Chapter 7

Signaling through immune system receptors
免疫系統受器的訊息傳遞

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Learning objectives

- General principles of transmembrane signaling
- Antigen receptor structure and signaling pathways
- Other signaling pathways related to lymphocyte behavior

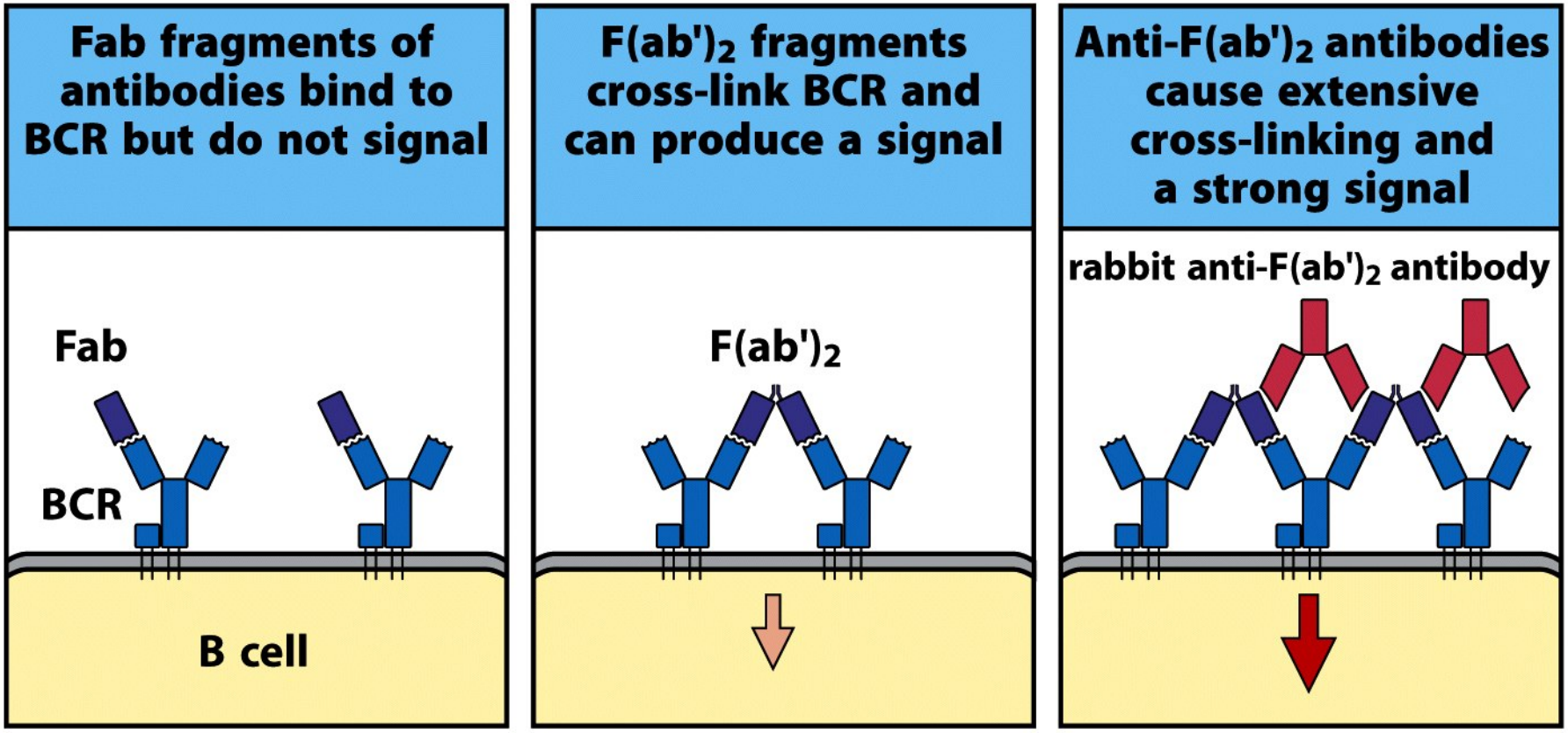
How do cells respond to external stimuli?

- Recognition phase
  - Ligand-receptor interaction
    - Ligand: antigen, pathogen, …etc.
    - Receptor: membrane-bound proteins
- Transmission phase
  - ‘signaling transduction’
    - Relays, sustains, and amplifies the signal onward
    - Lots of proteins involved
- Response phase
  - changes of cytoskeleton
  - activation of secretory apparatus
  - activation of effector cells (e.g. cytotoxic T cells)

Binding of Ag-receptors (early events)

- Conformational change of receptors
- Clustering of receptors
  - via cross-linking antigens
    - Anti-receptor Ab (at least bivalent is required)
    - Ag (e.g. bacteria) with repetitive epitopes
  - Degree of cross-linking determines the intensity of the signal transmitted
    - Proven true for B cells (Fig. 6.11)
    - Less certain for T cells (3 models) (Fig. 6.12 & 6.13)

Degree of cross-linking on receptor determines the signal intensity



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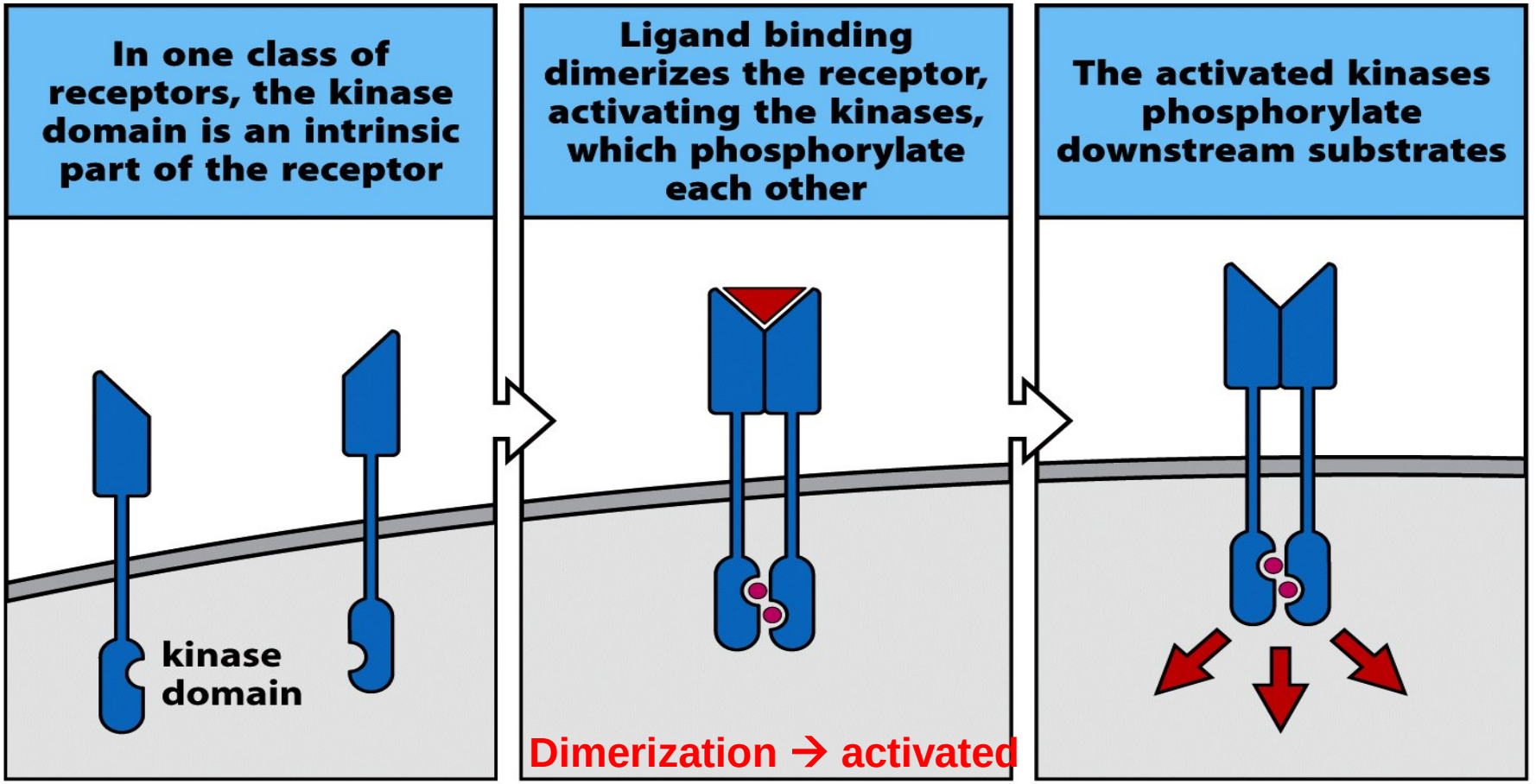
Signal intensity

None Weak Strong

Binding of Ag-receptors (late events)

- Activation of intracellular signal molecules
  - Protein tyrosine kinase (TK)
    - Phosphorylates tyrosine residue
  - Two types of TK
    - intrinsic TK (Fig 6.1)
      - Receptor itself possesses the TK activity
      - e.g. Kit receptor (CD117); although Kit is a non-Ag receptor
    - receptor-associated TK (non-receptor TK)
      - Activated receptor provides a docking site for other molecules that possess TK activity; e.g. receptor for TGF-β
- Final destination of the signal is the nucleus

(Fig. 7.1) Tyrosine kinase class I
(intrinsic)



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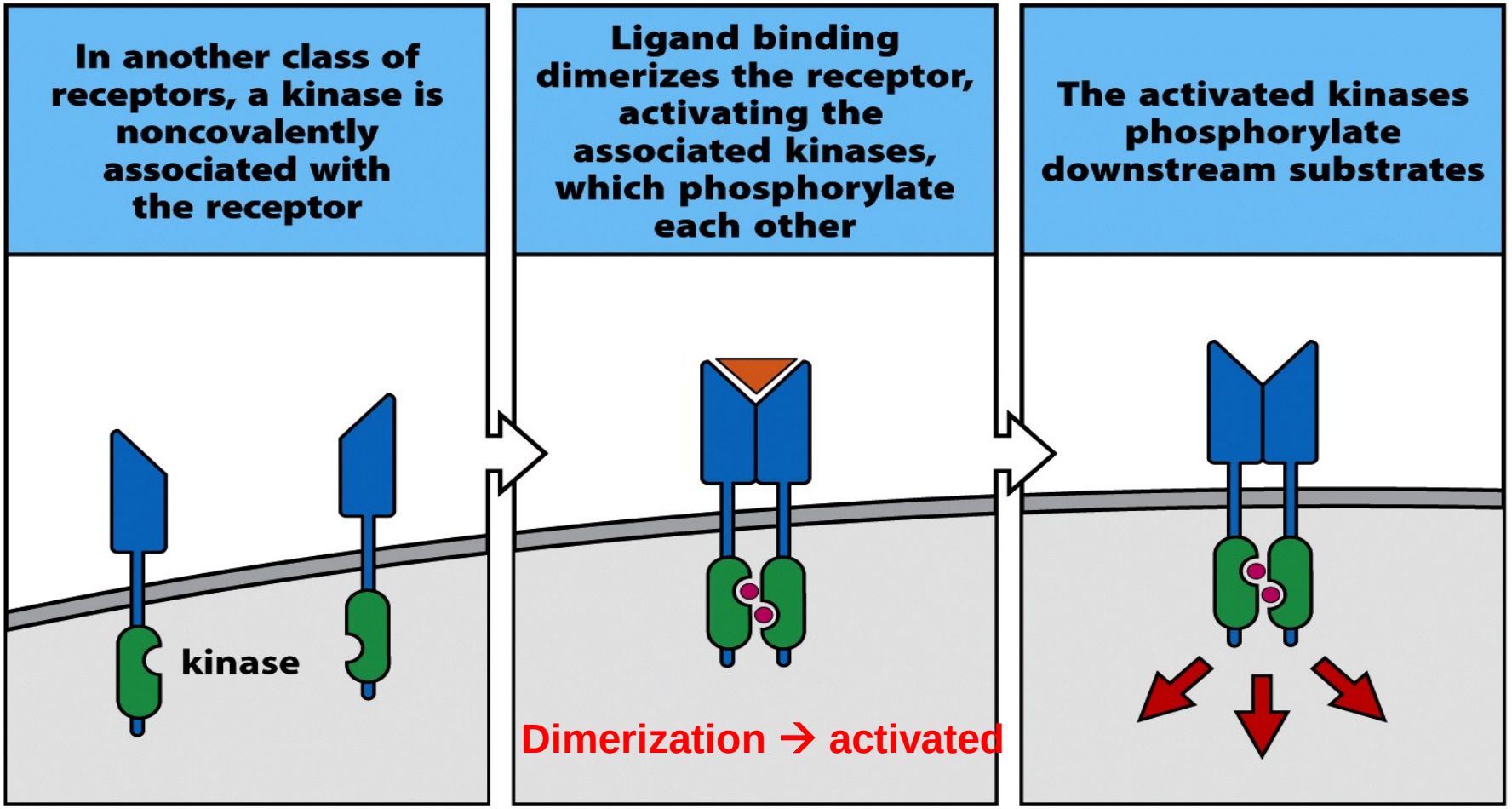
Tyrosine kinase class II
(receptor-associated)



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Phosphorylation vs. Dephosphorylation

- Adding vs removing phosphate group onto/from a protein
  - Amino acids:
    - Tyr, Ser/Thr → involved in immune signaling
    - His → not involved in immune signaling
  - Via the action of
    - Kinase or Phosphorylase (addition)
    - Phosphatase (removal)
- Proteins then become either activated or inactivated (or vice versa)

Raf (a MAPKKK): Ser/Thr kinase
Mek (a MAPKK): dual Tyr/Thr kinase

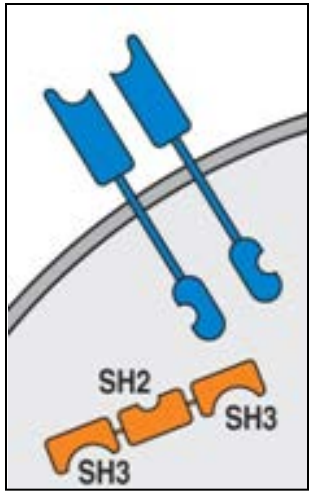
Kinase vs Phosphatase

Crucial outcomes of phosphrylation

- Activation of proteins
- Creation of binding sites on activated proteins
  - Recruitment of other non membrane-anchored signaling molecules involved in the cascade

Molecules in signaling pathways often contain the following structural domains:

1. SH2 → binds to Tyr-p
2. SH3 → bind to Proline-rich domain



(Fig. 7.2) Signaling proteins interact via their modular protein domains

(Signaling molecules) (scaffold or adaptor molecules)

Protein domain	Found in	Ligand class	Example of ligand
SH2	Lck, ZAP-70, Fyn, Src, Grb2, PLC-γ, STAT, Cbl, Btk, Itk, SHIP, Vav, SAP, PI3K	phosphotyrosine	pYXXZ
SH3	Lck, Fyn, Src, Grb2, Btk, Itk, Tec, Fyb, Nck, GADS	proline	PXXP
PH	Tec, PLC-γ, Akt, Btk, Itk, SOS	phosphoinositides	PIP_3
PX	$P40^{phox}$, $P47^{phox}$, PLD	phosphoinositides	PIP_2
PDZ	CARMA1	C termini of proteins	IESDV, VETDV

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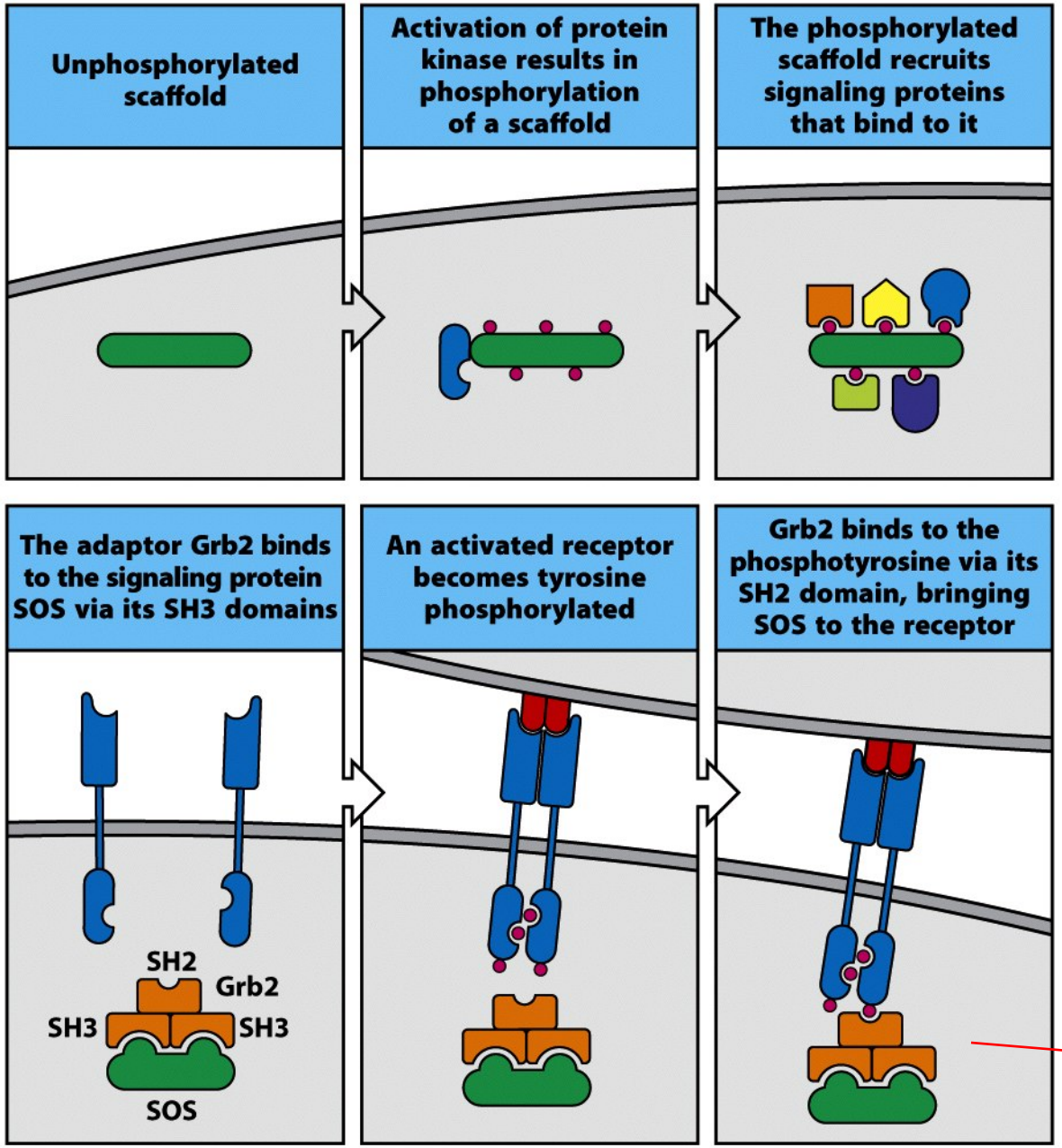
(Fig. 7.3) Formation of signaling complexes



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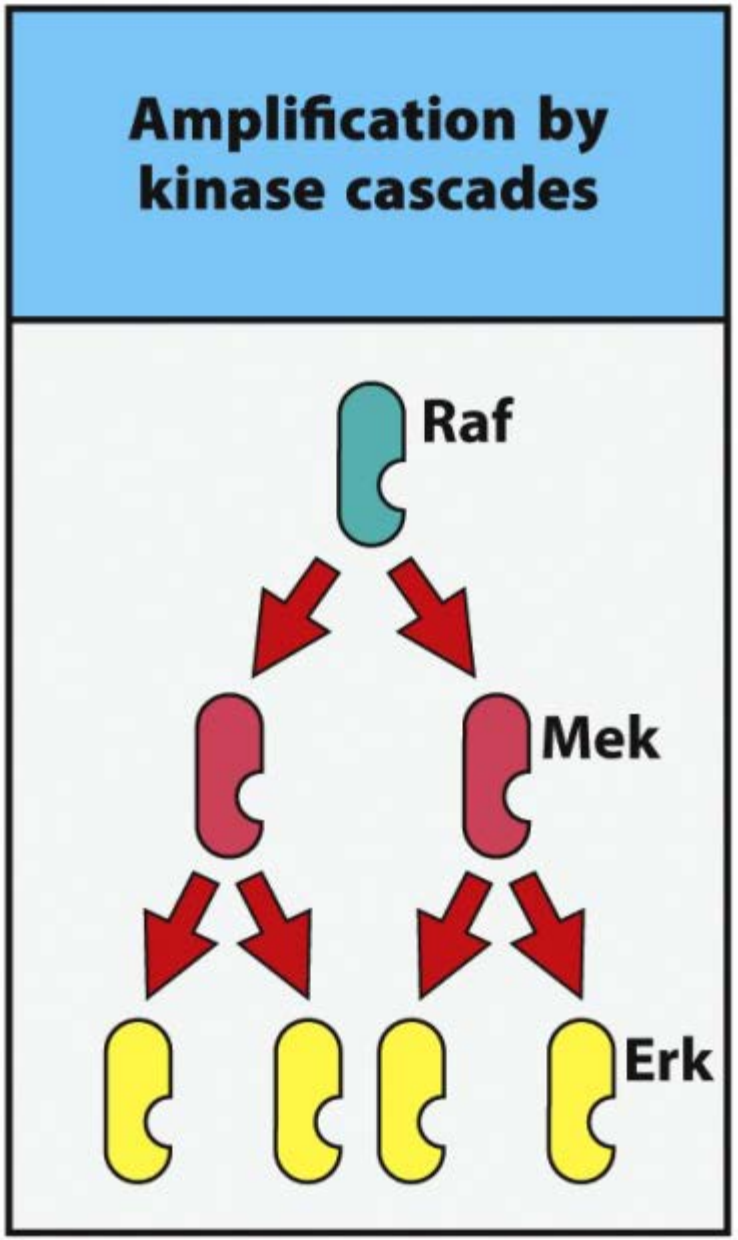
Via **scaffold protein**
→Phosphorylation first
→Recruitment of signaling proteins to its Tyr-p sites

Via **adaptor protein**
→Bound by other signaling protein (via SH3 domain)
→Binds to activated receptor (via its SH2 domain)

Signal is passed to downstream '**small G proteins**' (Fig. 6.5)

Signal amplification (1)

(Fig. 7.7) Small initial signal intensity can be amplified by relaying proteins



Small G proteins = Small GTPases = G-proteins (e.g. Ras GTPase)

MAPKKK (activated by small G proteins)

↓ phosphorylates (on Ser/Thr)

MAPKK

↓ phosphorylates (on Tyr/Thr)

MAPK (mitogen-activated protein kinase)

Fig. 6.4-L

Signaling causes the generation of small-molecule ('second messenger')

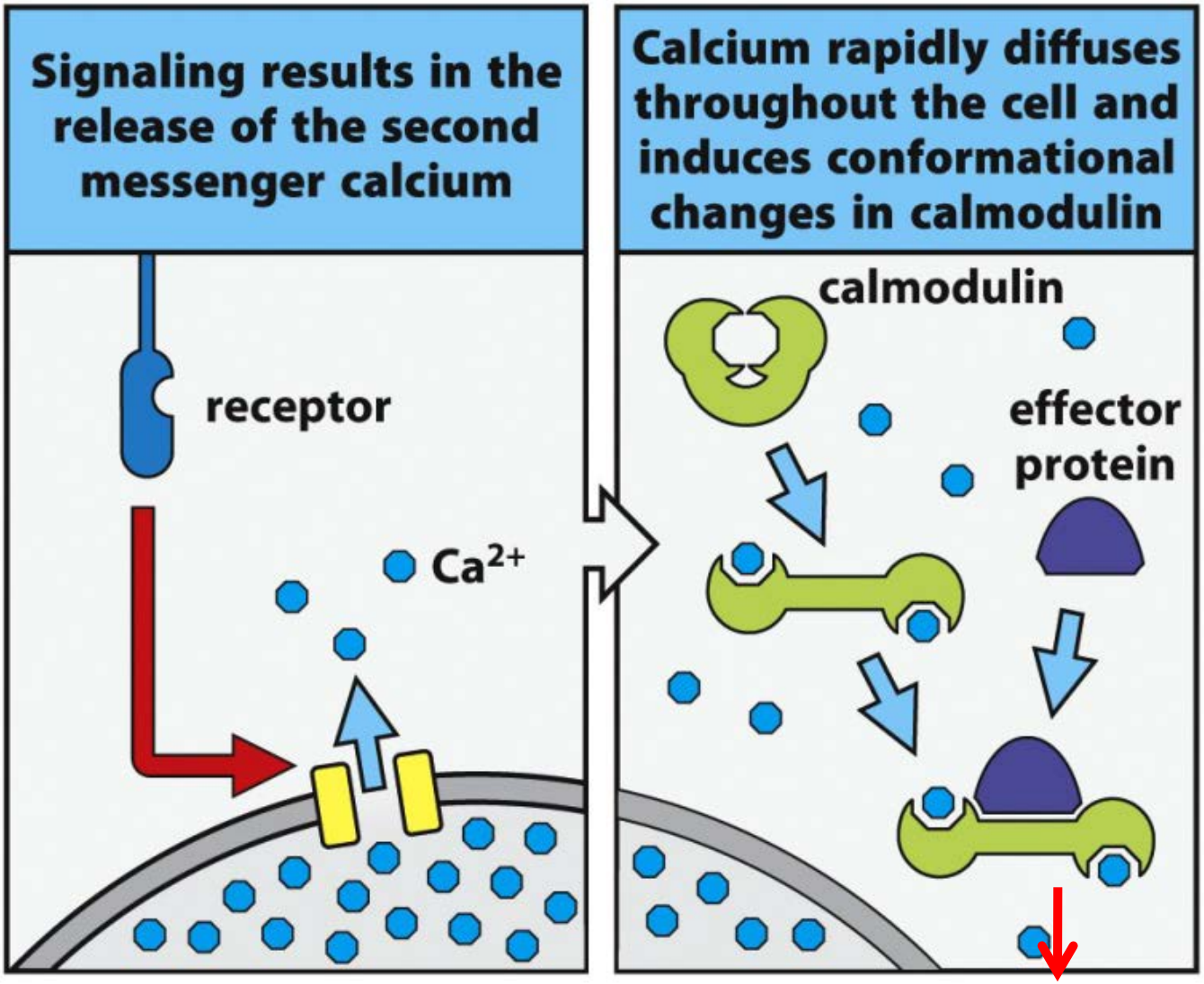
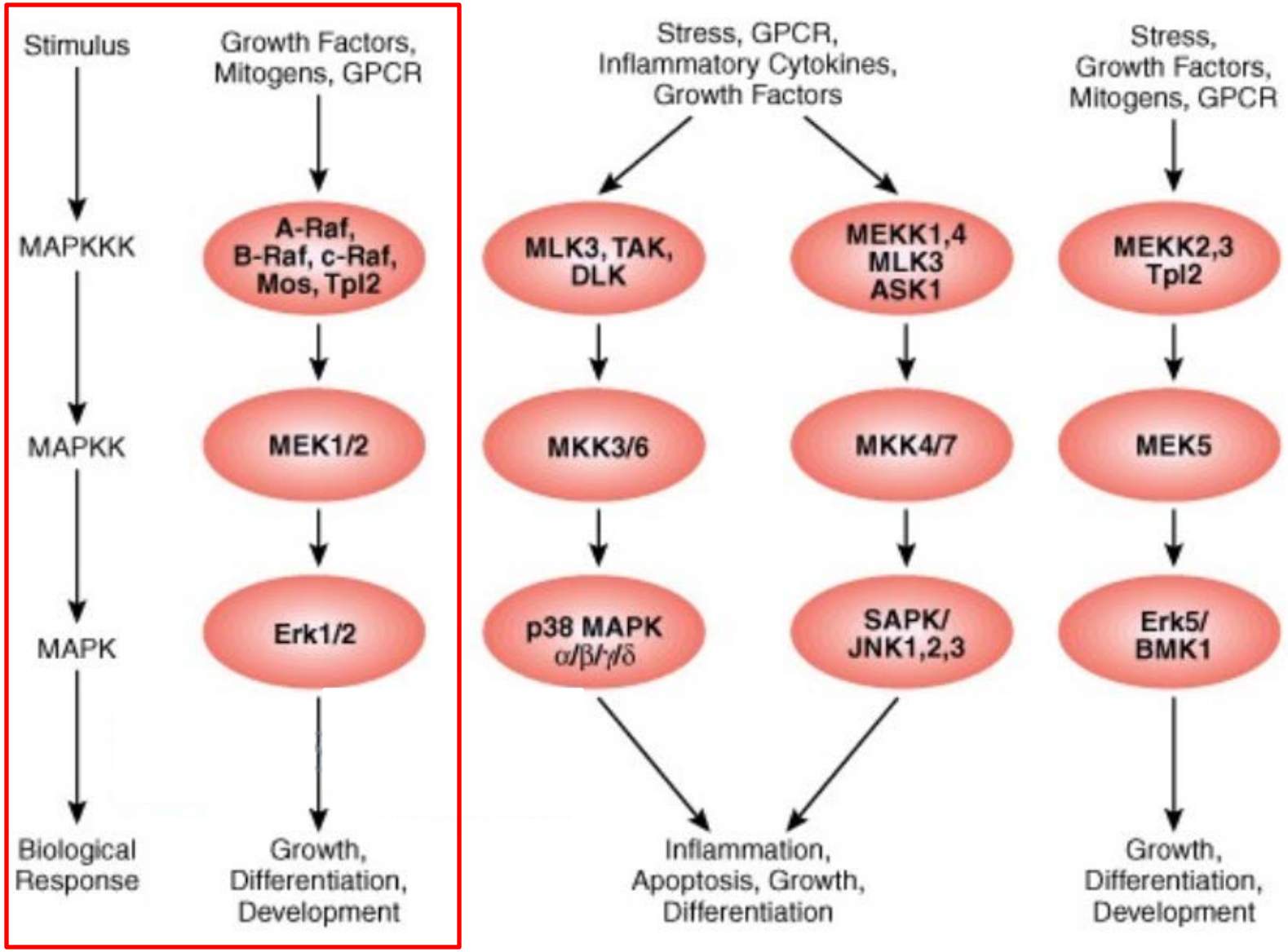


Fig. 7.7

e.g.
Calcineurin
(a phosphatase)

Signal amplification (2)

Calcineurin dephosphorylates NFATc


Stimulus
Growth Factors, Mitogens, GPCR
Stress, GPCR, Inflammatory Cytokines, Growth Factors
Stress, Growth Factors, Mitogens, GPCR
MAPKKK
A-Raf, B-Raf, c-Raf, Mos, Tpl2
MLK3, TAK, DLK
MEKK1,4 MLK3 ASK1
MEKK2,3 Tpl2
MAPKK
MEK1/2
MKK3/6
MKK4/7
MEK5
MAPK
Erk1/2
p38 MAPK α/β/γ/δ
SAPK/ JNK1,2,3
Erk5/ BMK1
Biological Response
Growth, Differentiation, Development
Inflammation, Apoptosis, Growth, Differentiation
Growth, Differentiation, Development

(Fig. 7.4) Small G proteins are downstream of tyrosine kinase-associated receptor

GEF (+) → Small G proteins (e.g. Ras, Rac) → MAPK → AP-1 activation (Fig. 6.19)

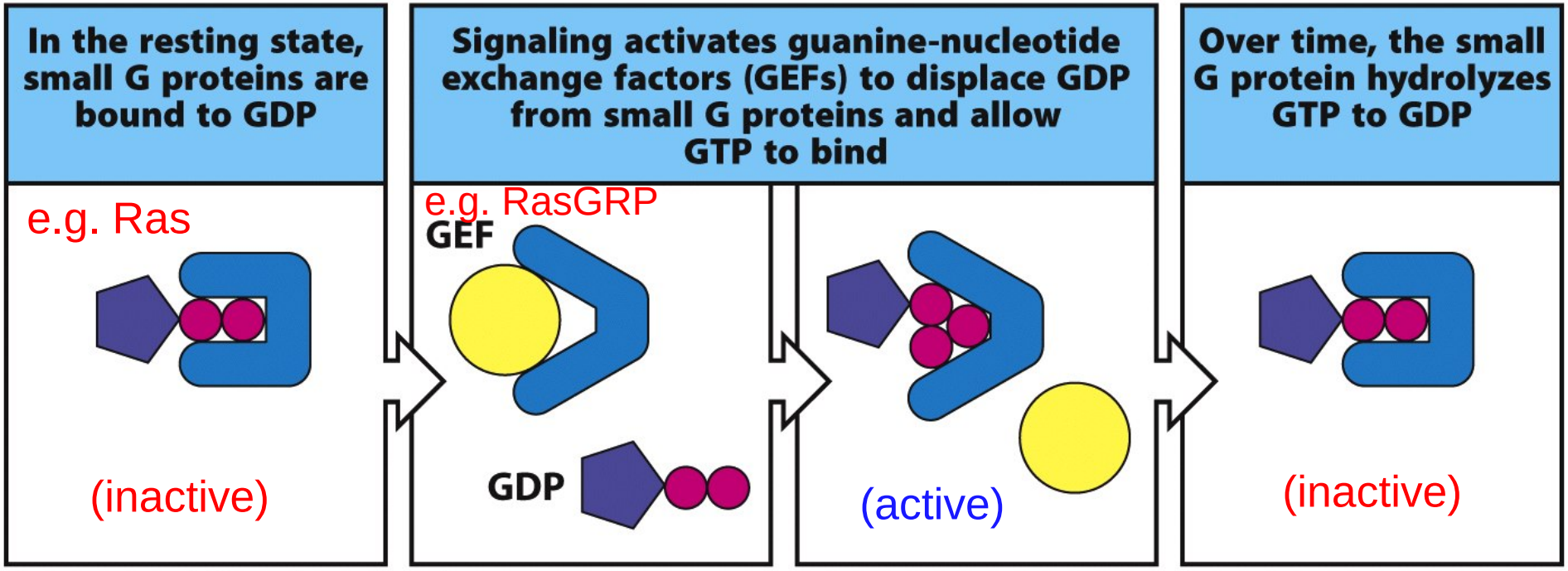


Figure 6-5 Immunobiology, 7ed. (© Garland Science 2008)

- Small G-GDP (inactive, resting state) v.s. small G-GTP (active, transient)
- GEF assists the –GDP to –GTP transformation
- Small G proteins have intrinsic GTPase activity
  - To ensure activation signal would only be present transiently!!
  - GTPase-activating proteins (GAPs) will accelerate this reaction..

(Fig. 7.5) Summary: 3 different ways to recruit signaling molecules to the membrane

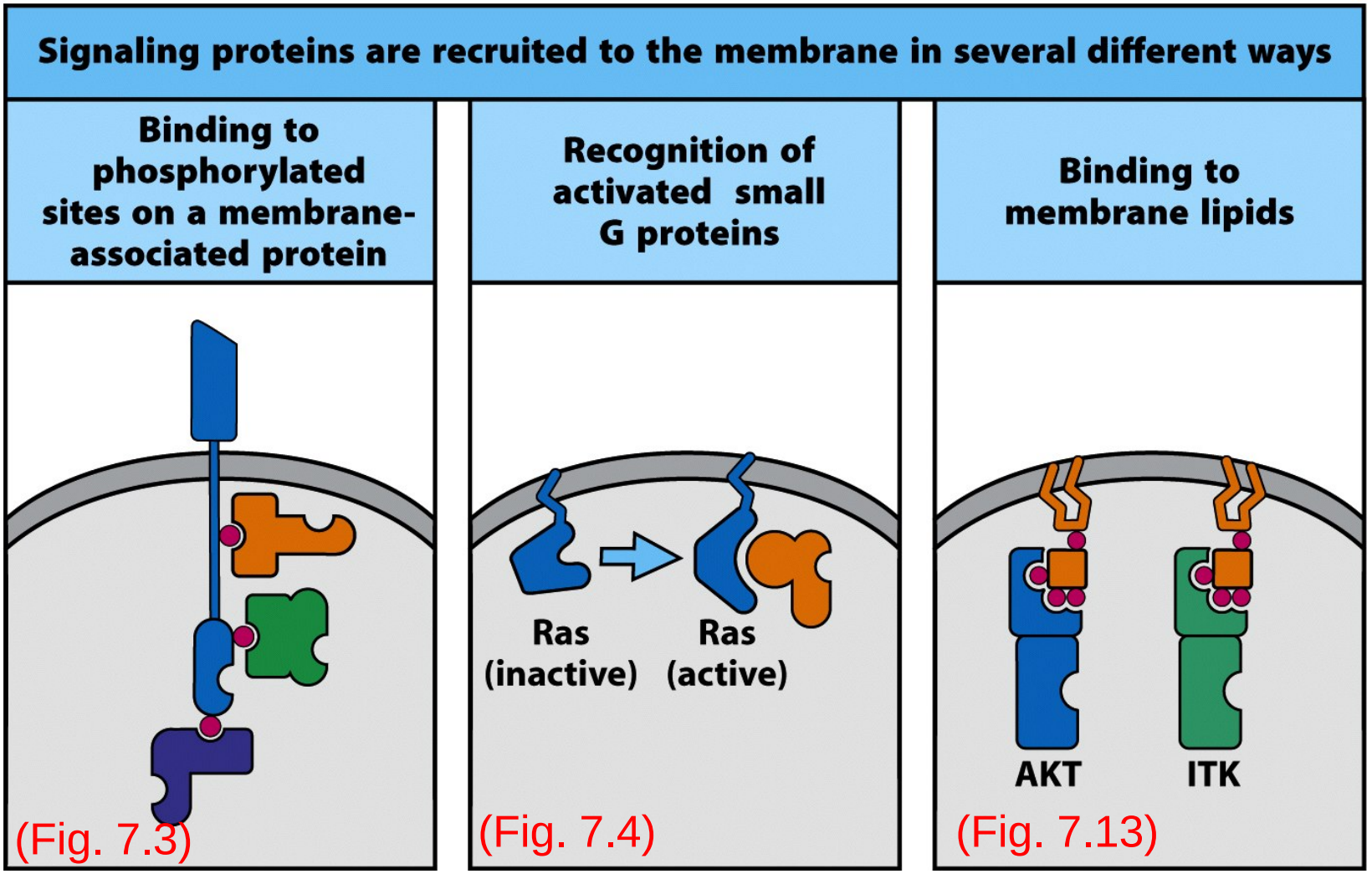


Figure 6-6 Immunobiology, 7ed. (© Garland Science 2008)

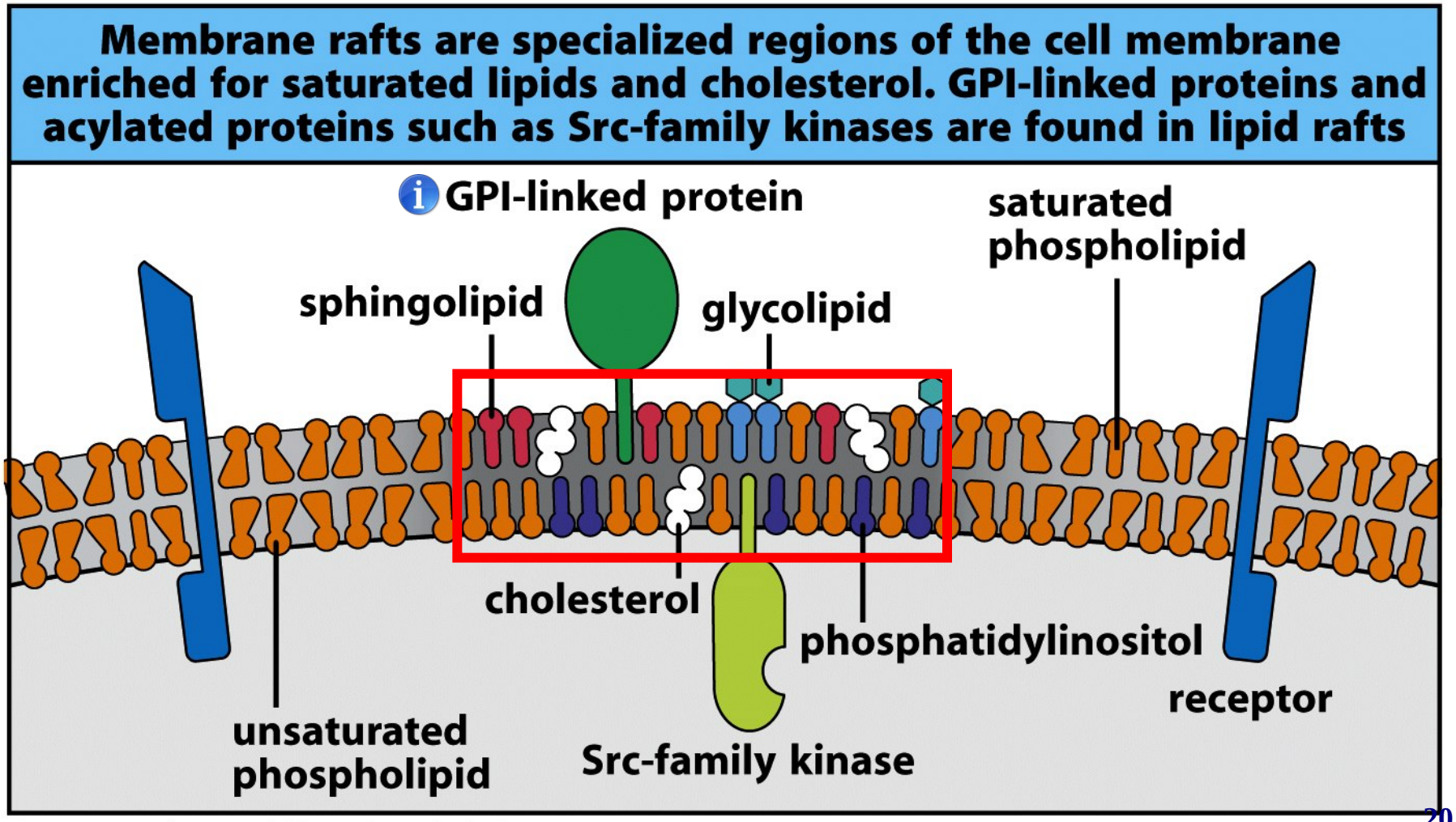
Membrane localization of signaling molecules

- Constrained in specific ‘microdomain’
  - Rich in lipids (e.g. sphingolipid, cholesterol)
  - Structurally more rigid than surrounding areas
  - Resistant to treatment by detergents
- Some common description of such feature
  - Lipid raft
  - glycolipid-enriched microdomain (GEM)
  - Detergent-insoluble glycolipid-rich domain (DIG)

Lipid raft

- More rigid (higher% of saturated phospholipids)
- Harbors molecules important in signaling events
  - e.g. Src-family kinase
- Dynamic structure
  - can change size
  - proteins can migrate in and out of raft
    - e.g. cross-linked T- or B-cell receptors migrate into raft
- Crucial for signaling events to occur
  - depletion of cholesterol disrupts T-cell activation

Association of signaling molecules with lipid raft



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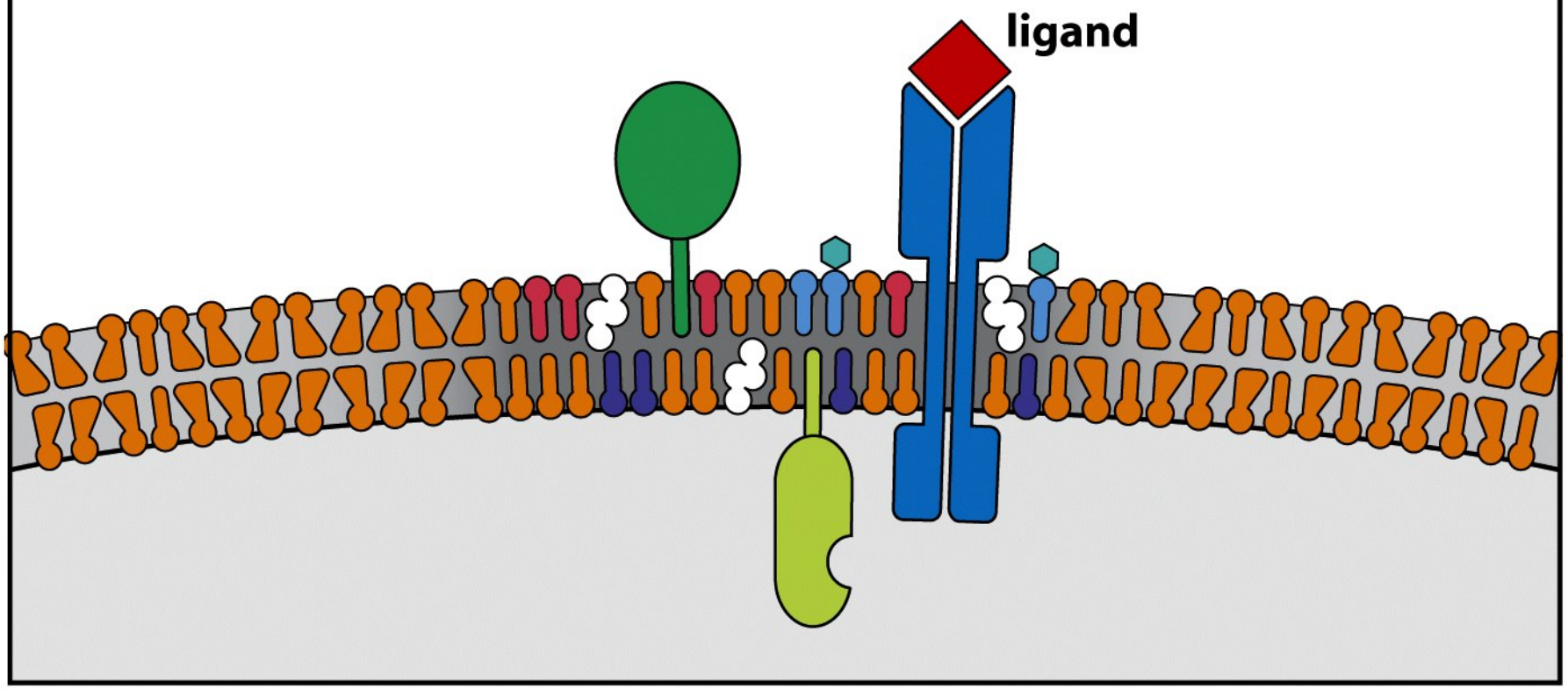
Lipid rafts are dynamic structures that can change size and protein content. Some proteins migrate into lipid rafts when they are oligomerized by binding ligand



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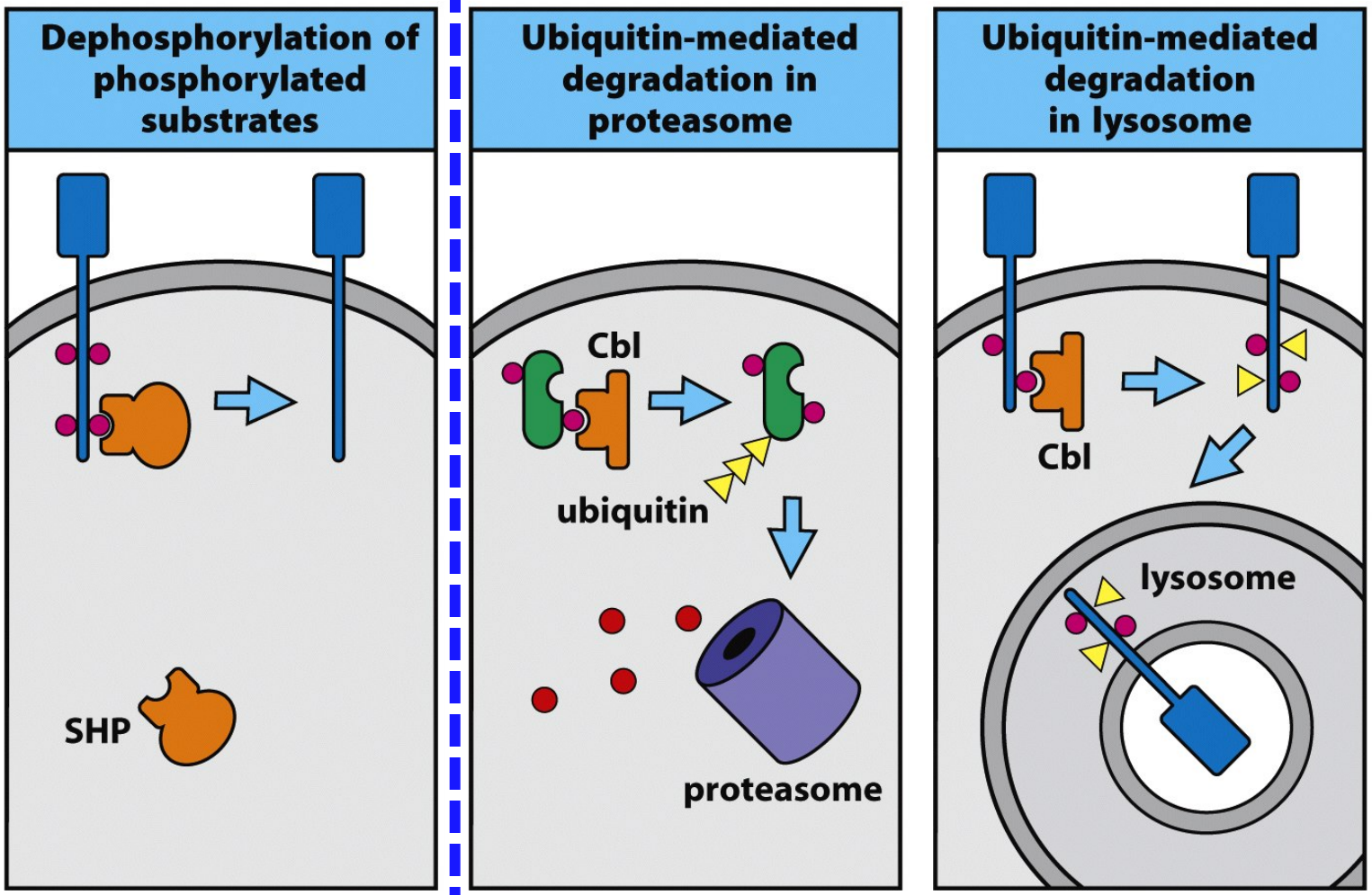
(Fig. 7.6) How are signals turned off?

(via phosphatase or Ubi. ligase)

SHP: phosphatase

Cbl: ubiquitin ligase



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Ag receptor signaling and lymphocyte activation

(Fig. 7.10) Antigen receptor complex (B cell receptor)

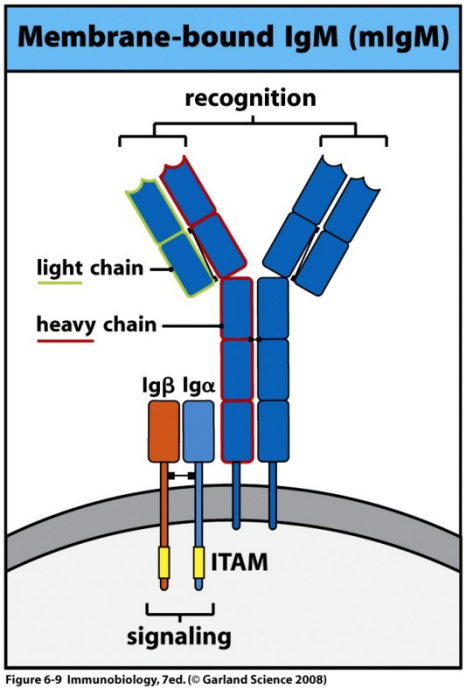


Figure 6-9 Immunobiology, 7ed. (© Garland Science 2008)

- BCR complex
  - B-cell receptor (membrane-bound Ig)
  - Accessory proteins
    - Igα and Igβ (heterodimer)
      - Both are required for complete surface expression of BCR complex
        - No Igα/Igβ → no surface BCR complex expressed
      - Crucial for signaling (due to the "ITAM motif")

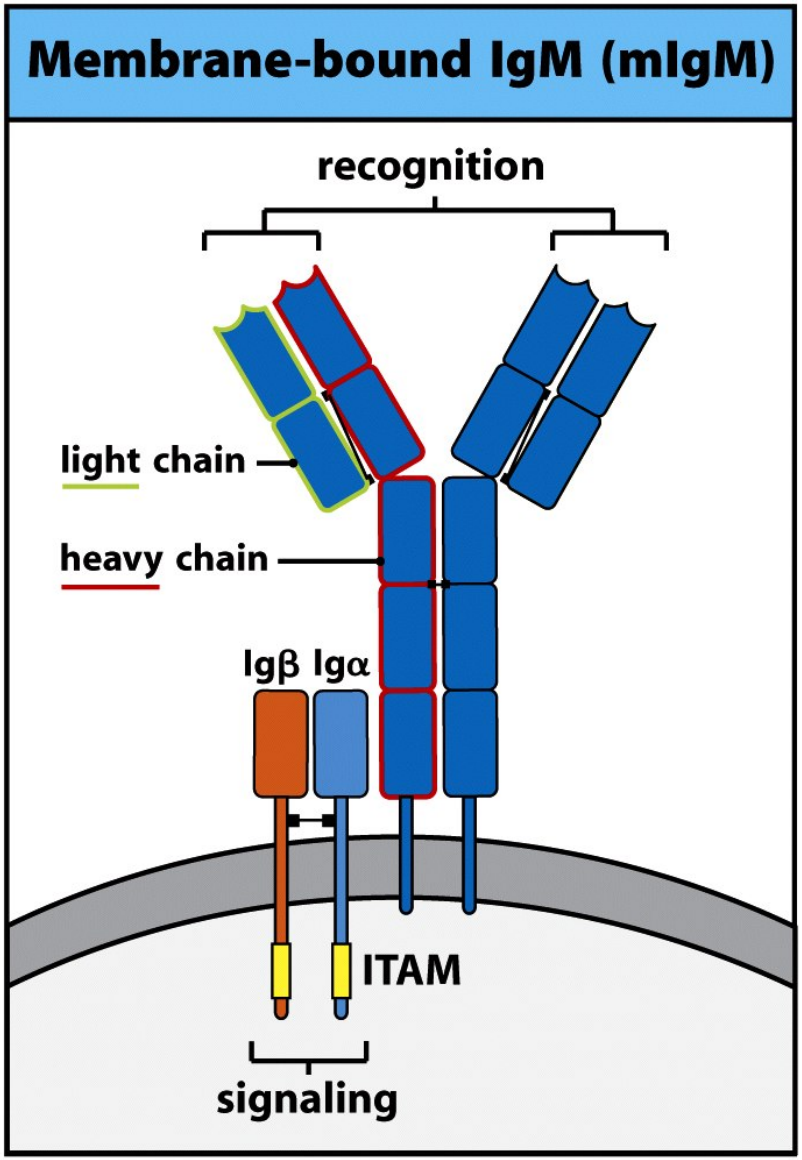


Figure 6-9 Immunobiology, 7ed. (© Garland Science 2008)

BCR complex

- ITAM motif
  - **I**mmunoreceptor **t**yrosine-based **a**ctivation **m**otif
  - Responsible for intracellular signaling
  - Canonical sequence
    - **Y**XX[L/I]$X_{6\text{-}9}$**Y**XXX[L/I]
  - Present in other immune cells
    - FcεRI, CD3, NK receptor, …etc.
    - vs. ITIM motif

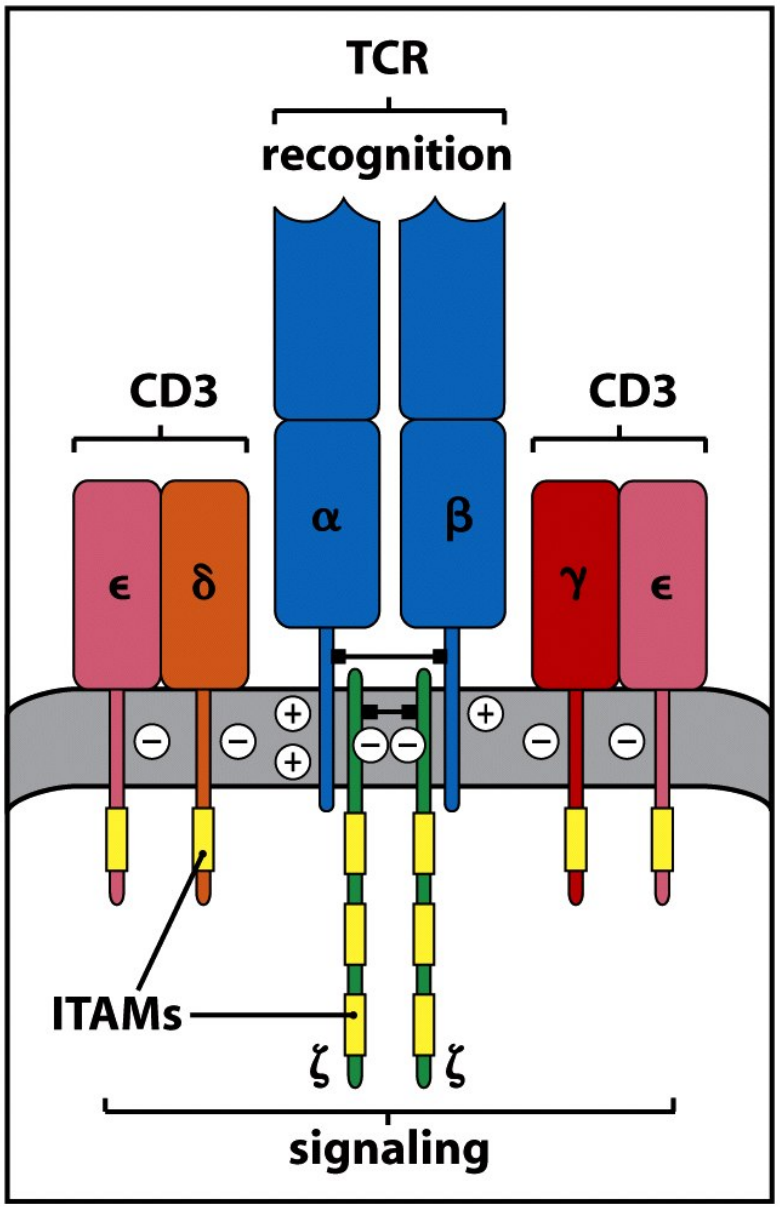


Figure 6-10 Immunobiology, 7ed. (© Garland Science 2008)

TCR complex

- Structural composition similar to BCR complex
- Genes for CD3 (ε/γ/δ)are clustered on chromosome
  - Required for expression of α/β dimer!!
- More ITAM motif
  - TCR (10) vs. BCR (2)
  - Greater ability/flexibility in signaling

(Fig. 7.9) ITAMs recruit signaling molecules with tandem SH2 domains

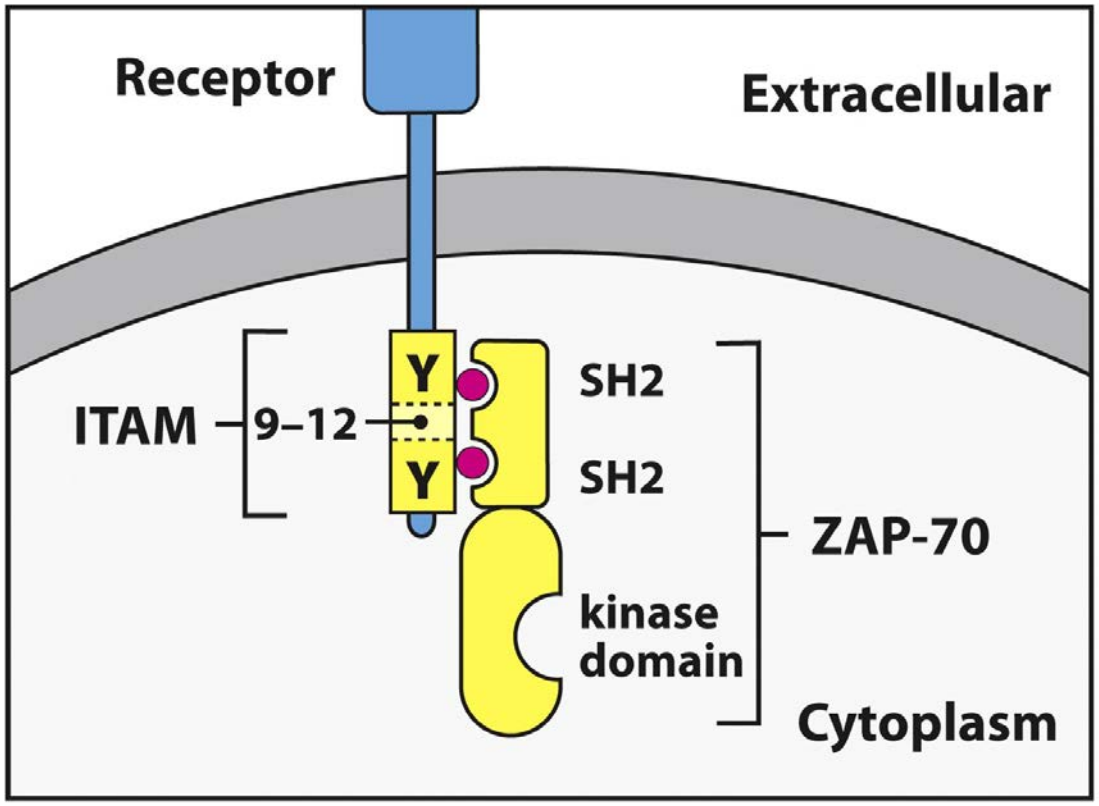
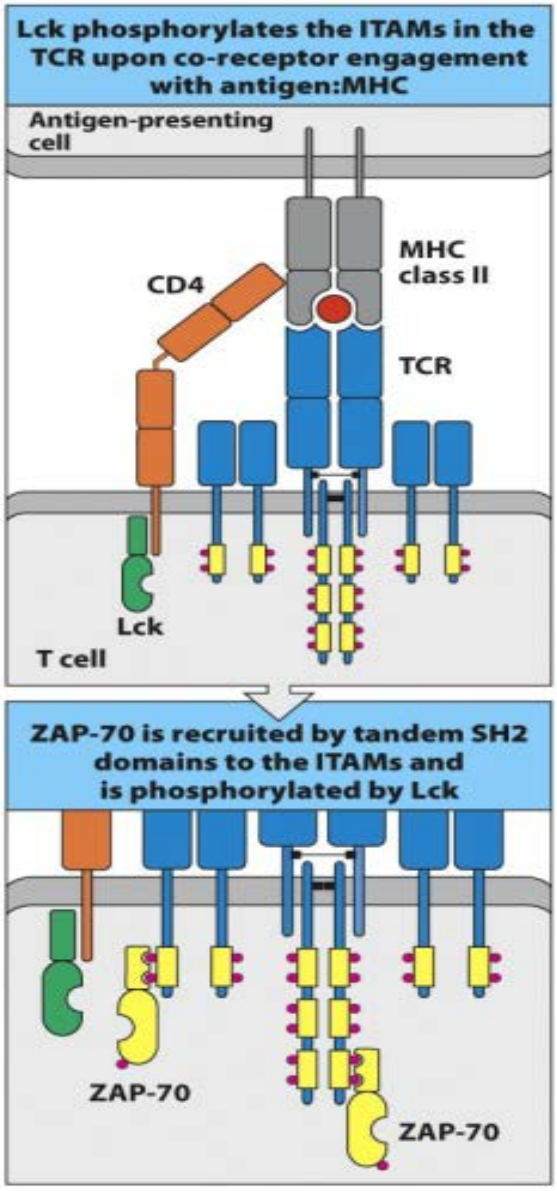


Figure 7.9 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

(Fig. 7.11) Antigen receptor complex (T cell receptor)

- TCR complex
  - T-cell receptor (α:β heterodimer)
    - Different from the Igα/Igβ in BCR complex!!
  - Accessory proteins
    - CD3 complex (2ε:δ:γ, or ε:δ/ε:γ)
    - ζ chains (homodimer)
    - ITAM motif responsible for relaying signals



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Ag-binding causes ITAM phosphorylation on Ag receptor

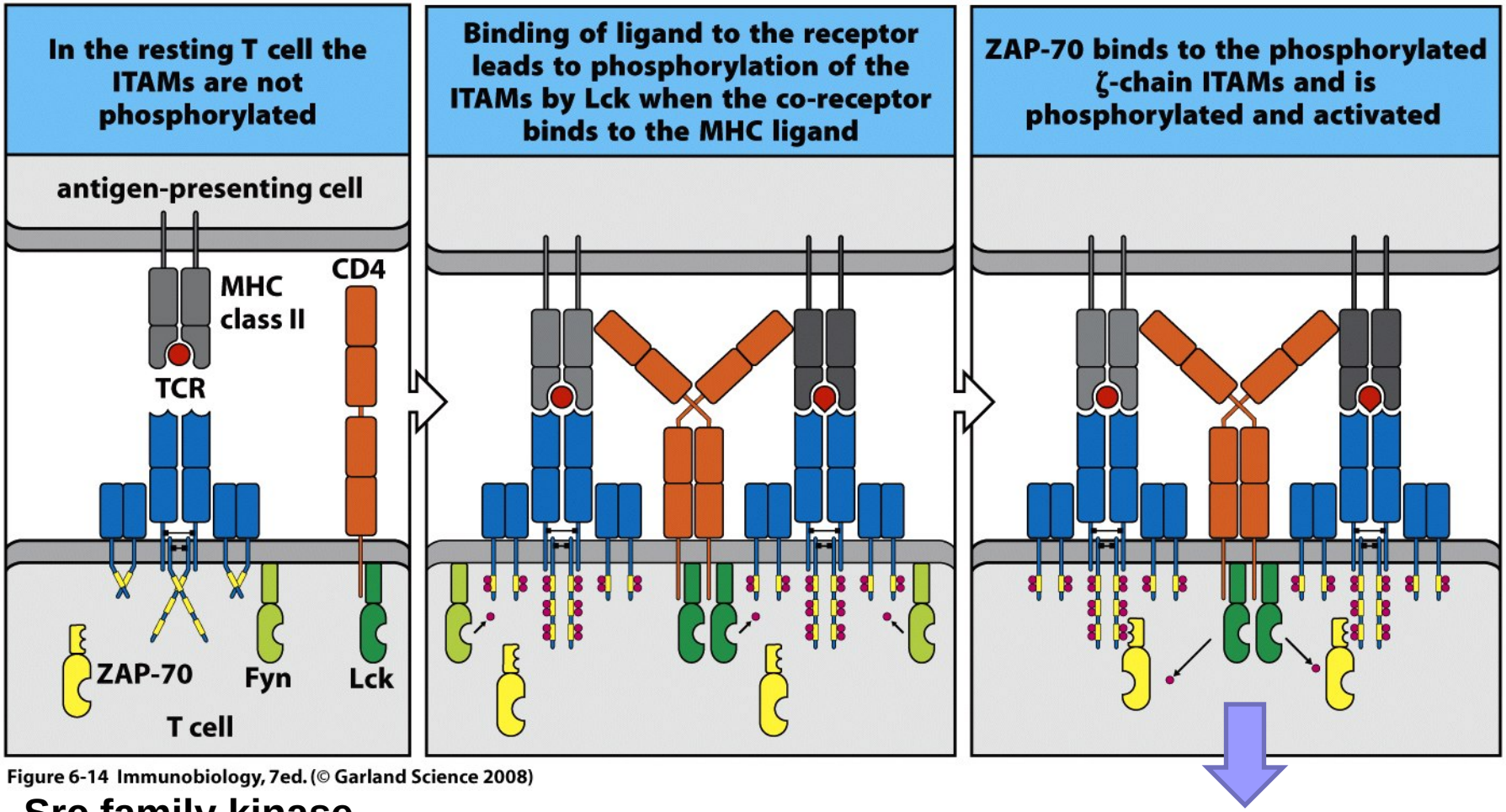


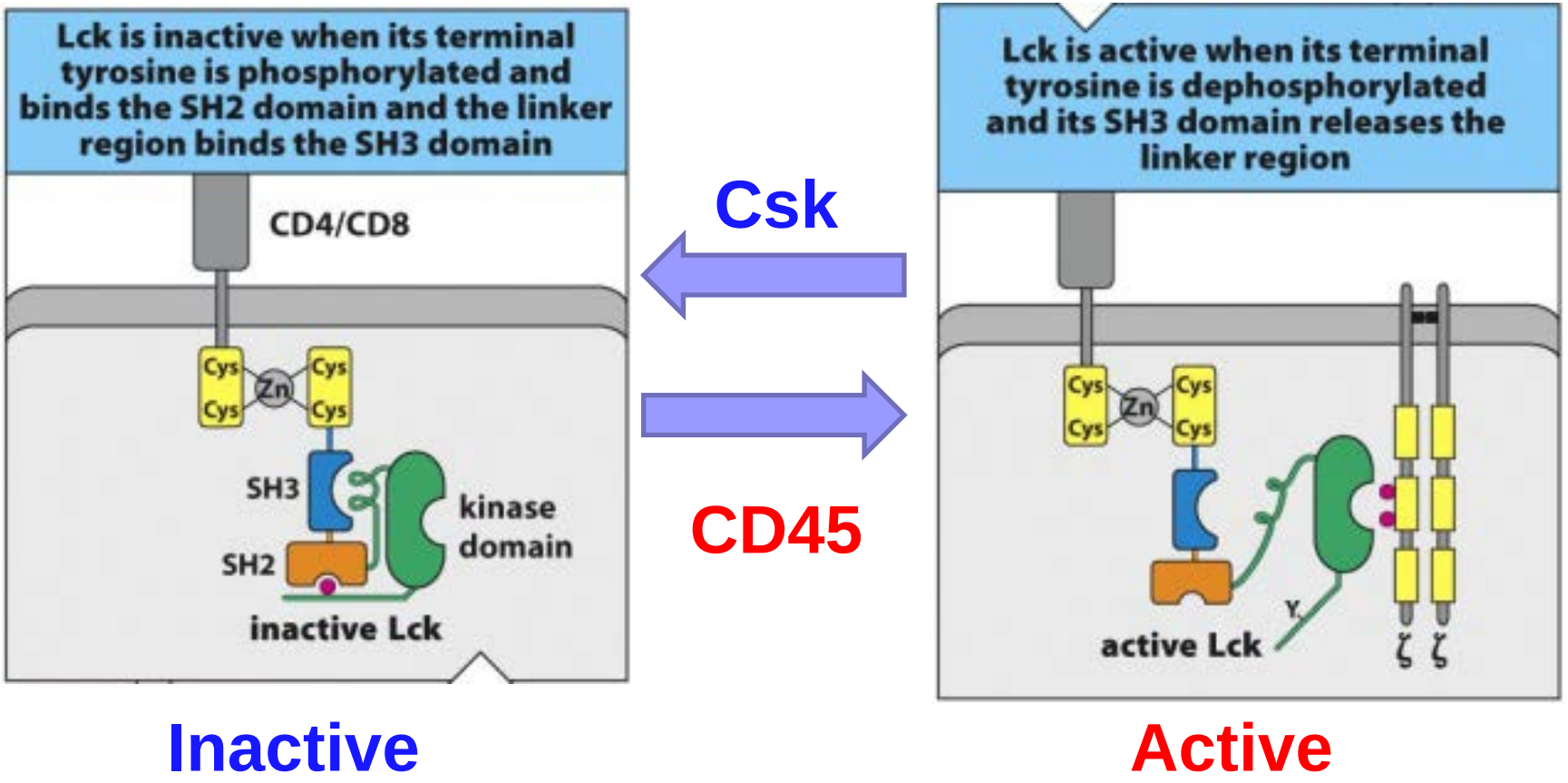
Figure 6-14 Immunobiology, 7ed. (© Garland Science 2008)

Src family kinase

1. Fyn: weakly associated with TCR ζ chain & CD3
2. Lck: constitutively associated with CD4 and CD8

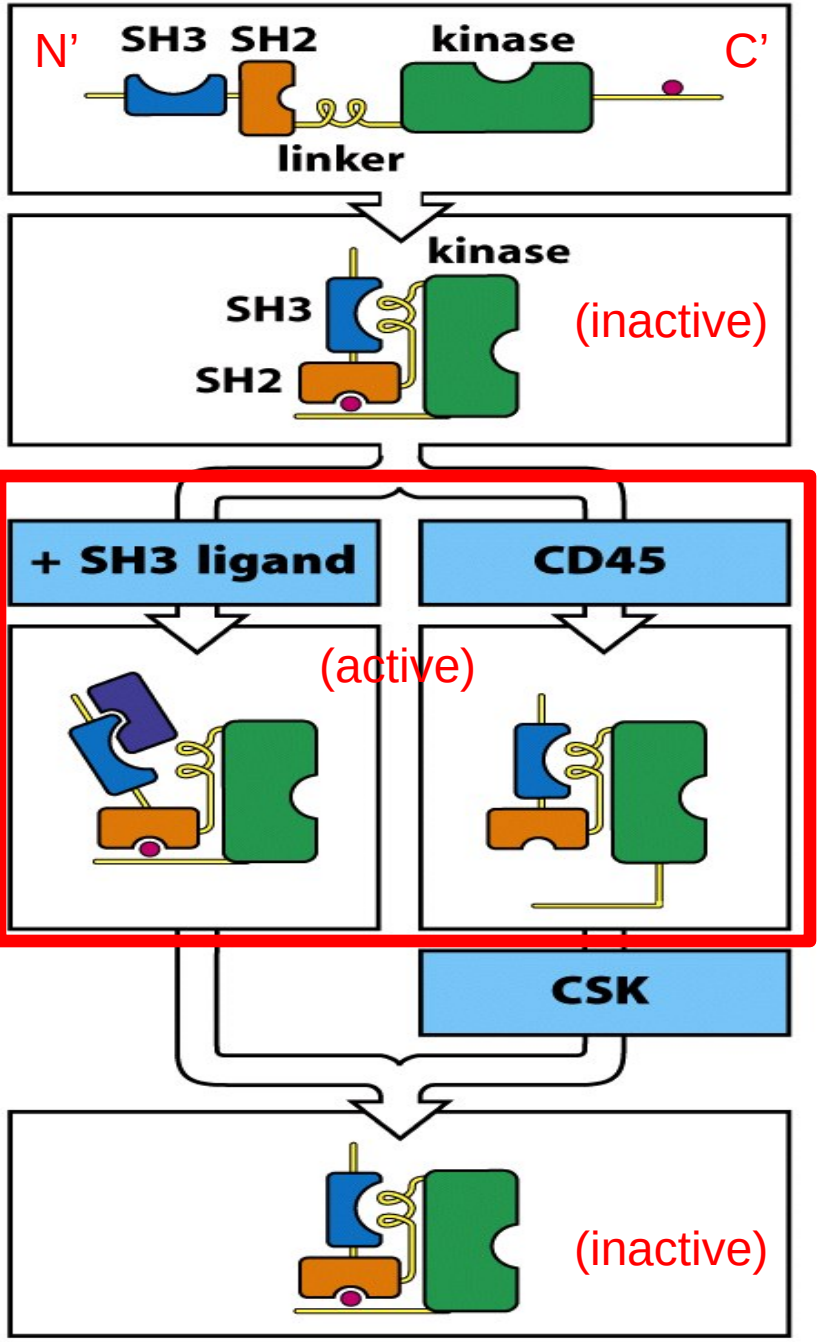
Lck phosphorylates ZAP-70

(Fig. 7.12) Lck activity is regulated by phosphorylation & dephosphorylation



Inactive

Active



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Regulation of Src-family kinases

CD45: phosphatase
CSK: C-terminal Src kinase

Relays signaling process

Lck can phosphorylate:
- ZAP-70
- CD3ε
- ζ chain
- Itk (Tec kinase)
- Phospholipase C γ (PLCγ)

(Fig. 7.13) The recruitment and activation of PLCγ is crucial in T-cell activation

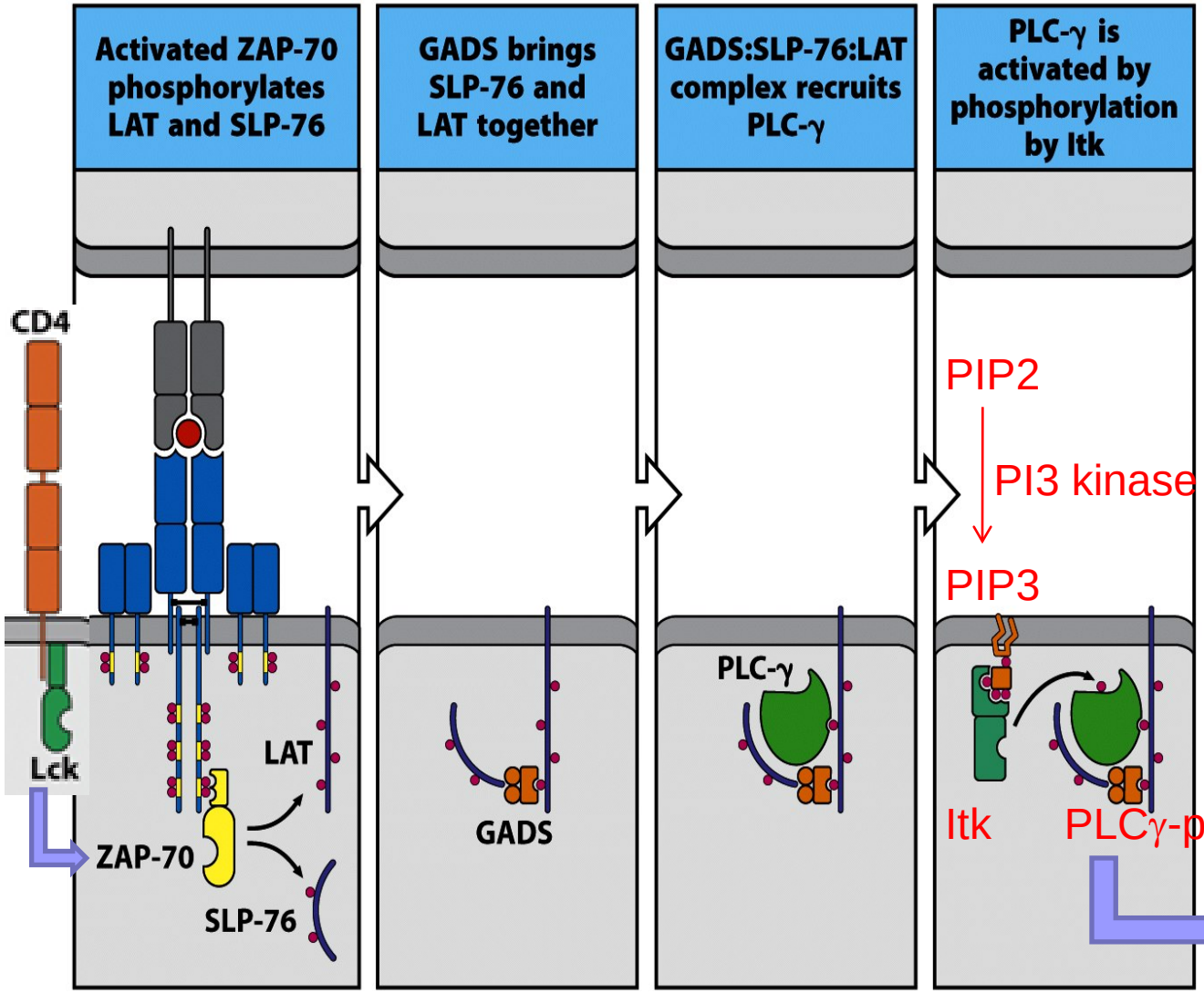
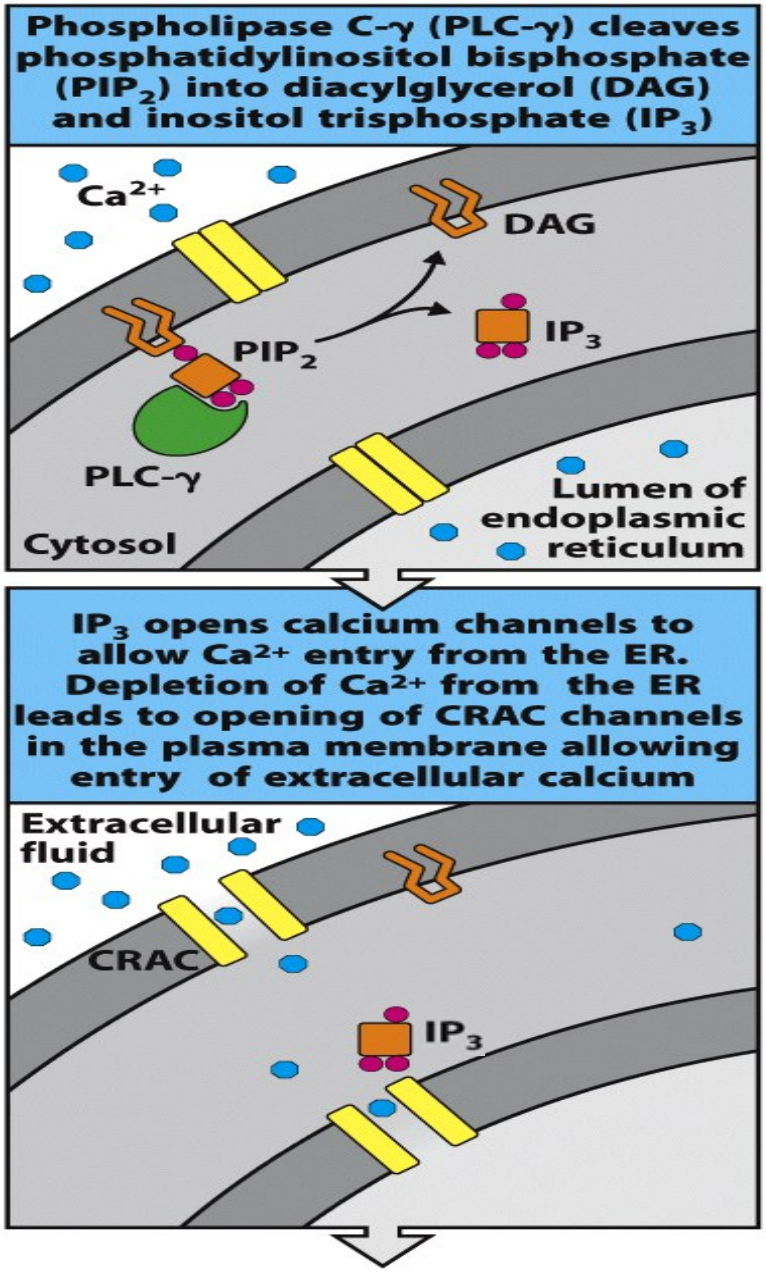


Figure 6-16 Immunobiology, 7ed. (© Garland Science 2008)

ZAP-70
(ζ-associated protein)
-Has high affinity w/ 2 Tyr-p on ζ-chain
-Recruited to ζ-chain
-Becomes –p by **Lck**
-In turn, phosphorylates
- **LAT**, **SLP-76**
- LAT-p/SLP76-p forms complex with **GADS**
-PLCγ becomes –p by **Tec** family kinase (e.g. **Itk**)

(Fig. 7.14) PLCγ cleaves PIP2 during signaling



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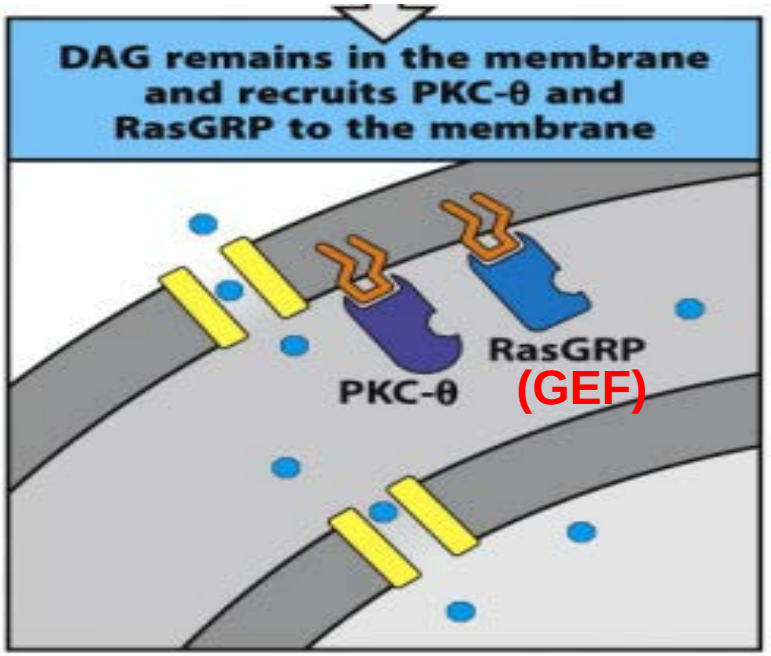

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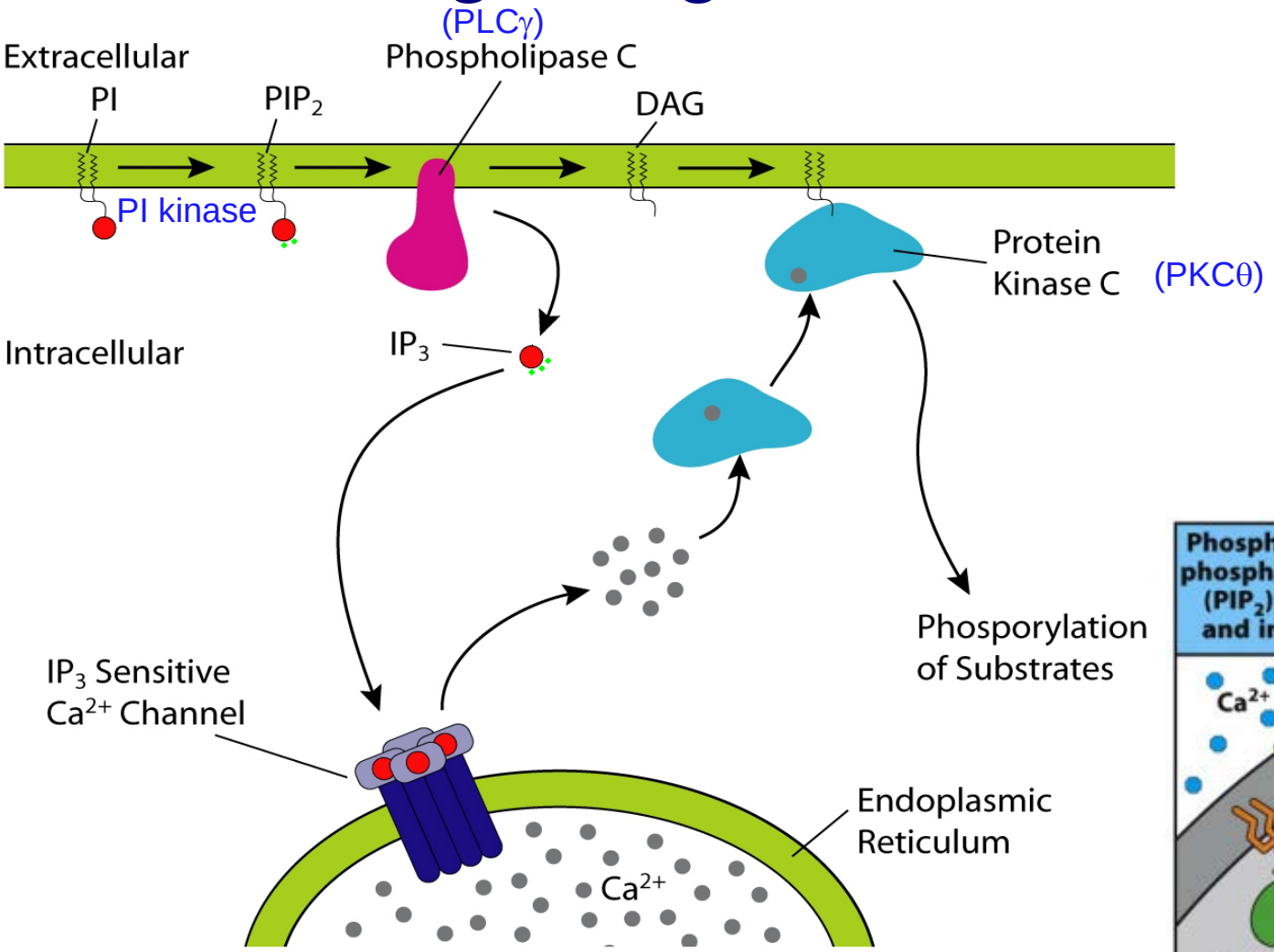
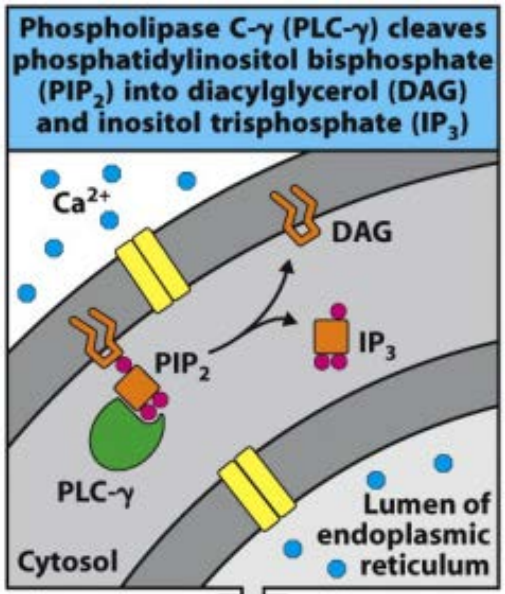
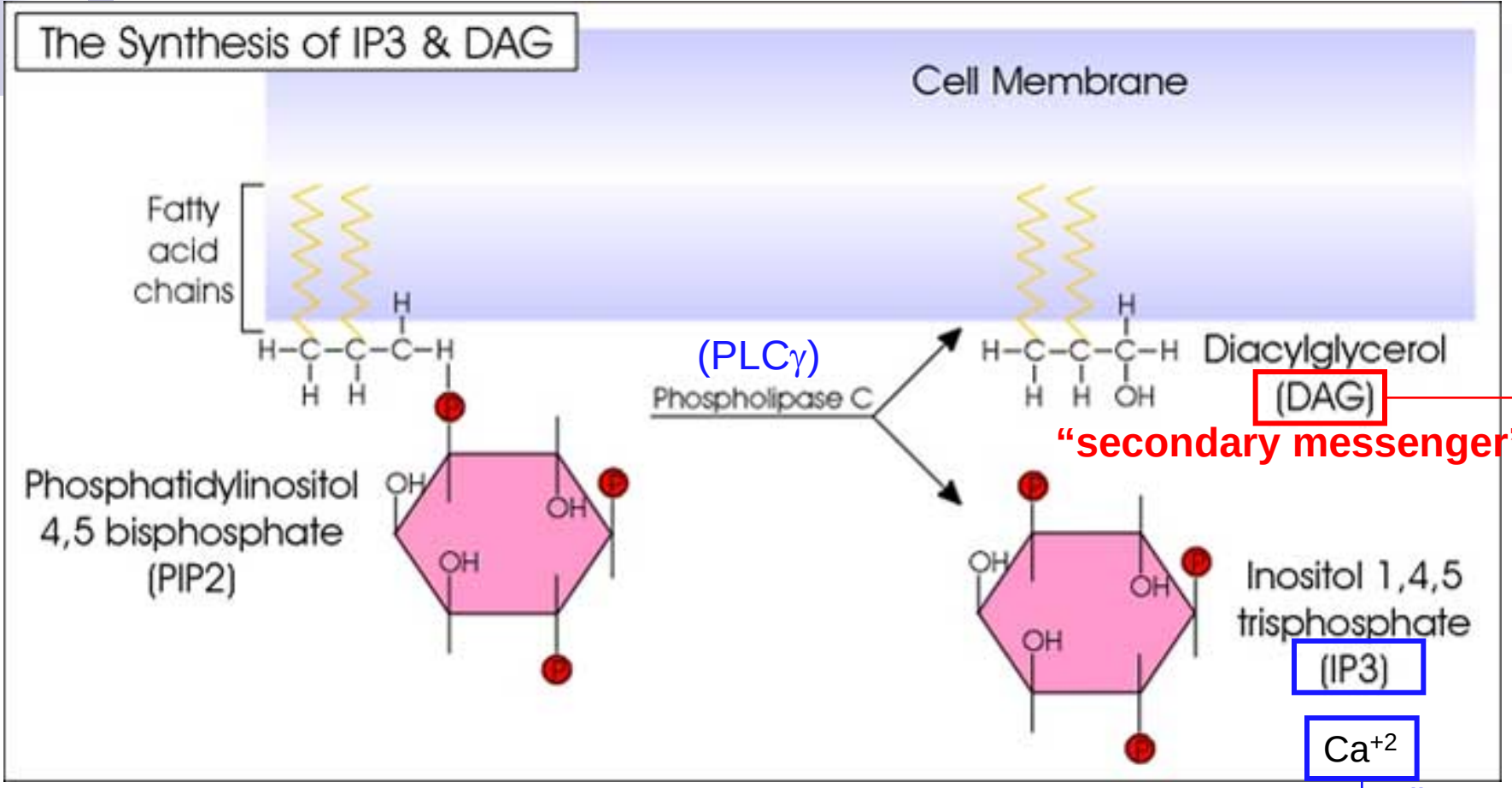
Secondary messengers:
IP3,
DAG, (PMA is its analog)
Ca++ (inomycin could mimic its action)

Lead to 3 signaling pathways (Fig. 7-15)

Activation of membrane lipids by signaling molecules





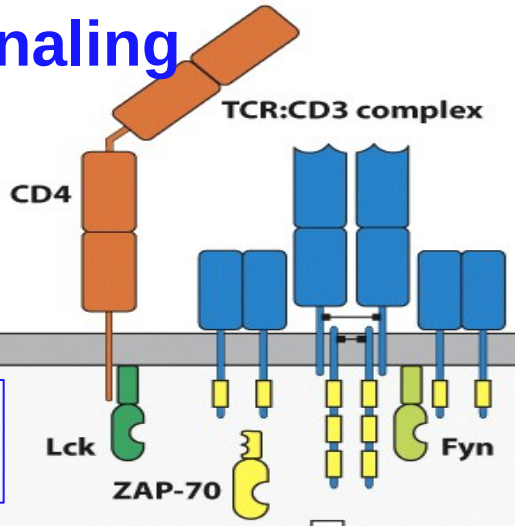


3. NFAT activation ← Activation of calcineurin

1. Activation of protein kinase C θ (PKCθ)
   → Phosphorylates Ser/Thr
2. Activation of GEF, and Ras
   → MAPK activation

1. **NFκB activation (by PKCθ)**
2. **AP-1 activation (by MAPK)**

T-cell receptor signaling



Co-receptor:
CD4 (T helper)
CD8 (T cytotoxic)

ZAP-70 needs to be activated by **Lck** !!

Lck phosphorylates tyrosine residues on the CD3 and ζ ITAMs, allowing ZAP-70 to bind

Lck activates ZAP-70, which in turn phosphorylates LAT and SLP-76. SLP-76 and LAT bind phospholipase C-γ (PLC-γ).

PLC-γ is activated by Itk, and cleaves phosphatidylinositol bisphosphate (PIP_2) to yield diacylglycerol (DAG) and inositol trisphosphate (IP_3)

(1) IP_3 increases intracellular Ca^{2+} concentration, activating a phosphatase, calcineurin

Calcineurin activates a transcription factor, NFAT (nuclear factor of activated T cells)

Fig. 7-16

(2) DAG recruits protein kinase C-θ

Protein kinase C-θ activates CARMA, which leads to activation of NFκB

Fig. 7-19

(3) DAG recruits RasGRP, which in turn activates Ras

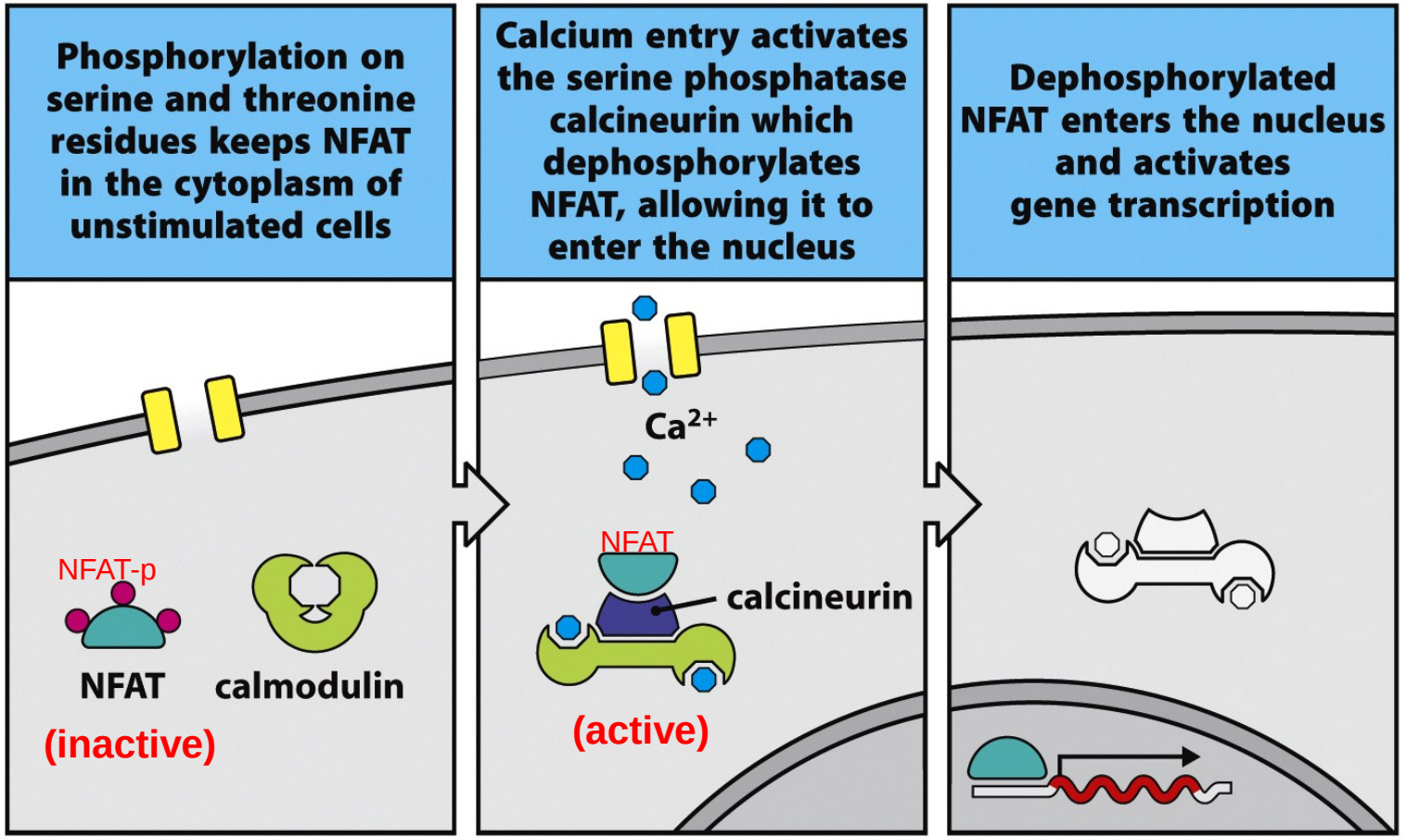
Ras activates the MAP kinase cascade, which activates Fos, a component of the AP-1 transcription factor

Fig. 7-17
Fig. 7-18

The transcription factors NFκB, NFAT, and AP-1 act to induce specific gene transcription, leading to cell proliferation and differentiation

Figure 7.15 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

(1) NFAT activation by Ca^{++} signaling



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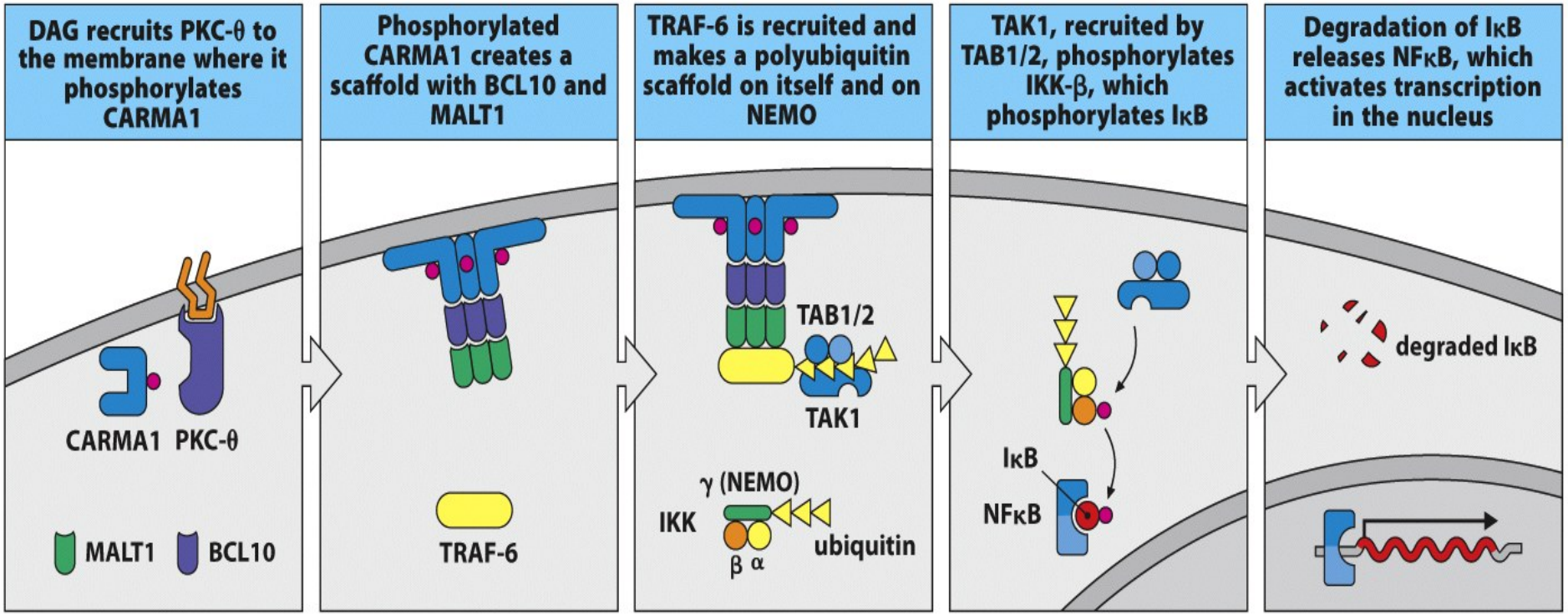
NFAT-p: inactive, phosphate can be removed by **calcineurin** (Ca^{2+}-dependent)
NFAT: active, can bind to active Ca^{2+}-bound calmodulin
Calcineurin: Serine phosphotase

(2) PKCθ activates NFκB through DAG signaling



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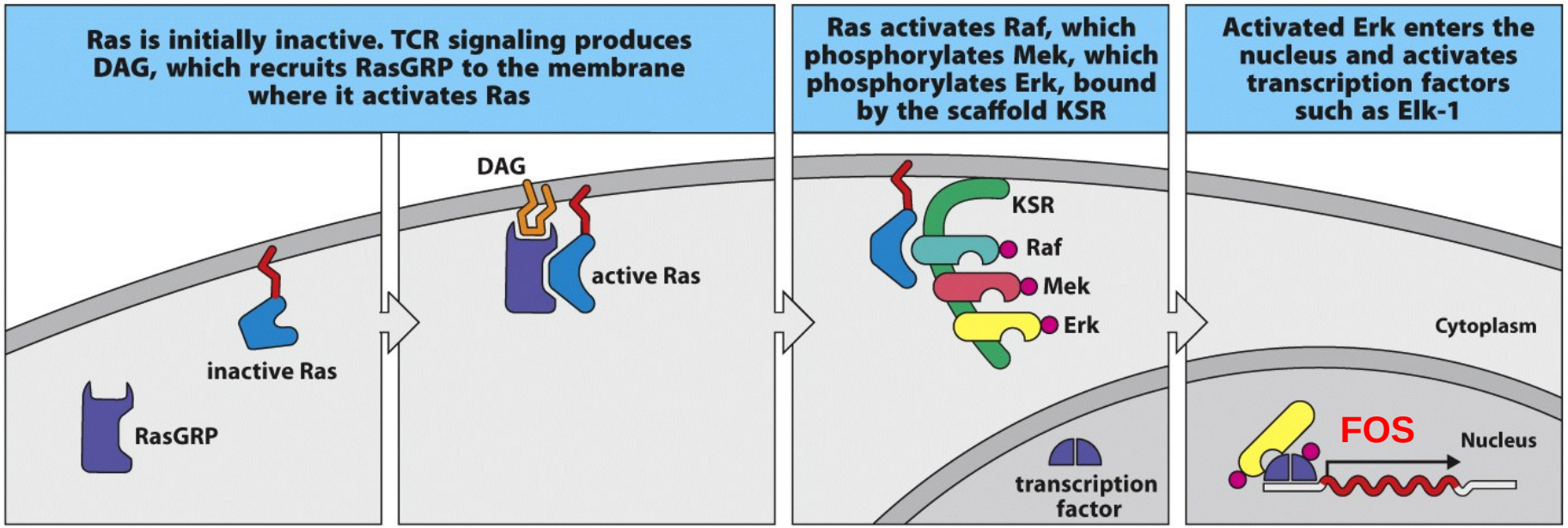
CARMA1: scaffold protein, binds to other adaptor proteins (e.g. BcL10, MALT1)
Ikβ kinase complex (IKKα:IKKβ:IKKγ): phophorylates Ikβ → NFκB translocation

(3) Formation of AP-1 transcription factor by the MAPK pathway

[Step 1] Activation of small G protein (Ras) leads to MAP kinase (MAPK) activation



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KSR: scaffold protein

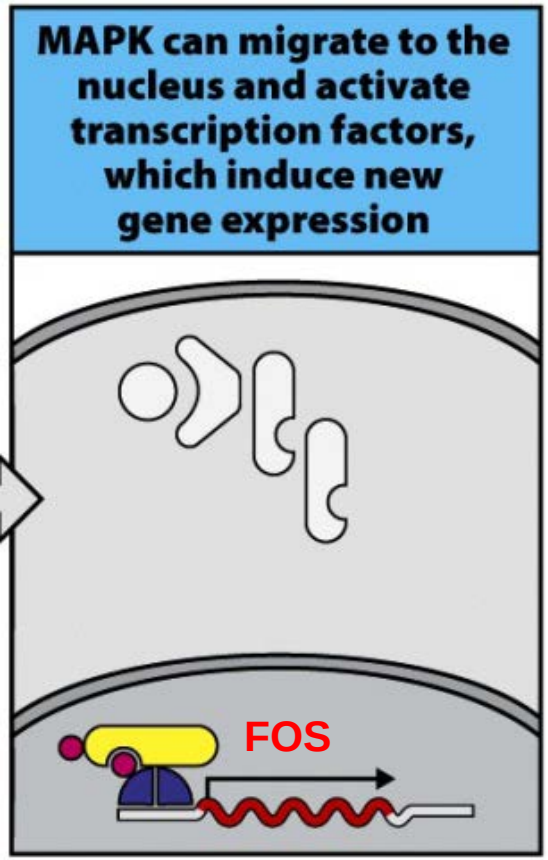
Raf: MAPKKK
Mek: MAPKK　上游依序磷酸化下游 → activation
Erk: MAPK

(3) Formation of AP-1 transcription factor by the MAPK pathway

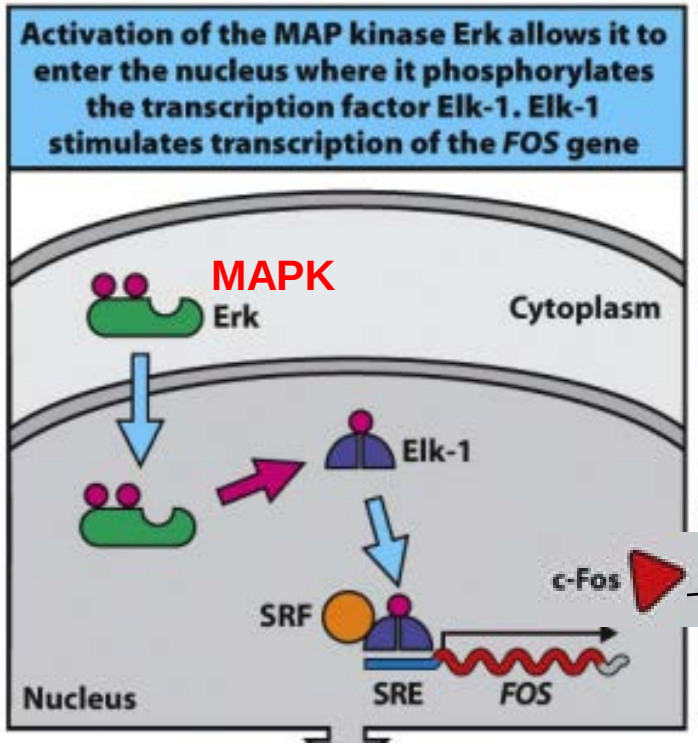
[Step 2] Formation of AP-1 by joining of c-Fos and c-Jun

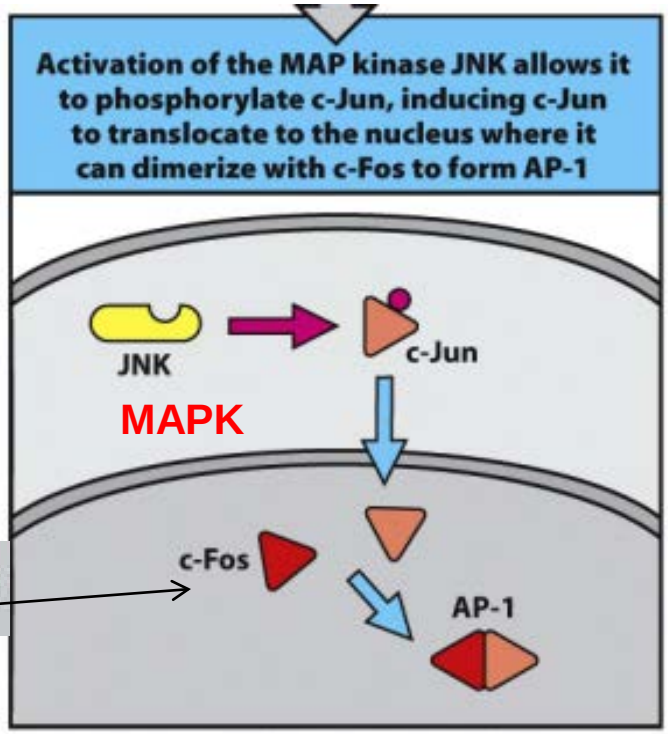
- Erk-p phosphorylates Elk1
- Elk1-p increases *FOS* transcription

- JNK phosphorylates c-Jun
- c-Jun-p nuclear translocation
- Formation of AP-1



Erk-p translocated into the nucleus





(Fig. 7.20) T cell activation also requires co-stimulatory signal

APCs:
B7.1 (CD80)
B7.2 (CD86)

Naïve T cells:
CD28

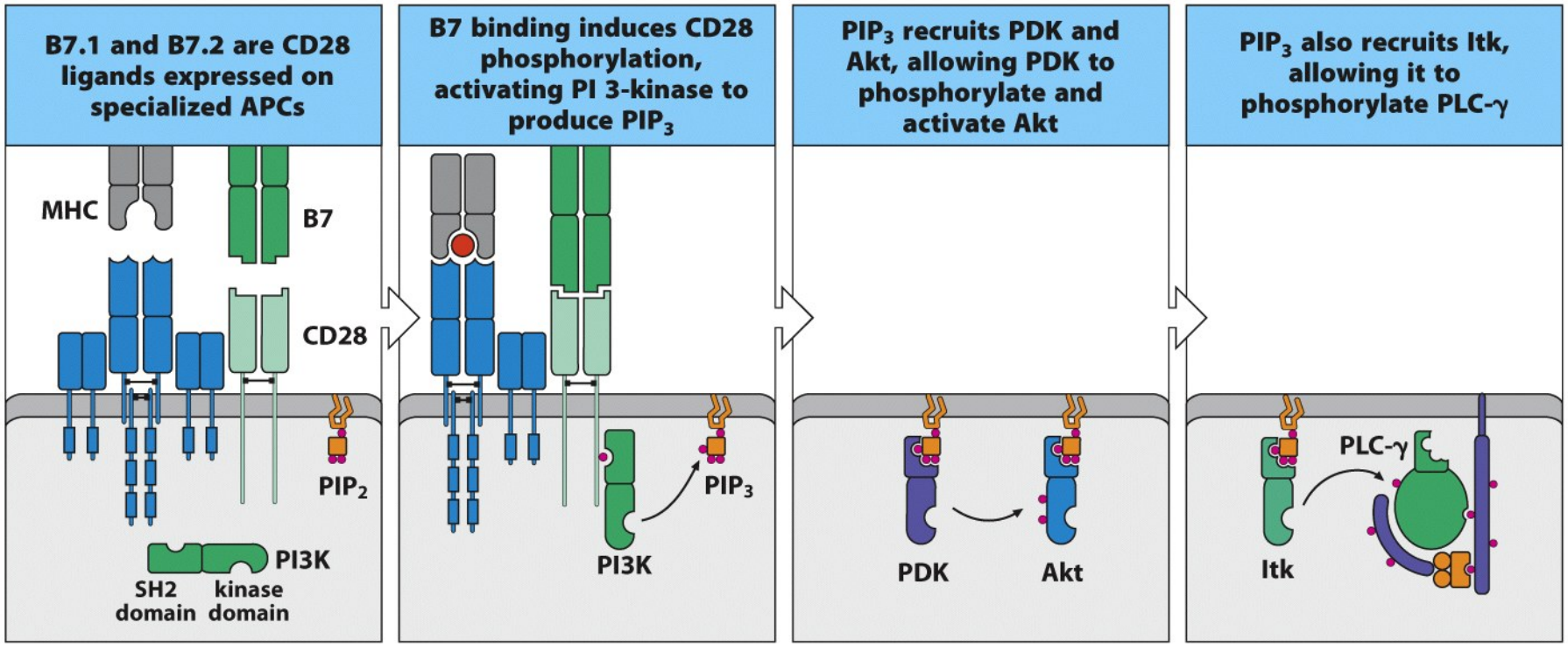


Figure 7.20 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

IL-2 transcription requires converge of multiple signaling pathways

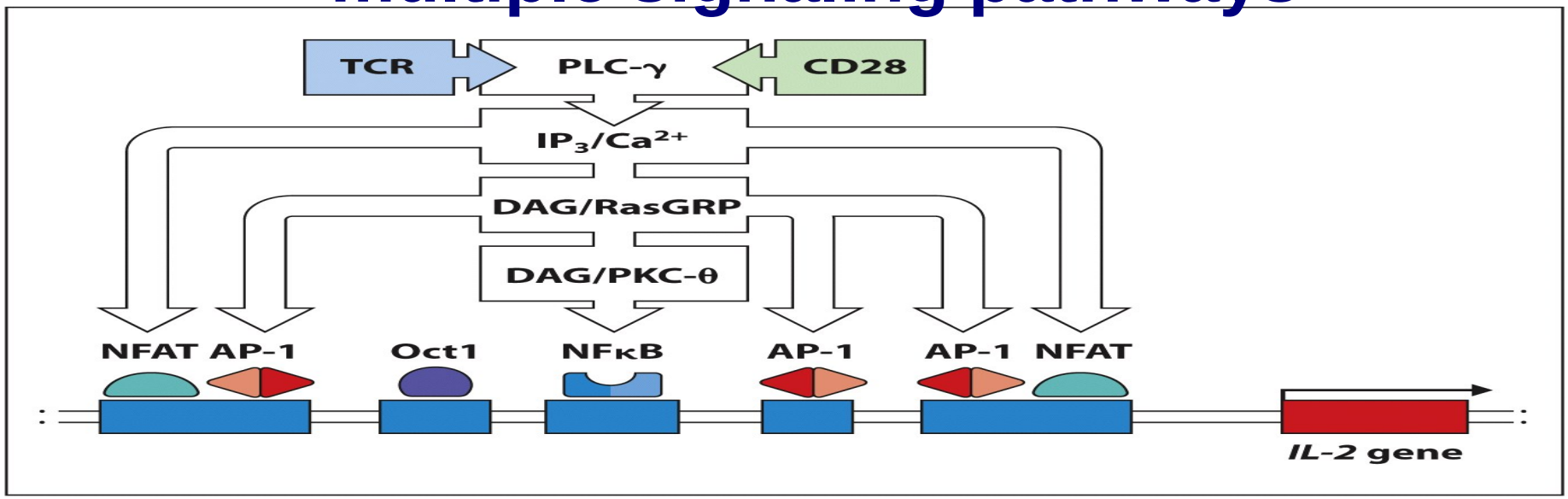


Figure 7.21 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

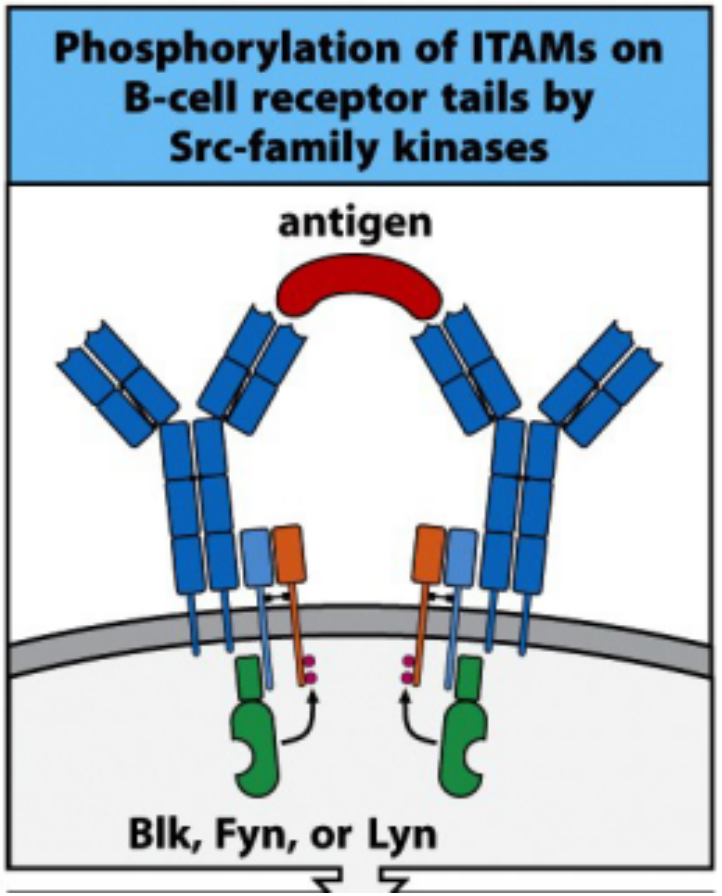
- AP-1: MAPK signaling (Fig. 6-20)
- NFAT: calcium signaling (Fig. 6-21)
- NFκB: DAG/PKCθ signaling (Fig. 6-22)
- Oct-1: constitutively bound, so not regulated by signaling

IL-2 = T-cell growth factor

- ALL are required for IL-2 transcription!!

B-cell receptor signaling

1. BCR clustering by Ag
2. Activation of receptor-associated Src-family kinases
   - e.g. Blk, Fyn, Lyn
   - Activation of ITAMs on Igα/Igβ
3. Recruitment of cytosolic adaptor
   - **Syk** (B activation) by Igβ
     - vs. **ZAP-70** (T activation)
4. Binding of Syk to the Tyr-p on the ITAM motif of BCR
   - e.g. Syk has two SH2 domains



B-cell receptor signaling

5. Bound Syk kinases (2 on BCR) are activated
   - via transphosphorylation
6. Syk-p activates CD19/BLNK/PLCγ/…etc.

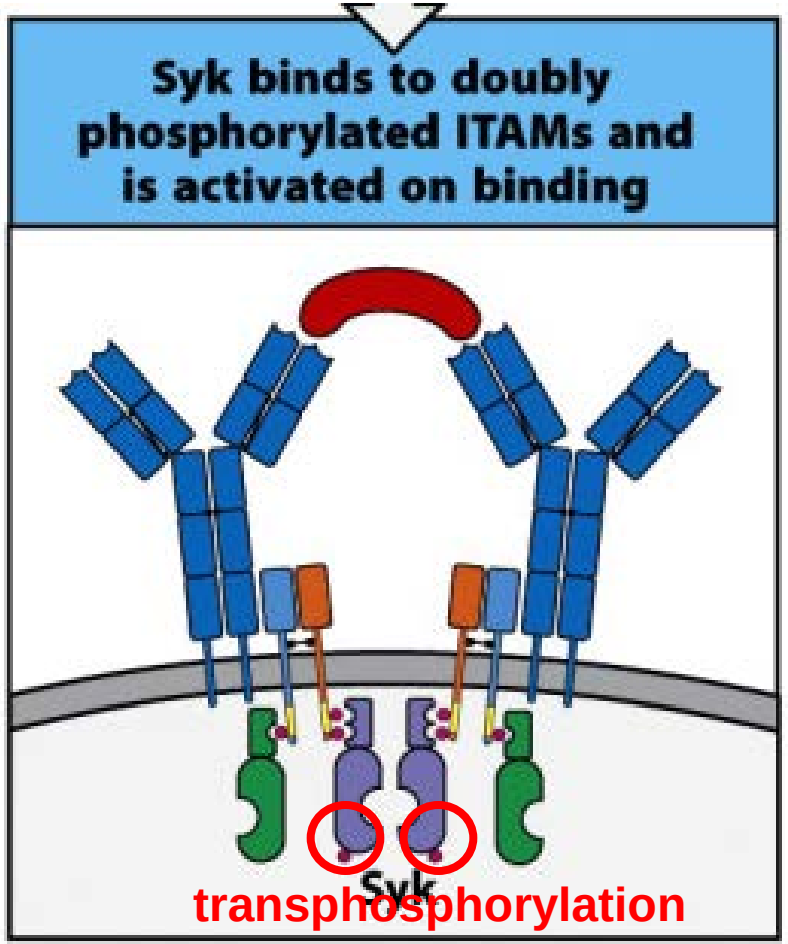


Figure 6-24 Immunobiology, 7ed. (© Garland Scie

Definition: Transphosphorylation

- When two activated kinases are very close to each other, they can phosphorylate each other.

(Fig. 7.23) B-cell co-receptors

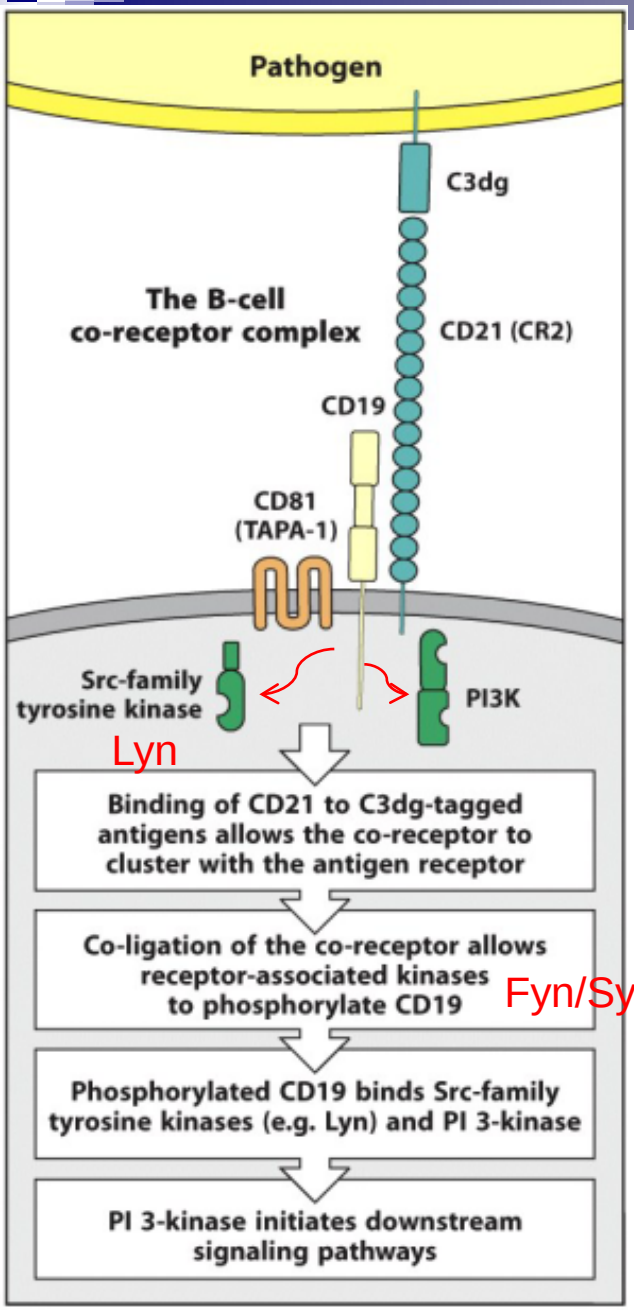


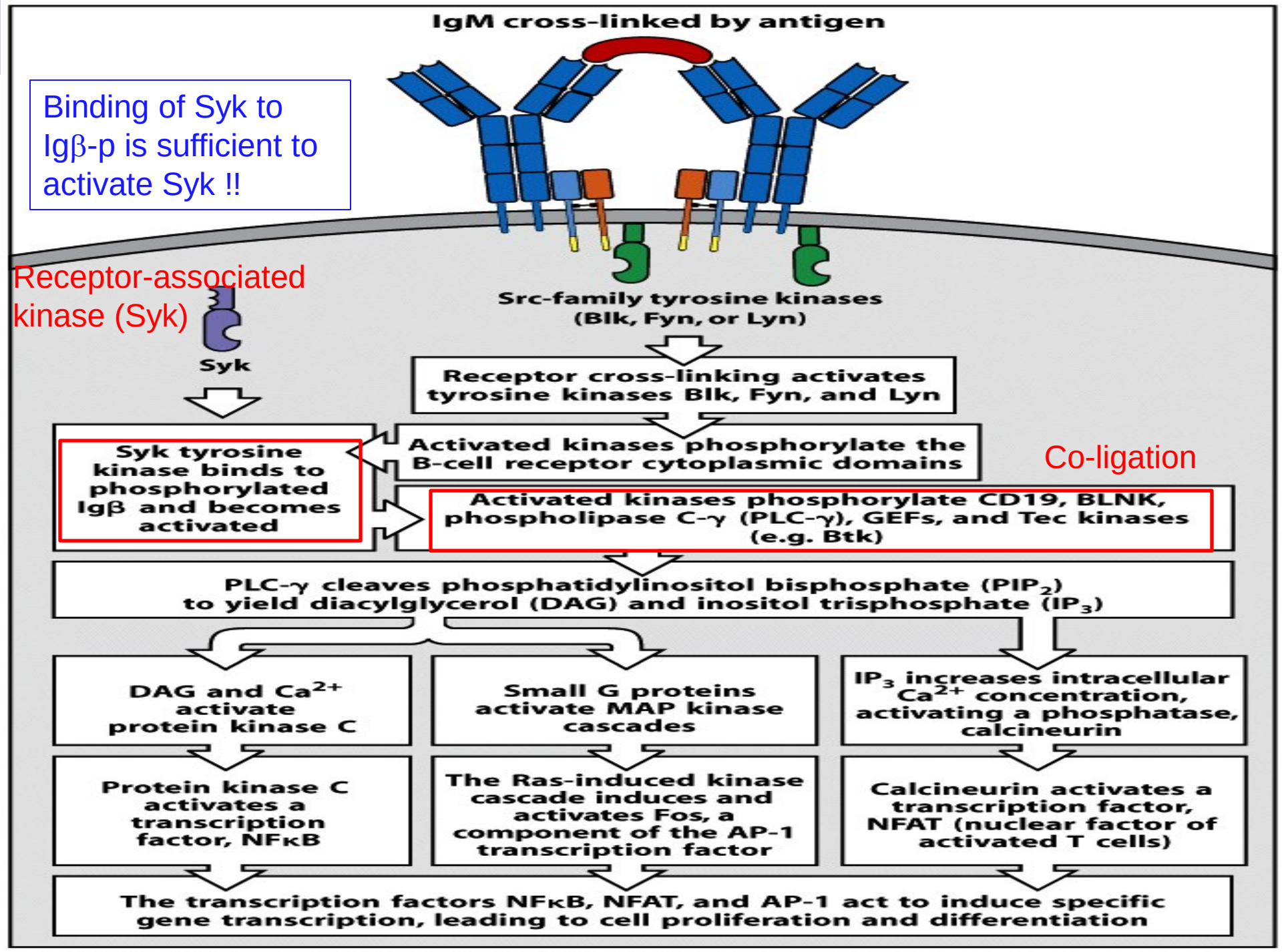
Figure 7.23 Janeway's Immunobiology, 8ed. (© Garland S

- Consisted of

  - CD19, CD21, CD81

- Expressed on mature B cells
- Clustering (Co-ligation) leads to activation of CD19 (phosphorylation)
  - By receptor-associated kinase (e.g Fyn-p)
  - Or, by other Src-family kinase (e.g. Syk-p)
- Activated CD19 binds to and phosphyrylates Src-family kinases (e.g. Lyn/Blk) & PI-3 kinase
- Clustering ensures at least 1000-fold increase in signaling intensity

  → Amplification of BCR signals!!


IgM cross-linked by antigen
Binding of Syk to Igβ-p is sufficient to activate Syk !!
Receptor-associated kinase (Syk)
Syk
Src-family tyrosine kinases (Blk, Fyn, or Lyn)
Receptor cross-linking activates tyrosine kinases Blk, Fyn, and Lyn
Activated kinases phosphorylate the B-cell receptor cytoplasmic domains
Co-ligation
Syk tyrosine kinase binds to phosphorylated Igβ and becomes activated
Activated kinases phosphorylate CD19, BLNK, phospholipase C-γ (PLC-γ), GEFs, and Tec kinases (e.g. Btk)
PLC-γ cleaves phosphatidylinositol bisphosphate (PIP_2) to yield diacylglycerol (DAG) and inositol trisphosphate (IP_3)
DAG and Ca^{2+} activate protein kinase C
Small G proteins activate MAP kinase cascades
IP_3 increases intracellular Ca^{2+} concentration, activating a phosphatase, calcineurin
Protein kinase C activates a transcription factor, NFκB
The Ras-induced kinase cascade induces and activates Fos, a component of the AP-1 transcription factor
Calcineurin activates a transcription factor, NFAT (nuclear factor of activated T cells)
The transcription factors NFκB, NFAT, and AP-1 act to induce specific gene transcription, leading to cell proliferation and differentiation

Clustering with co-receptors is crucial
in
both BCR and TCR signaling events

“Co-receptors”

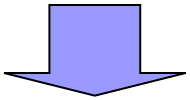
Co-receptors of BCR and TCR

- BCR
  - CD19, CD21(CR2), and CD81(TAPA-1)
- TCR
  - CD4 (T_H)
  - CD8 (T_C)

- Purpose: Co-receptors can **enhance** BCR/TCR signaling by **aggregating (clustering)** with either BCR/TCR

Outcome of Ag recognition

Activation of transcription factors



Induction of new gene synthesis (for survival and proliferation)

(Fig. 7.25) ITAMs are also present in immune cells other than B and T cells

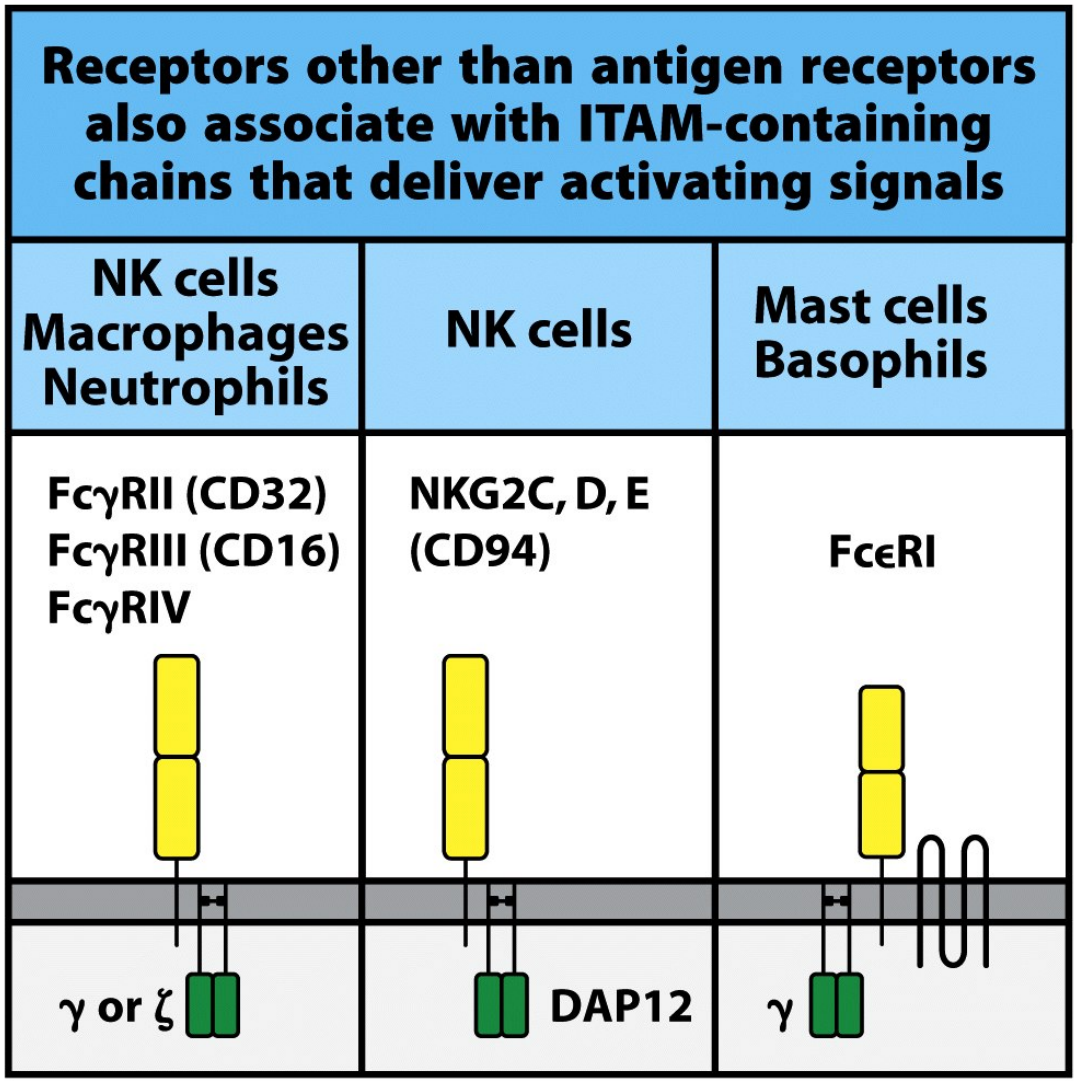


Figure 6-27 Immunobiology, 7ed. (© Garland Science 2008)

(Fig. 7.27) Immunoreceptor tyrosine-based inhibitory motif (ITIM)

- Canonical sequence
  - [I/V]XYXXL
- Opposite function as to ITAM
- Recruits inhibitory phosphatase
  - SHP-1: removes phosphate group added by tyrosine kinase
  - SHIP: removes 5'-P on PIP3
- Transduces (-) signal for the inactivation of the receptors

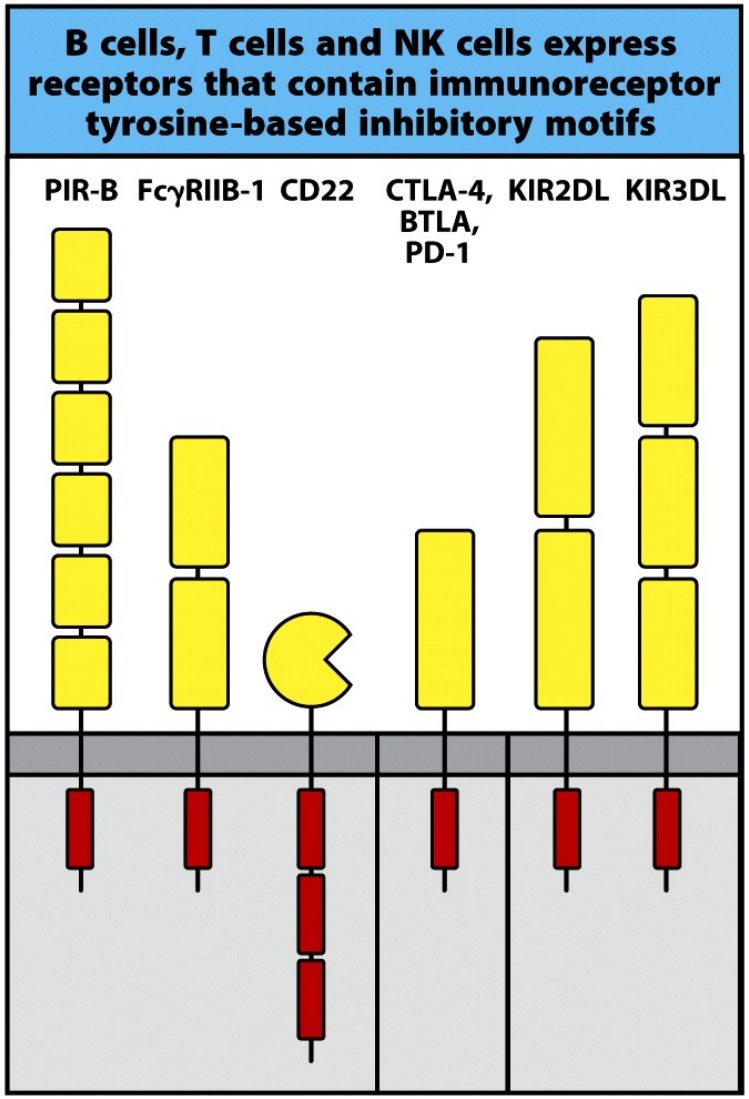


Figure 6-29 Immunobiology, 7ed. (© Garland Science 2008)

Other receptors
and
signaling pathways

Cytokine-activated Janus kinases (JAKs) → the "JAK-STAT pathway"

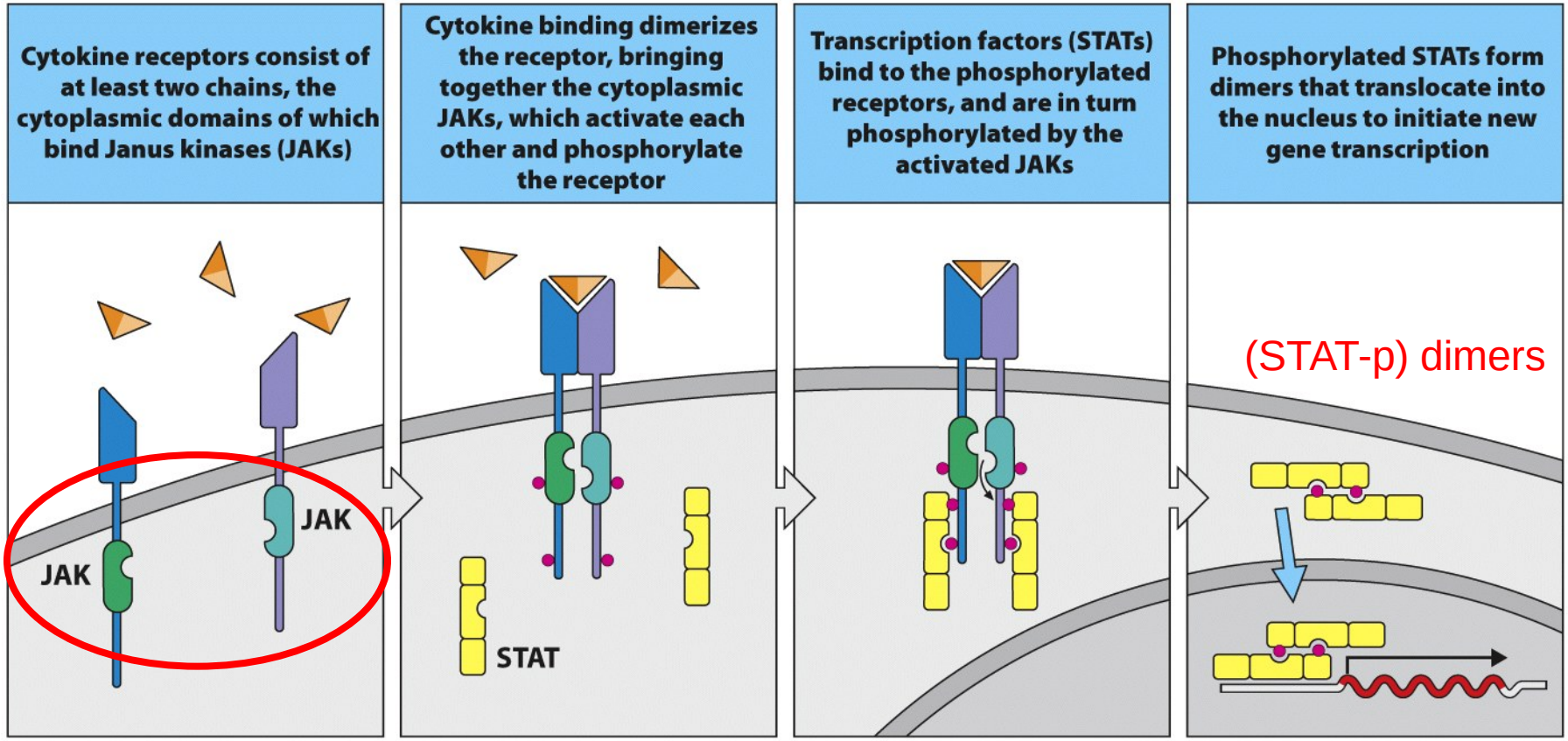


Figure 7.29 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

"**S**ignal **t**ransducers and **a**ctivators of **t**ranscription" (STAT)

(Fig. 7.30) Fas-FasL pathway

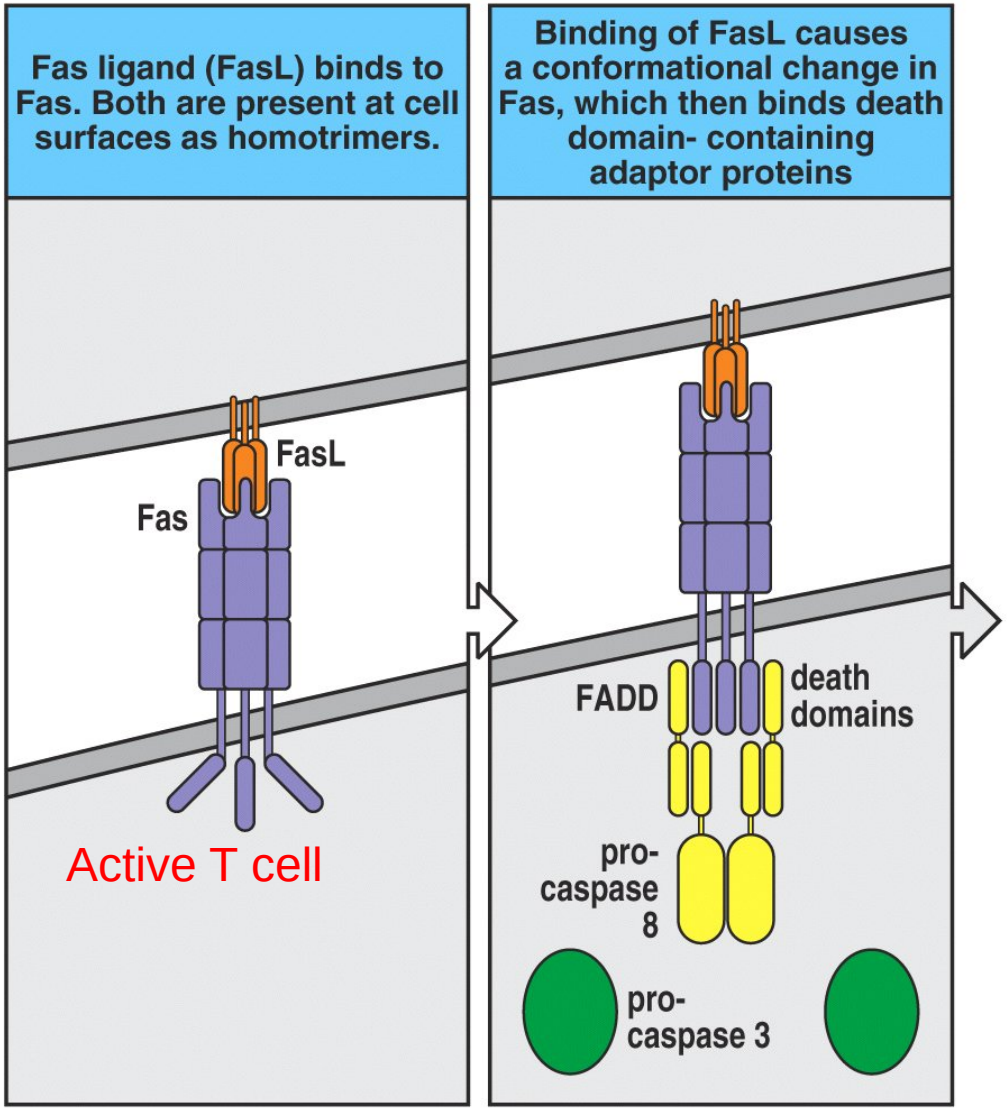


Figure 6-24 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Apoptosis:
Purpose → To 'inactivate' activated T cells once the infection is gone!!

Pathway involves the activation of a series of cysteine protease that cleave at the C-terminus of the subsequent protein in play.

Apoptosis

Extrinsic Intrinsic

(initiator caspase)

caspase 8 caspase 9

(effector caspases)

3

6

7

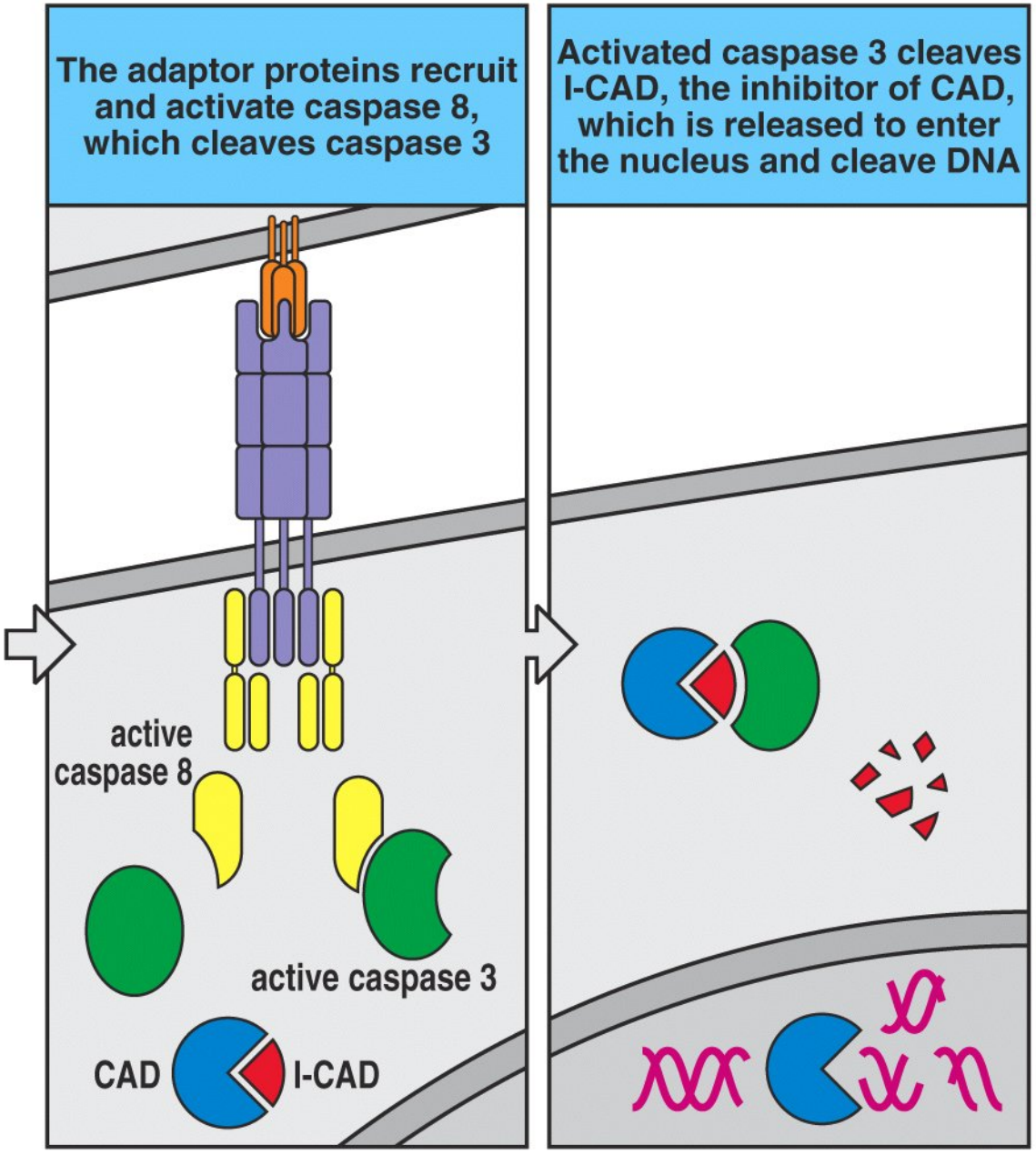


Figure 6-24 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Caspase-activated DNase (CAD)

(Fig. 7.32) Swelling/Leaking of mitochondria can induce the CAD-mediated cell death

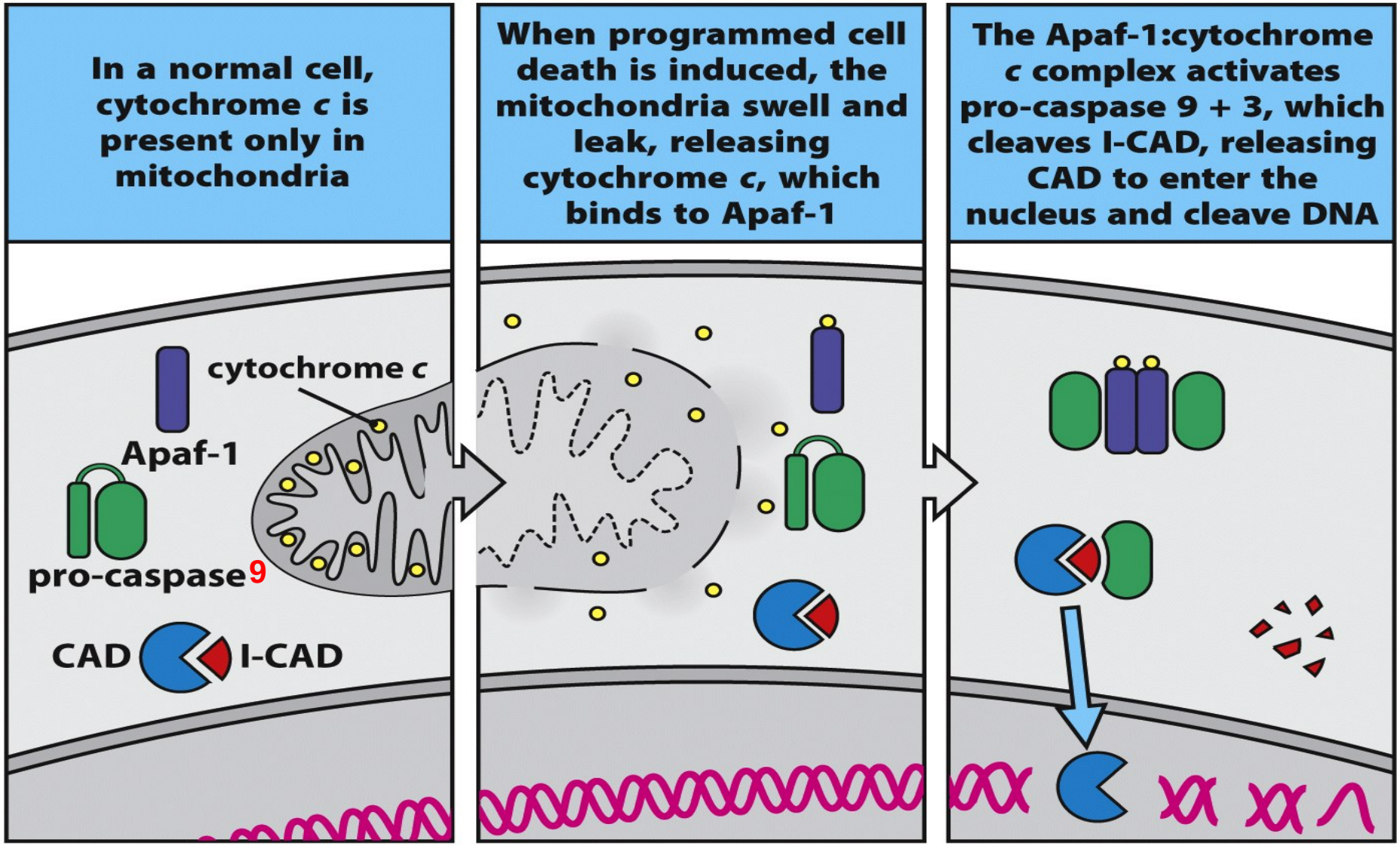


Figure 6-33 Immunobiology, 7ed. (© Garland Science 2008)

Death-inhibiting gene family

- First identified from B-cell lymphoma
  - *bcl-2* (encodes Bcl-2)
    - Belonged to a gene family containing BOTH death-promoting and death-inhibiting genes
      - Inhibiting: *bcl-2*, *bcl-XL*
      - Promoting: *bax*, *bad*
      - Requires dimerization to become active
      - All members possess the Bcl-2 homology (BH2) domains
    - Cell lives or dies is dependent upon the abundance of either death-inhibiting or -promoting protein products

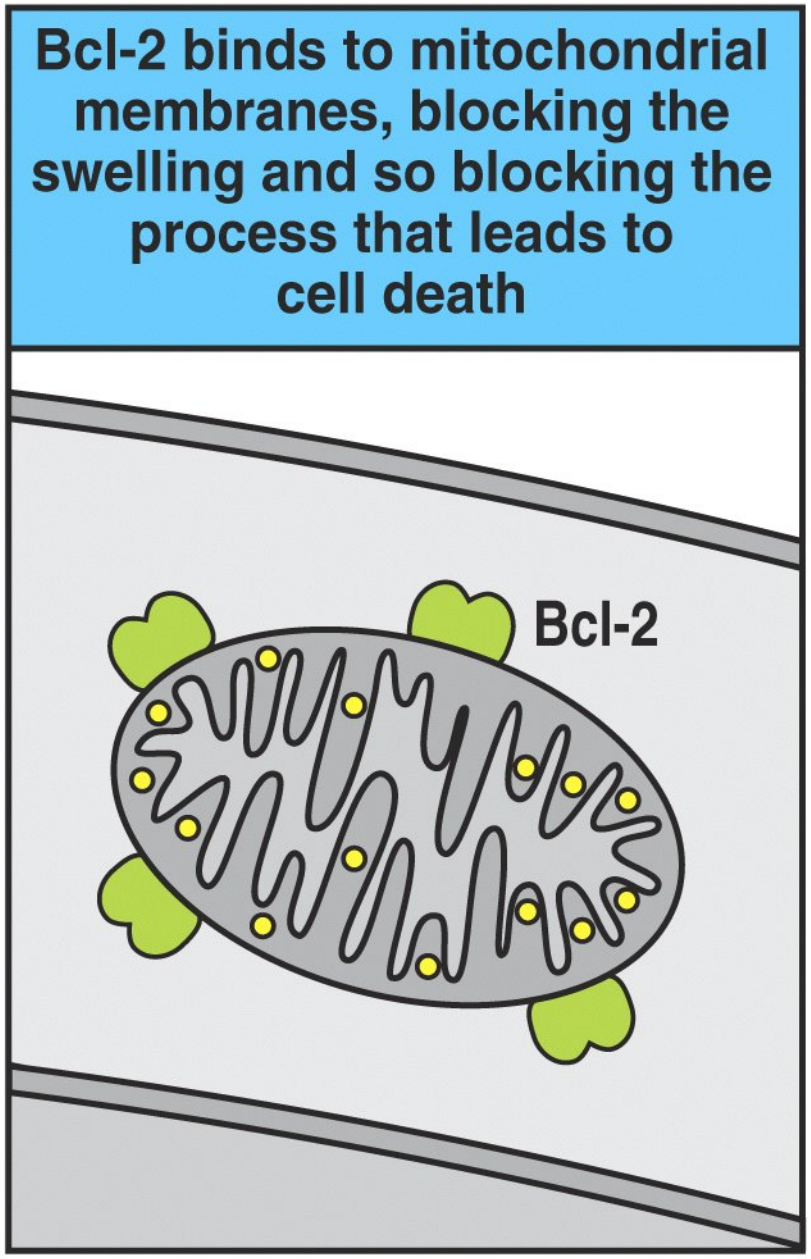


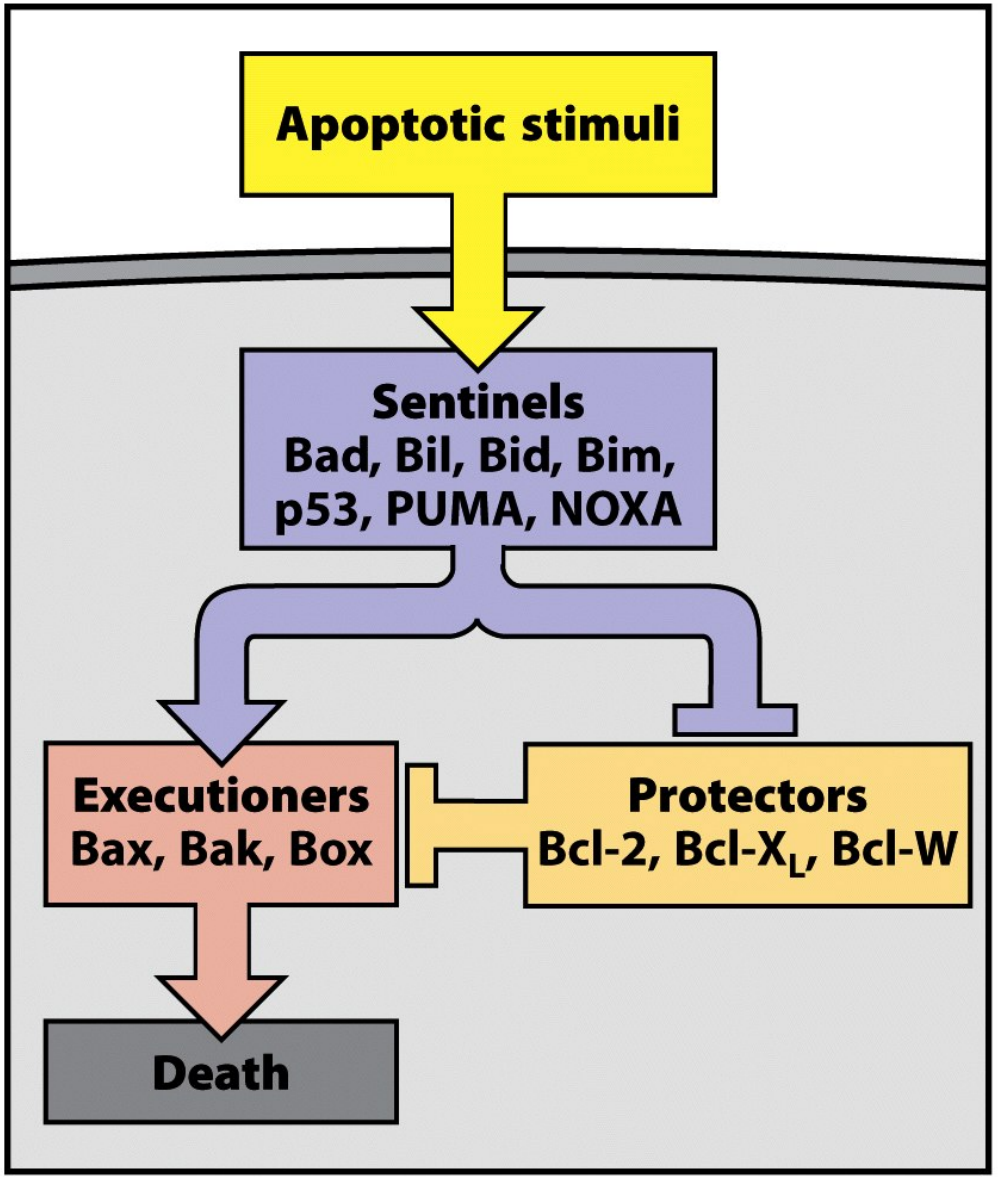
Figure 6-25 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Bcl-2 can inhibit cell death by binding to and maintaining the integrity of mitochondria

(Fig. 7.33) Two Bcl-2 family members

1. Pro-apoptotic

1. Pro-apoptotic



2. **Anti-apoptotic**
(or pro-survival)

Figure 6-34 Immunobiology, 7ed. (© Garland Science 2008)

Summary

- Both B and T lymphocytes require signaling through their membrane-bound receptors for activation.
- Multiple signaling pathways contribute to lymphocyte behavior.

End of Chapter