Signal Transduction in Immune Cells

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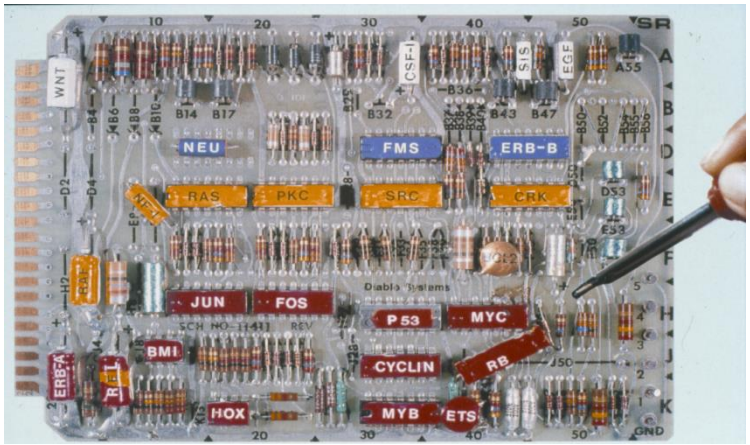
RUB

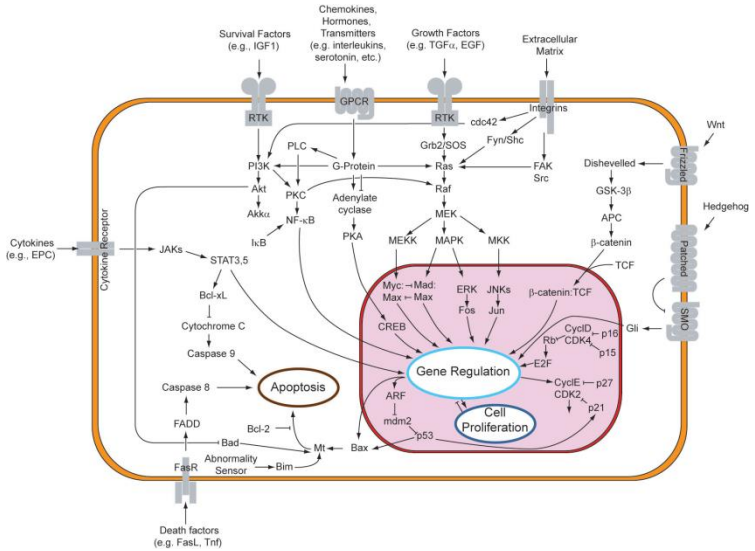
Molecular Immunology

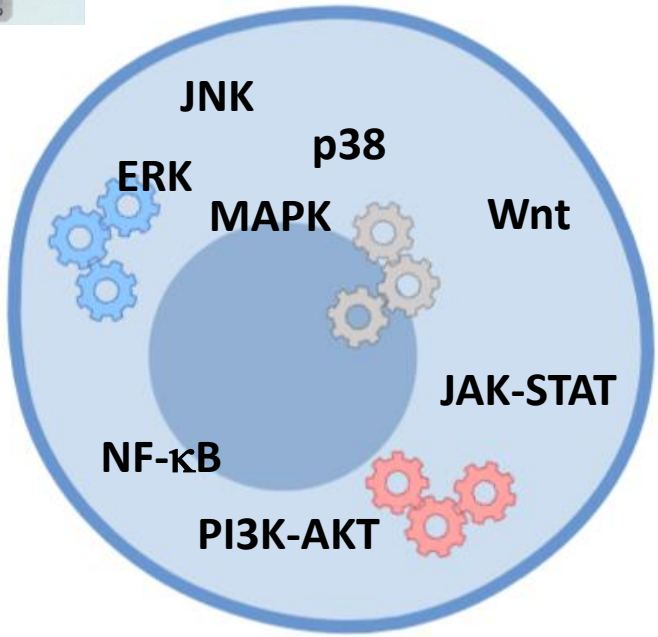
Index

- General Principles of Signal Transduction
  - Introduction
  - Signals
  - Receptors
  - Mediators
- T cell receptor (TCR) signaling
- NFAT signaling pathway
- IL-2 signaling pathway

Introduction







Introduction

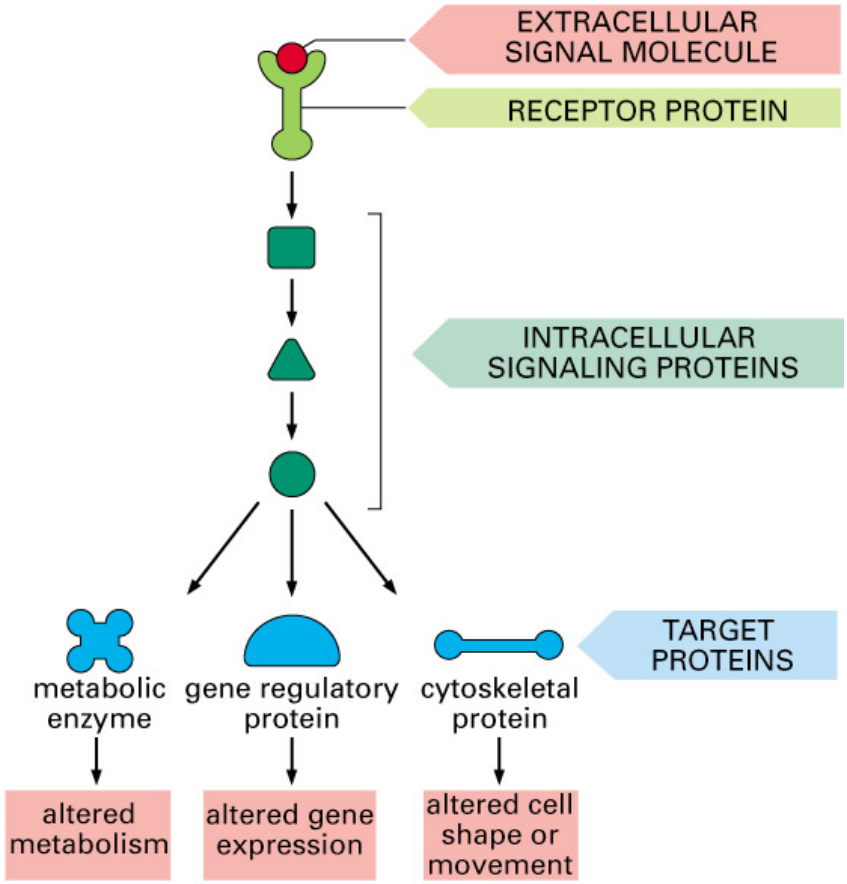
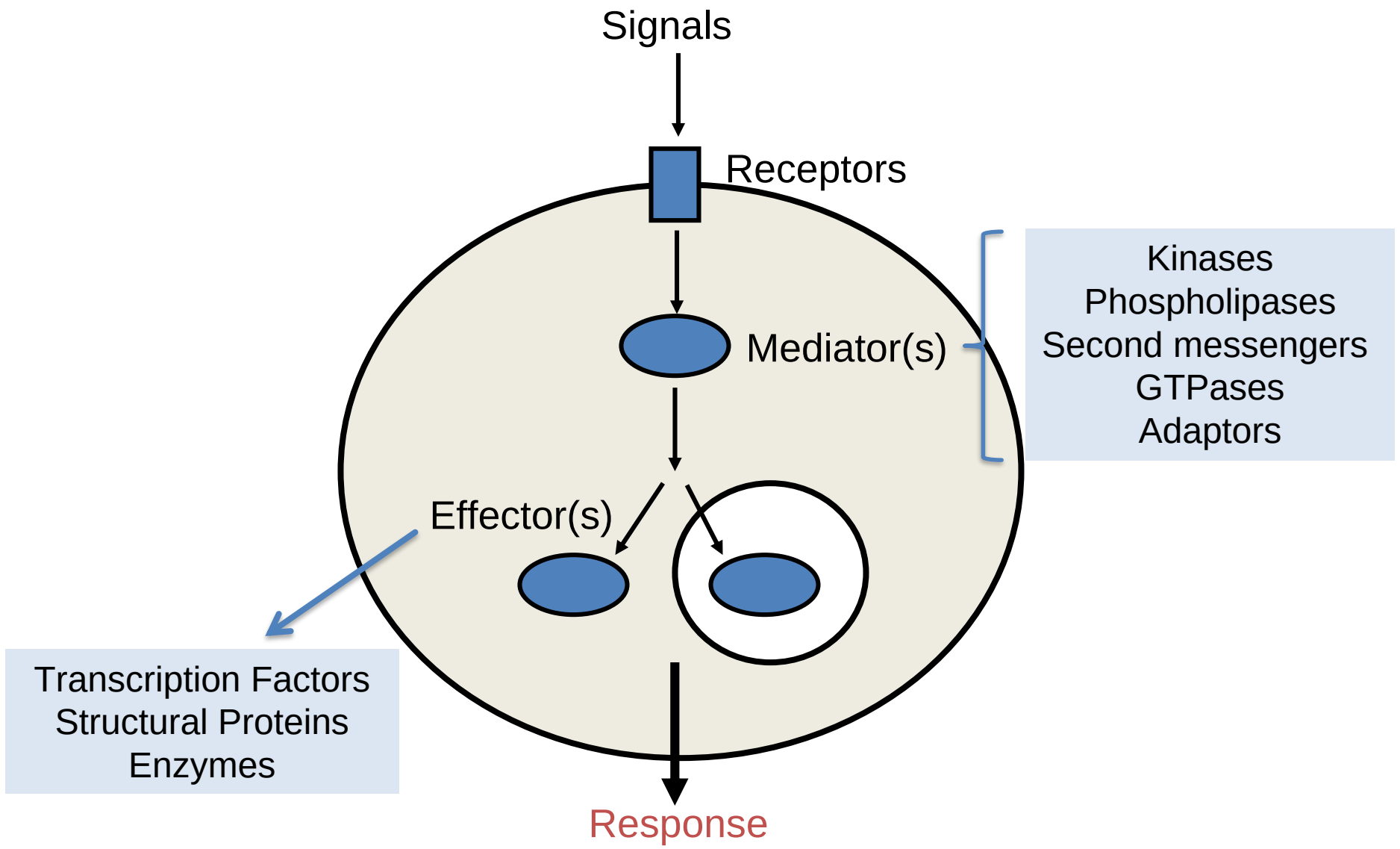


Figure 15–1. Molecular Biology of the Cell, 4th Edition.

Introduction



Response

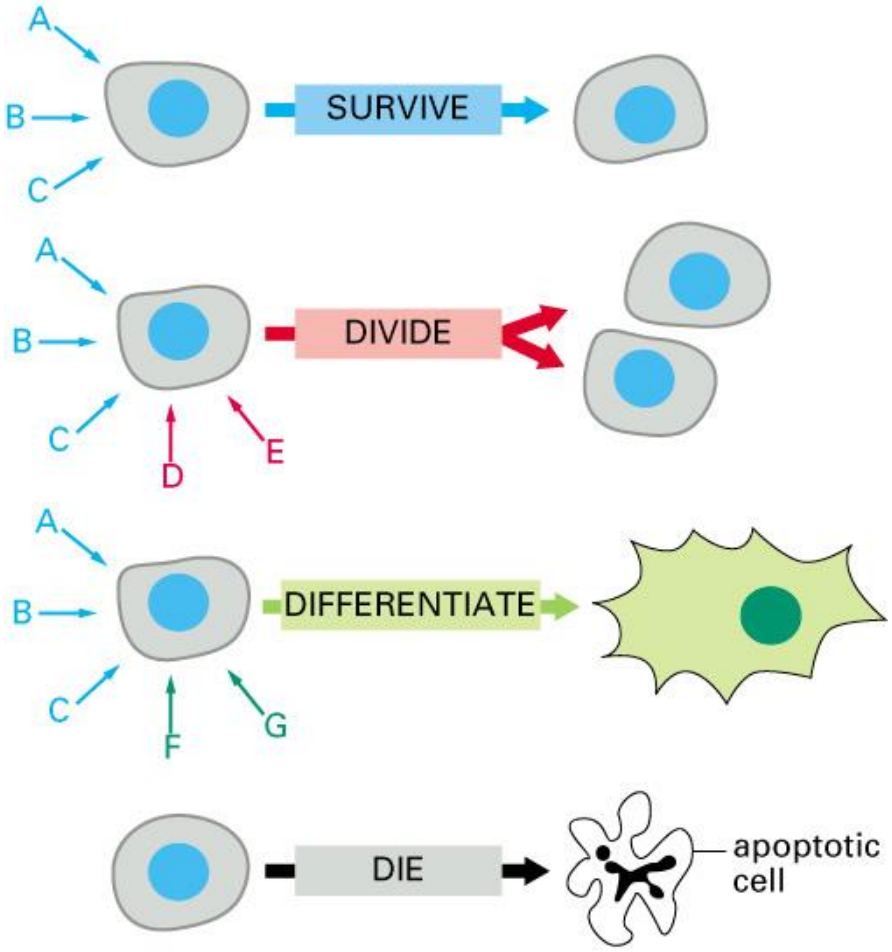
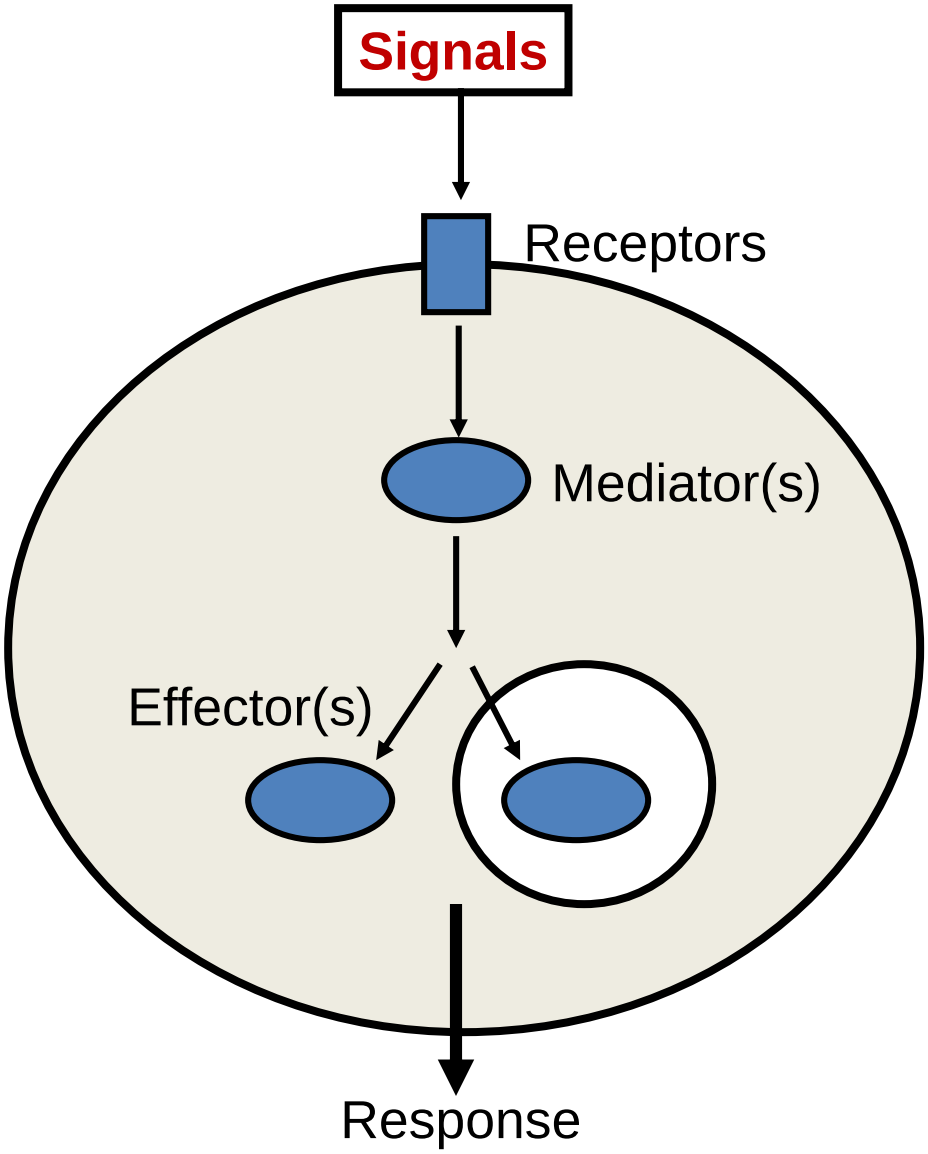


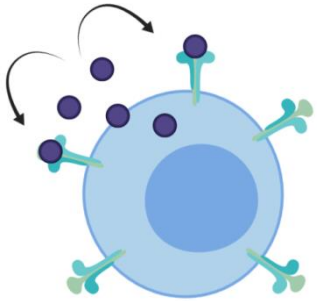
Figure 15–8. Molecular Biology of the Cell, 4th Edition.

Signals

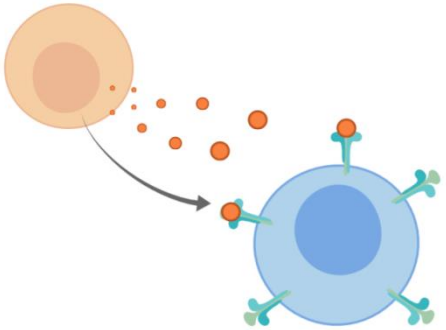


Signals

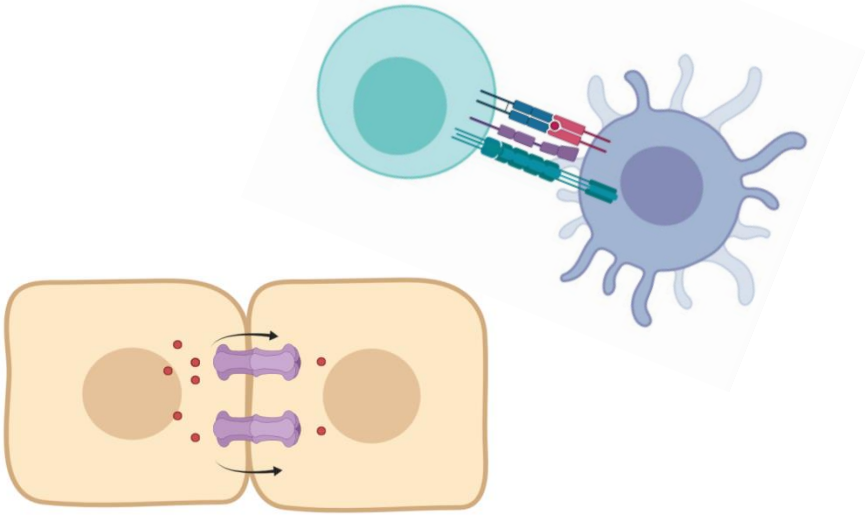
Autocrine



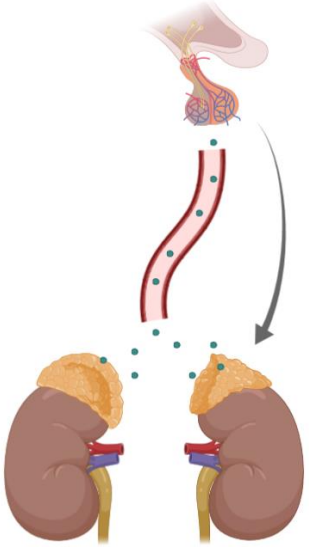
Paracrine



Juxtacrine



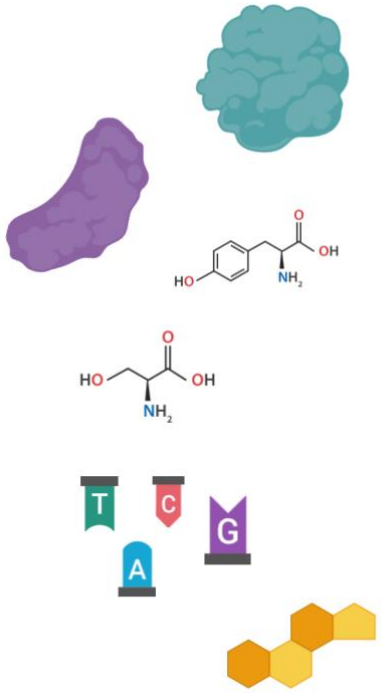
Endocrine



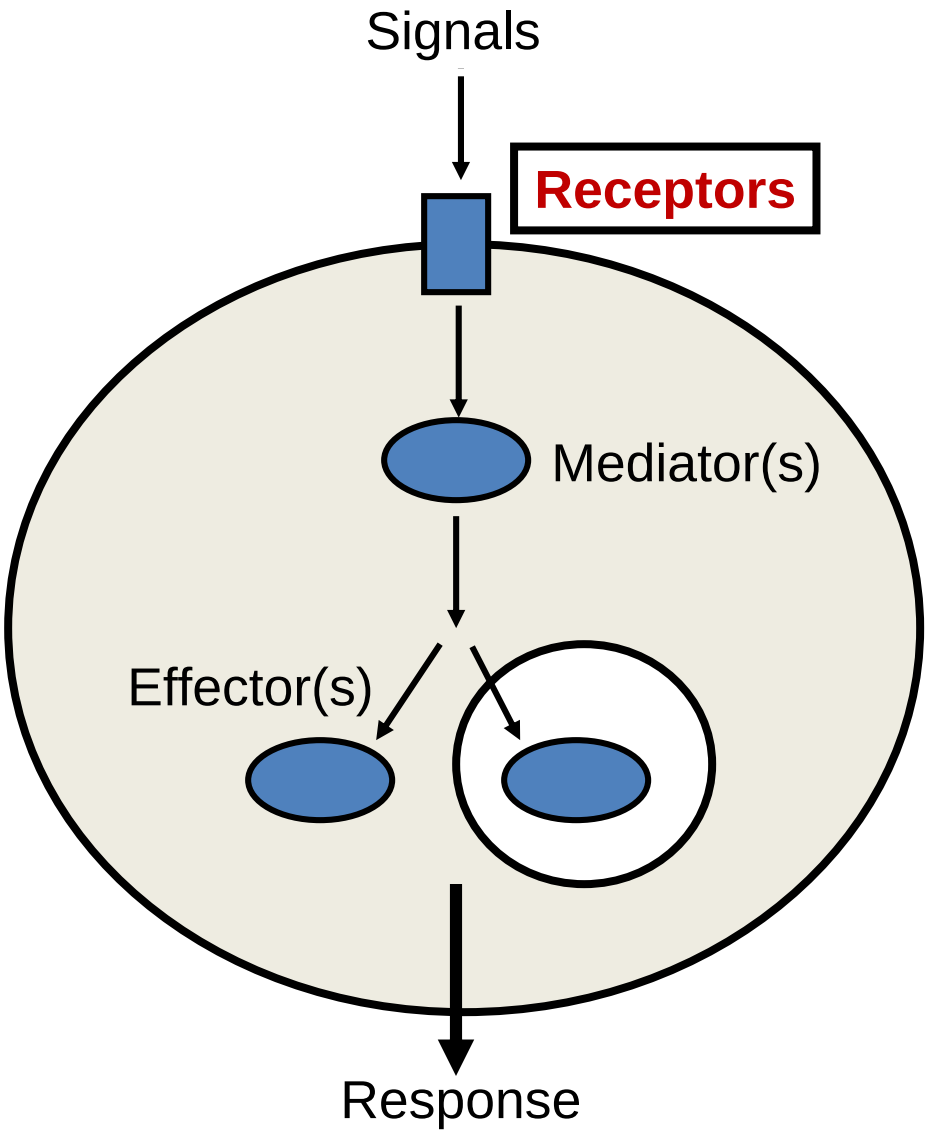
Signals

Ligands

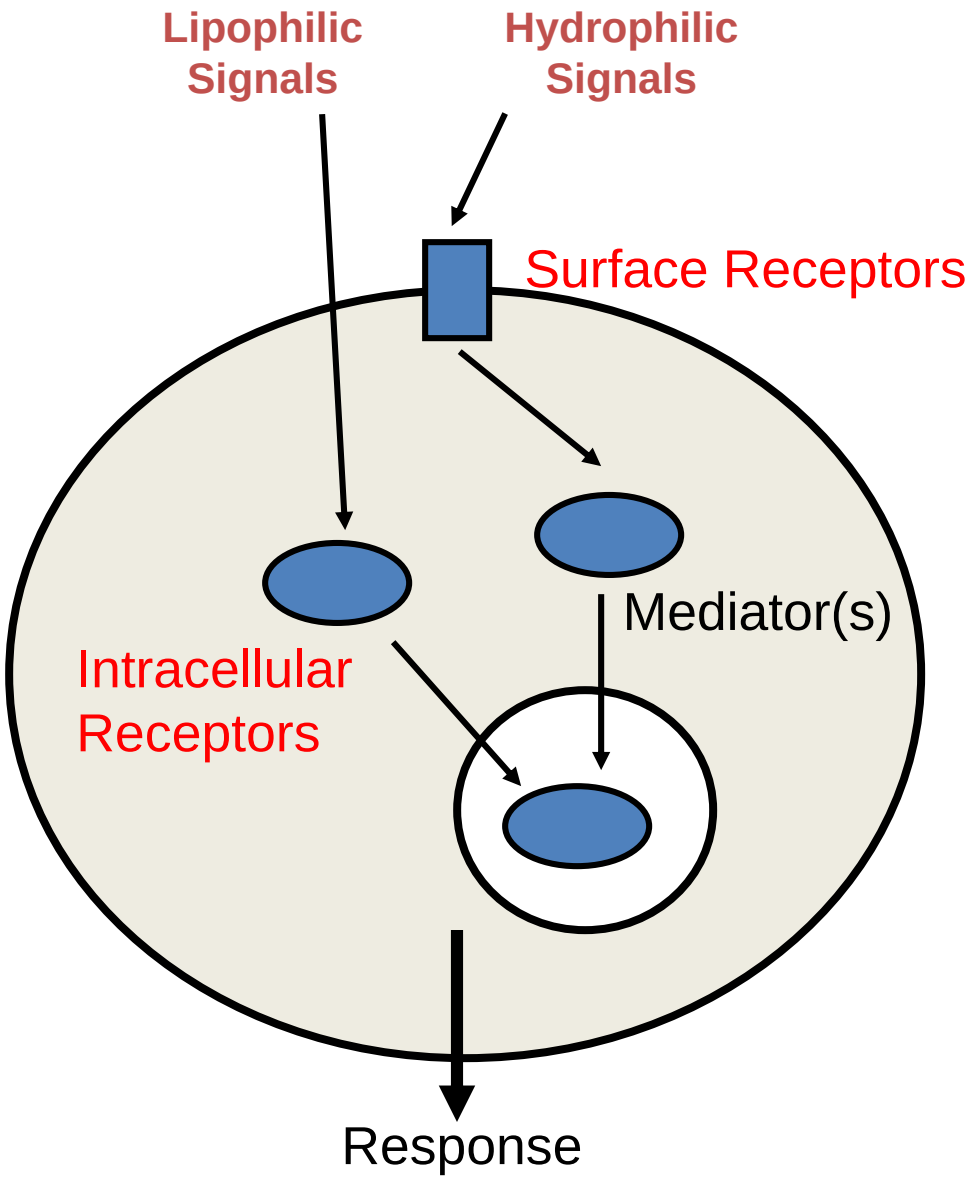
Proteins
Small peptides
Aminoacids
Nucleotides
Steroids
Retinoids
Fatty acids derivatives
NO, CO



Receptors



Receptors

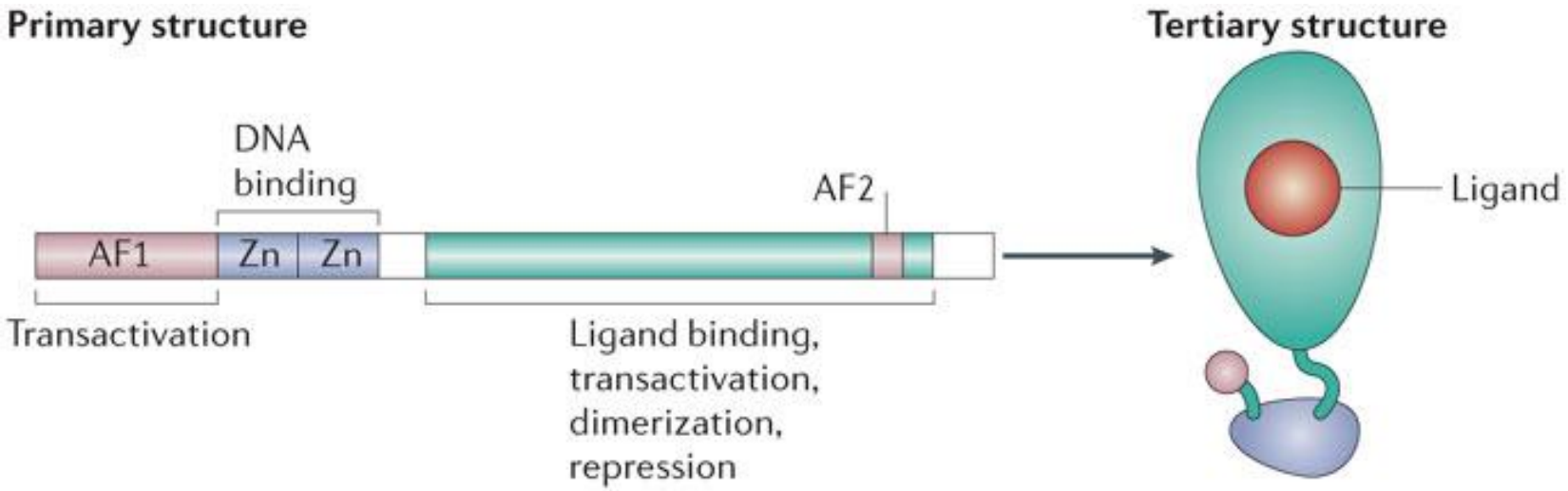

Lipophilic Signals
Hydrophilic Signals
Surface Receptors
Mediator(s)
Intracellular Receptors
Response

Intracellular Receptors

Lipophilic ligands

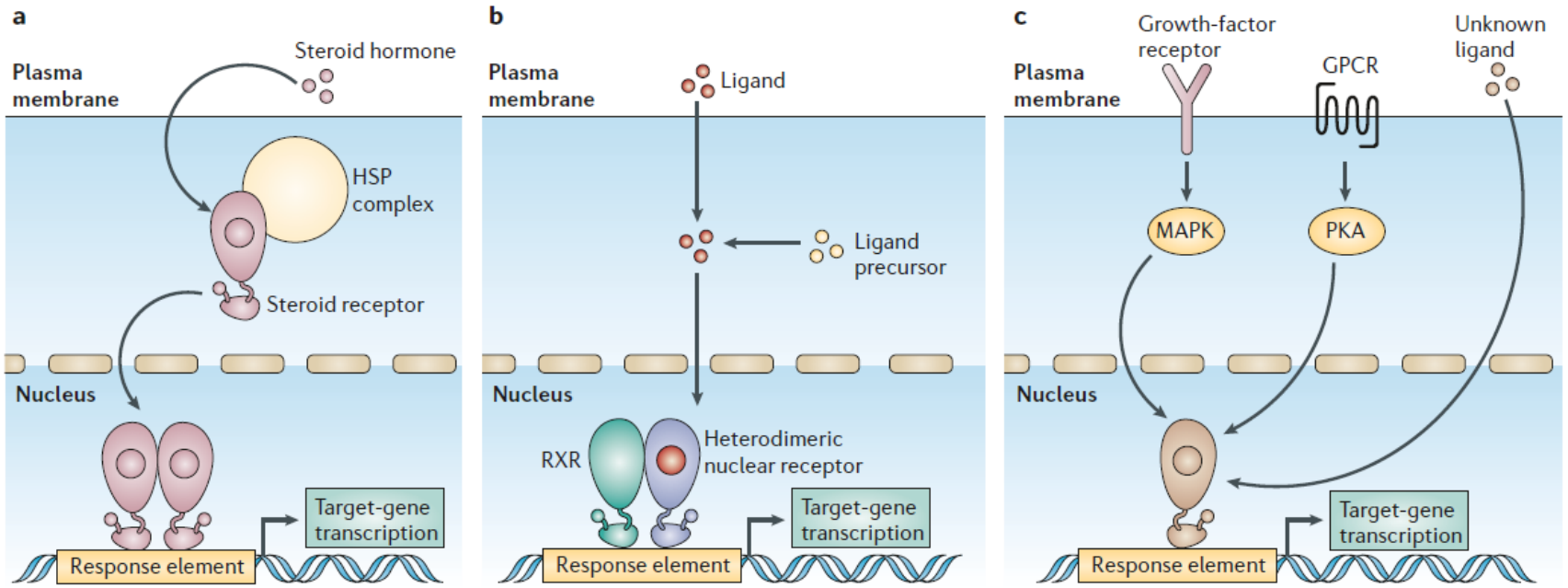
- Estrogen, progesterone, testosterone
- Vitamin D
- Glucocorticoids and mineralocorticoids
- Thyroid hormone

Domain structure of nuclear receptors



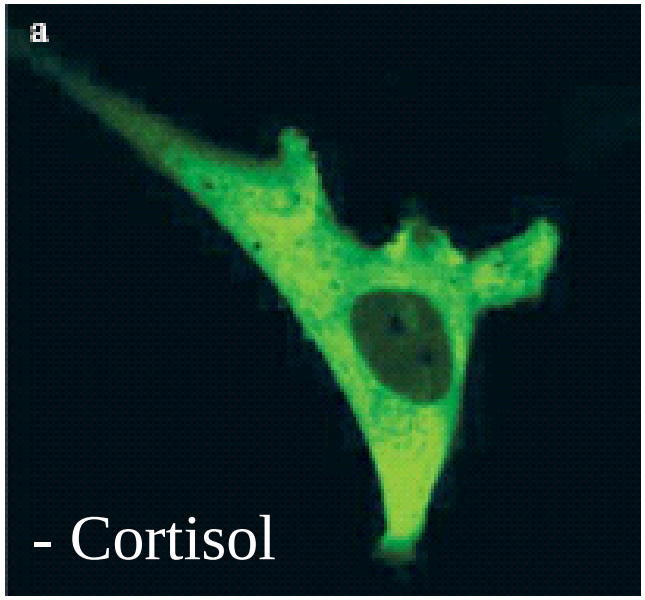
Christopher KG & Sumito O (2006) Combinatorial roles of nuclear receptors in inflammation and immunity
Nat Rev Immu 6: 44–55 doi:10.1038/nri1748

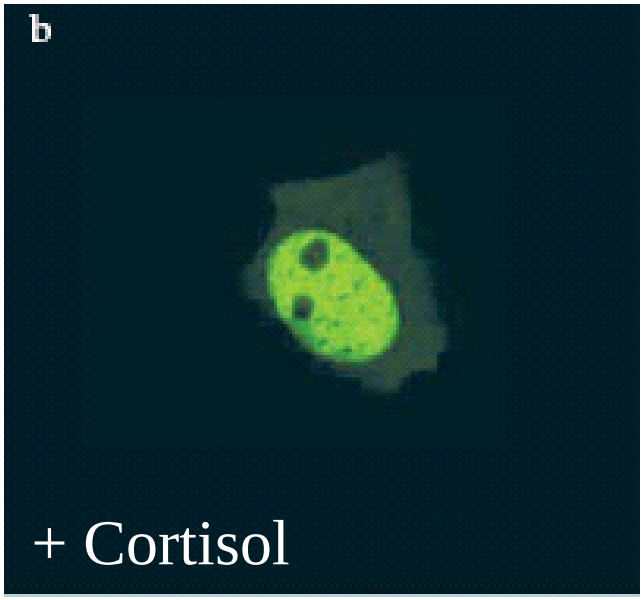
Nuclear-receptor classes



Christopher KG & Sumito O (2006) Combinatorial roles of nuclear receptors in inflammation and immunity
Nat Rev Immu 6: 44–55 doi:10.1038/nri1748

Intracellular dynamic of GFP-labelled glucocorticoid receptor





Anti-inflammatory properties of Glucocorticoids

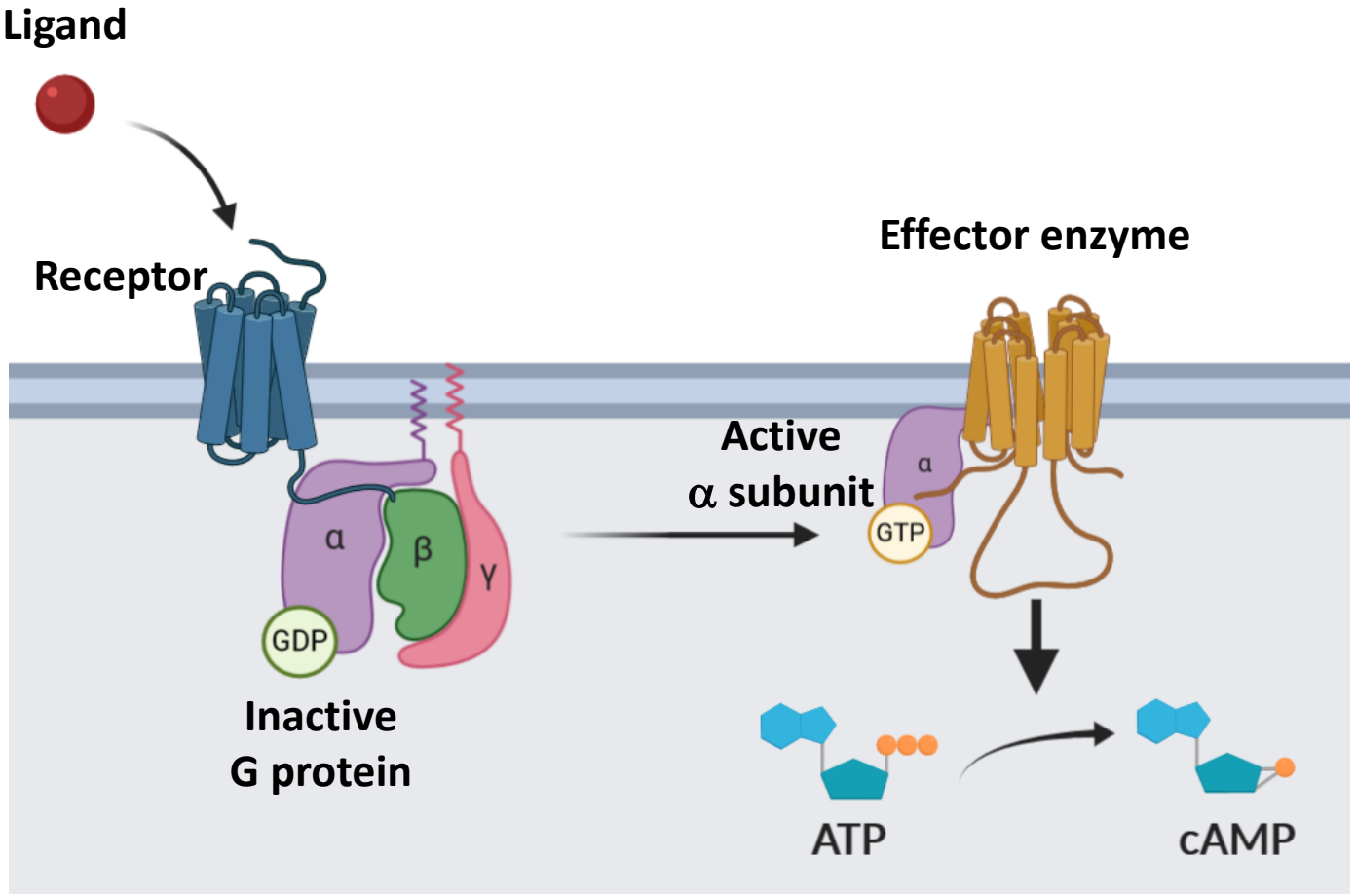
- Glucocorticoids inhibit NF-κB and AP-1 pathway activation preventing expression of inflammatory genes

- Glucocorticoids induce apoptosis of immature ($CD4^+$ $CD8^+$ SP) thymocytes

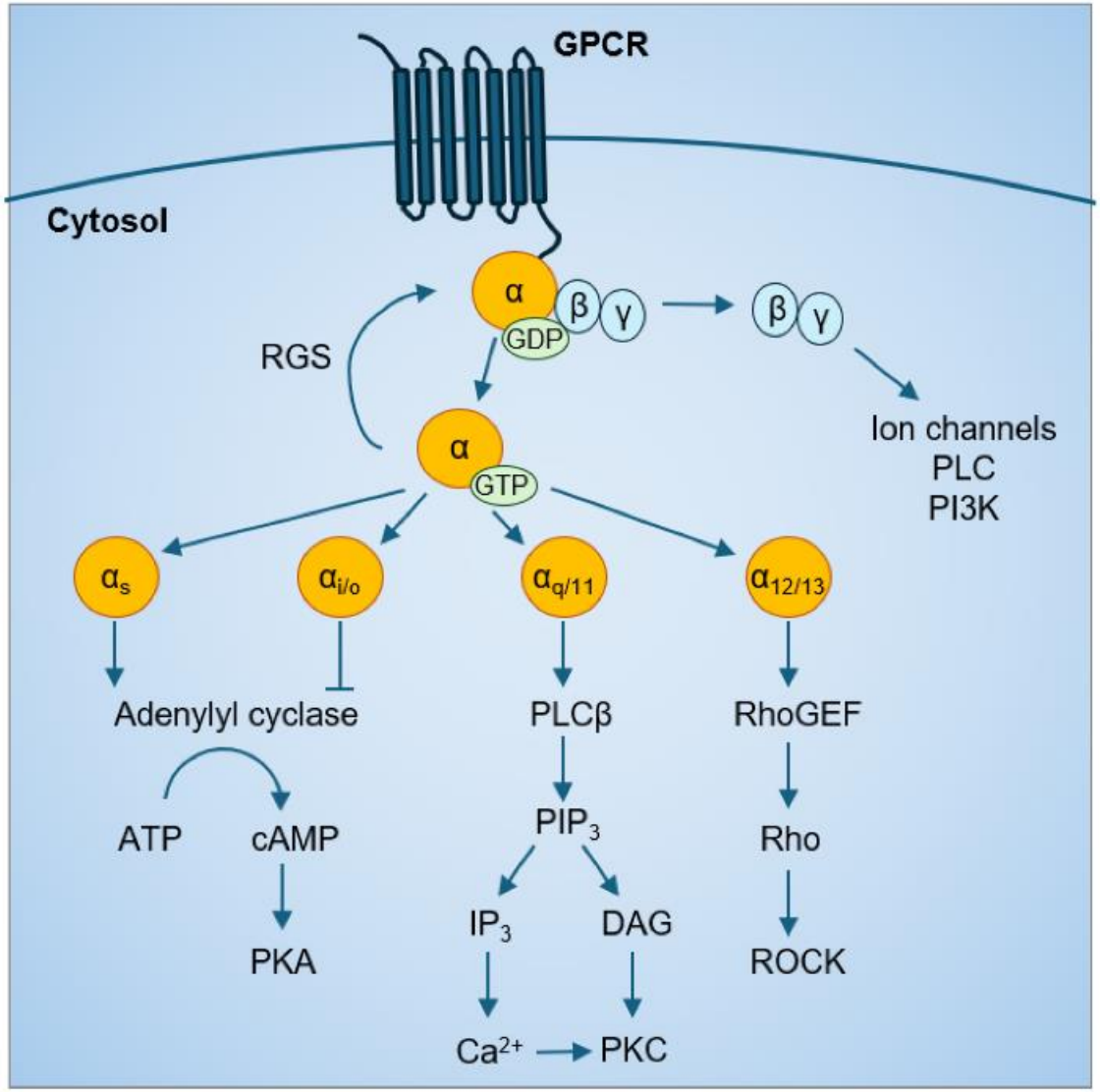
Surface Receptors

- G-protein coupled receptors (GPCR)
- Ion channel receptors
- Tyrosine kinase-linked receptors
- Receptors with intrinsic enzymatic activity

G-protein coupled receptors (GPCR)

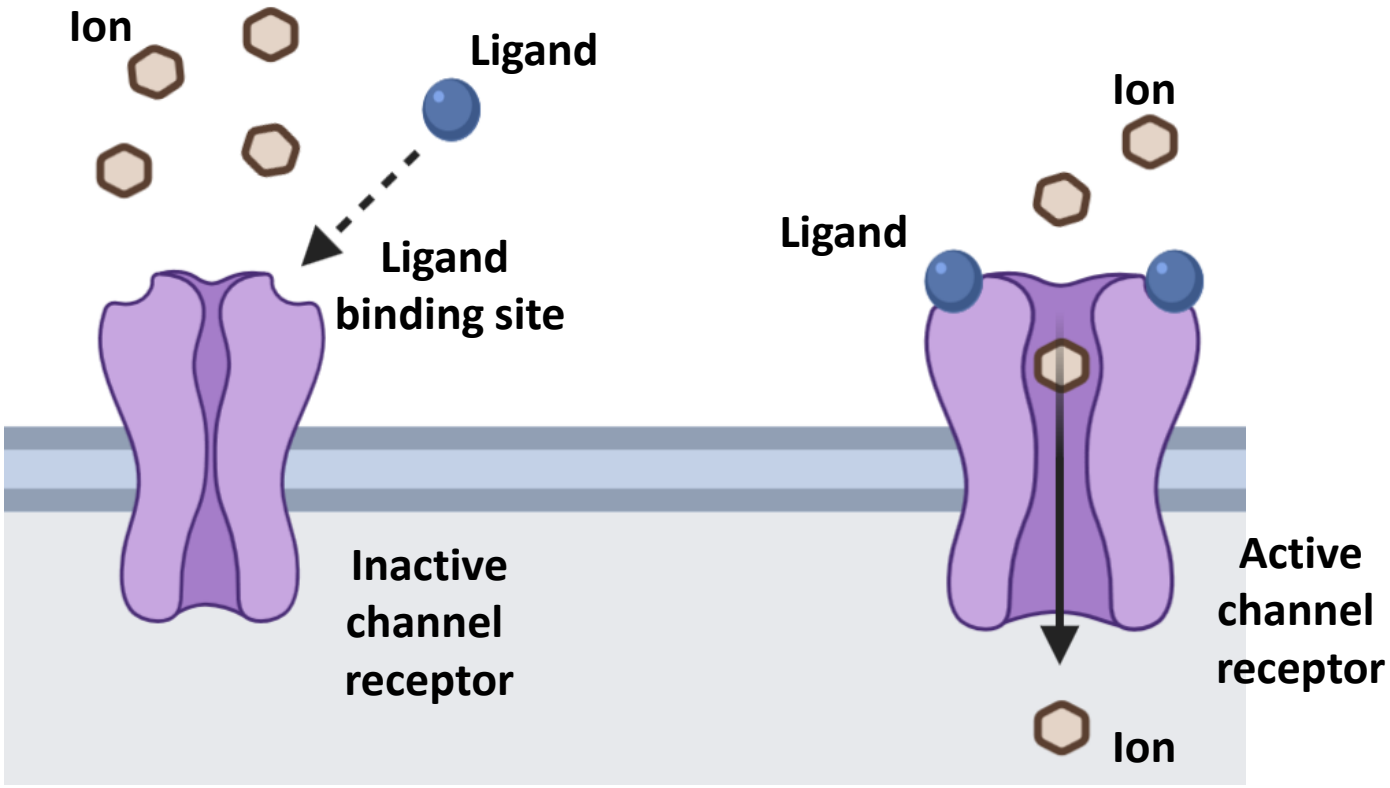


G-protein coupled receptors (GPCR)

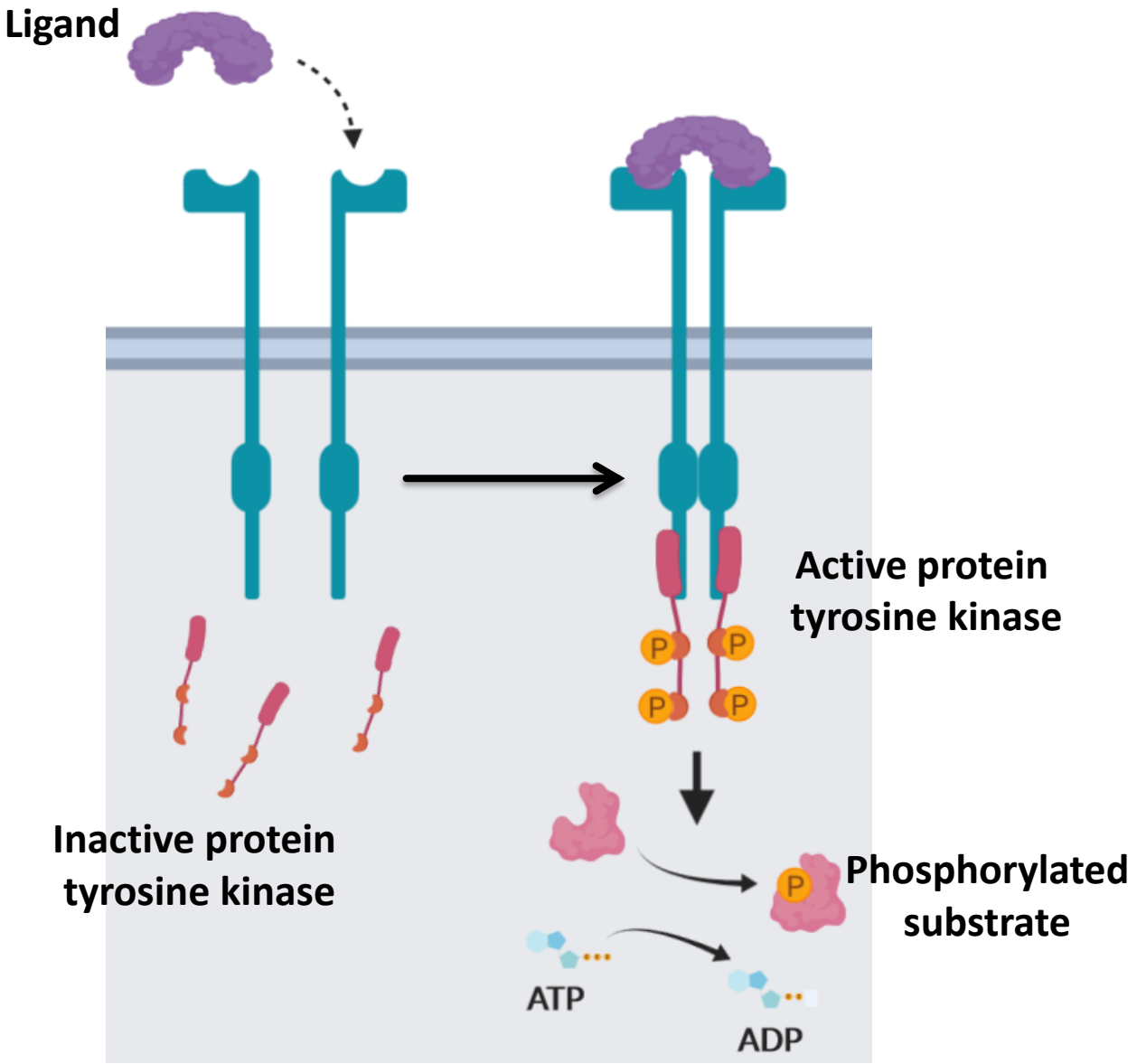


https://doi.org/10.3390/ijms25137162

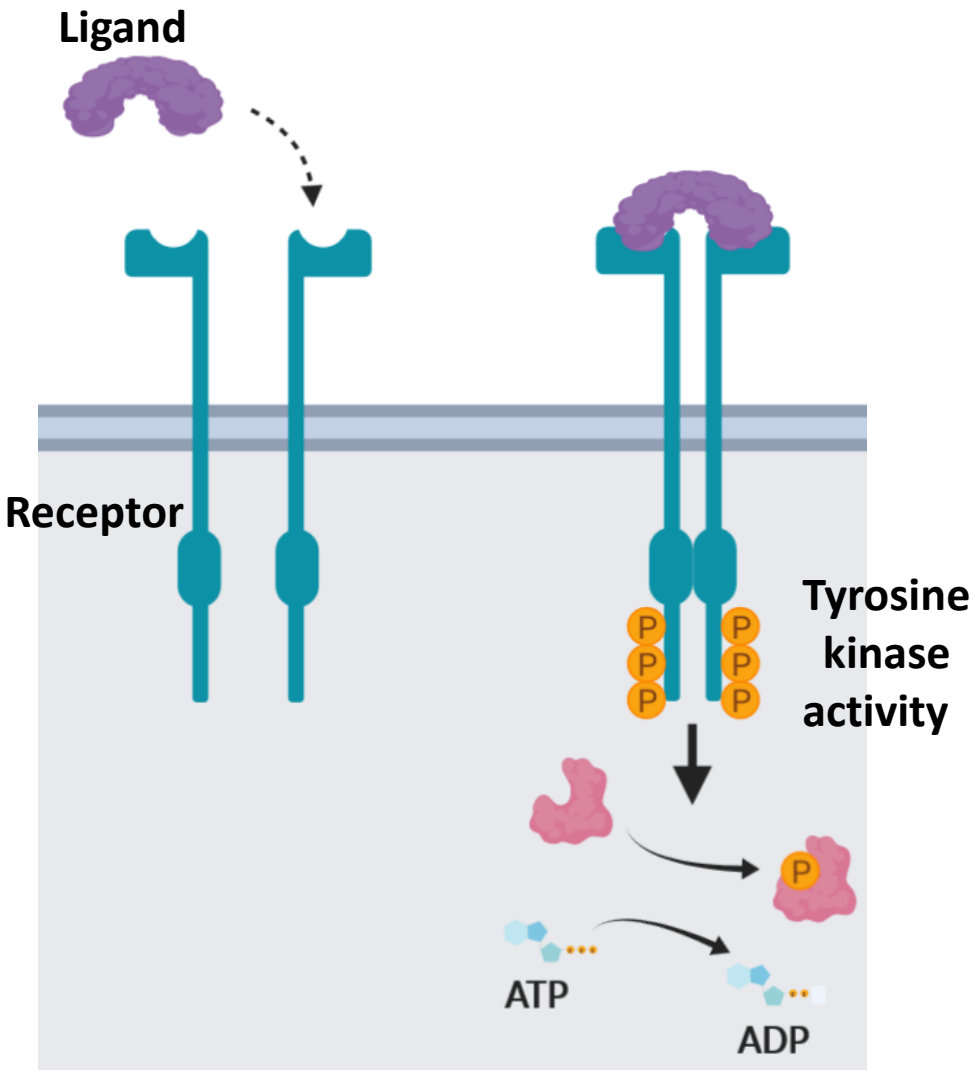
Ion channel Receptor



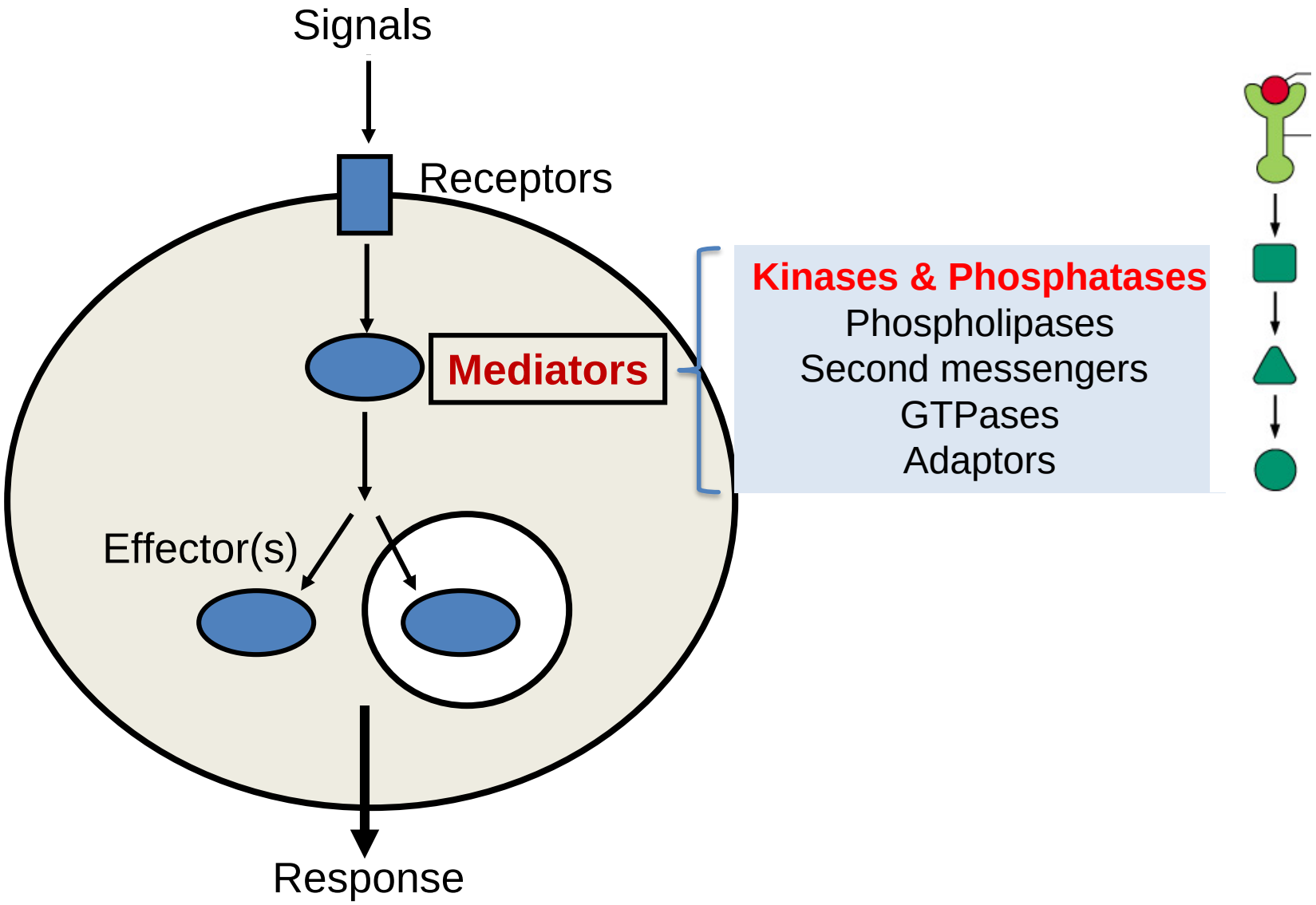
Tyrosine kinase-linked receptors



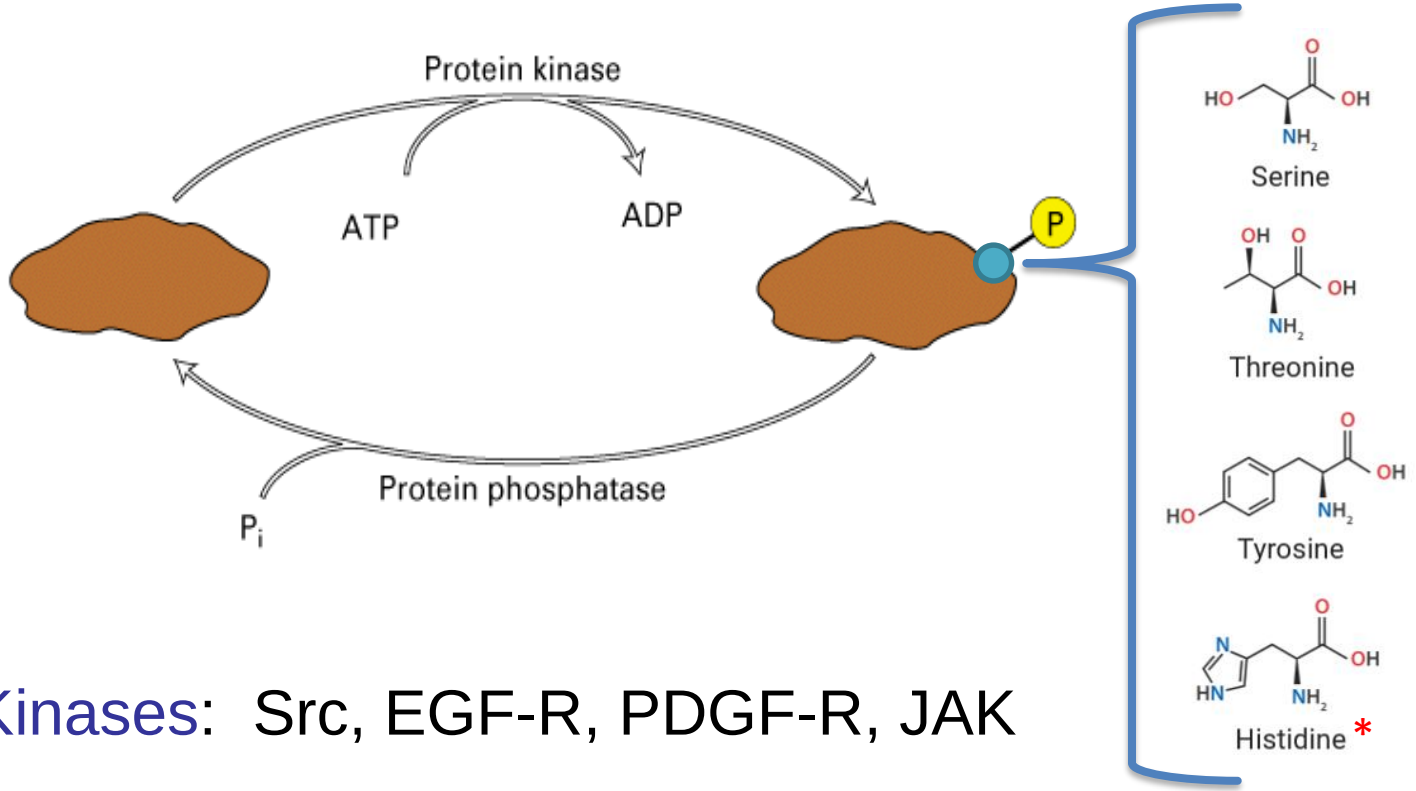
Receptors with intrinsic enzymatic activity



Mediators


Signals
Receptors
Mediators
Effector(s)
Response
Kinases & Phosphatases
Phospholipases
Second messengers
GTPases
Adaptors

Protein Kinases & Phosphatases



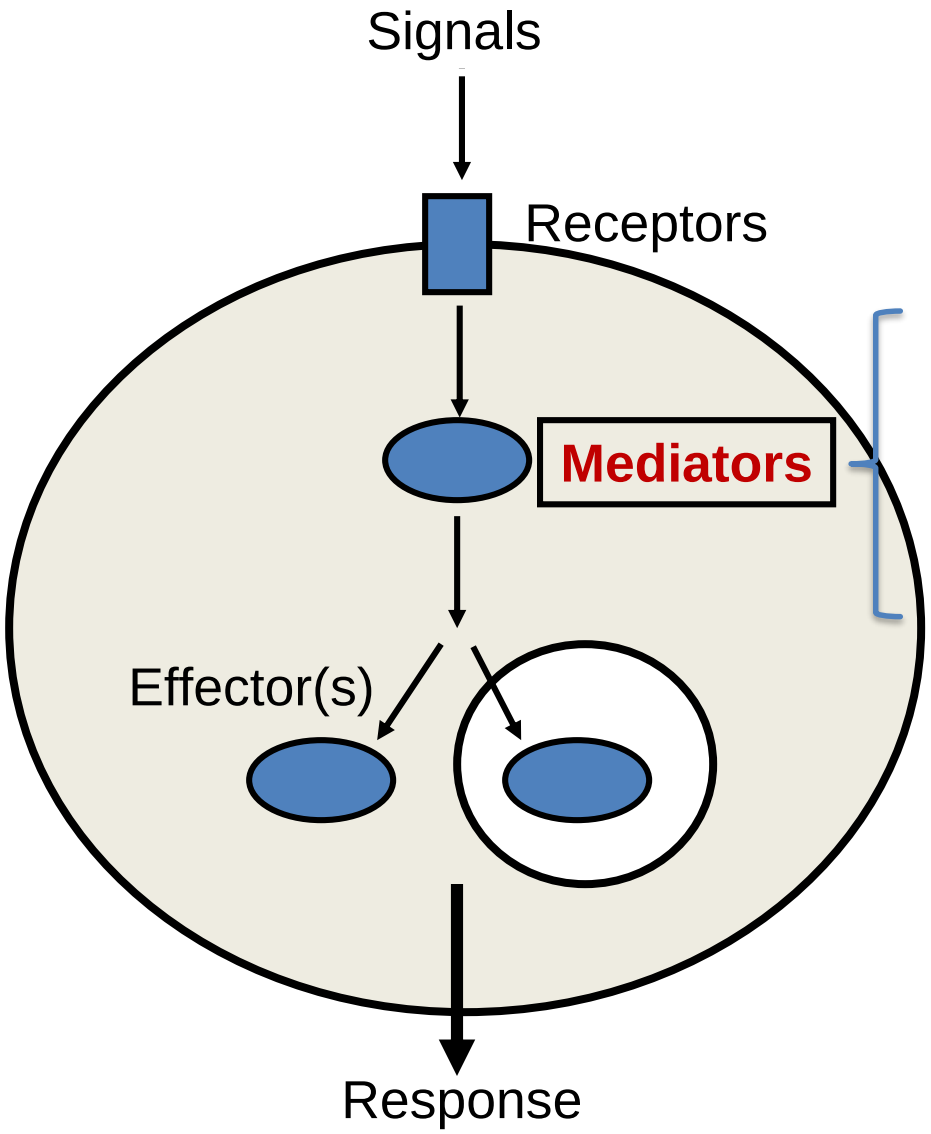
Tyr Kinases: Src, EGF-R, PDGF-R, JAK

Ser/Thr Kinases: PKC, Raf, MAPK, Akt

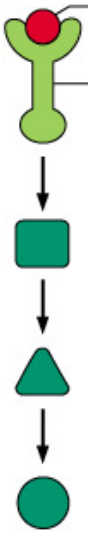
Dual-specificity kinases: MEK, MKK

Lipid Kinase: PI3K

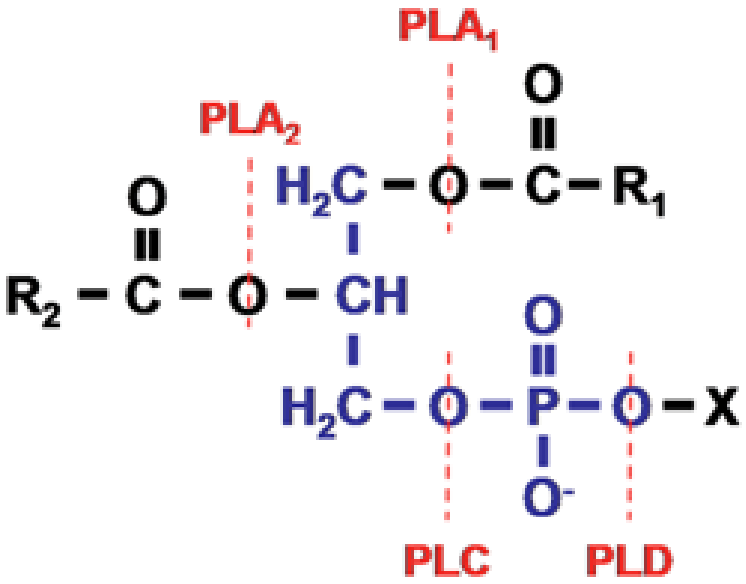
Mediators



Kinases & Phosphatases
Phospholipases
Second messengers
GTPases
Adaptors

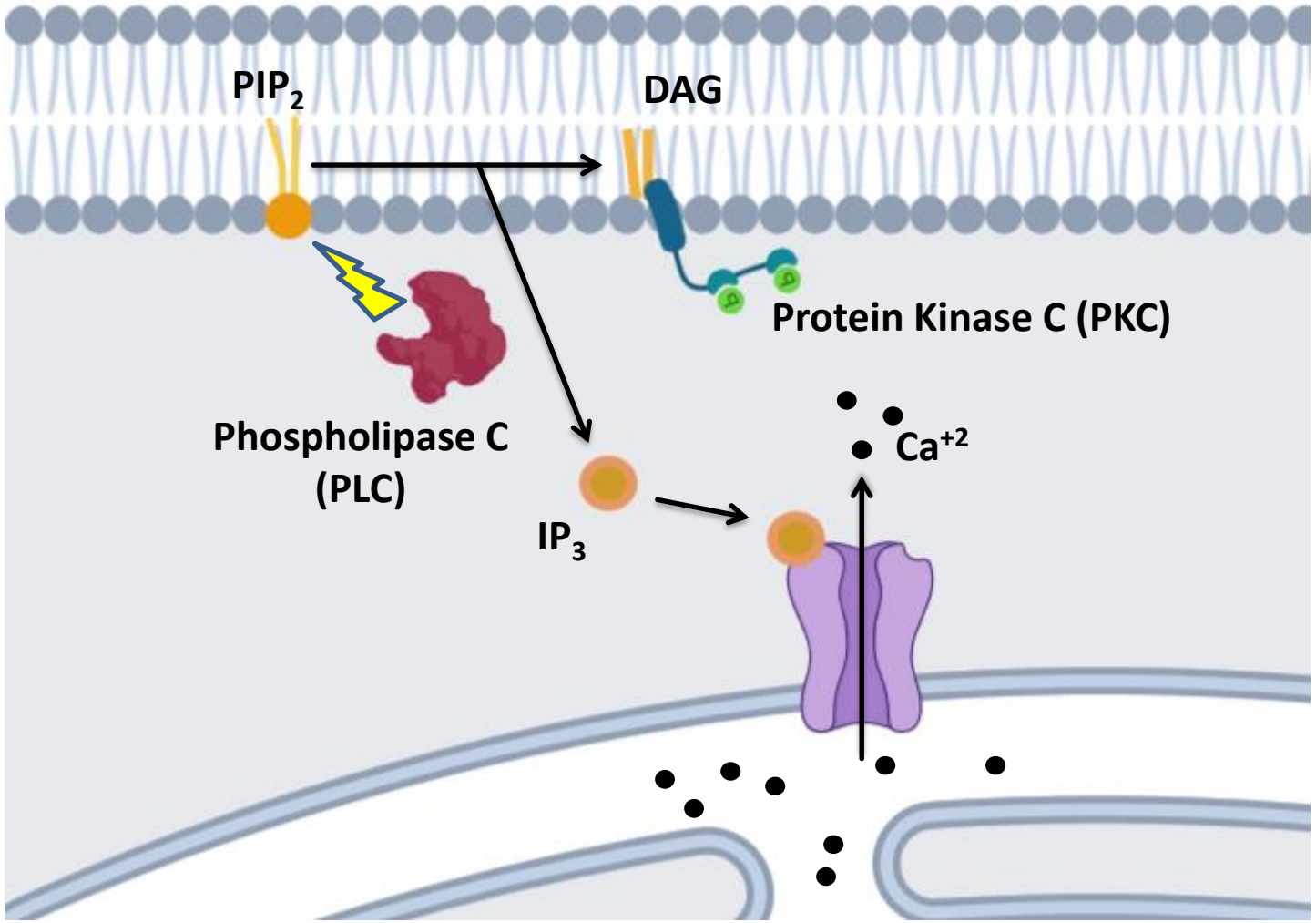


Phospholipases

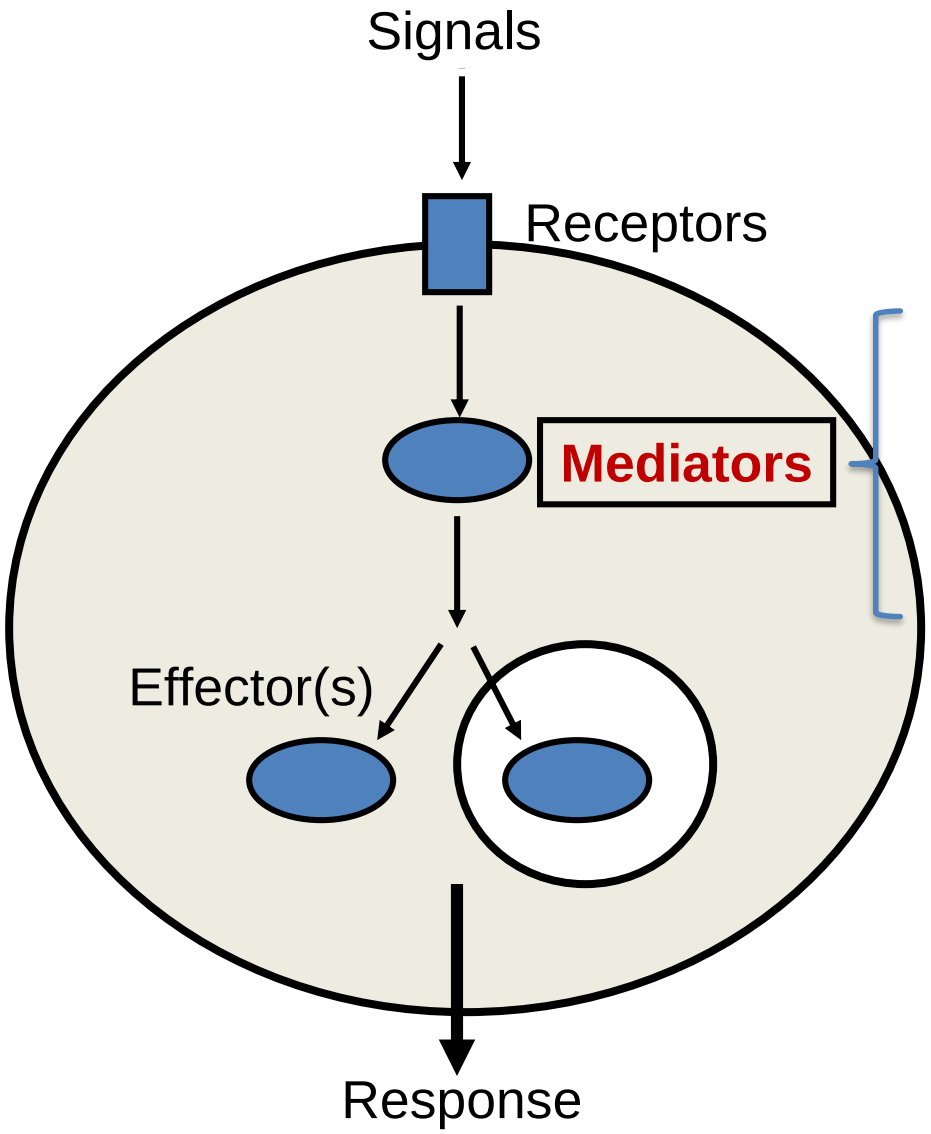


Hydrolyze phospholipids

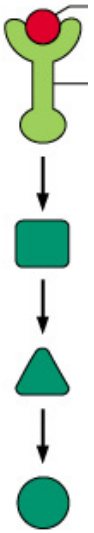
Phospholipases



Mediators

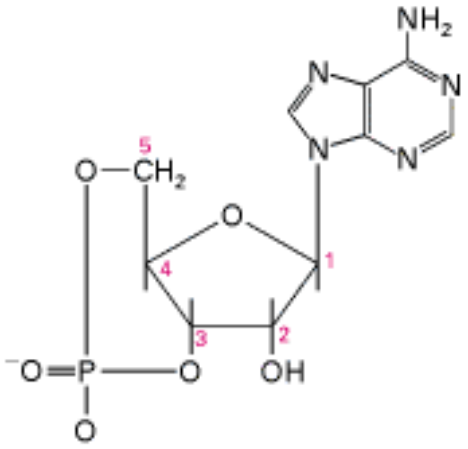


Kinases & Phosphatases
Phospholipases
Second messengers
GTPases
Adaptors

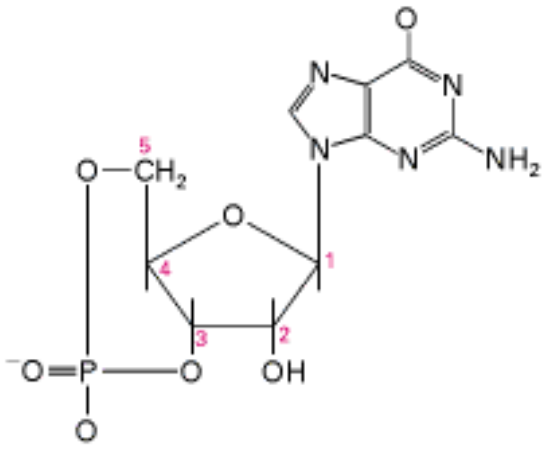


Second Messengers

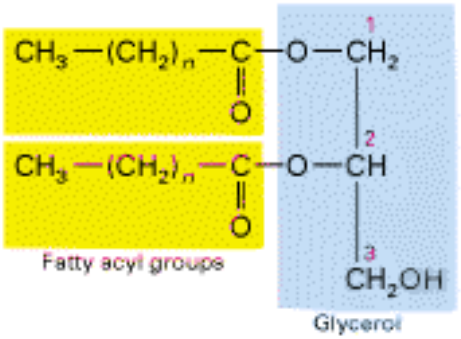
- Cyclic nucleotides
  - Cyclic AMP and cyclic GMP
- Lipid metabolites
  - Diacylglycerol
  - Inositol triphosphate
- Calcium



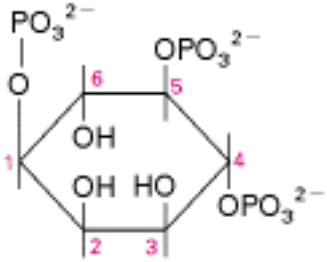
3′,5′-Cyclic AMP
(cAMP)



3′,5′-Cyclic GMP
(cGMP)

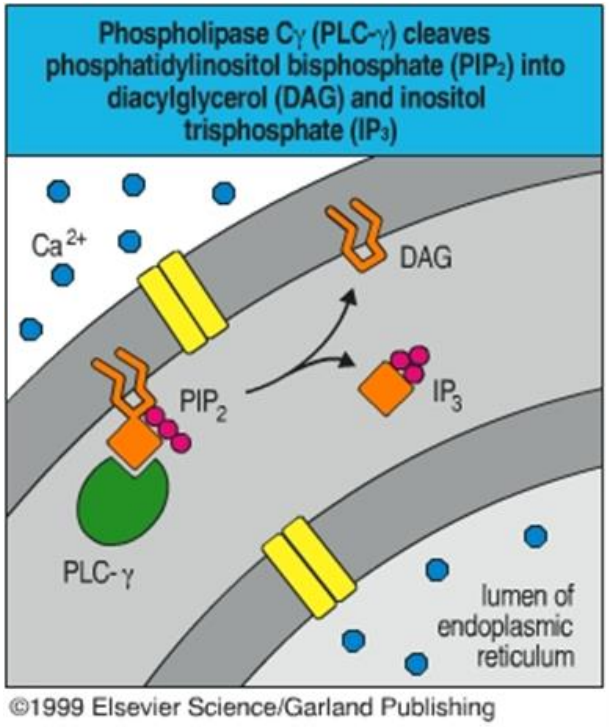


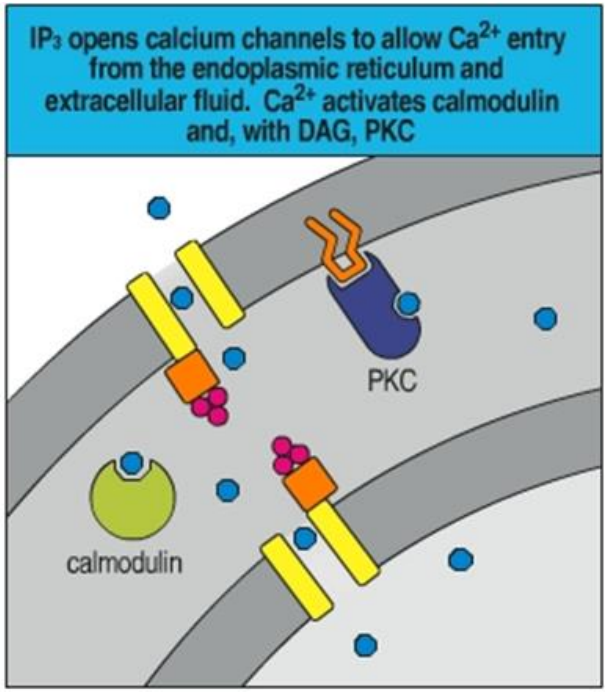
1,2-Diacylglycerol
(DAG)



Inositol
1,4,5-trisphosphate
(IP_3)

Second Messengers

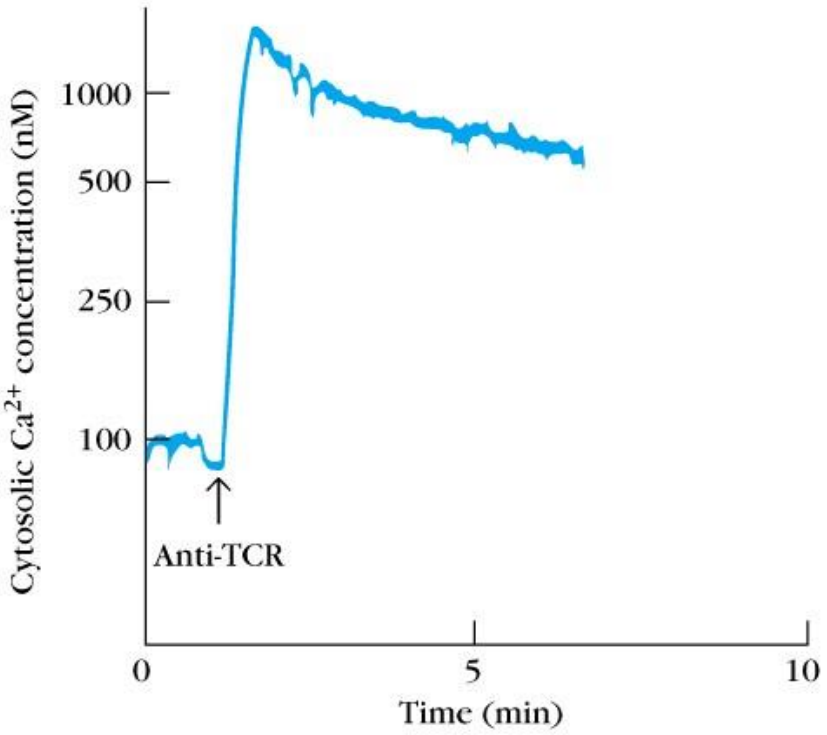




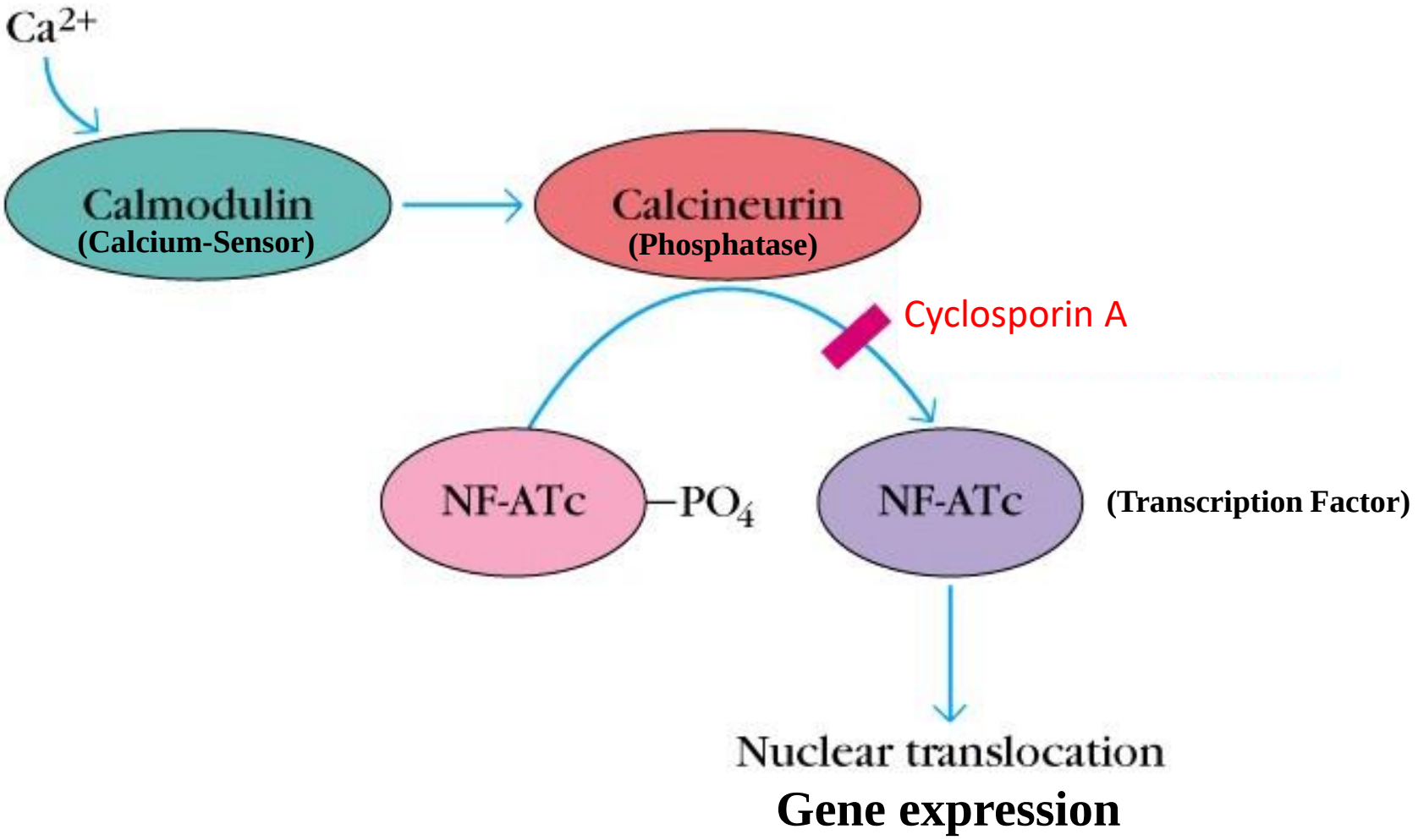
Calcium

Cellular calcium concentrations:

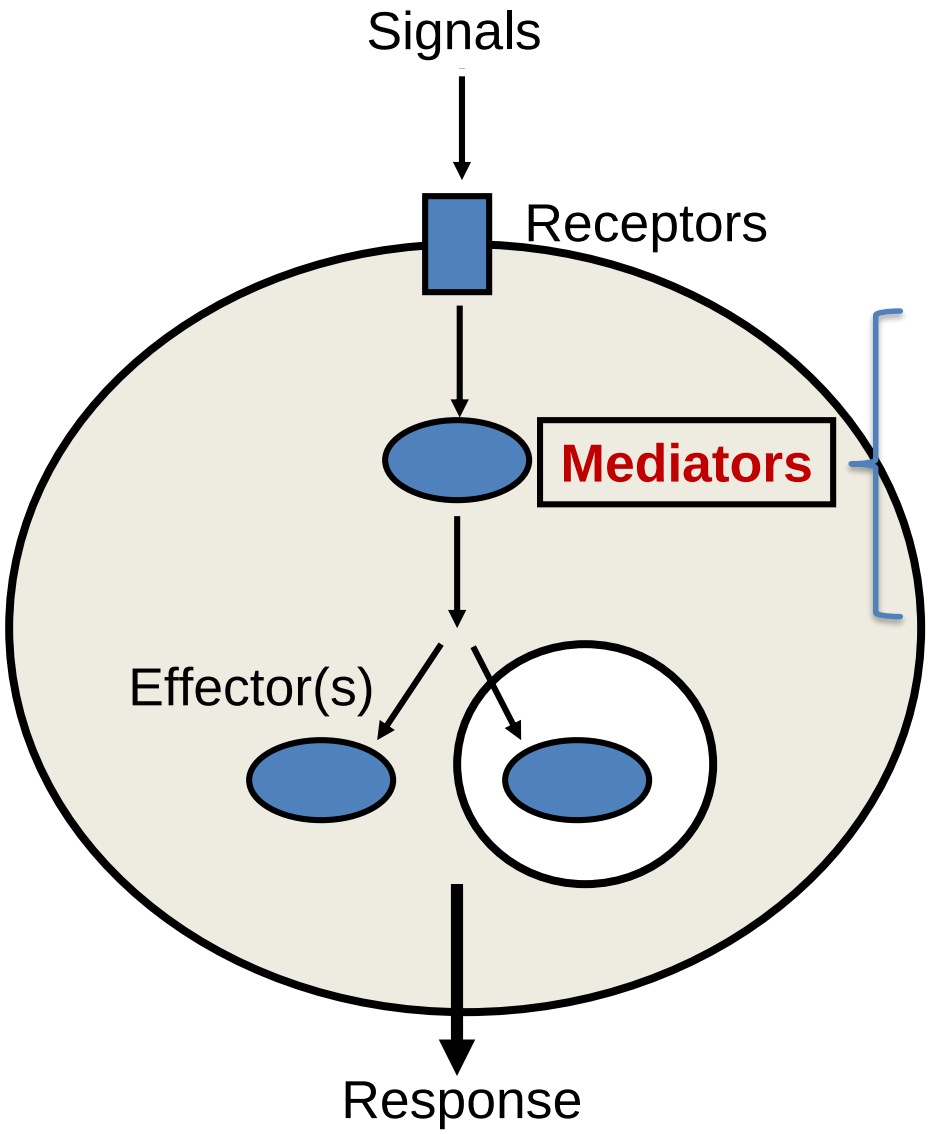
- extracellular: 1-2 mM
- cytosolic: 50 - 100 nM
- intracellular lumenal (ER etc.): 30 - 300 μM



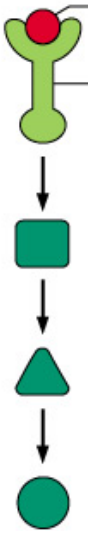
Calcium


Ca2+
Calmodulin
(Calcium-Sensor)
Calcineurin
(Phosphatase)
Cyclosporin A
NF-ATc
PO4
NF-ATc
(Transcription Factor)
Nuclear translocation
Gene expression

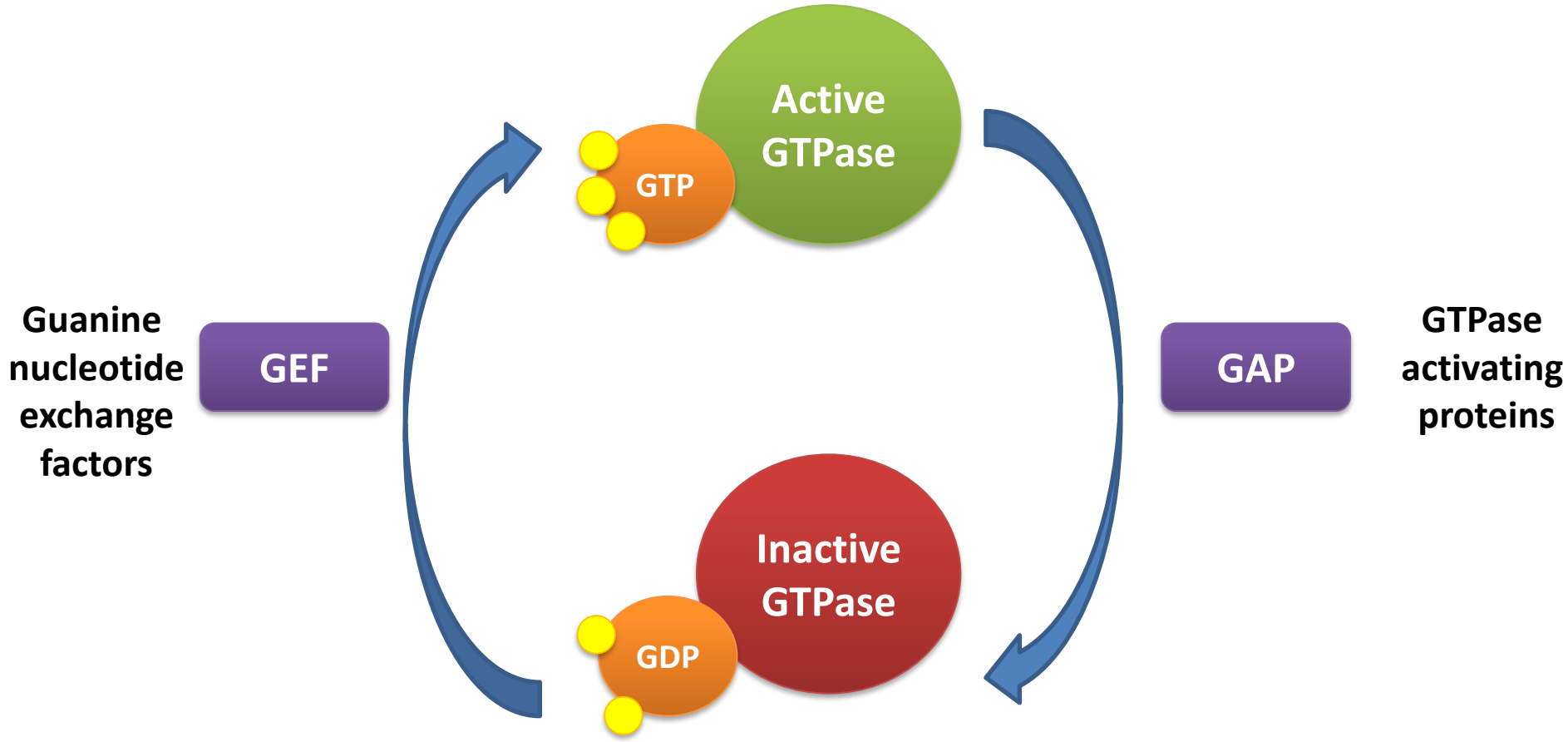
Mediators



Kinases & Phosphatases
Phospholipases
Second messengers
GTPases
Adaptors



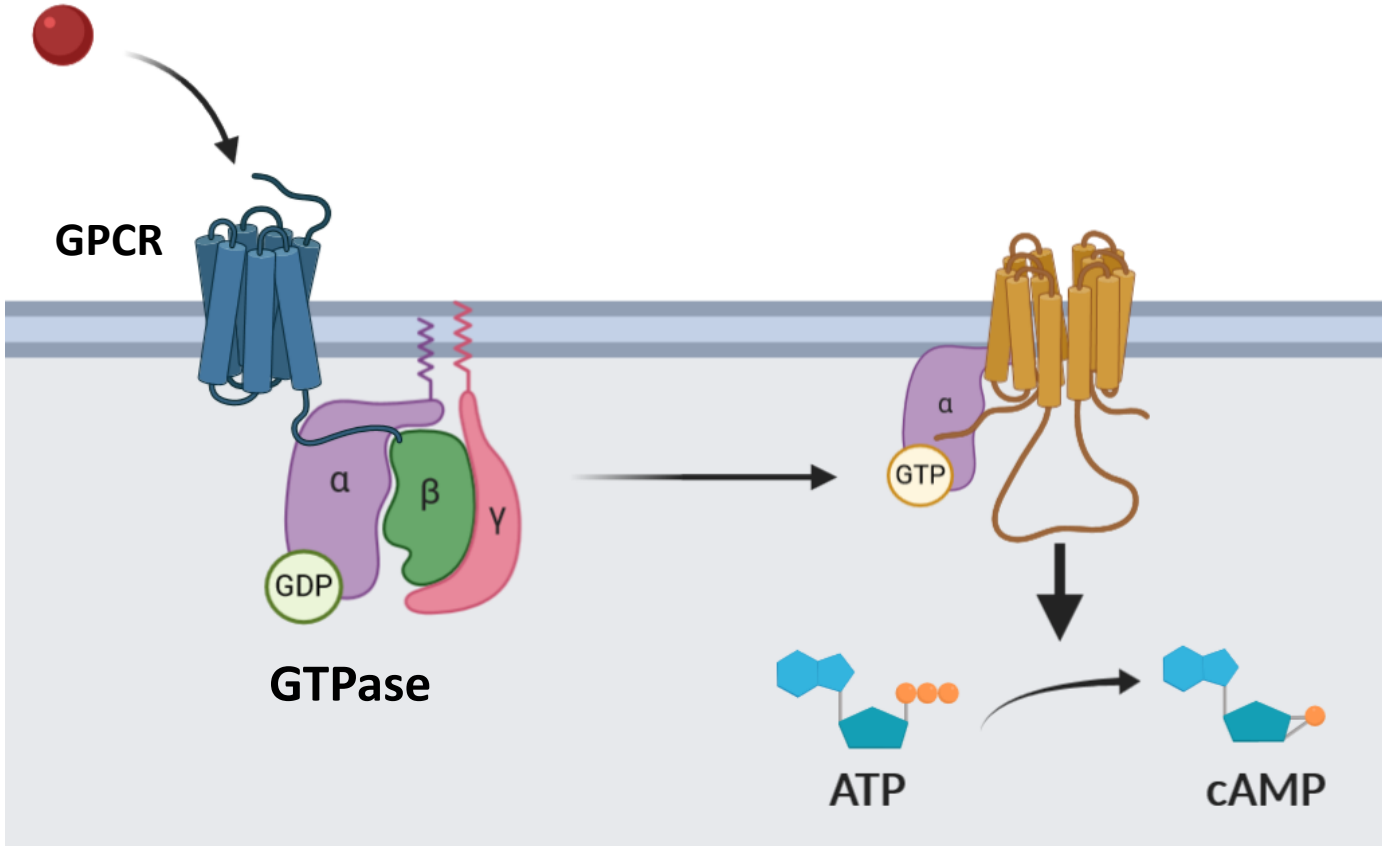
GTPases


Active GTPase
GTP
Guanine nucleotide exchange factors
GEF
GAP
GTPase activating proteins
Inactive GTPase
GDP

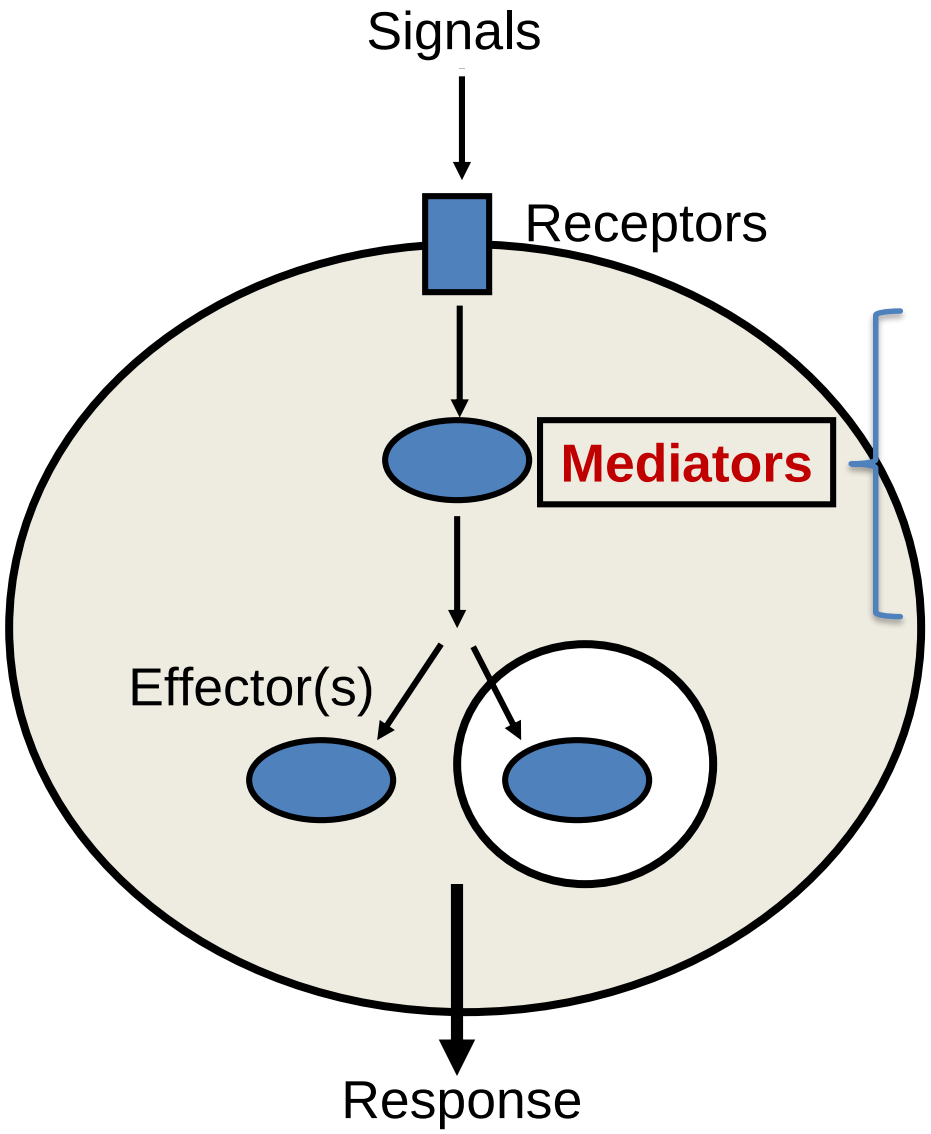
Small GTPases superfamily

- ***Ras family:*** cell proliferation, differentiation and survival.
- ***Rho family:*** actin reorganization.
- ***Rab family:*** vesicle transport and membrane trafficking in secretory and endocytic pathways.
- ***Ran family:*** nucleocytoplasmic transport of RNA and proteins through the nuclear pore.

Heterotrimeric GTPases



Mediators



Kinases & Phosphatases
Phospholipases
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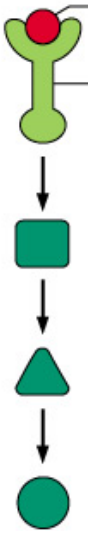
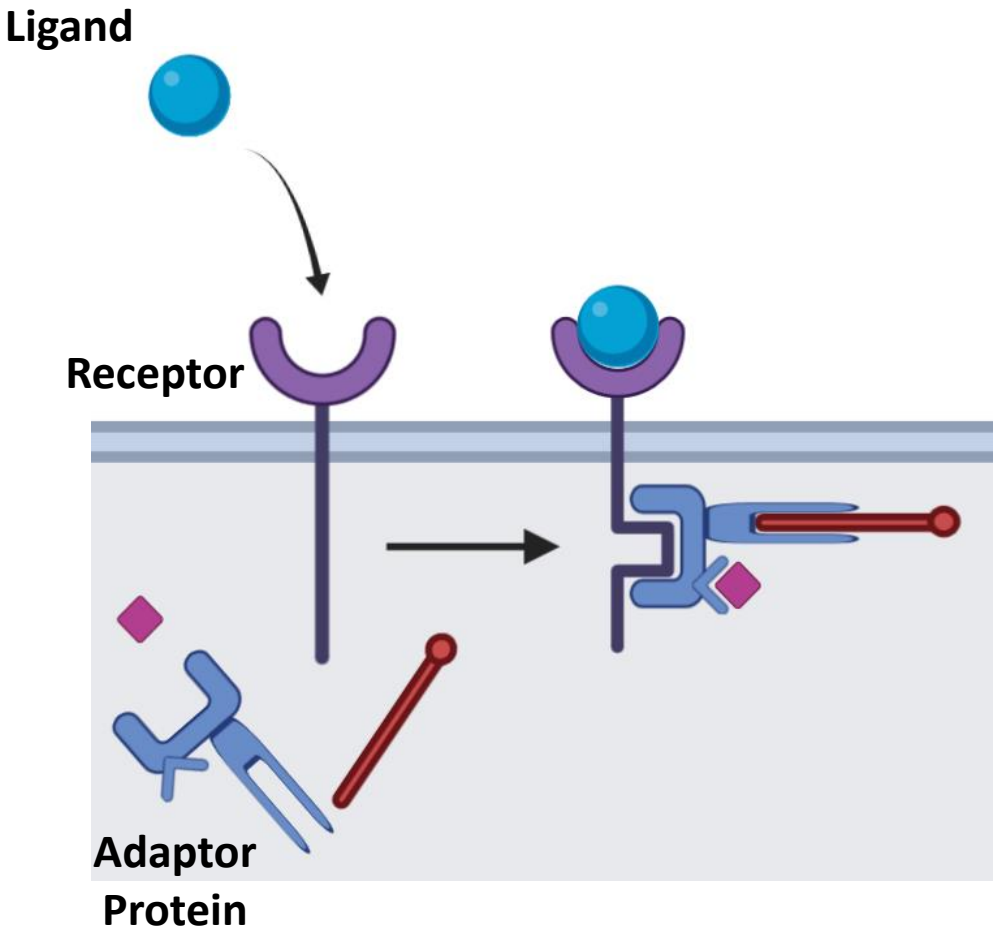
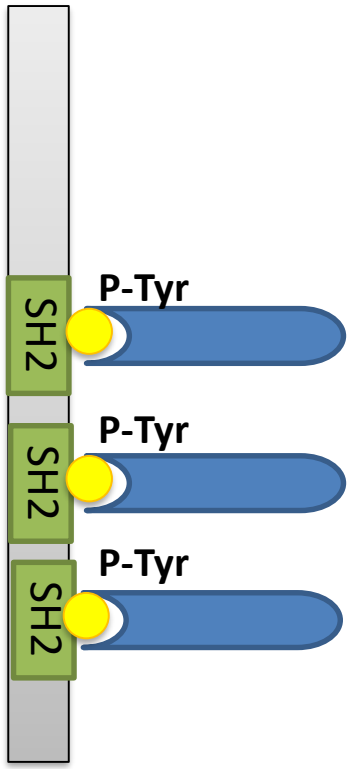


Adaptor proteins



Adaptor proteins

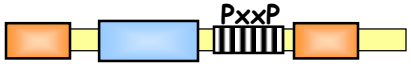
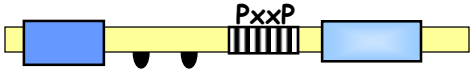
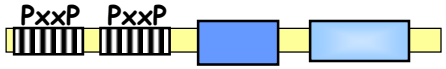
SH2 Adaptor Proteins



- SH2 domains bind phosphorylated tyrosine residues.
- SH2 domains recruit proteins phosphorylated by tyrosine kinases

Adaptor proteins

Cytoplasmic adaptor proteins

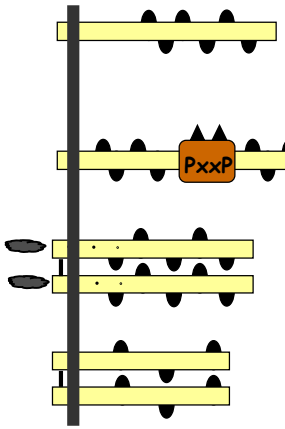
Name	Structure	MW (kD)	Interaction partners	Expression
Grb2		28	Sos,LAT,c-Cbl,Shc,SLP-76, Vav,SHP-2,WASP,HPK1	ubiquitous
Grap		28	Sos,LAT,Shc,SAM68	B-Cells, T-Cells
Gads		40	LAT,Shc,SLP-76,HPK1	T-Cells, NK Cells Mast cells. Macrophages, Thrombocytes
Nck-1		47	Sos,SLP-76,WASP,PAK,Cbl	ubiquitous
Shb		55,66	Grb2,LAT,PI3K,Eps8, PLCγ1,CD3ζ,Src	ubiquitous
Shc		46,52,66	Grb2,SHIP,ZAP-70,CD3ζ, Igα/β,RasGAP	ubiquitous

SH2-Binding Site
PxxP SH3-Binding Site
SH3-Domain
PTB-Domain
SH2-Domain

A. Leo and B.Schraven, Current Opinion in Immunology, 2001

Adaptor proteins

Transmembrane adaptor proteins

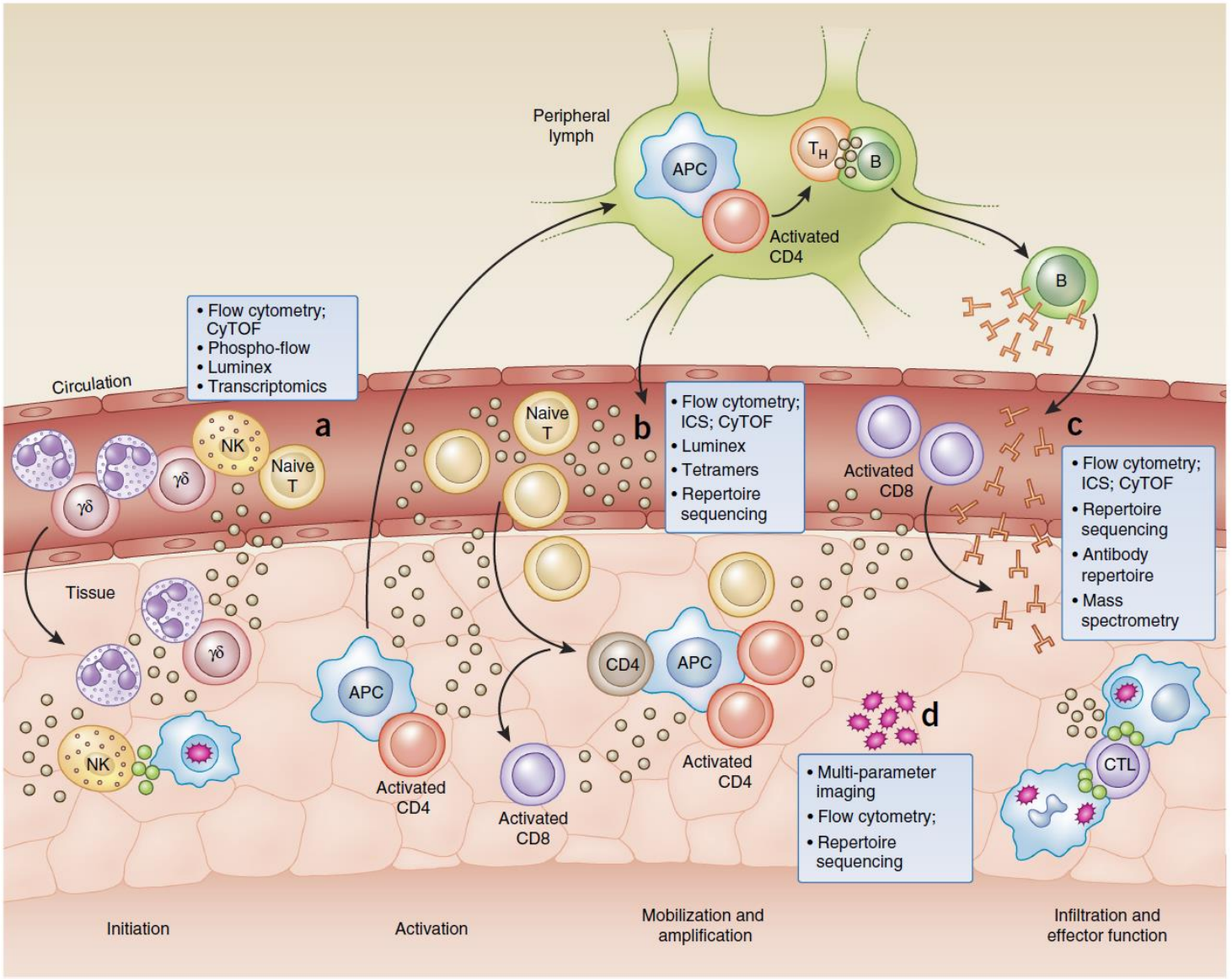
Name	Structure	MW (kD)	Interaction partners	Expression
LAT		36-38	Grb2,Gads,SLP-76, PLCγ1/2,c-Cbl,PI3K	T-Cells, NK Cells, Mast cells, Platelets
PAG/Cbp		75-85	Csk,Fyn	Ubiquitous
SIT		30-40	SHP2	B-Cells, T-Cells
TRIM		29-30	PI3K	T-Cells, NK Cells

● SH2-Binding Site

PxxP SH3-Binding Site

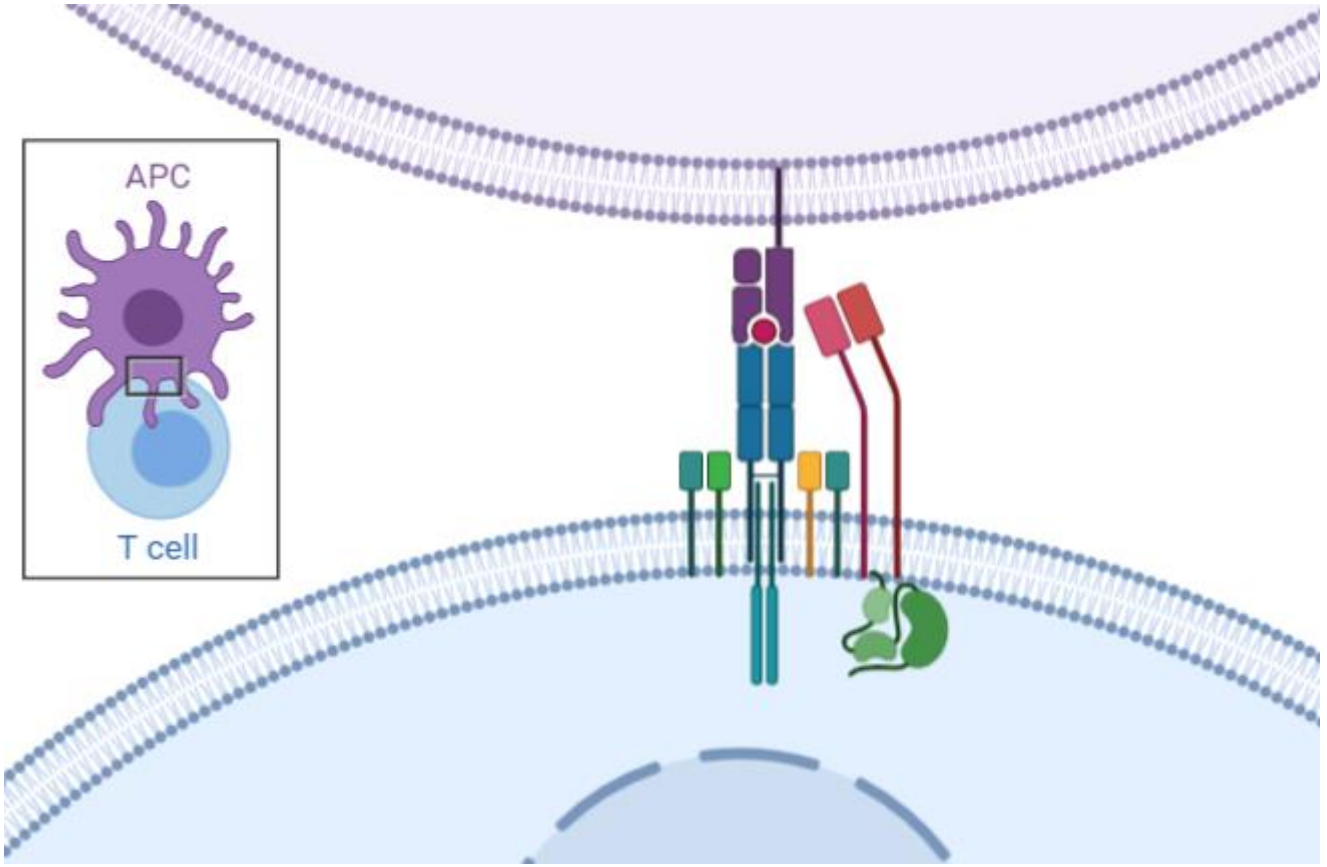
A. Leo and B.Schraven, Current Opinion in Immunology, 2001

T cell receptor (TCR) signaling



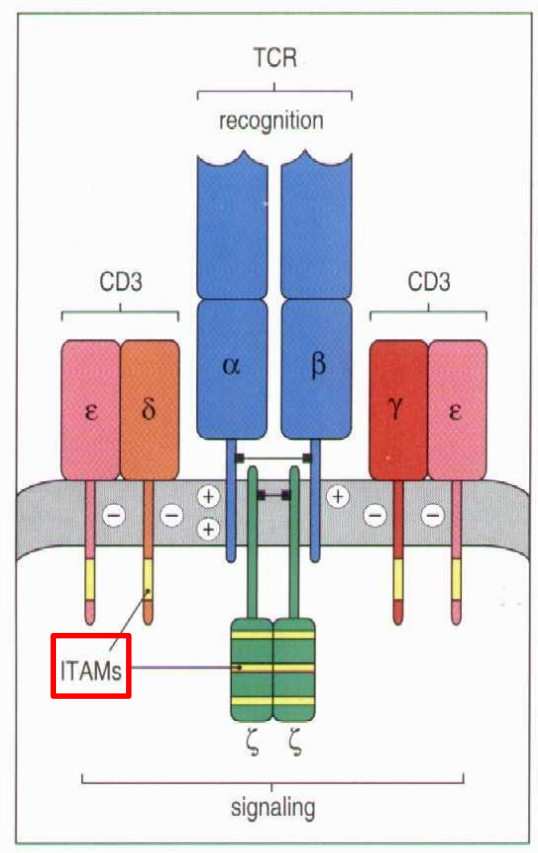
Davis MM, Tato CM, Furman D. Systems immunology: just getting started. *Nat Immunol.*

Signal transduction induced by T cell receptor engagement



TCR structure

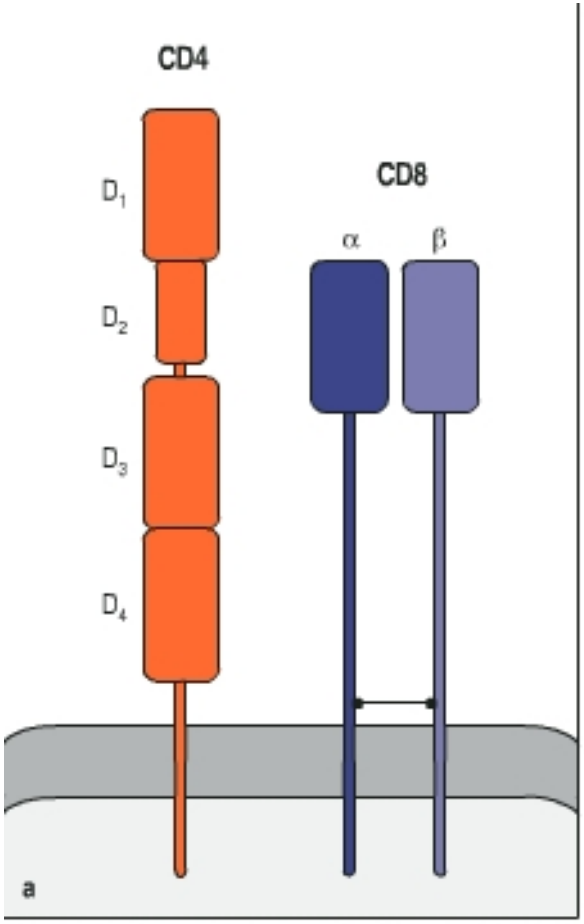
T cell receptor:CD3 complex



- T cell receptor
  - α and β chain heterodimer
  - antigen recognition
- CD3
  - transmembrane proteins with extracellular domains and cytoplasmic tails
    - two ε-chains
    - one δ-chain
    - one γ-chain
  - transmembrane/cytoplasmic ζ-homodimers

ITAM: immunoreceptor tyrosine-based activation motifs

Co-Receptors of the TCR



- CD4 and CD8 recognize invariant amino acids on MHCII and MHCI, respectively.
- CD4 and CD8 are constitutively associated with **Lck** (Tyr-kinase)
- CD4/CD8 concentrate **Lck** at the site of TCR-MHC interaction

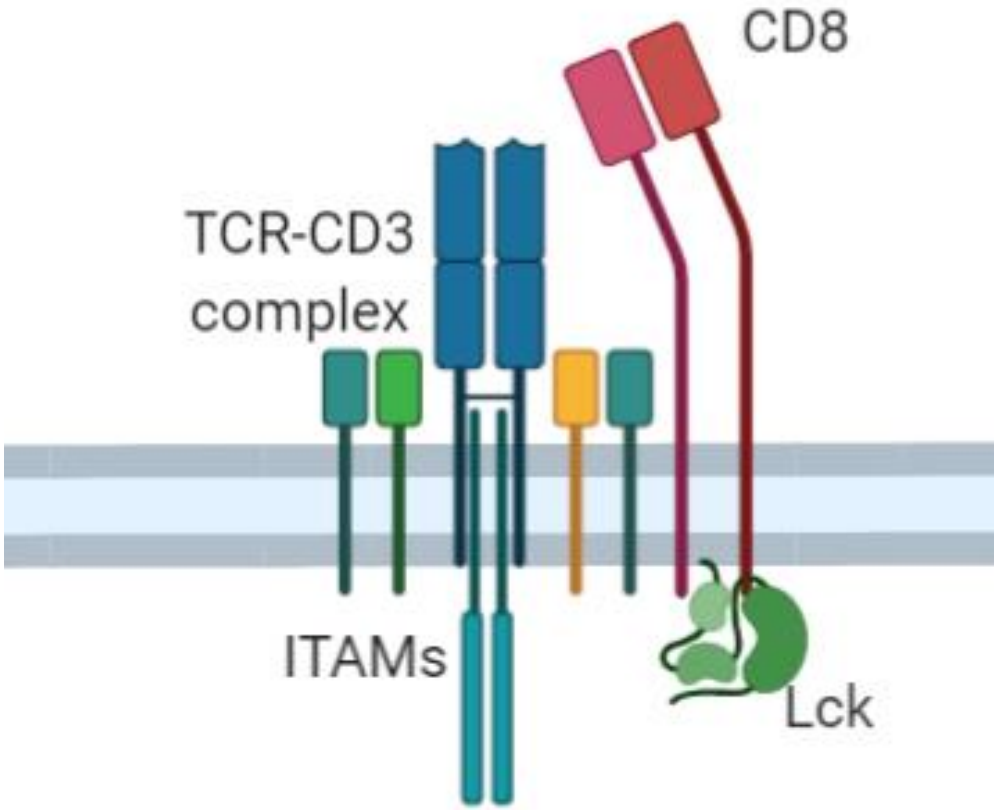
Co-receptors function: increasing efficiency of lymphocyte activation

Lipid Rafts

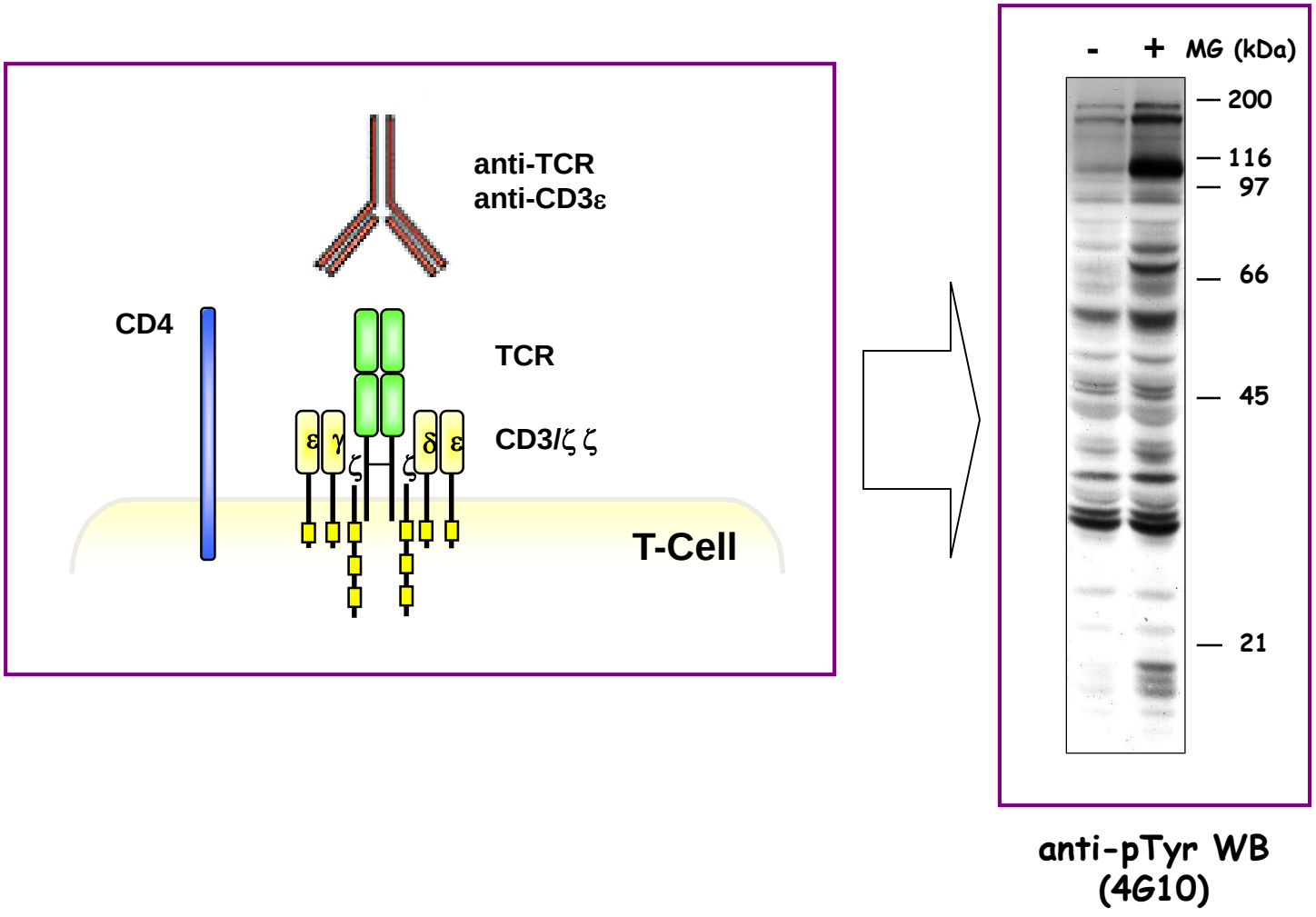
- Membrane compartments enriched with cholesterol, glycosphingolipids and sphingomyelin
- Selectively concentrate membrane proteins with lipid anchor of saturated acyl chains
- Contain lipid modified signal proteins
  - Src kinases (Lck, Fyn)
  - GTPases (Ras proteins, G-proteins)
  - Adaptor proteins (LAT)

TCR signaling

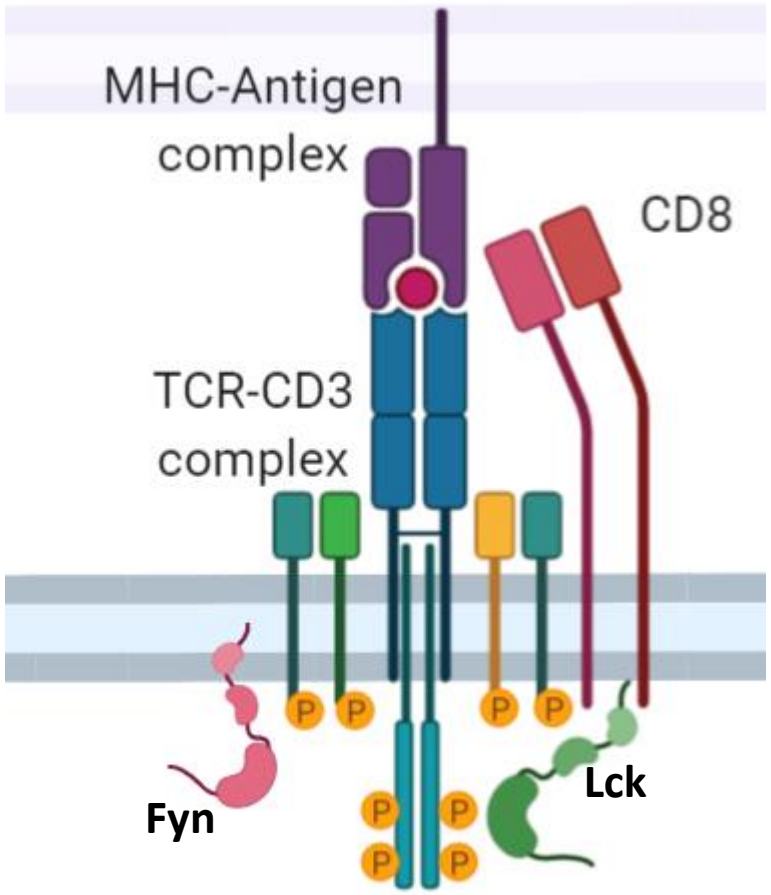
Inactive TCR



Induction of a tyrosine phosphorylation wave

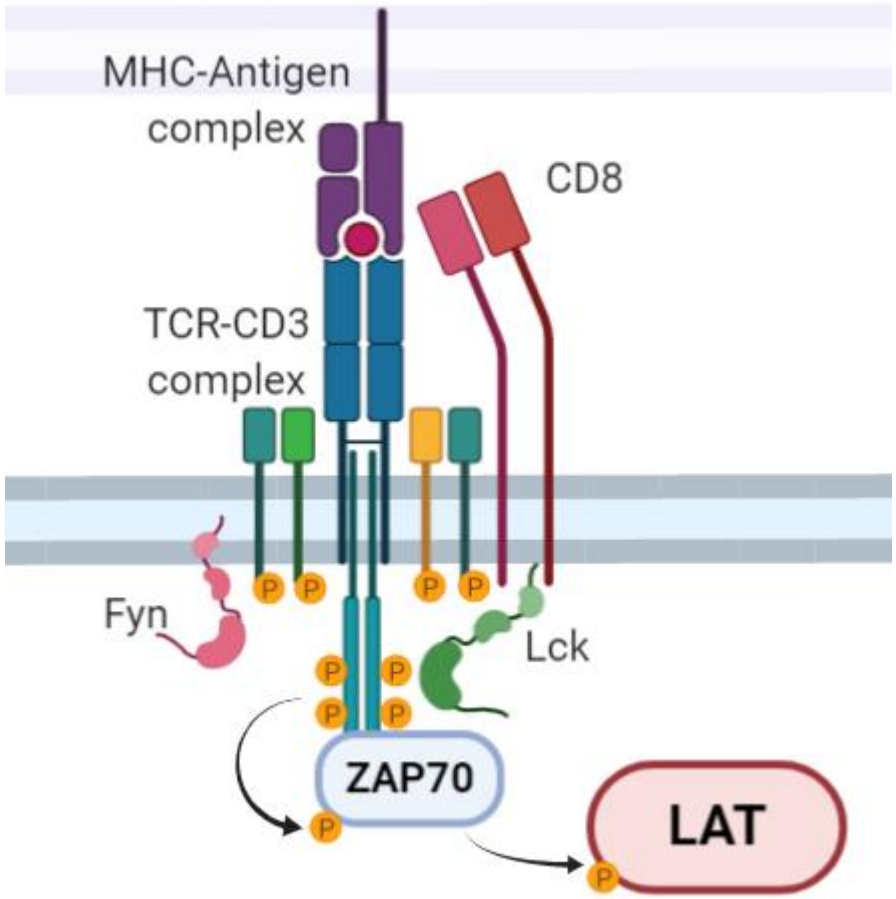


ITAMs phosphorylation



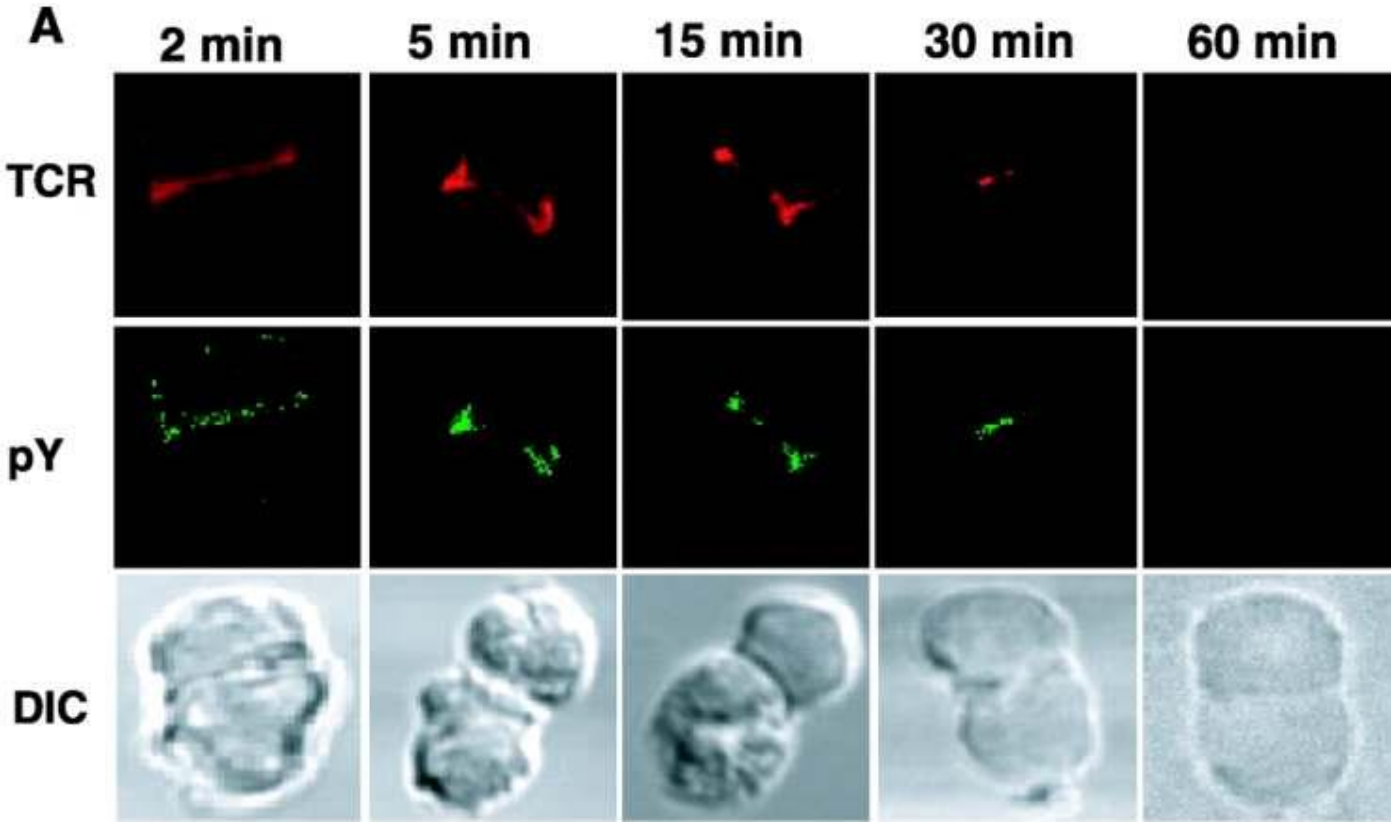
- Recruitment and activation of Lck and Fyn (Src-family tyrosine kinases)
- Lck and Fyn phosphorylate ITAMs of TCR-CD3 complex

ZAP70 and LAT



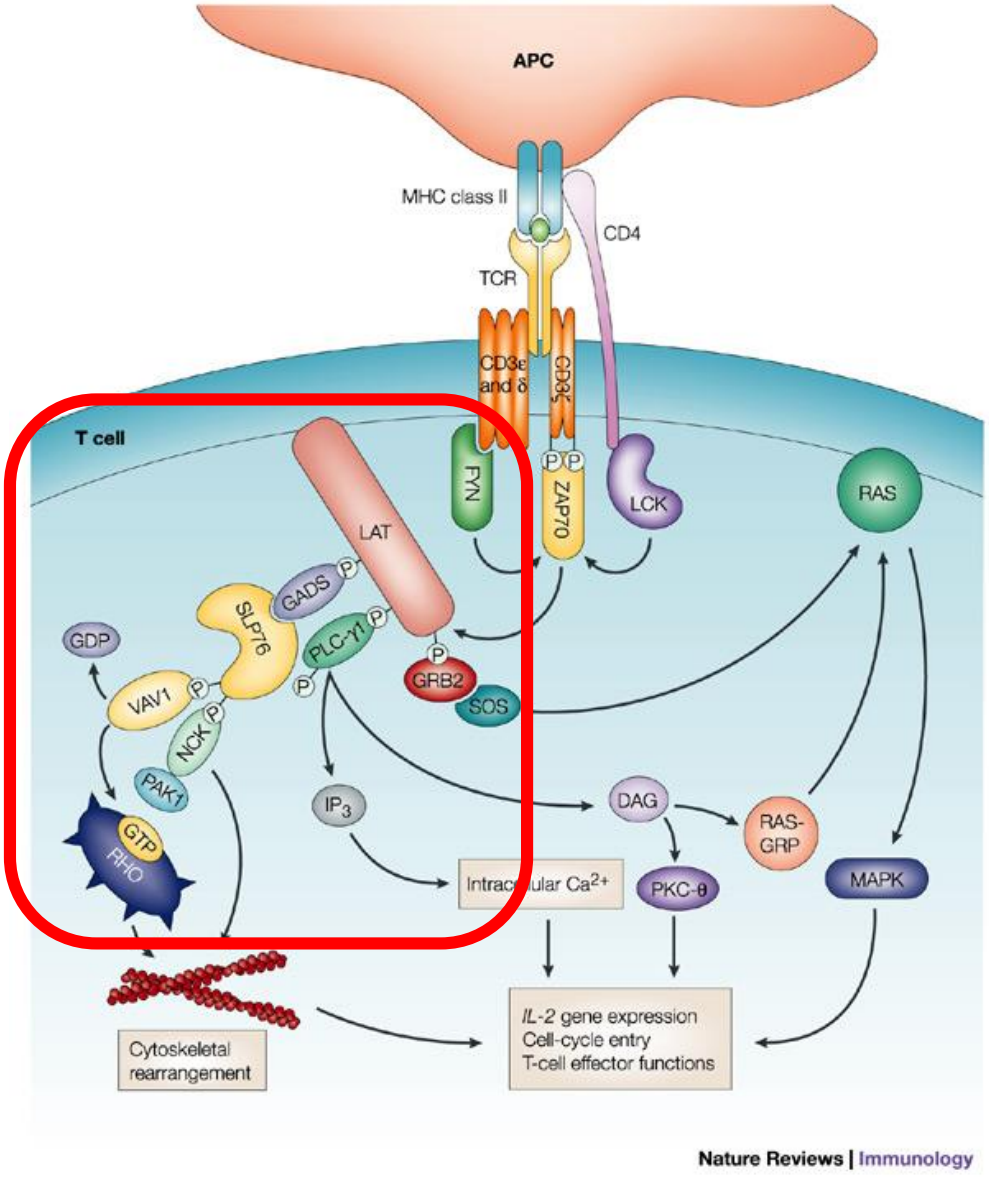
- Recruitment and phosphorylation of ZAP70 (Tyr-kinase)
- Recruitment of and phosphorylation of LAT (adaptor protein)

Time course of pTyr migration in T cell:APC conjugates



Lee et al, 2002

LAT complex

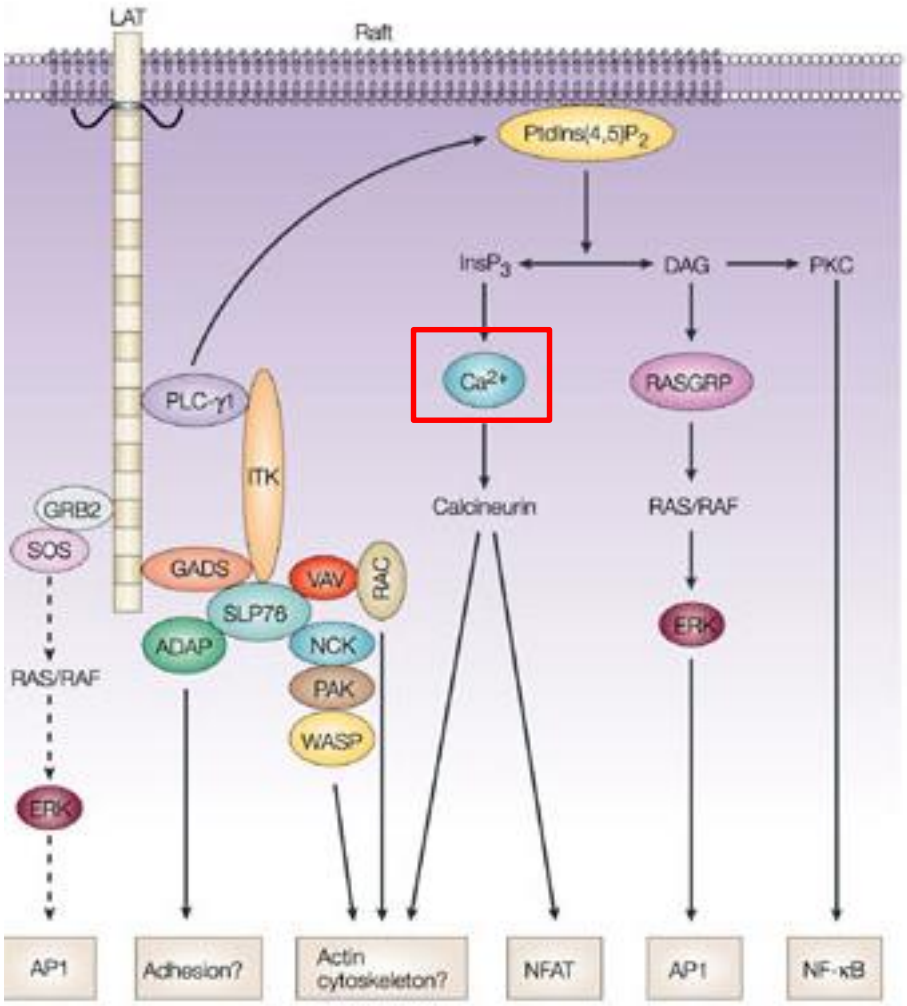


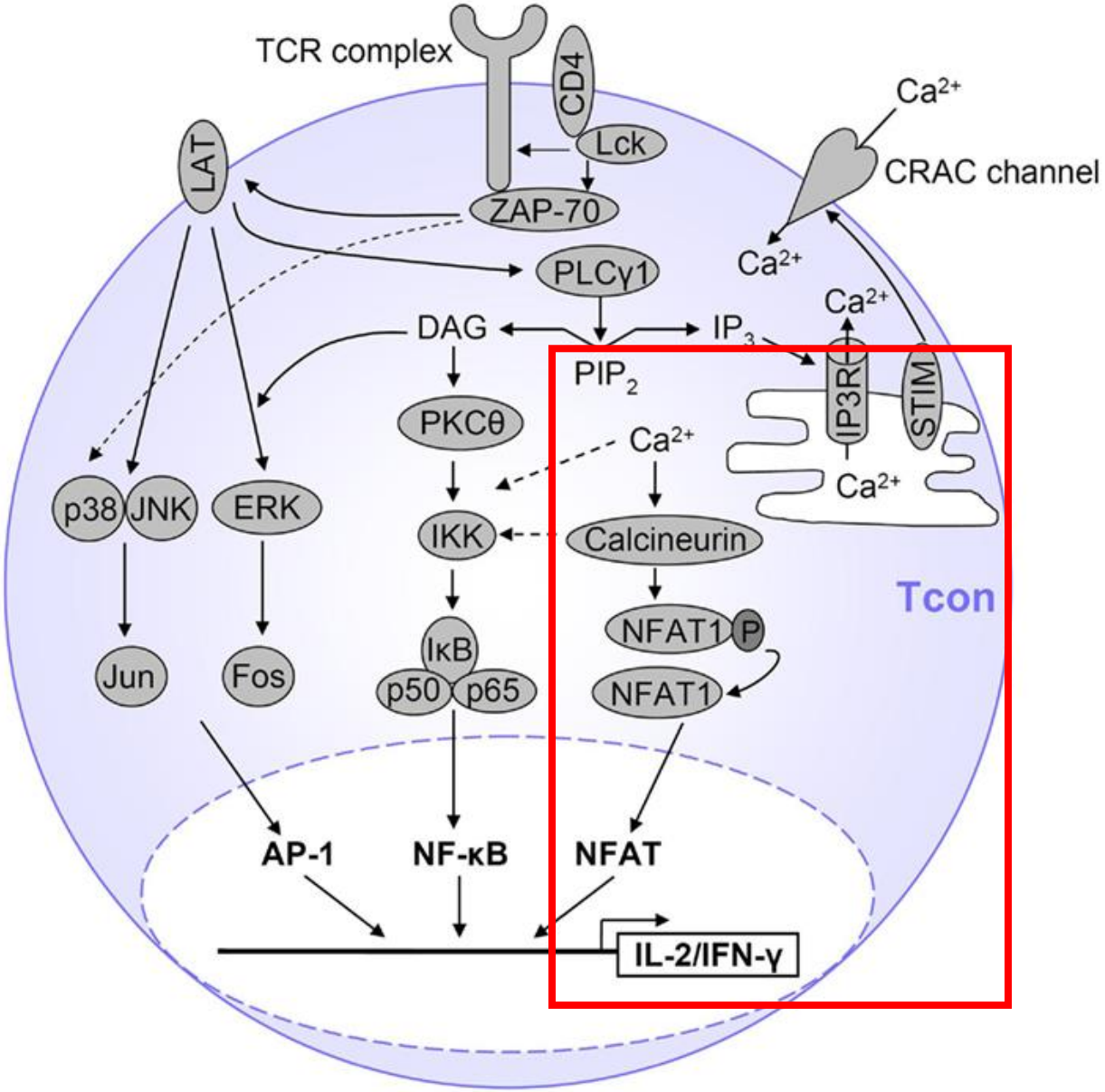
- LAT phosphorylation triggers the formation of multi-protein signaling complexes.

LAT complex include:

- Adaptor proteins
  - (SLP-76, Grb-2, and Gads)
- SLP-76 recruits Nck, Vav, and Itk to the LAT complex
- Grb-2 recruits SOS
- Phospholipase C (PLC) γ1

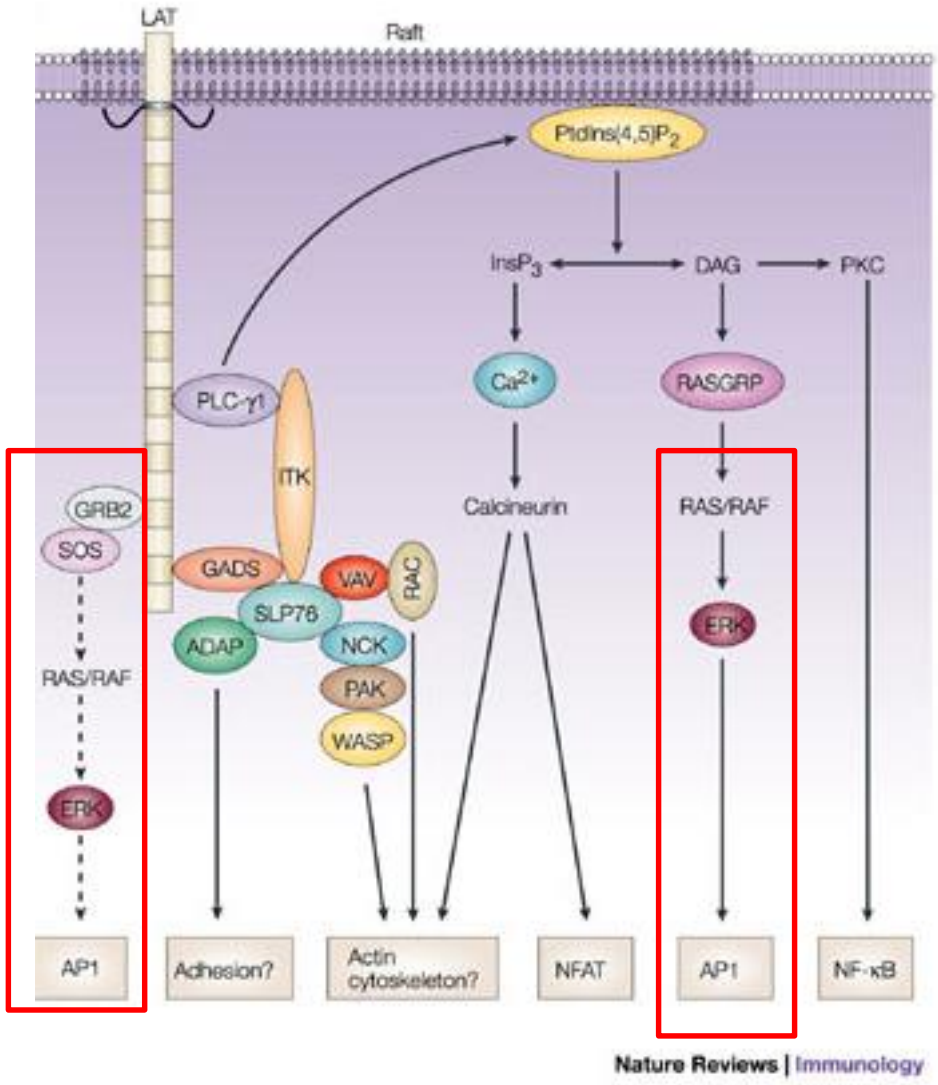
Calcium-NFAT signaling

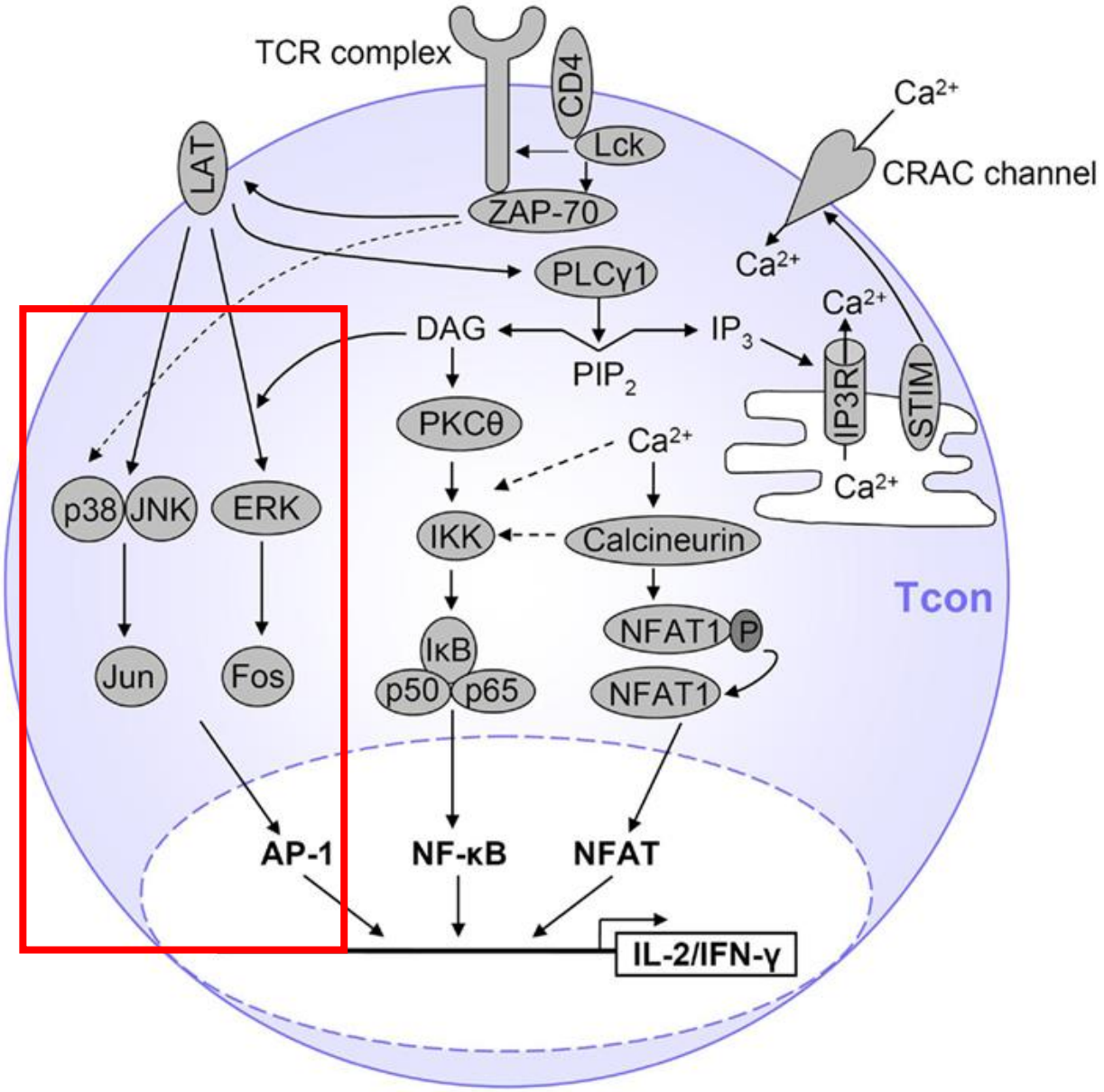




Schmidt A, Oberle N, Krammer PH. *Front Immunol*. 2012

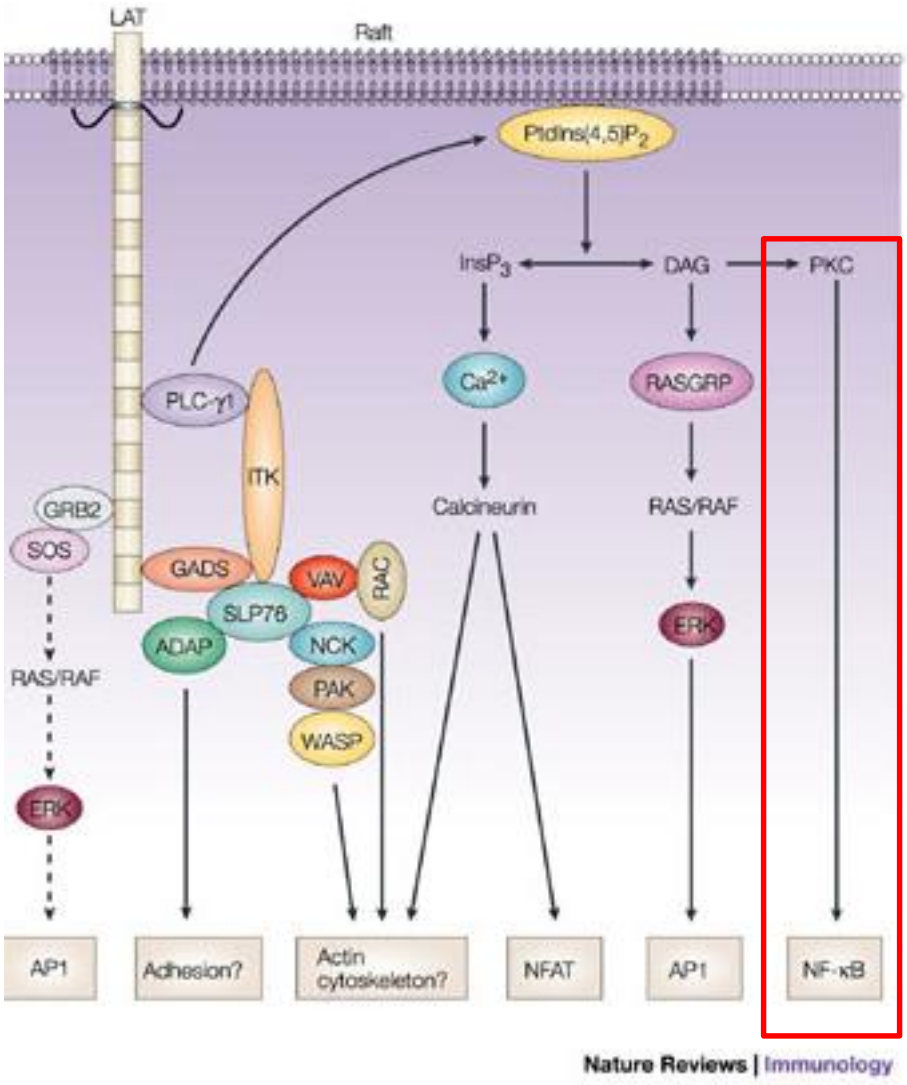
AP-1 pathway activation

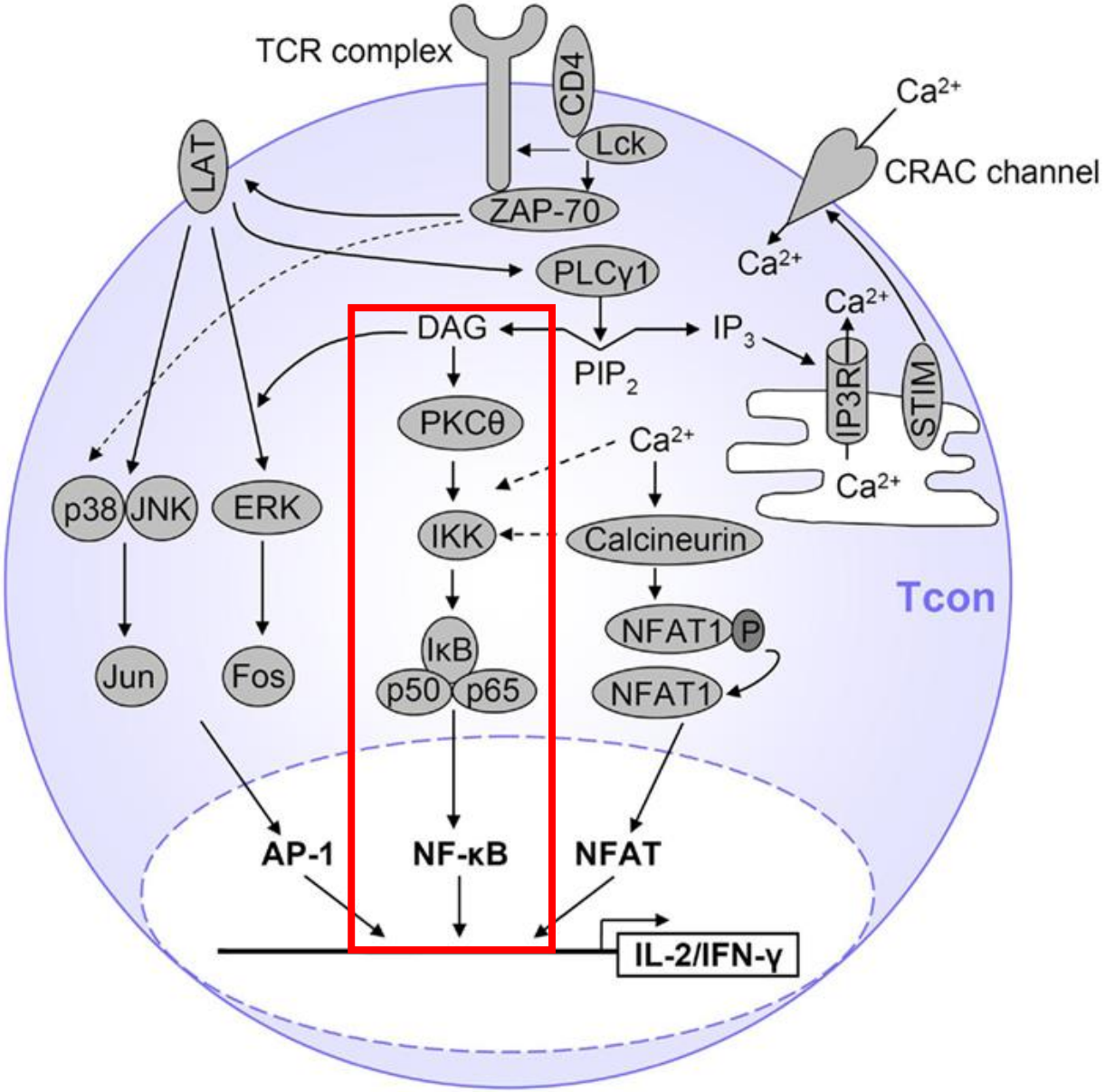




Schmidt A, Oberle N, Krammer PH. *Front Immunol*. 2012

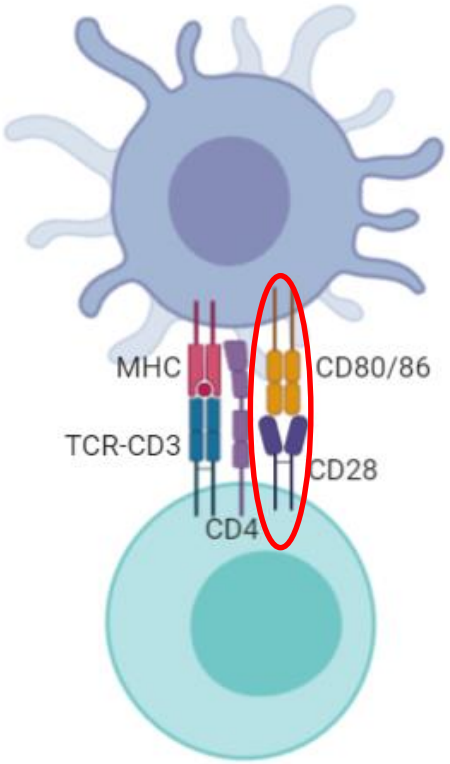
NF-κB pathway activation

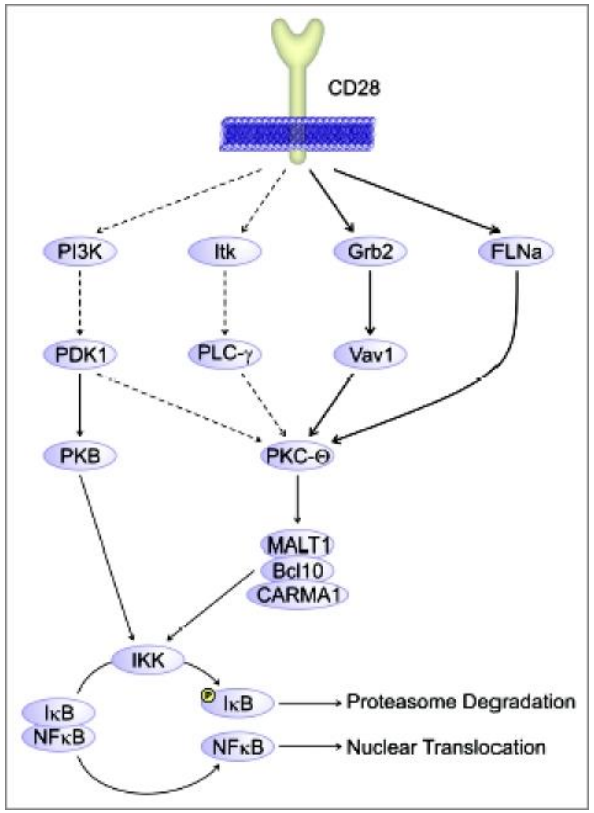




Schmidt A, Oberle N, Krammer PH. *Front Immunol*. 2012

CD28 costimulation



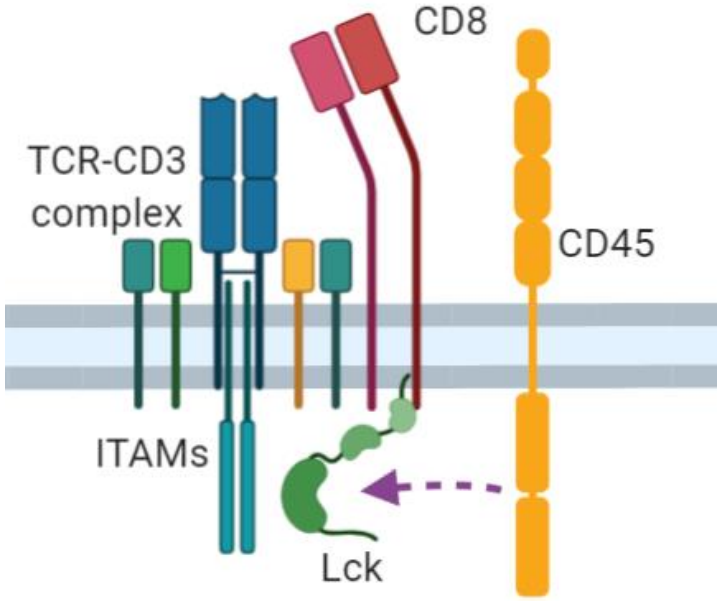


Riha P, Rudd CE. 2010

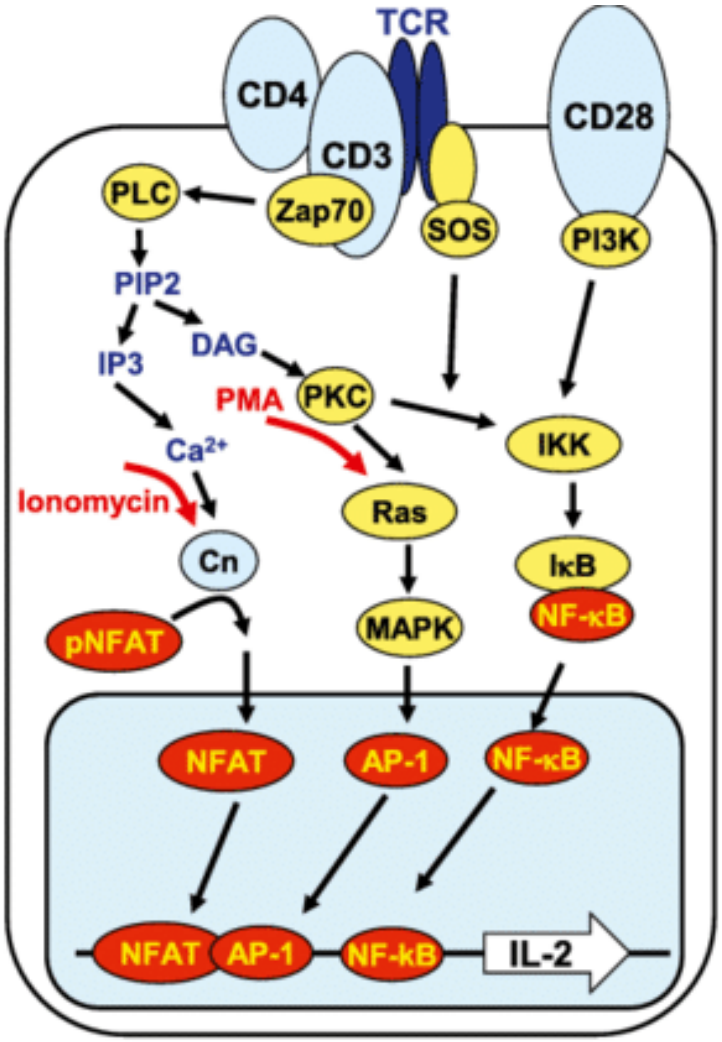
- CD28 stimulation is necessary to induce T cell activation
- CD28 stimulation potentiates TCR signaling and triggers NF-κB activation

CD45

- CD45 tyrosine phosphatase promotes Lck activity by de-phosphorylating the negative regulatory carboxy-terminal tyrosine on Lck, maintaining Lck in an open active configuration.



PMA / Ionomycin

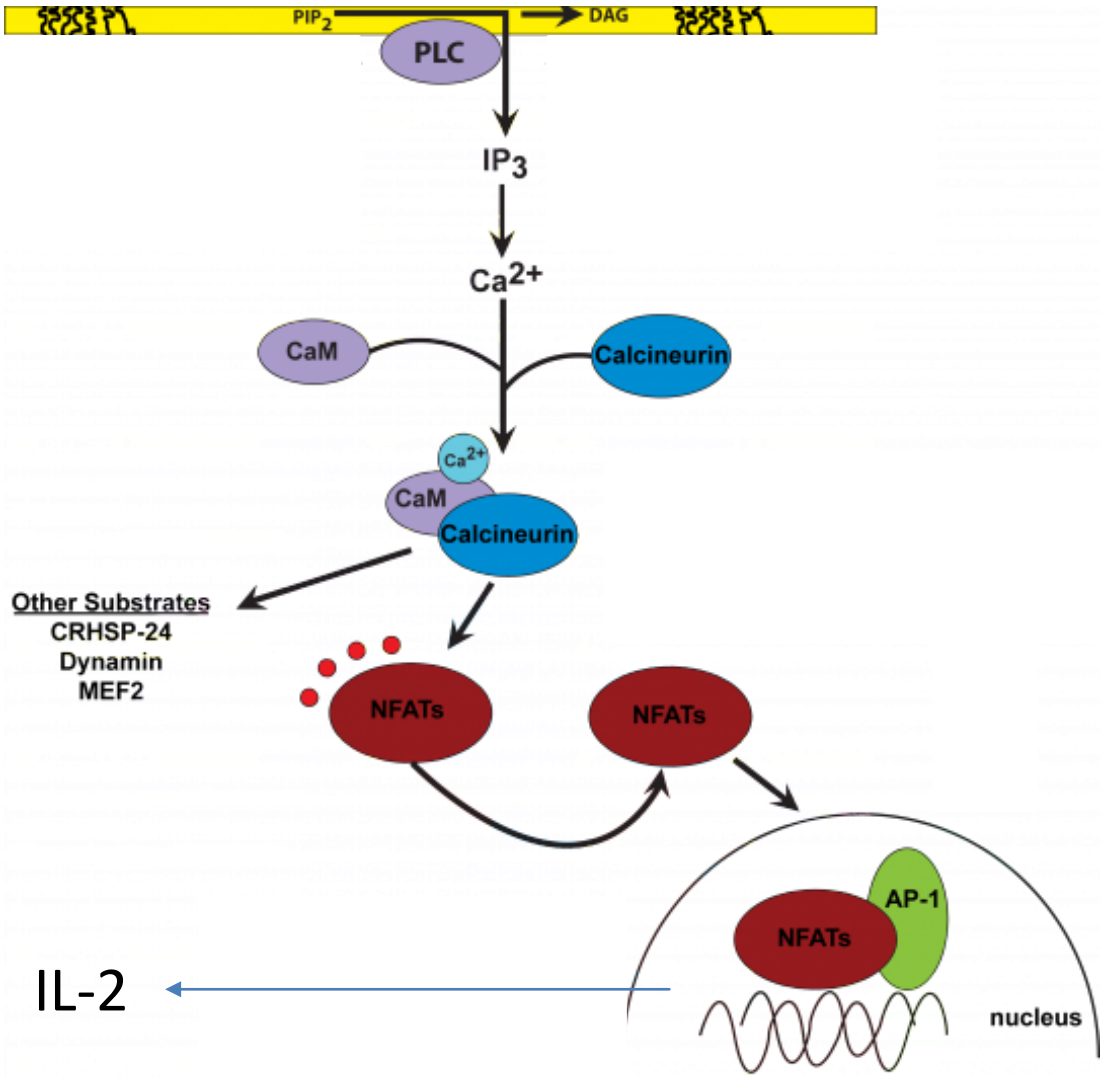


TCR dysregulation

- CD45 misexpression: immune deficiency or autoimmunity
- CD45 polymorphisms: Multiple sclerosis
- CD3 mutations: Severe combined immunodeficiency (SCID)
- CD3ζ reduced expression: Rheumatoid arthritis and SLE
- ZAP-70 absence: T cell development blocked at DP (CD4+ CD8+) stage in thymus. Complete absence of CD4+ and CD8+ cells
- ZAP-70 mutation: Absence of CD8+ T cells and functionally impaired CD4+ Tcells
- Mutations of SH3 domain in ZAP-70: Rheumatoid arthritis
- Mutations in TCR signaling components: T cell malignancies

NFAT signaling pathway

NFAT (nuclear factor of activated T cells) pathway



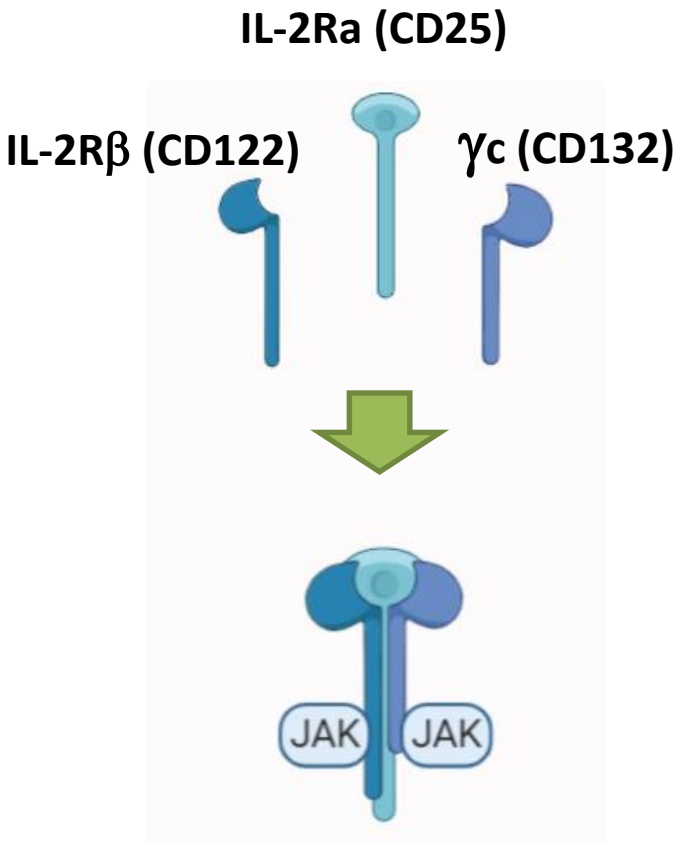
IL-2R signaling pathway

IL-2R signaling pathway

- IL-2 is promotes T cell survival and differentiation.

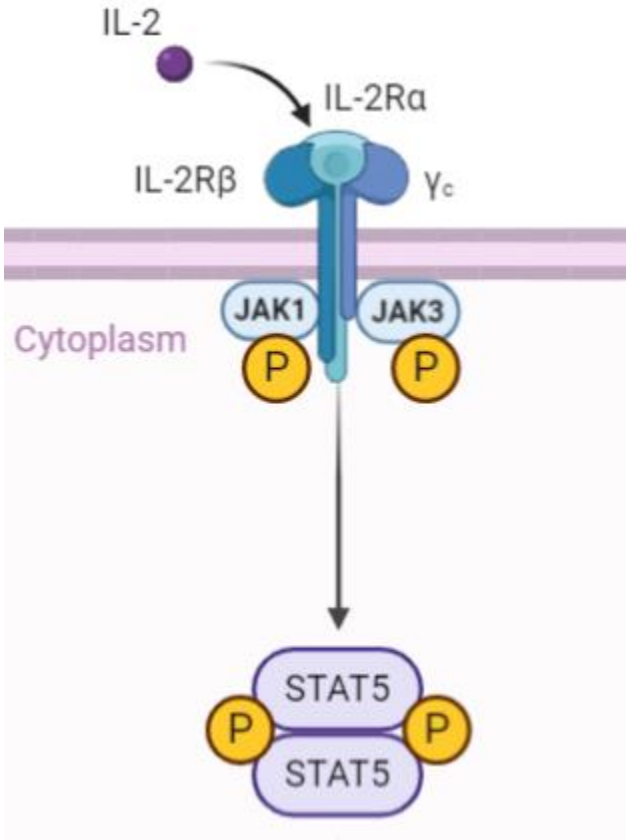
- JAK/STAT signaling pathway:
  - JAK: Janus Kinase (JAK1, JAK2, JAK3, TYK2)
    - Tyrosine kinase activity
    - SH2 domain
  - STAT: Signal Transducer and Activators of Transcription
    - Transcription factor

IL-2 receptor (IL-2R)



- Trimeric receptor:
  - α chain: increases receptor affinity
  - β chain and γc subunit: couple to JAK

JAK/STAT signaling

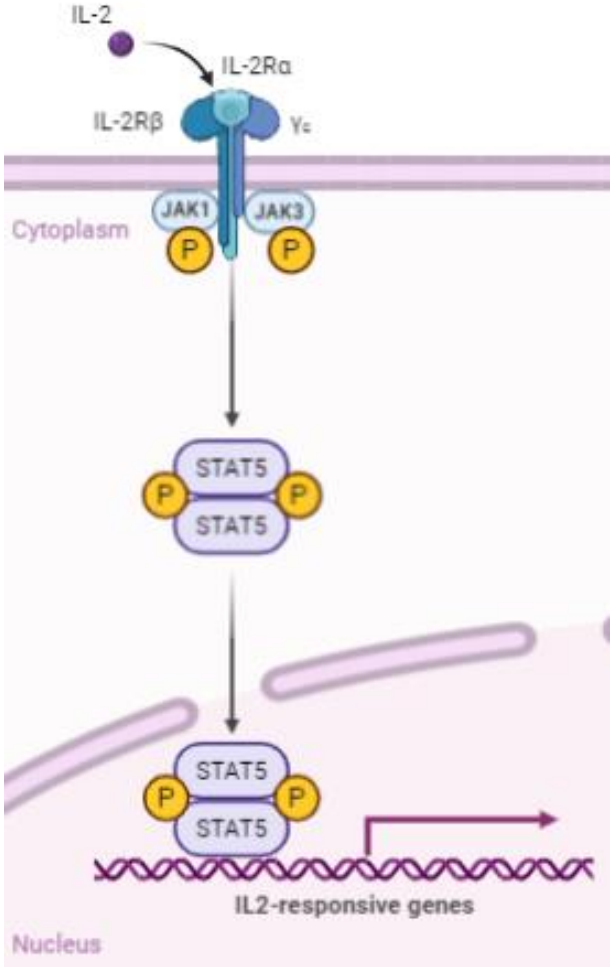


IL-2Rβ chain - JAK1

γ_c subunit - JAK3

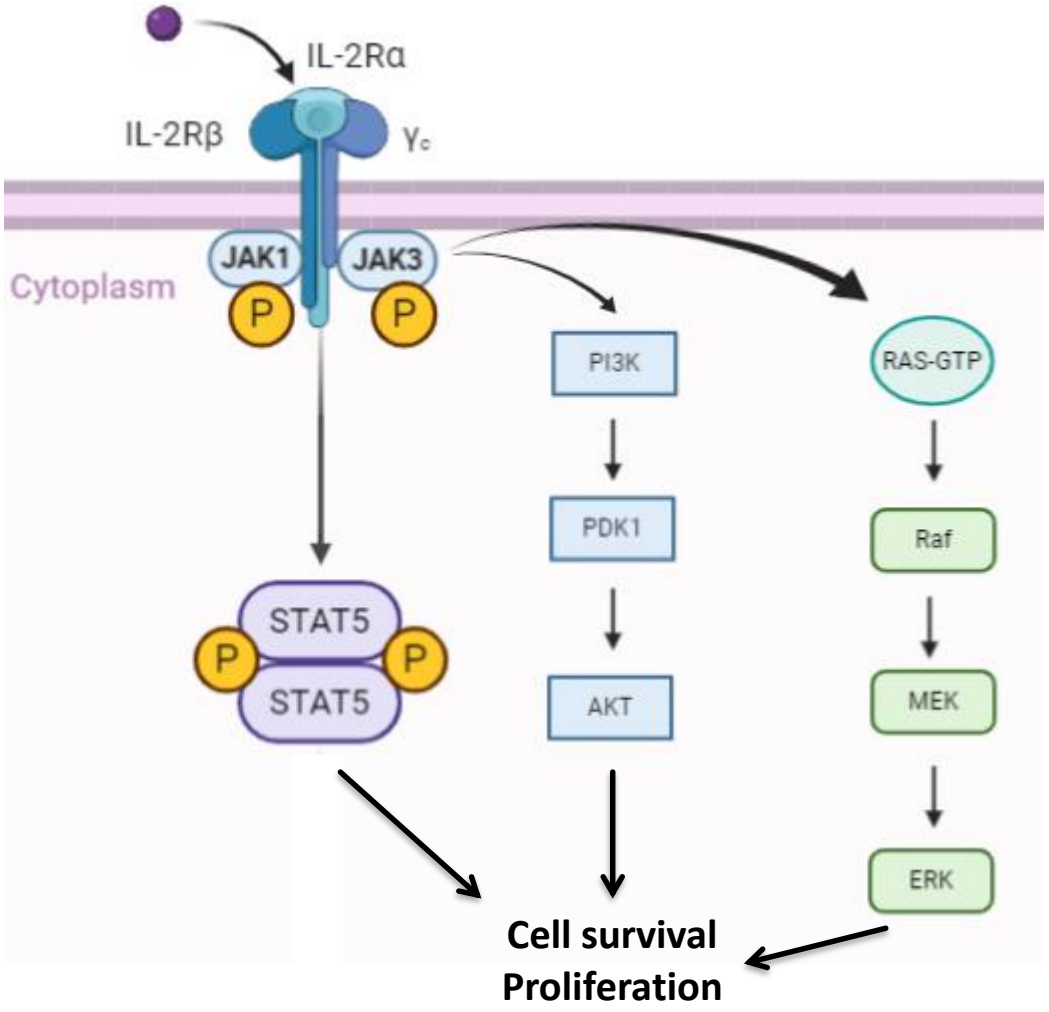
- JAK activation results in IL-2Rβ and γc subunit phosphorylation
- Tyrosine phosphorylation permits the recruitment of STAT5A, STAT5B, and STAT3

JAK/STAT signaling



- Tyrosine phosphorylation of STAT induces dimerization, nuclear translocation, and STAT-mediated transcription.

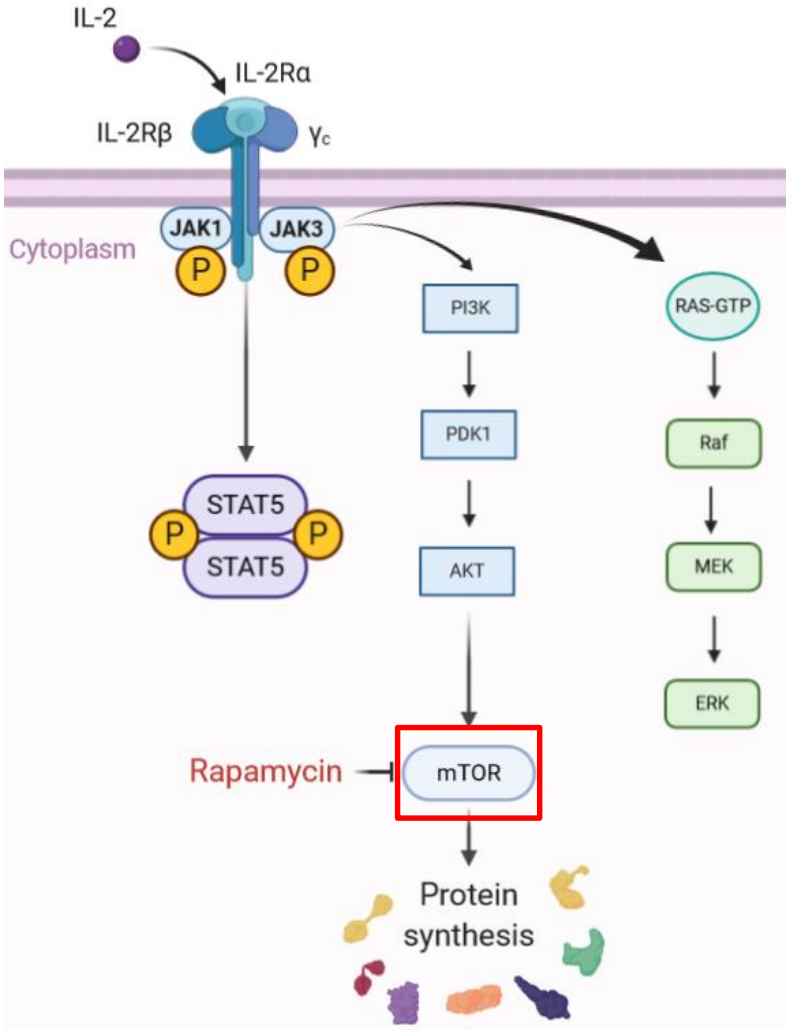
IL-2R signal transduction



IL-2R:

- STAT phosphorylation
- Activation of:
  - PI3K/AKT pathway
  - MAPK cascade

Immunosuppression via IL-2R signaling inhibition



- IL-2R signaling promotes protein synthesis via mTOR activation
- mTOR inhibitor Rapamycin blocks cell cycle progression in IL-2 stimulated T cells

IL-2R dysregulation

- IL-2α chain mutations: Decrease numbers of peripheral T cells. Extensive lymphocytic infiltration of tissues. Severe Combined Immuno Deficiency-like (SCID-like) features and overwhelming autoimmunity.

- IL-2Rβ chain mutations: Severe immune dysregulation autoantibodies, hypergammaglobulinemia, bowel inflammation, dermatological abnormalities, lymphadenopathy.

- IL-2Rγ chain deletion: Absence of peripheral T cells. X-linked severe combined immunodeficiency (SCID)

Thank you for your attention!

Questions?

Please write to carlos.plazasirvent@rub.de