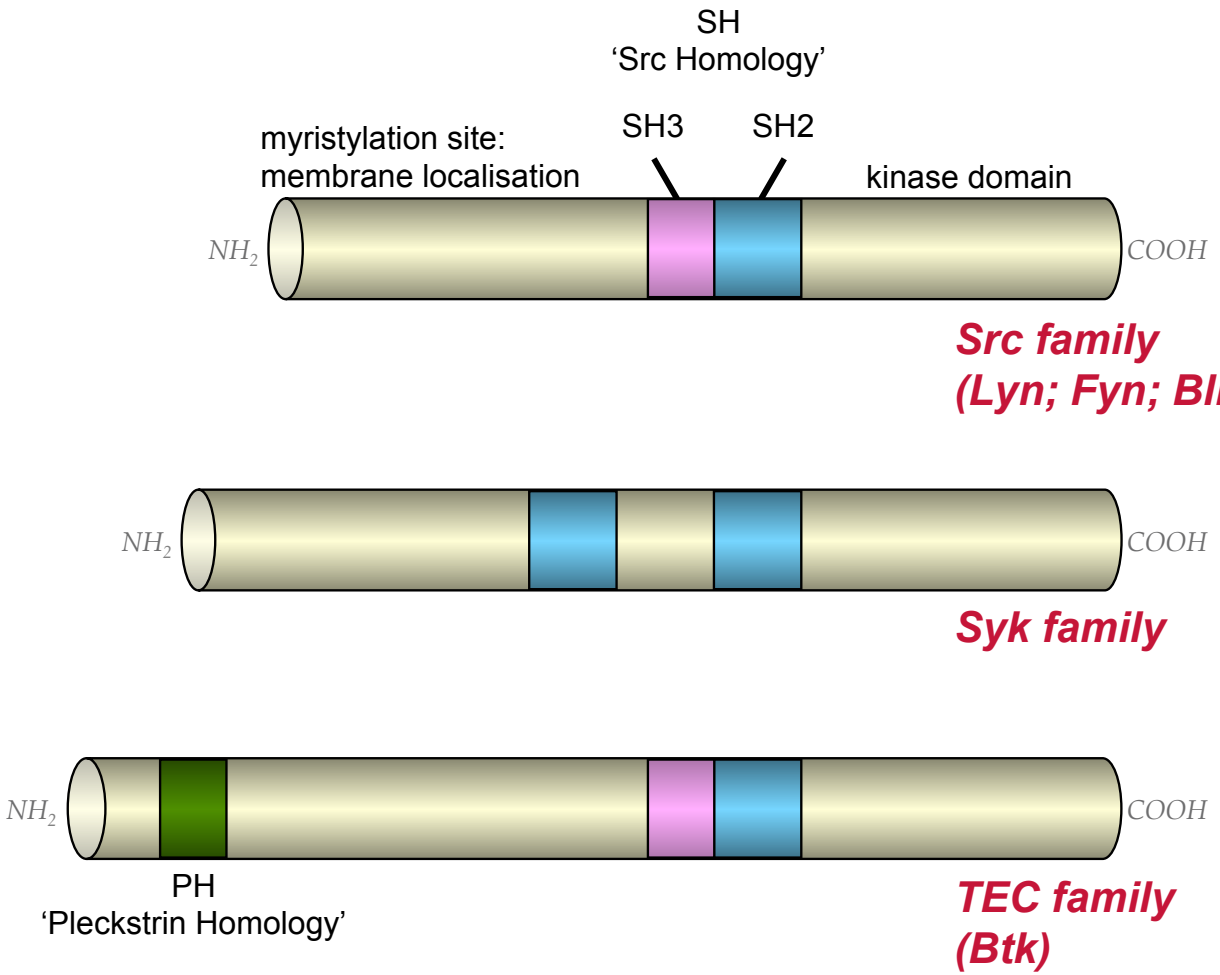
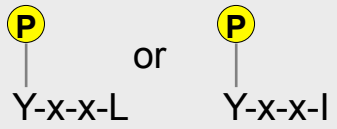
Protein Tyrosine kinases in B cell activation



SH2 domain recognises



SH3 domain recognises

- Proline rich regions

PH recognises

- phosphorilated lipids

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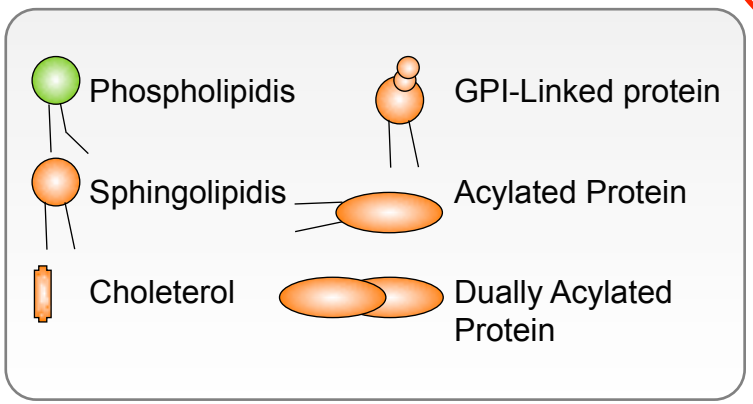
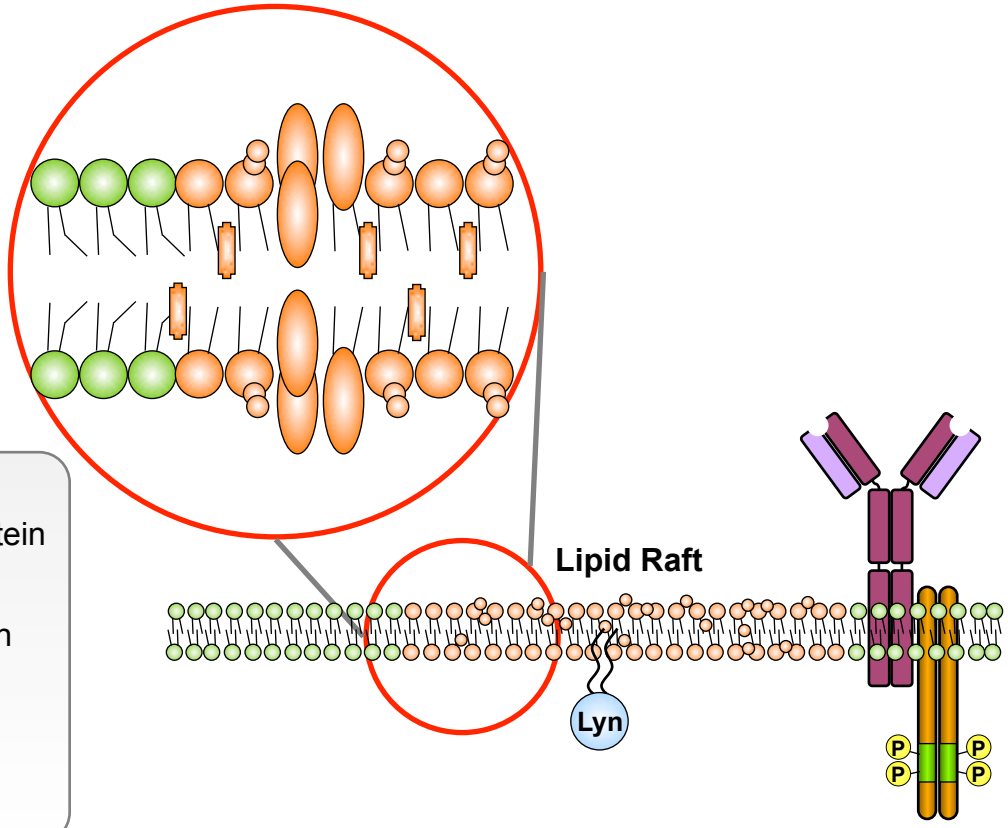
Lipid Raft: Definition & characteristics

- **Lipid rafts are shingolipid- and chleterol-rich membrane microdomains, which creates a liquid-ordered phase that is fluid, in which there is a very little lateral movement.**

- **By contrast, phospholipids are packed loosely into a liquid-disorder phase, allowing rapid movement withi the bilayer.**

- **The role of the rafts is to concentrate certain protein and exclude others.**





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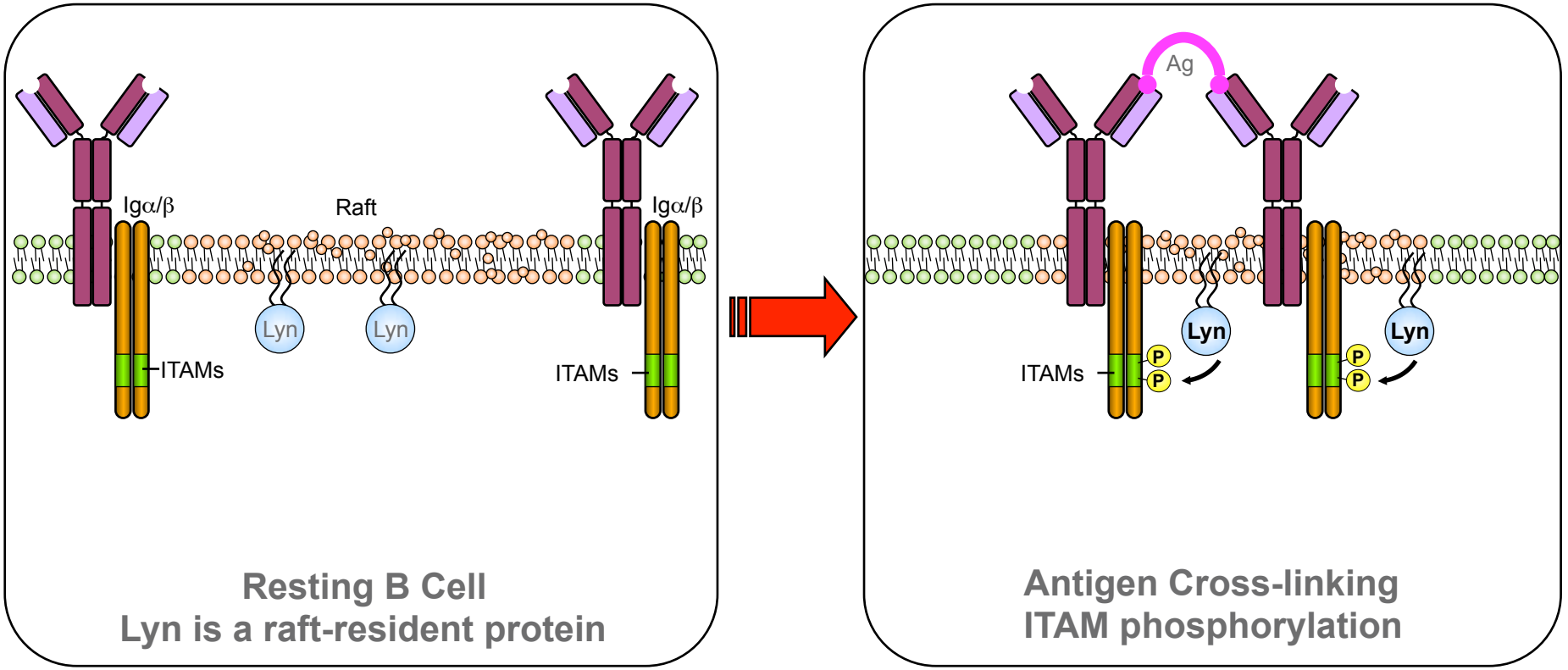
STEP 1: Antigen cross-linking and ITAMs phosphorylation



Step 1:

Antigen binding oligomerizes the BCR, increasing its affinity for the lipid rafts where come across with the Src-family kinase Lyn. BCR can associates with Lyn, which phosphorylates ITAMs within the Igα/β complex.

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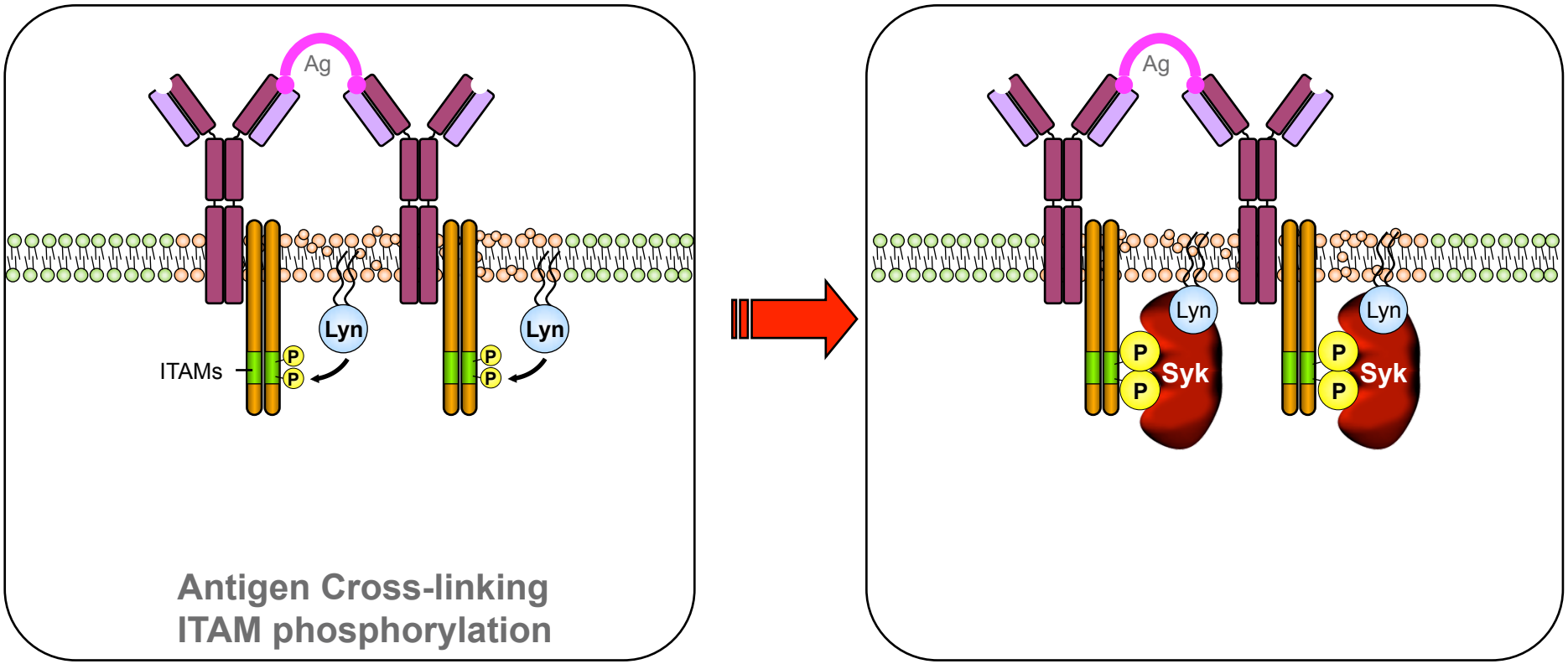
STEP 2: Syk binding to the activated ITAMs



Step 2:
Phosphorylated ITAM recruits Syk to the phosphorylation site, where it can be phosphorylated by itself or by other Src-kinases.

Note: Syk has 2 SH2 domains, therefore it shows high affinity binding to phosphorylated ITAM

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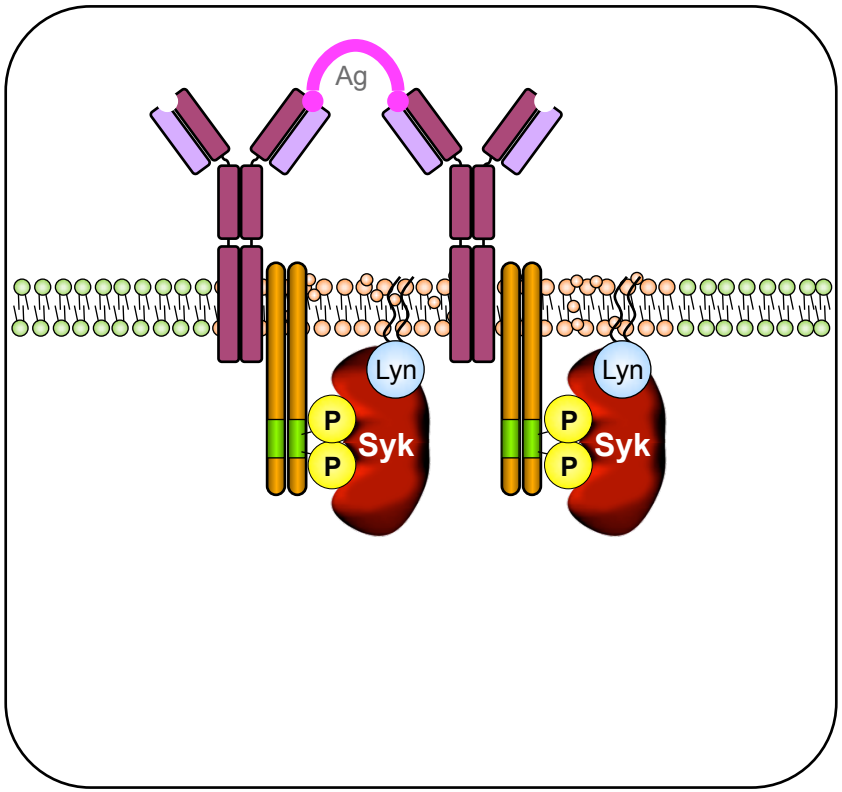
STEP 3: Activation of the ITAM-bound kinases



Step 3:
Syk undergoes conformational changes on binding to ITAM, increasing its kinase activity. Other Src-kinases also enhance their activity after binding.

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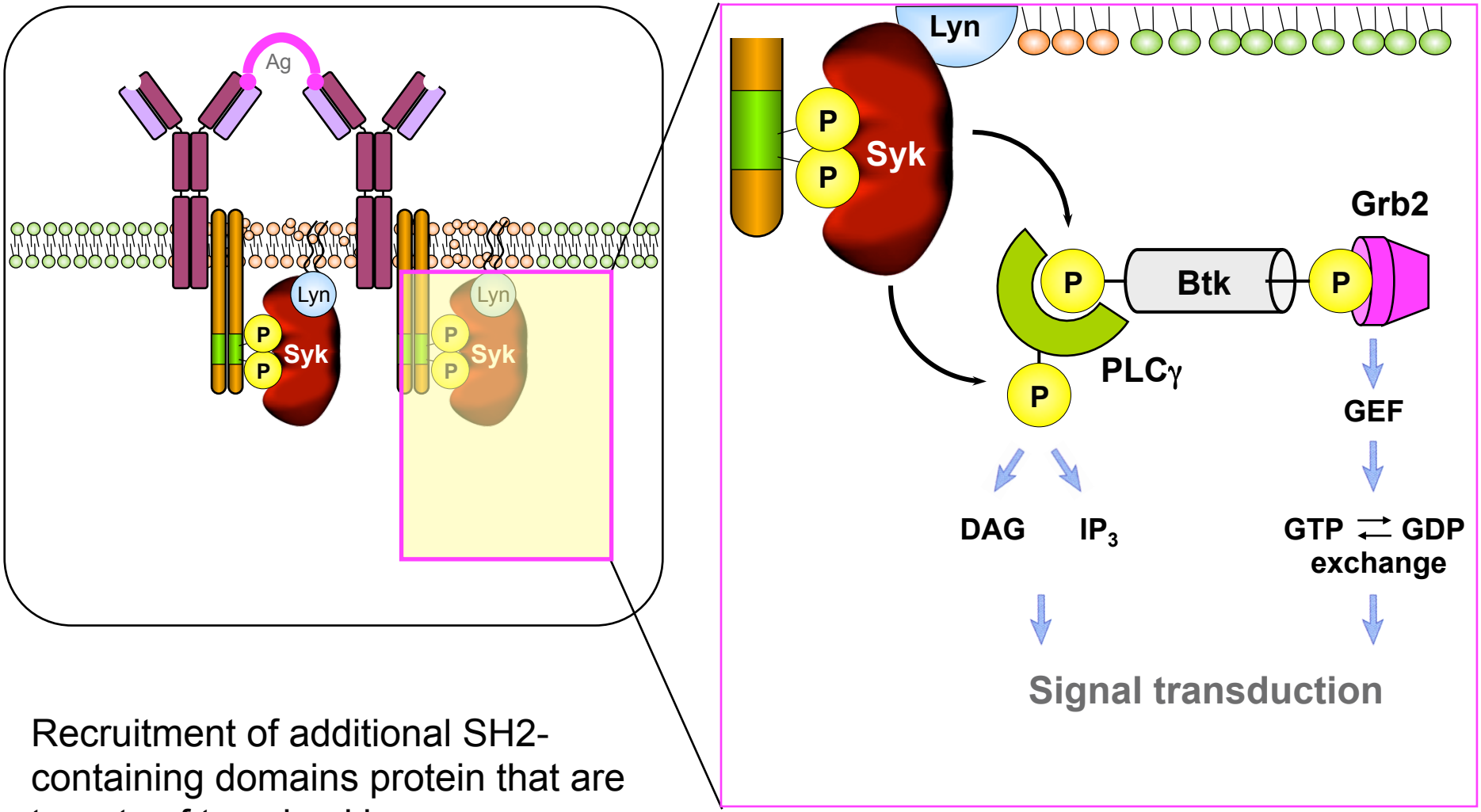
STEP 3: Activation of the ITAM-bound kinases



Recruitment of additional SH2-containing domains protein that are targets of tyrosine kinases

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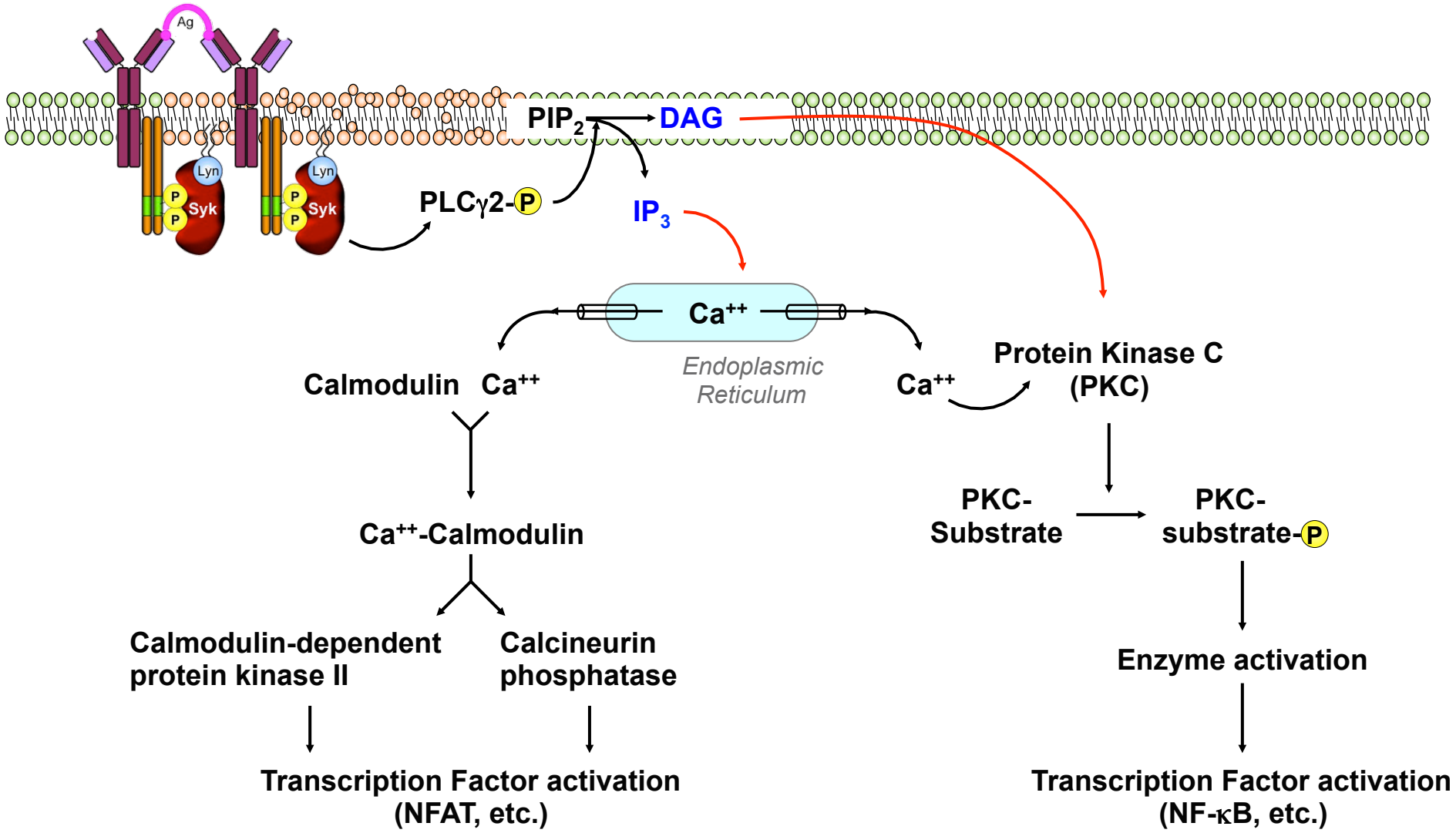
Second messengers: IP_3 and DAG



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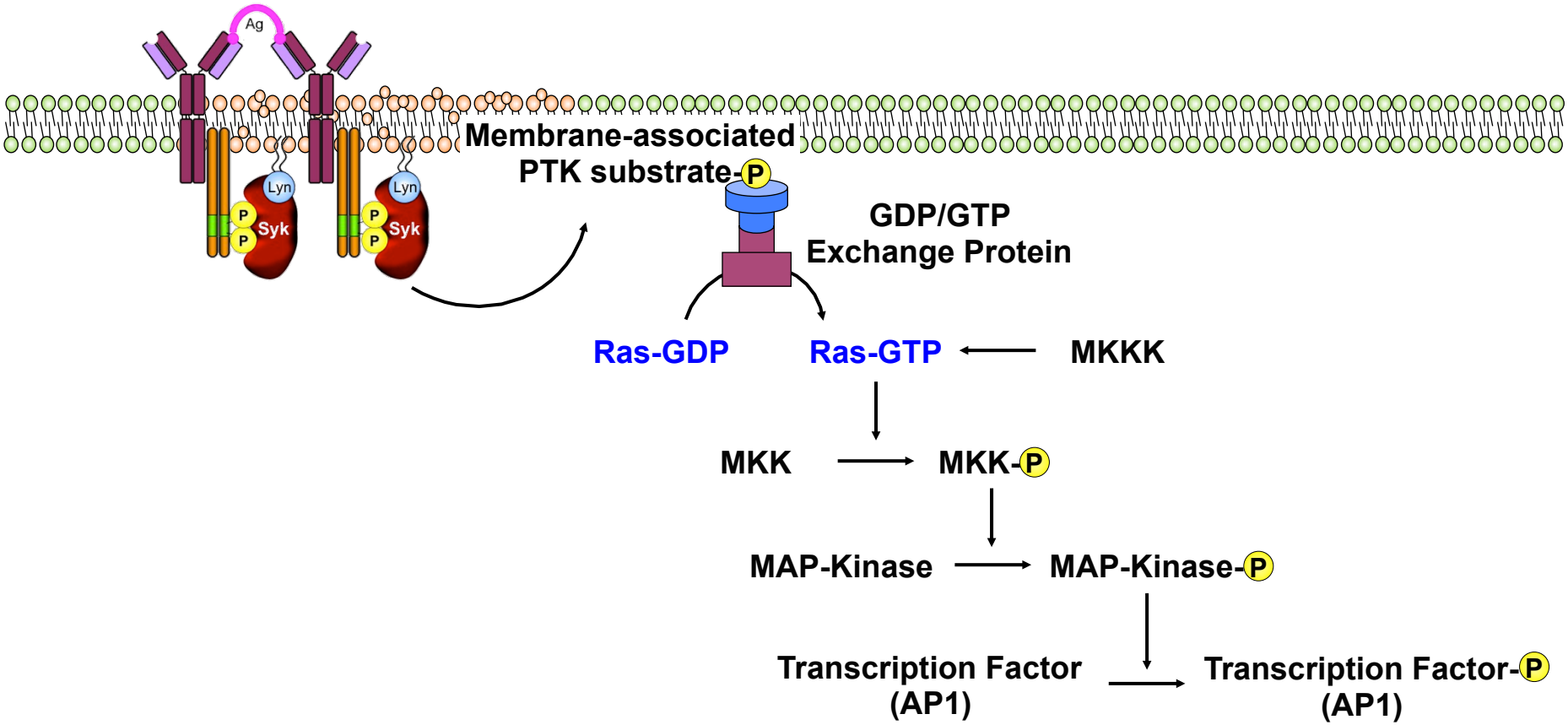
Second messengers: small G proteins



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Summary: Part I

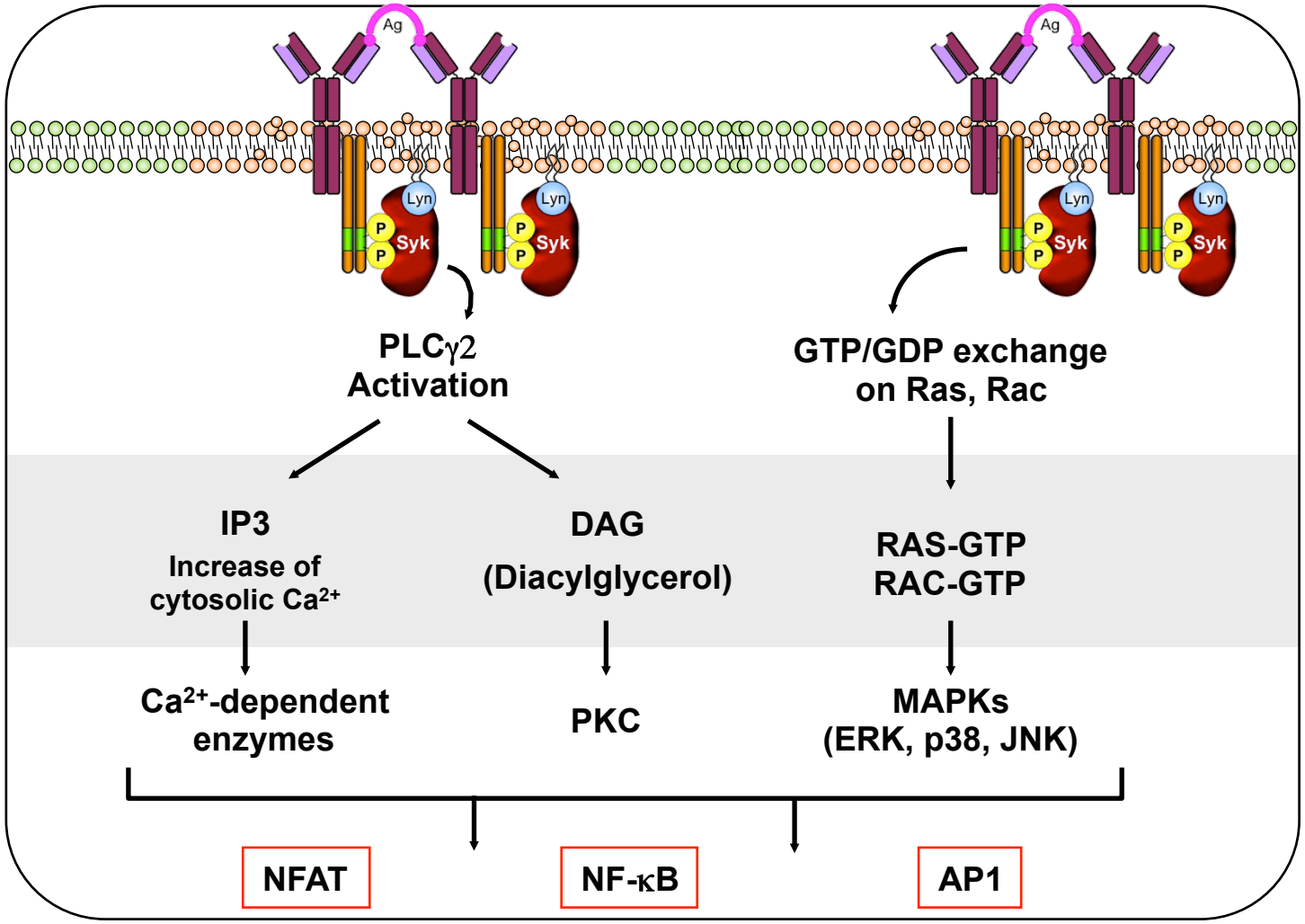
Cross-linking of membrane Ig by antigen

Tyrosine phosphorylation events

Second Messenger

Active enzymes

Transcription factors



Modified from: *Cellular and Molecular Immunology*; Authors: Abbas AK, Lichtman AH, and Pillai S (Saunders Elsevier).

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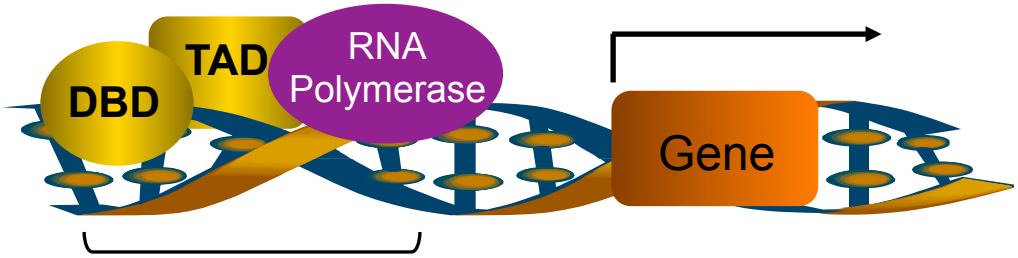
Transcription factor: Definition

Transcription factors are proteins involved in the regulation of gene expression that bind to the regulatory regions upstream of genes and either facilitate or inhibit transcription.

Transcription factors are composed of two essential functional regions: The DNA-binding domain (DBD) consists of amino acids that recognize specific DNA bases near the start of transcription.

The activator domains of transcription factors (TAD) interact with the components of the transcriptional apparatus (RNA Polymerase) and with other regulatory proteins, thereby affecting the efficiency of DNA binding



DBD = DNA-Binding Domain
TAD = Transactivation Domain

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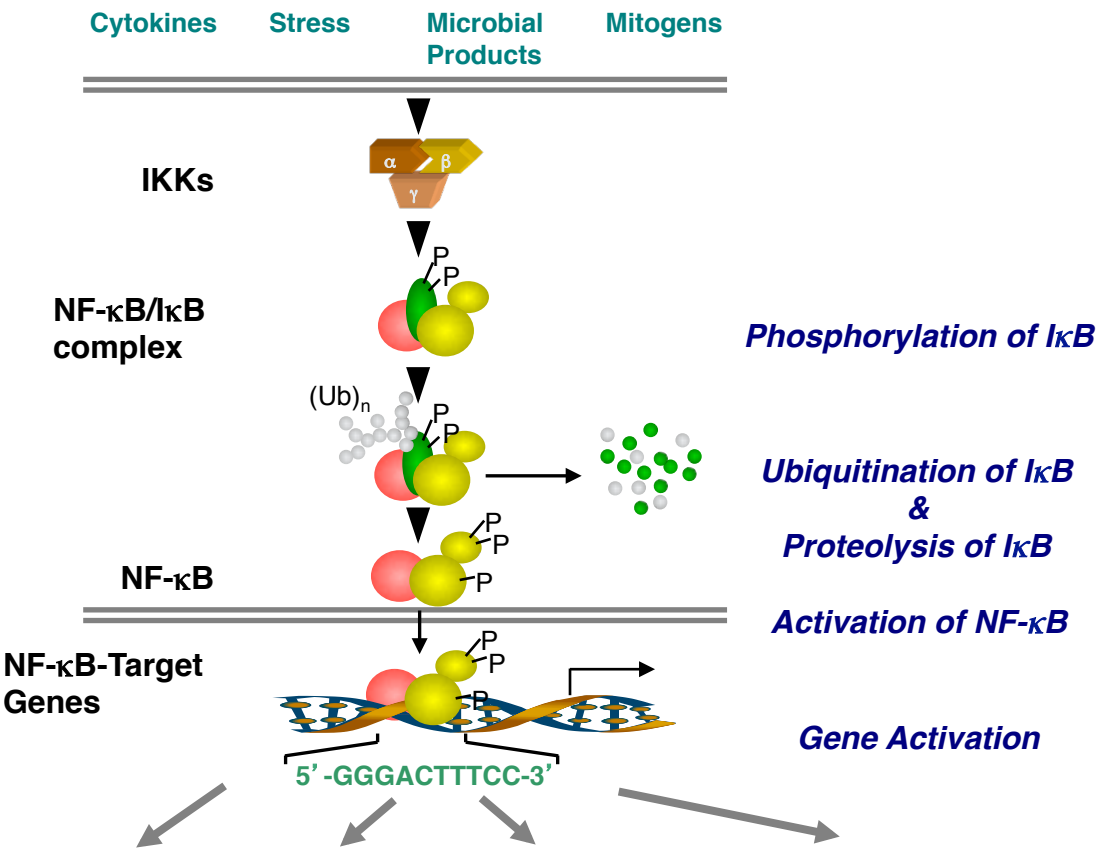
Examples of Transcription factors in B cells: Nuclear Factor-kappaB



Inflammatory Factors:
Angiotensinogen, C3 and B compl., SAA, iNOS, COX-2

Surface Molecules:
Ig-κLC, IL-2Rα, MHC-I, B7, β2-micr., VCAM-1, ICAM-1

Cytokines:
IL-1, IL-2, IL-6, IL-8, MIP-1α, GM-CSF, G-CSF, TNFα, IFNβ, LT

Pro-Survival Factors:
Gadd45b, FCH, A20, A1, Bcl-X_L, XIAP, c-IAP1/2, TRAF1/2

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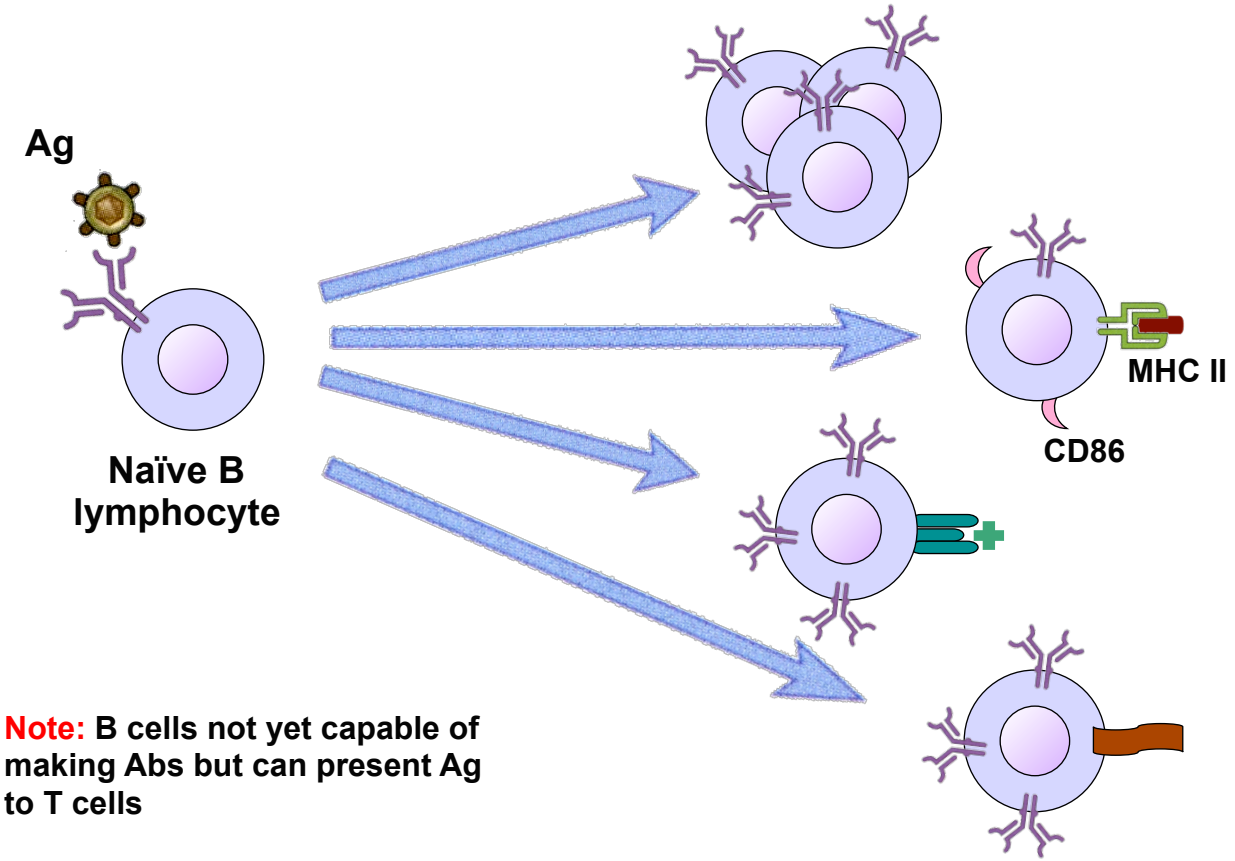
Effects of BCR signalling

Antigen binding to and cross-linking of membrane Ig	Activation of B lymphocytes	Changes in B cells phenotype and function



- Increased survival
- Proliferation

Increased expression of MHC class II and CD86

Increased expression of cytokine receptors

Increased expression of CCR7 and migration from Follicle to T cell areas

Note: B cells not yet capable of making Abs but can present Ag to T cells

Modified from: *Cellular and Molecular Immunology*; Authors: Abbas AK, Lichtman AH, and Pillai S (Saunders Elsevier).

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BCR co-receptors

Co-receptors expressed on B-cell modulate BCR signalling either positively or negatively

Positive modulators

- CD21 (CR2)/CD19/CD81 complex

Negative modulators

- FCgRIIb (CD32)
- CD22
- CD72

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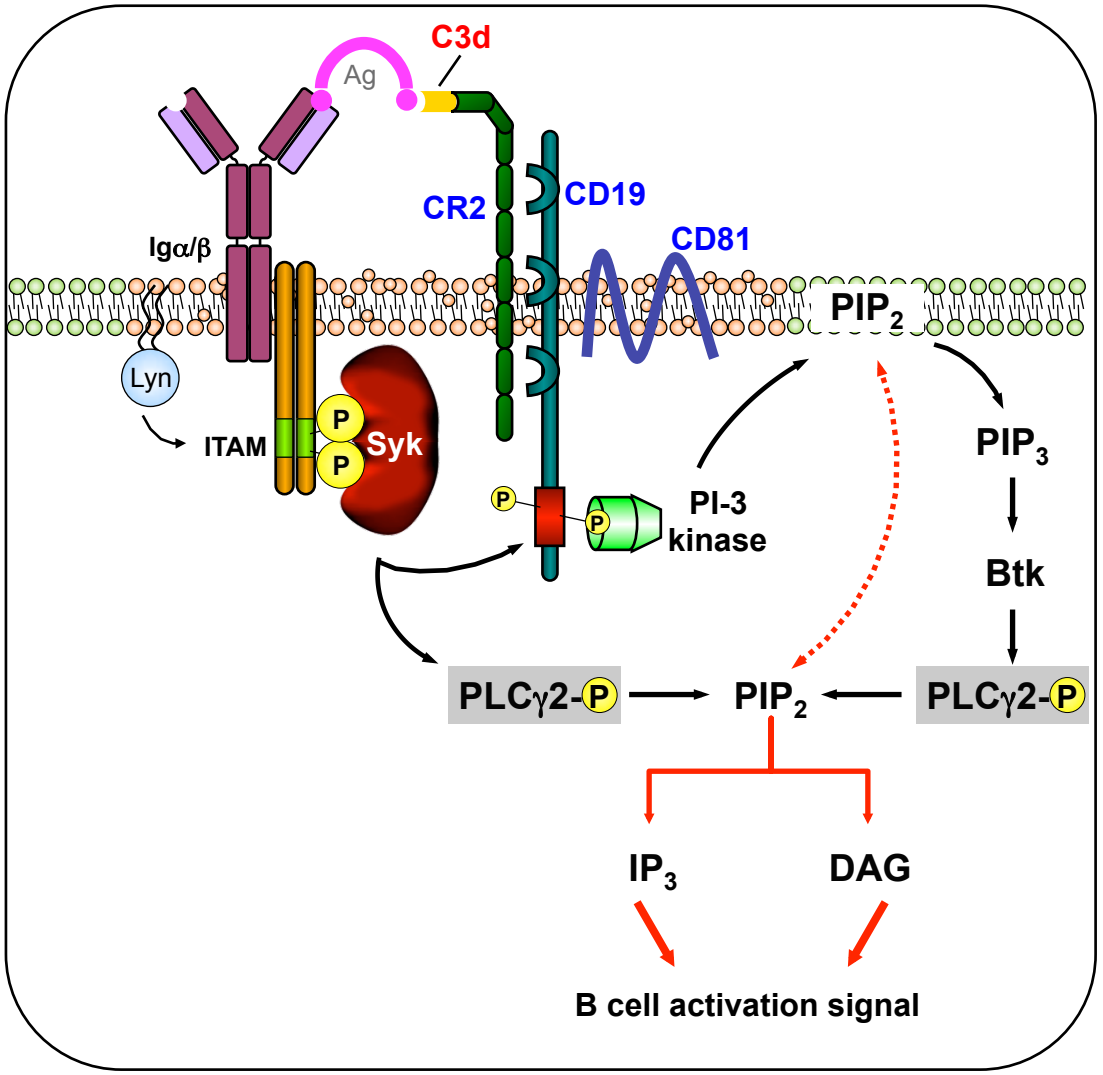
CD21 (CR2)/CD19/CD81 co-receptors complex

- **CD21 is a receptor for Cd3-tagged antigens (C3 > C3b > C3b-Ag > C3d-Ag)**
- **CD19 cytoplasmic region has 9 tyrosines, which are phosphorylated when cross-linked with BCR**
- **CD81 is required for localization of CD19/ CD21 into lipid rafts**

Note: *CD21- and CD-19-deficient mice have 10-100x reduction in intensity of Ab response to T-dependent Ag*



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FcγRIIB (CD32) co-receptor

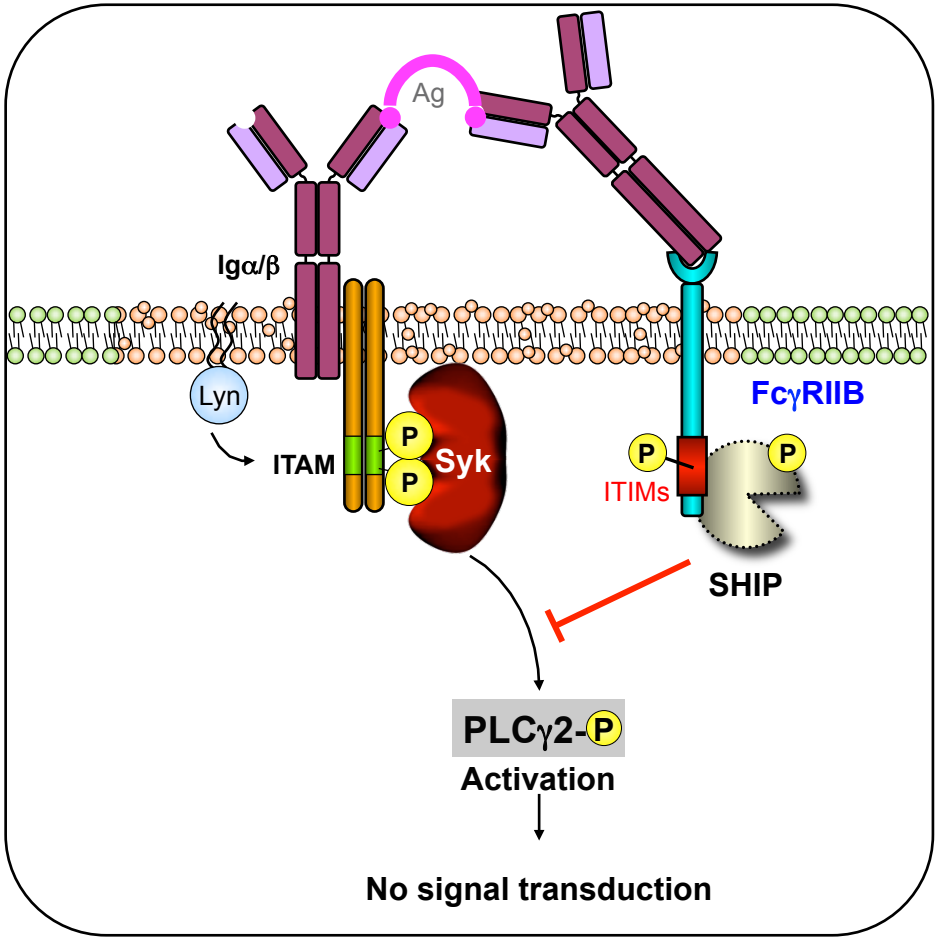
- **FcγRIIB is a transmembrane protein that contains Immunorecptor Tyrosine-based Inhibitory Motifs (ITIMs)**

- **ITIMs are targets of Src-family PTKs and recruit protein tyrosine phoshatases (such as SHP-1 and SHP-2) or inositol-phosphatases (such as SHIP).**

  - SHIP inhibits the activation of PLCγ > DAG > IP3 > Ca^{2+}

- **FcRγIIB signalling is crosslinked by immune comlexes and blocks further Ab production.**

- **FcγRIIB signalling promotes apoptosis**



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Other important B cell co-receptors

CD22: role still not certain

Immunology 123: 314-25 (2008).

CD72: inhibitor of BCR signals like FcRII

Trends in Immunology 25: 543-550 (2004)

TACI, BCMA and BAFF-R: all are receptors for B-cell activation factor (BAFF) - important survival and differentiation signals

Cytokine Growth Factor Rev. 19: 263-276 (2008).

CD27 - marker of memory B cells

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B cell activation

Requires 2 signals:

1. Signal 1 occurs through the B-cell receptor

- Ag engagement of the BCR initiates signal cascades that result in the transcription of variety of genes associated with B-cell activation

2. Signal 2 is derived from cell-cell interactions, cytokine stimulation (T-dependent) or from Ag directly

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B cells can be activated by two different routes

- **Thymus-independent activation which requires only antigen cells**

  - Antigens that activate in this way are named thymus-independent antigens

- **Thymus-dependent activation which requires antigen and direct interaction with Helper T cells**

  - Antigens that activate in this way are named thymus-dependent antigens

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Thymus-independent activation

- **Type 1 antigens are bacterial cell wall components (e.g., LPS) that acts as mitogens**
  - Non-specific activators
  - Type 1 antigens results in polyclonal activation
  - Activate both immature and mature B cells

- **Type 2 antigens are polymeric protein antigens with higly repetitive sequences, and can cause extensive cross-linking of membrane IgM**
  - interaction is antibody specific and consequently does not result in polyclonal activation
  - Activate only mature B cells

Note: *Humoral immune responses are characterized by IgM production (no class switching), generally lower Ab levels and failure to form memory cells.*

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Thymus-dependent activation

- Requires cooperation between T_H and B cells
- T_H cells stimulate B cells
  - Clonal expansion
  - Isotype switching
  - Affinity maturation
  - Differentiation

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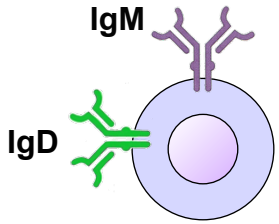
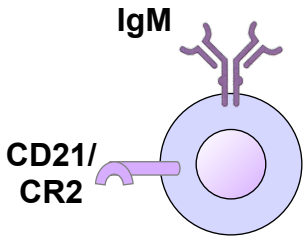
Peripheral B cell subsets



Follicular B cell

Follicular zone B cells:

- Preferentially produced after birth
- Replaced from bone marrow
- Respond to protein-antigens, requiring T cell help
- More than 95% of naïve B cells found in perypheral lymph nodes
- Typically are (m)IgM$^+$ and (m)IgD$^+$
- Re-circulating B cells



Marginal zone B cell

Marginal zone B cells:

- Occupy space between red and white pulp of the spleen
- Express high levels CD21 of complement receptor
- Respond quickly to antigens (e.g. LPS), mediating T cell-independent immune response (differentiate into plasma cells without T cell help)

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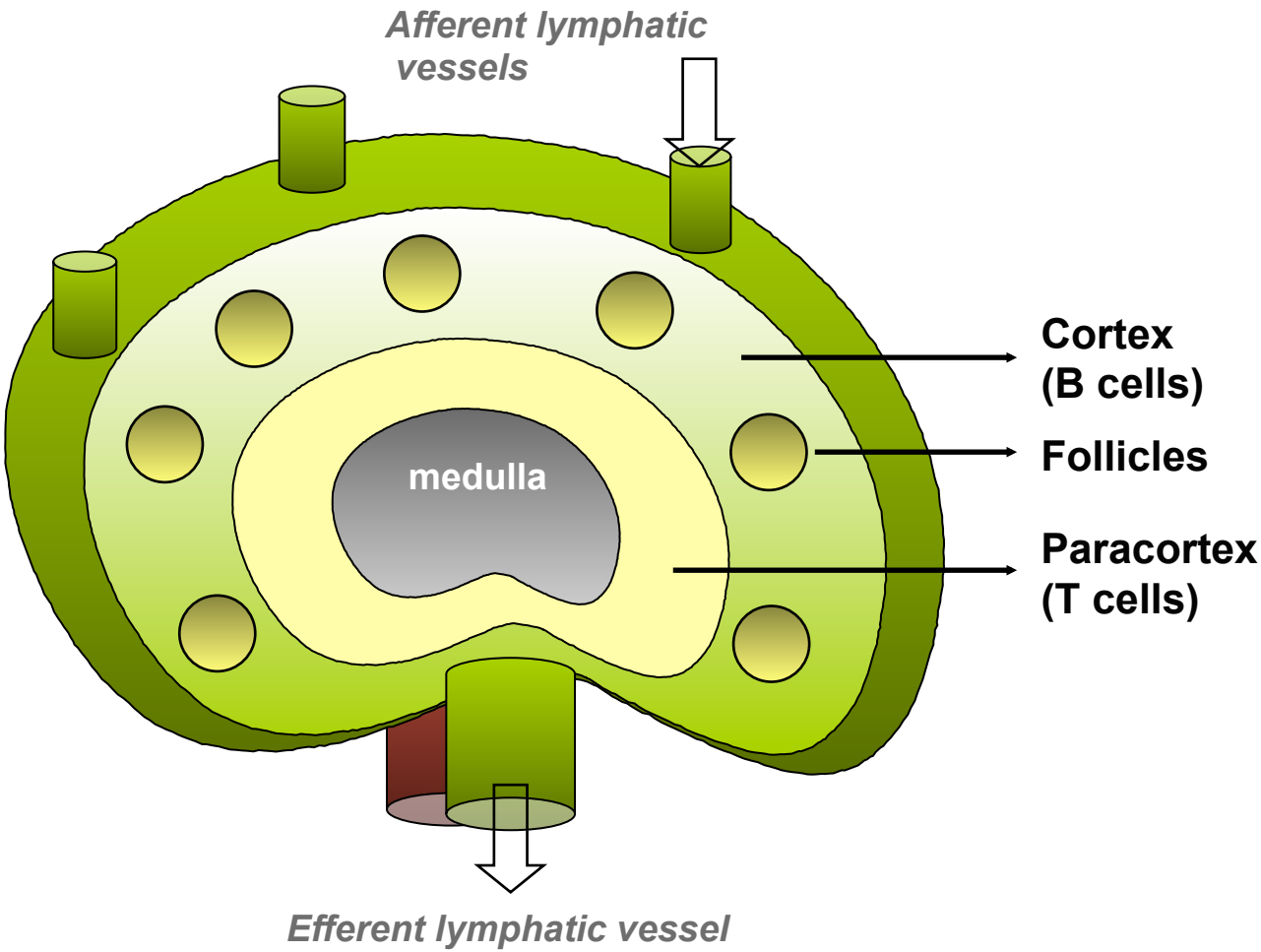
Schematic view of a lymph node

The Lymph node is made up of three components

- Lymphatic sinuses
- Blood vessels (red)
- parenchyma (cortex, paracortex, medulla)



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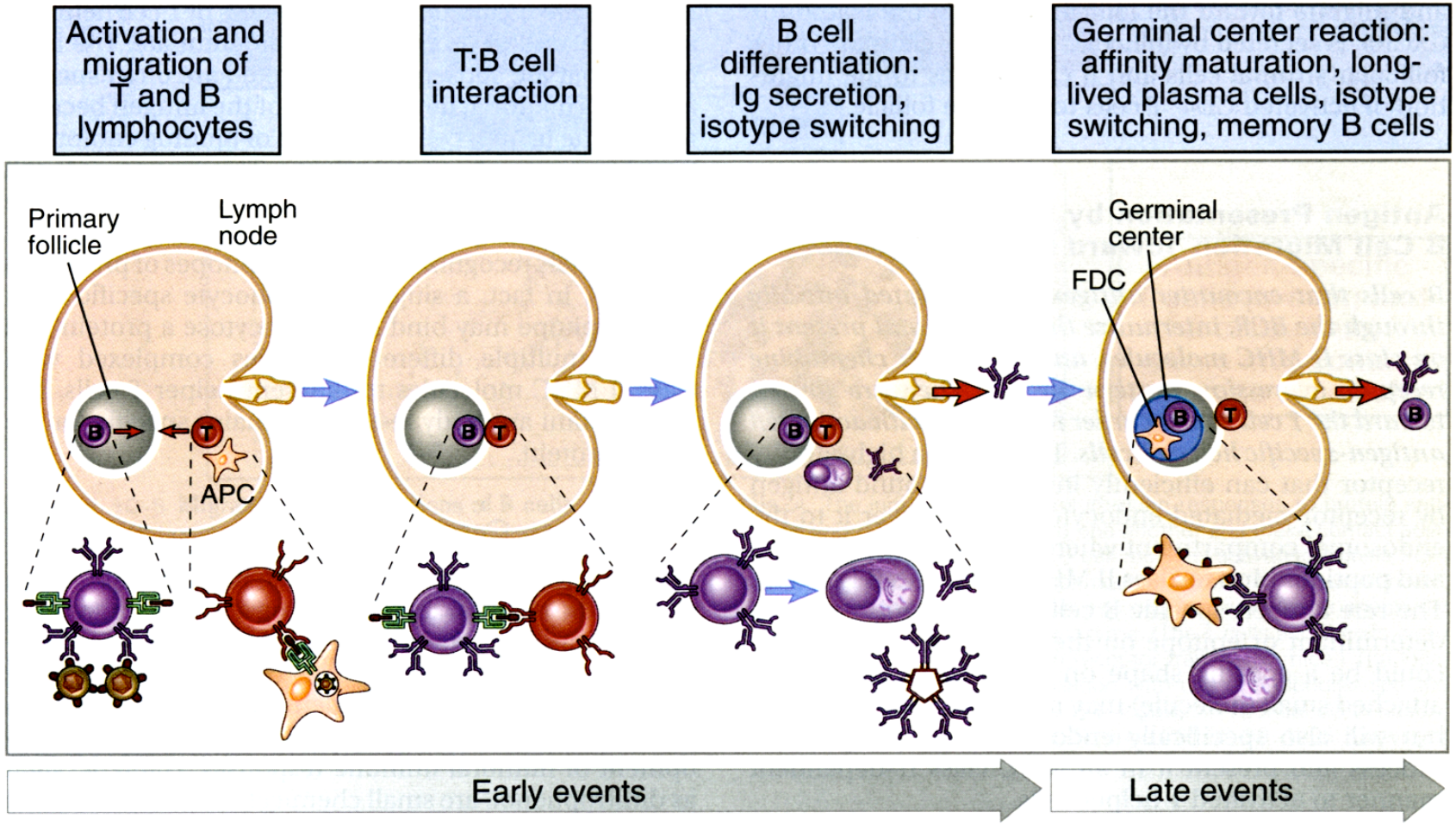
Events in humoral immune response to T cell-dependent antigens



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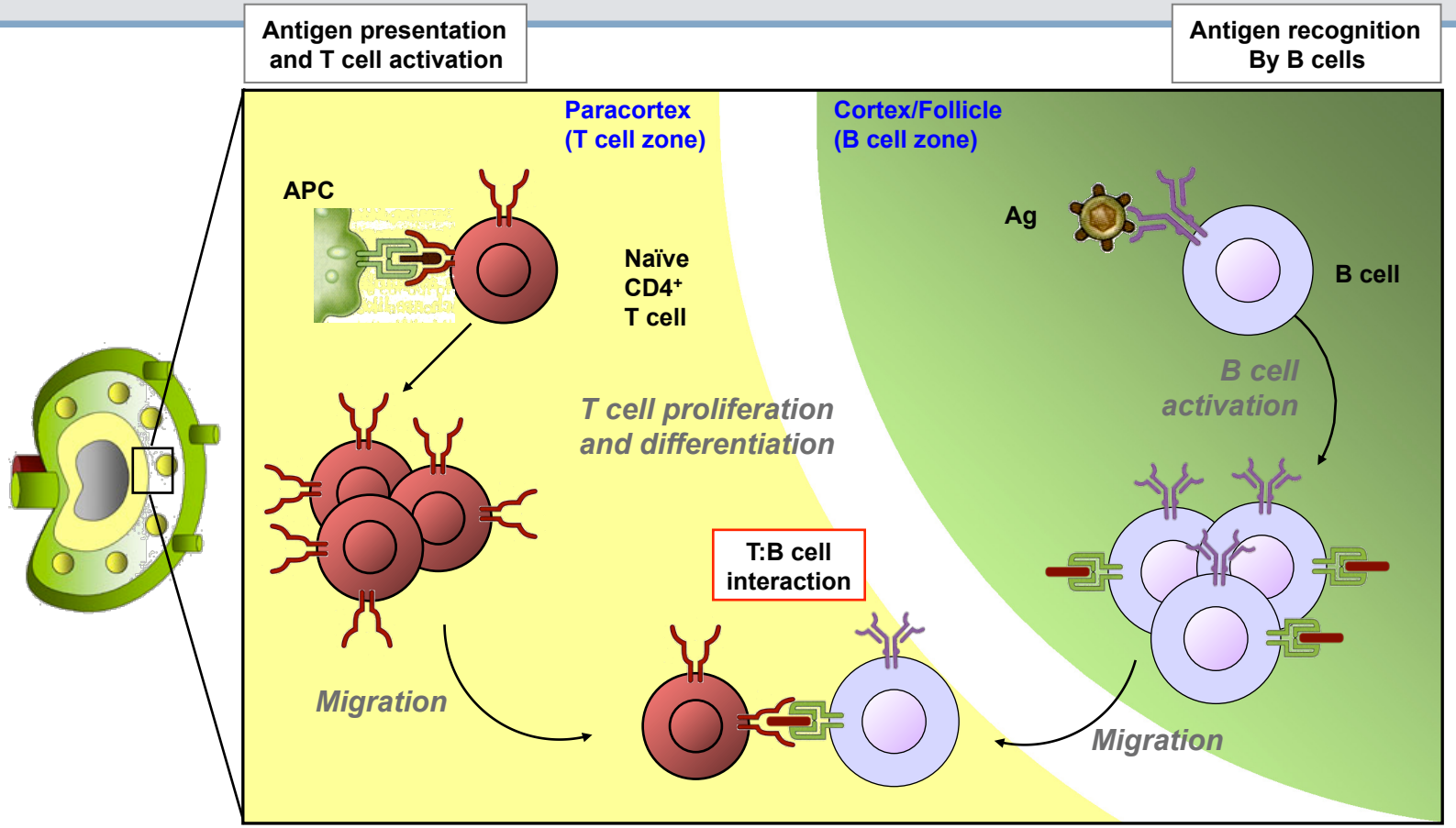
Sequence of events in B cell activation by T-dependent antigen



Modified from: *Cellular and Molecular Immunology*; Authors: Abbas AK, Lichtman AH, and Pillai S (Saunders Elsevier).

- Antigen is taken up by DC and presents to T_H cells
- TH cells are activated and move towards follicle

- B cells are activated by soluble Antigen
  - Antigen uptake/delivery to MHC class II
  - Antigen presentation is enhanced by BCR signalling
- B cells migrate toward T cell zone

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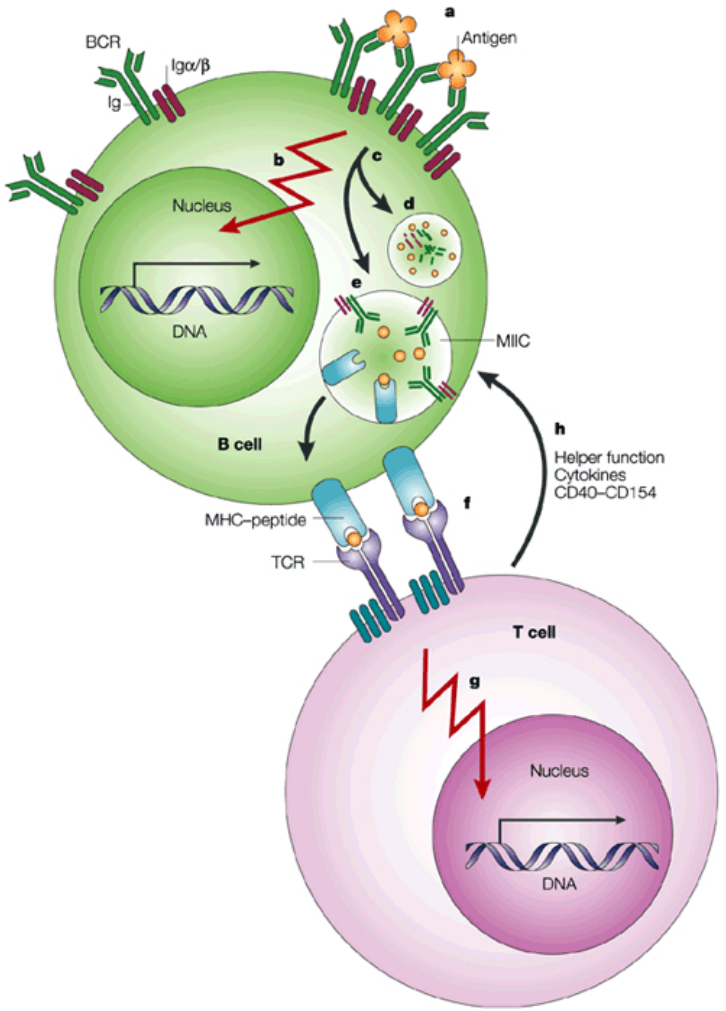
Antigen uptake and presentation



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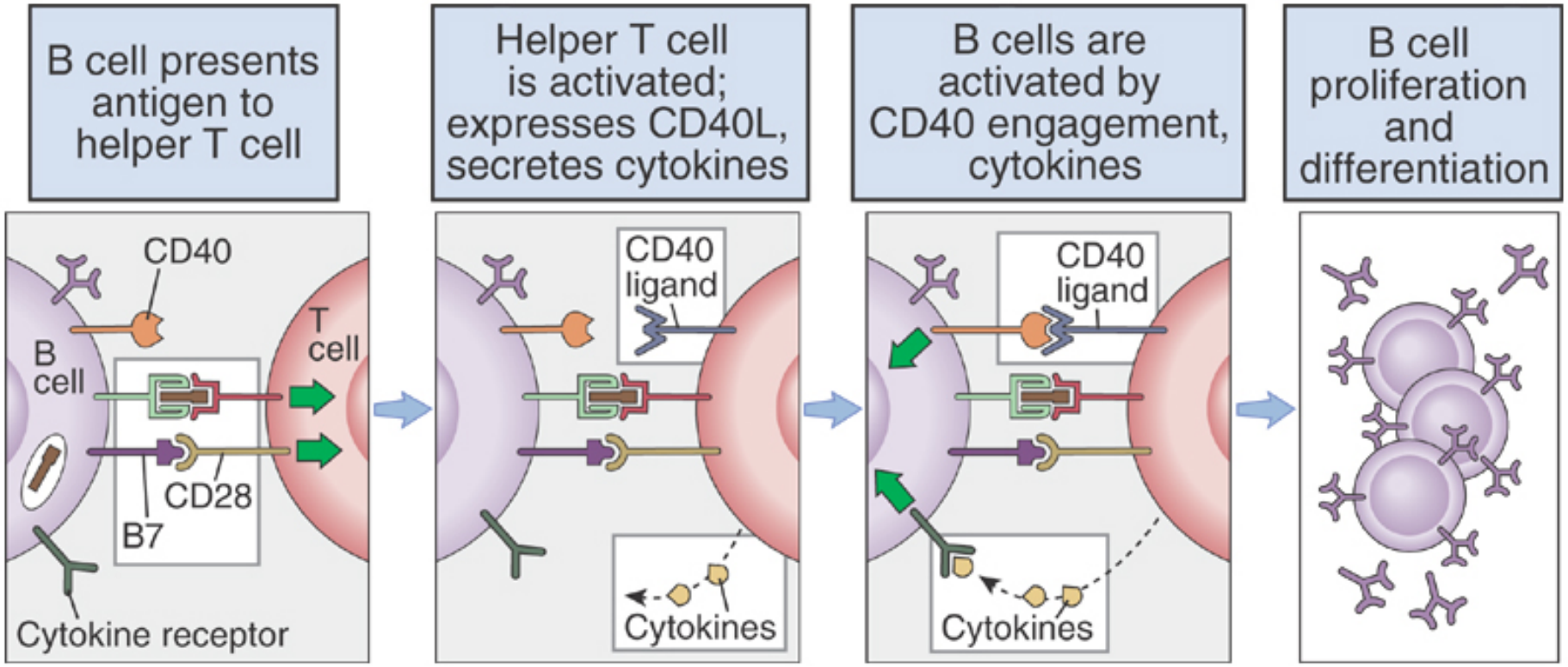
T:B cell interaction



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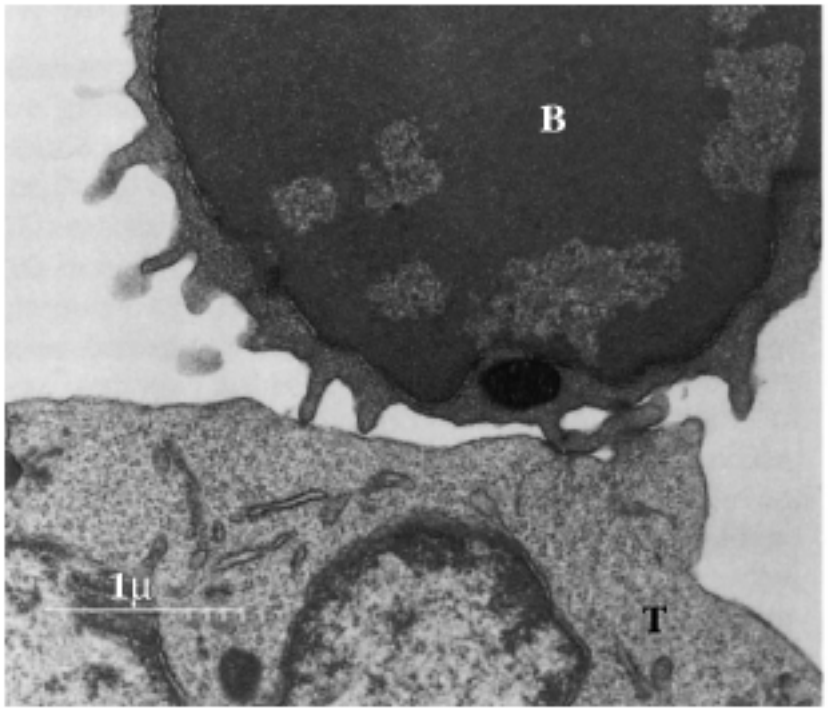
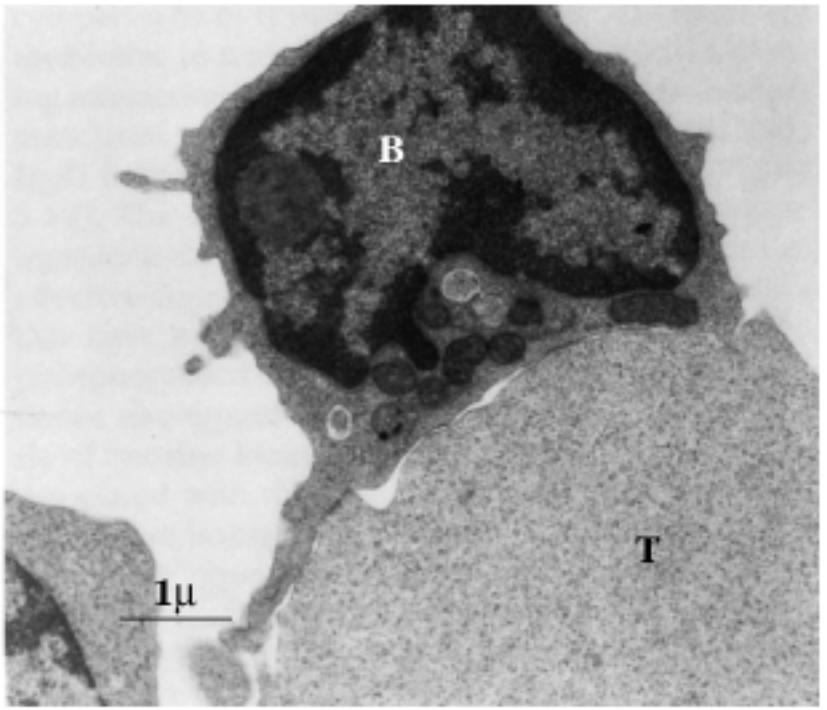
T:B cell interaction





http://www.vetmed.wisc.edu/data/coursematerial/suresh/Lecture15.ppt.

• **Both membrane contact and cytokine signals are necessary to induce B-cell proliferation and differentiation**

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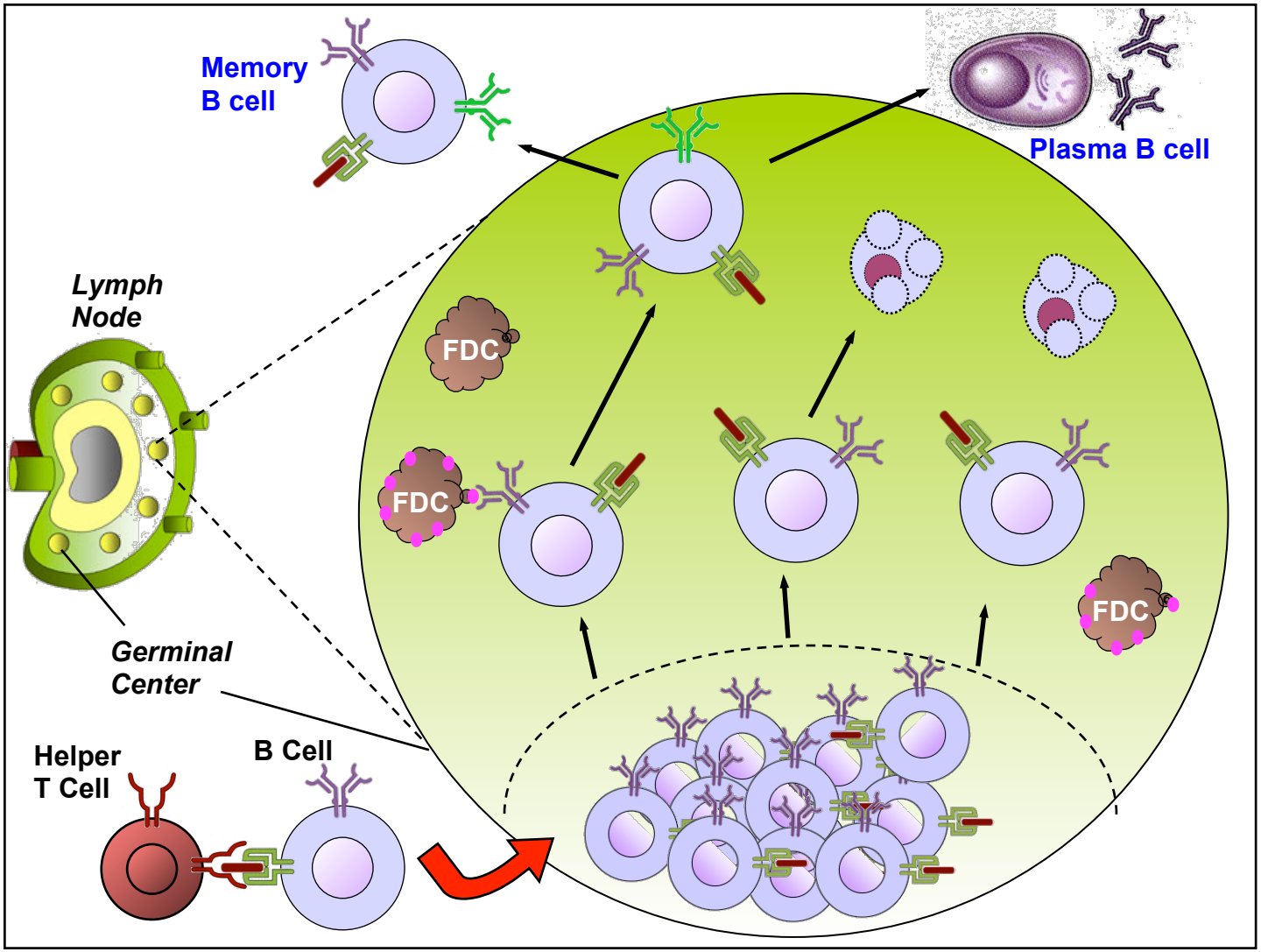
Germinal Center reaction



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

↑

Death of B cell that do not bind Ag

↑

B cell recognition of Ag on follicular dendritic cells; selection of high-affinity B cells

↑

Hypermutation of Ig V genes

↑

B cell proliferation

↑

Activation of B cells and migration into germinal center

Modified from: *Cellular and Molecular Immunology*; Authors: Abbas AK, Lichtman AH, and Pillai S (Saunders Elsevier).

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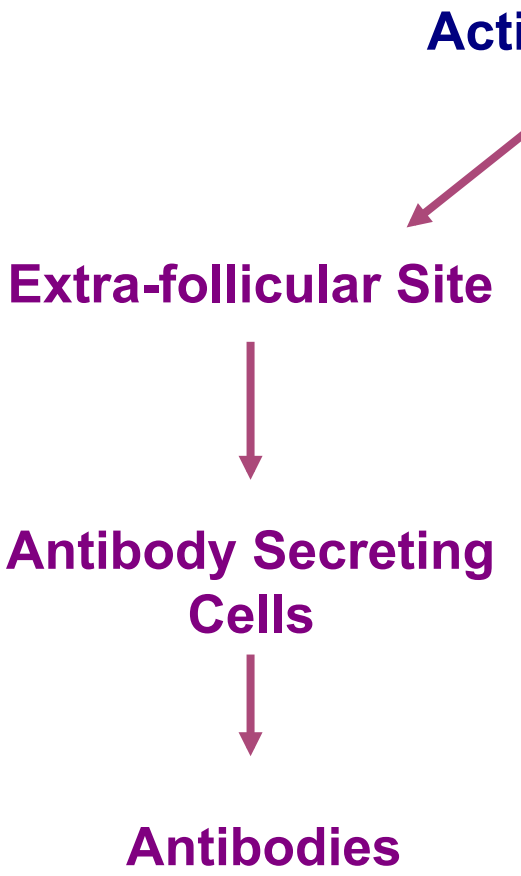
Summary: Part II



Follicle

Germinal Center

Affinity Maturation - Somatic Hypermutation

Generation of Memory B Cells

Note: Germinal center reaction is dependent on CD40/CD40L interaction

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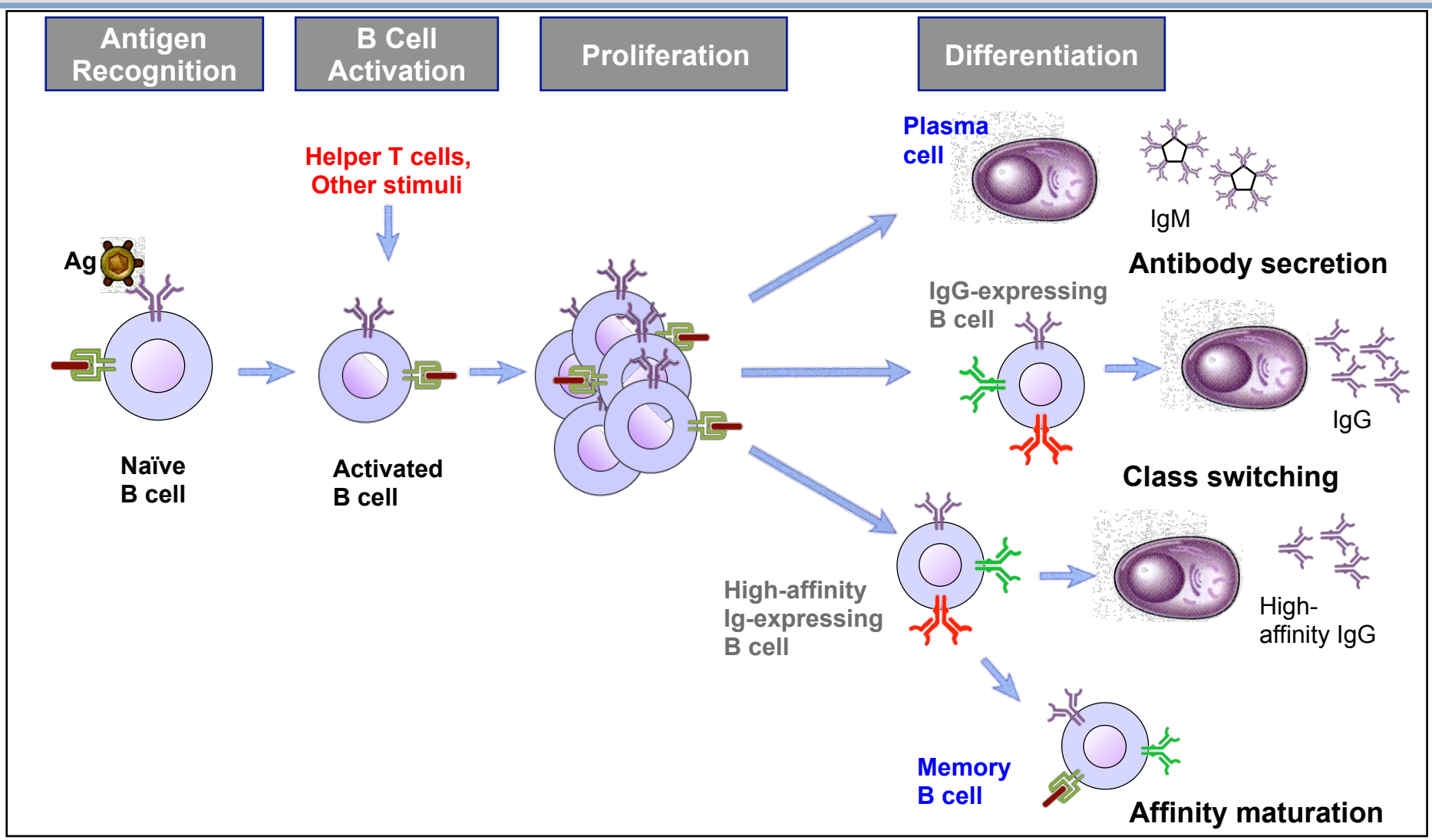
Conclusions:



Modified from: *Cellular and Molecular Immunology*; Authors: Abbas AK, Lichtman AH, and Pillai S (Saunders Elsevier).

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Learning objectives

To be able to:

- **Describe the key stages of the signalling through the BCR from the antigen recognition to the early response of B cell**:
  - *Define the B cell receptor*
  - *Identify the key events at each phosphorylation step*
  - *List the transcription factors and the second messenger pathways involved in the signal transduction*
  - *Outline the changes in B cell phenotype and function*
- **Give at least one example of B cell co-receptor and describe how this modulate the BCR signalling**
- **Describe the sequence of events in T-dependent B cell activation and relate them to the microanatomy within the peripheral lymphoid organs**

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References

Reviews:

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Nitschke L., **The role of CD22 and other inhbitory co-receptors in B cell activation.** *Curr Opin Immunol* **17:** 290-297 (2005).

Rickert R., **Regulation of B lymphocyte activation by complement C3 and the B cell coreceptor complex.** *Curr Opin Immunol* **17:** 237-243 (2005).

Cozine CL *et al.,* **The primary germinal center response in mice.** *Curr Opin Immunol* **17:** 298-302 (2005).

Harwood NE, Batista FD. **Early events in B cell activation.** Annu Rev Immunol. 28:185-210 (2010).

Books:

Basic immunology - Abbas and Lichtman 2006-2007 Elsevier
Immunobiology 6th Edition - Janeway et al., Garland Publishing