# Signal Transduction in Immune Cells

-1-

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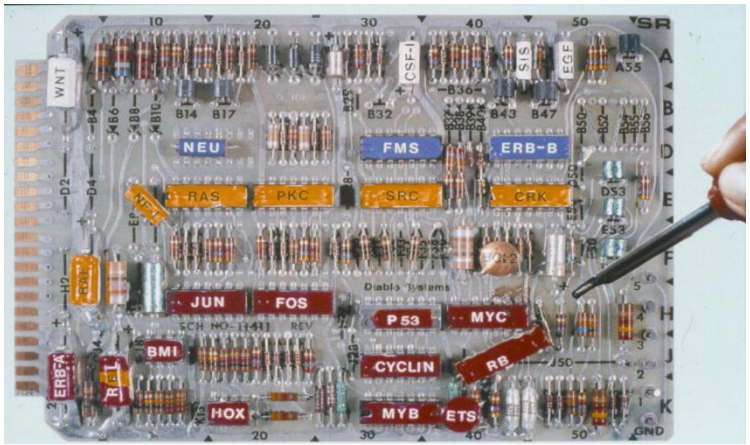
RUB

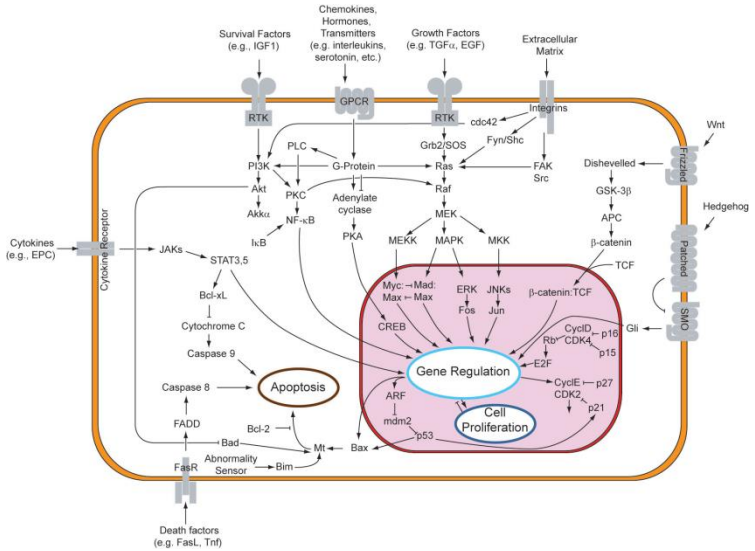
Molecular Immunology

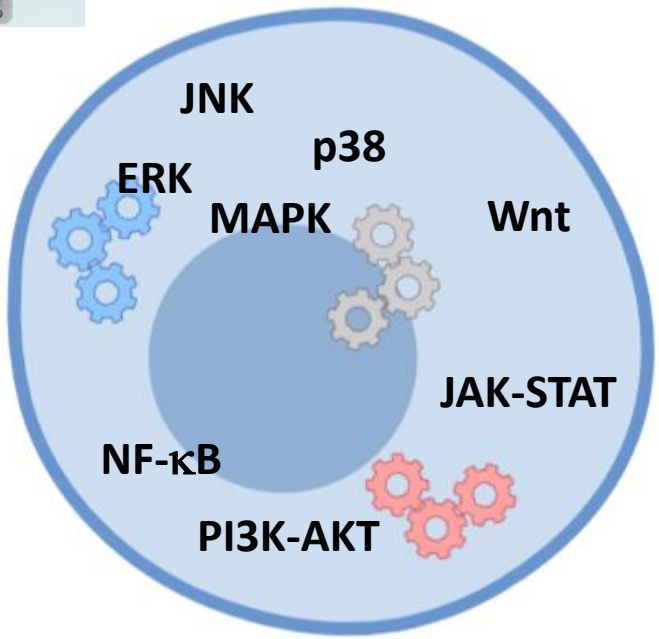
# Index

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

# Introduction



# Introduction

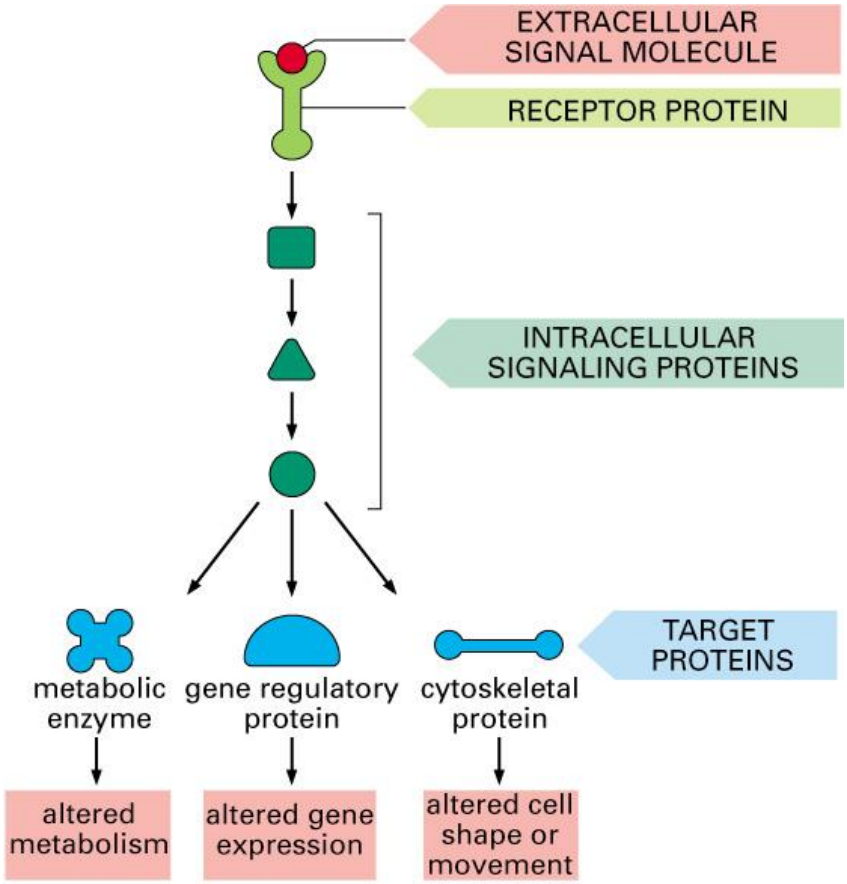
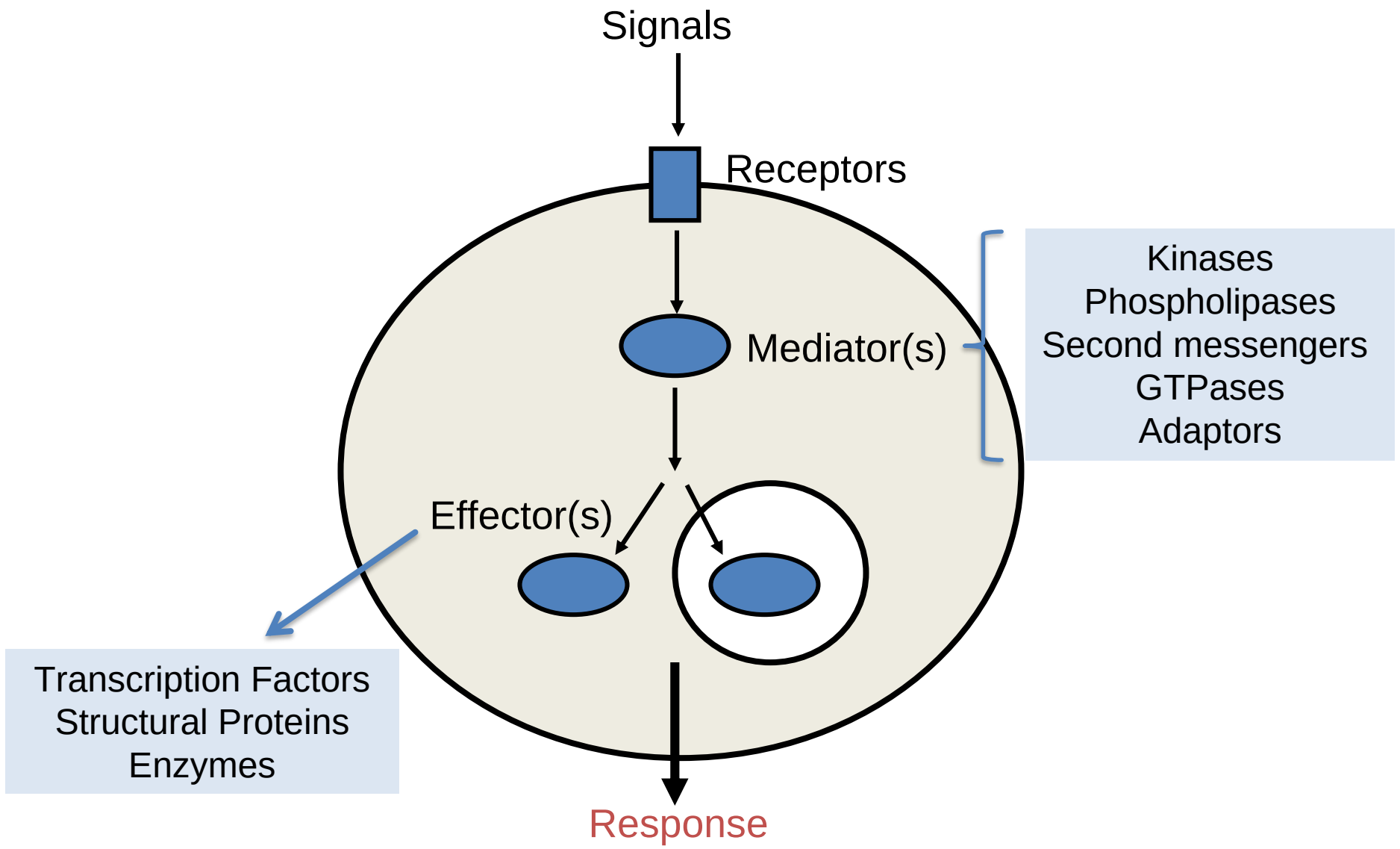



Figure 15–1. Molecular Biology of the Cell, 4th Edition.

# Introduction



# Response

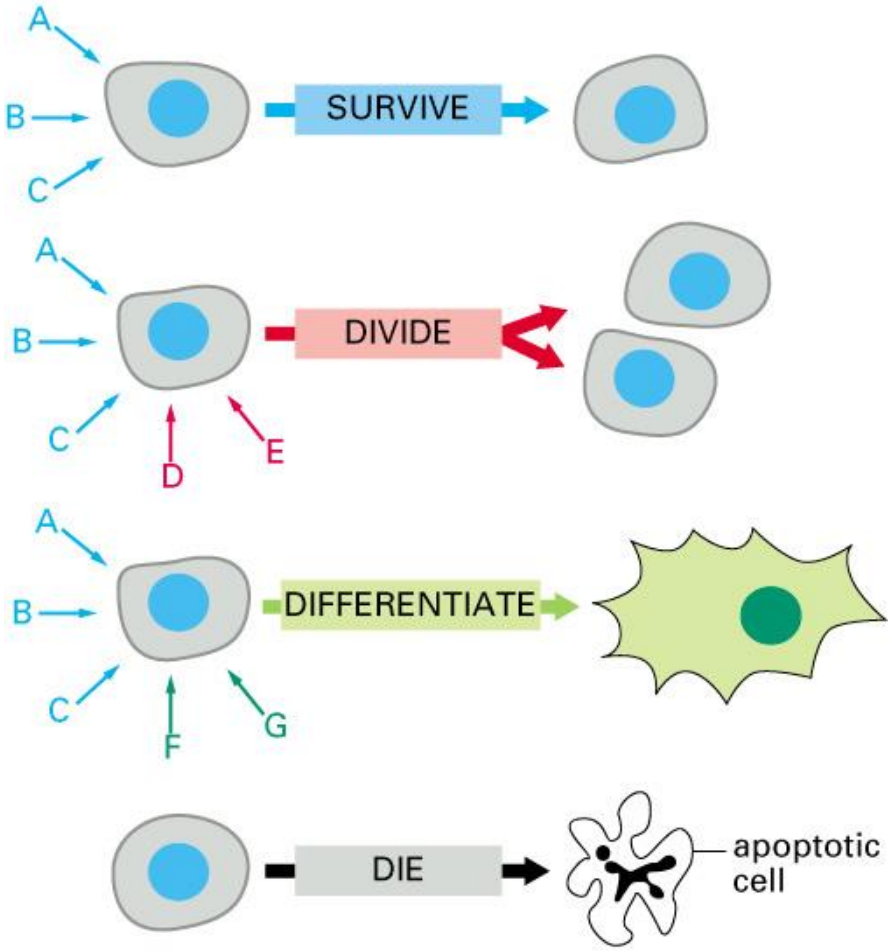
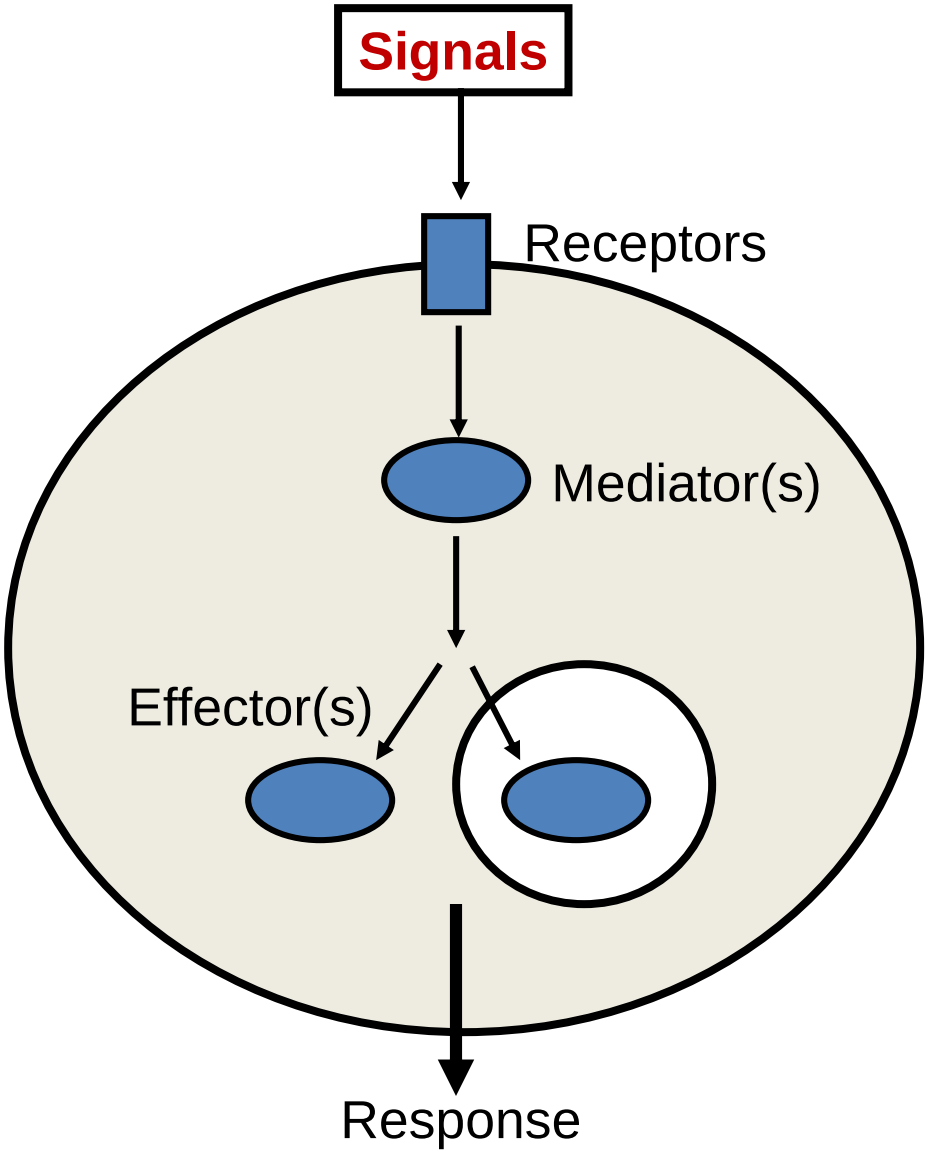


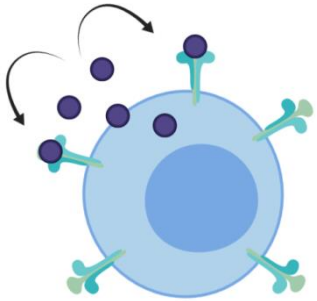

Figure 15–8. Molecular Biology of the Cell, 4th Edition.

# Signals

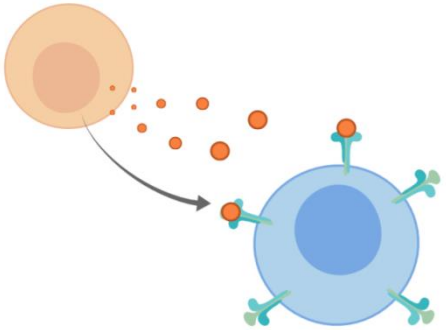


# Signals

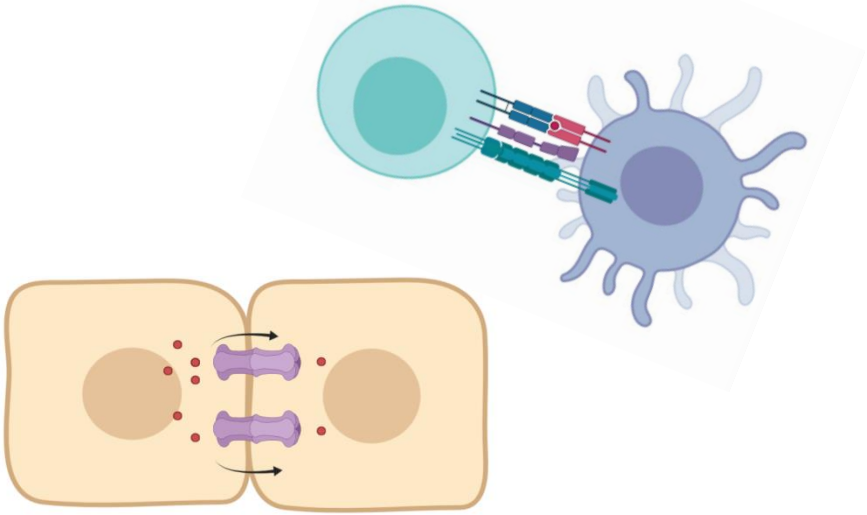
**Autocrine**



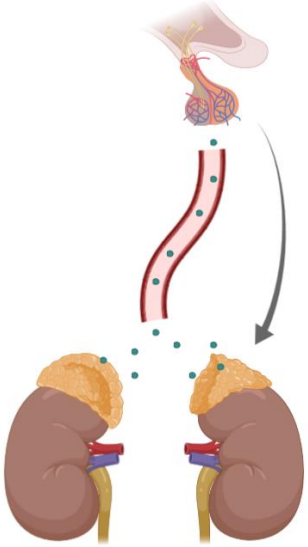
**Paracrine**



**Juxtacrine**



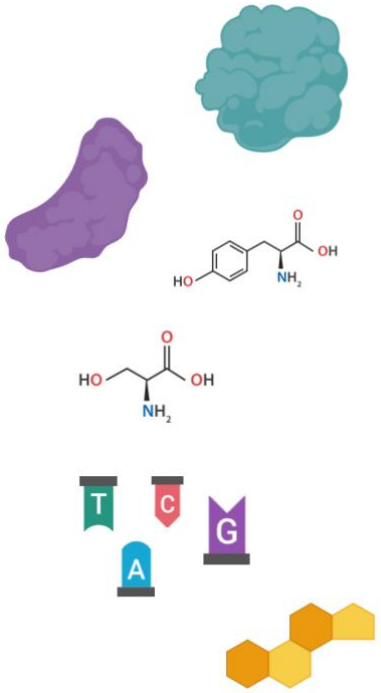
**Endocrine**



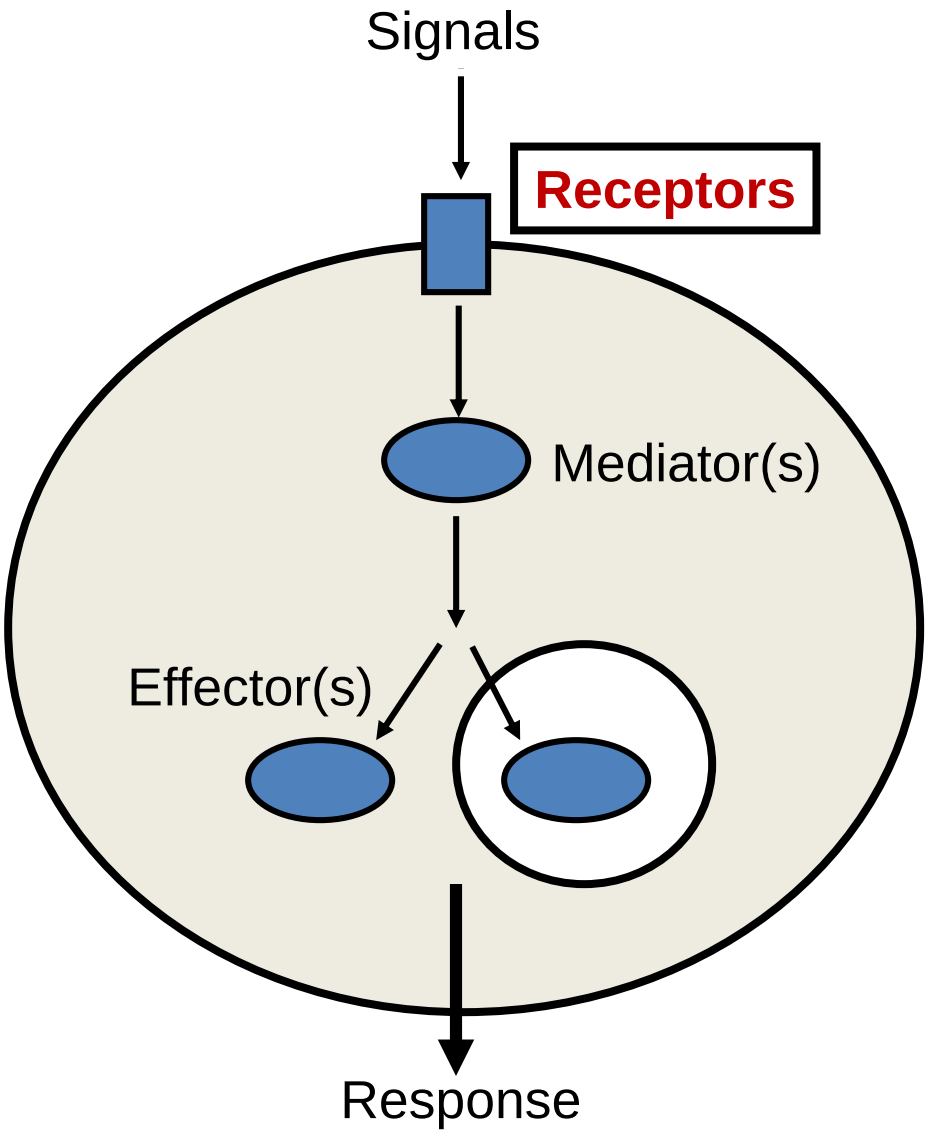
# Signals

## Ligands

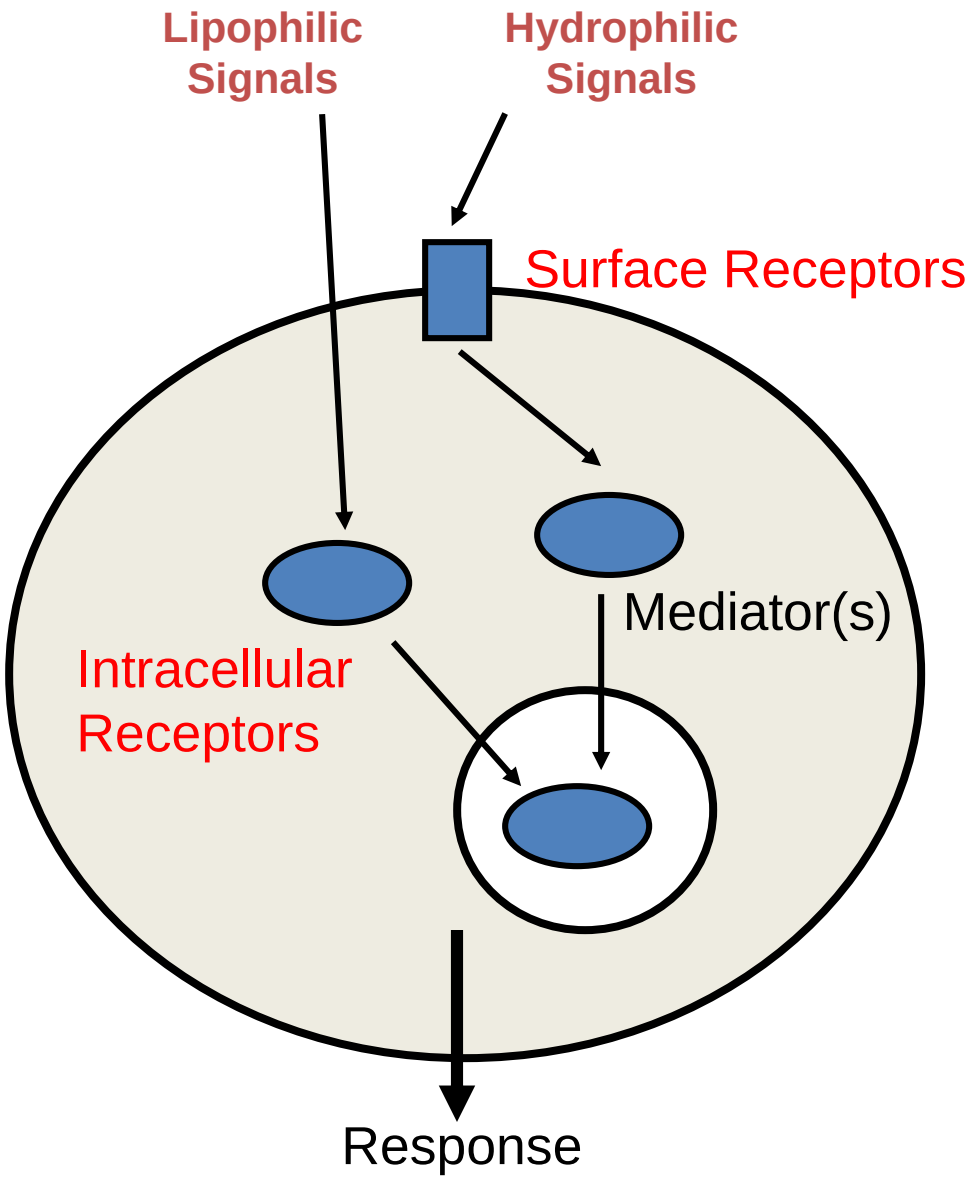
Proteins
Small peptides
Aminoacids
Nucleotides
Steroids
Retinoids
Fatty acids derivatives
NO, CO



# Receptors



# Receptors

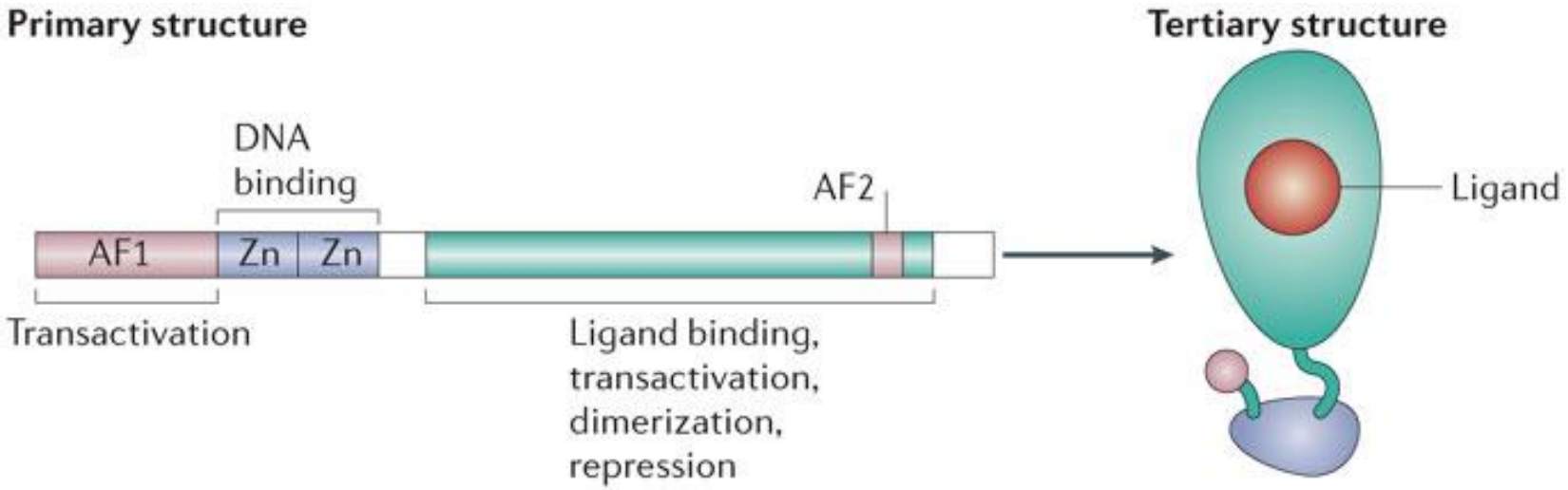

Lipophilic Signals
Hydrophilic Signals
Surface Receptors
Mediator(s)
Intracellular Receptors
Response

# Intracellular Receptors

Lipophilic ligands

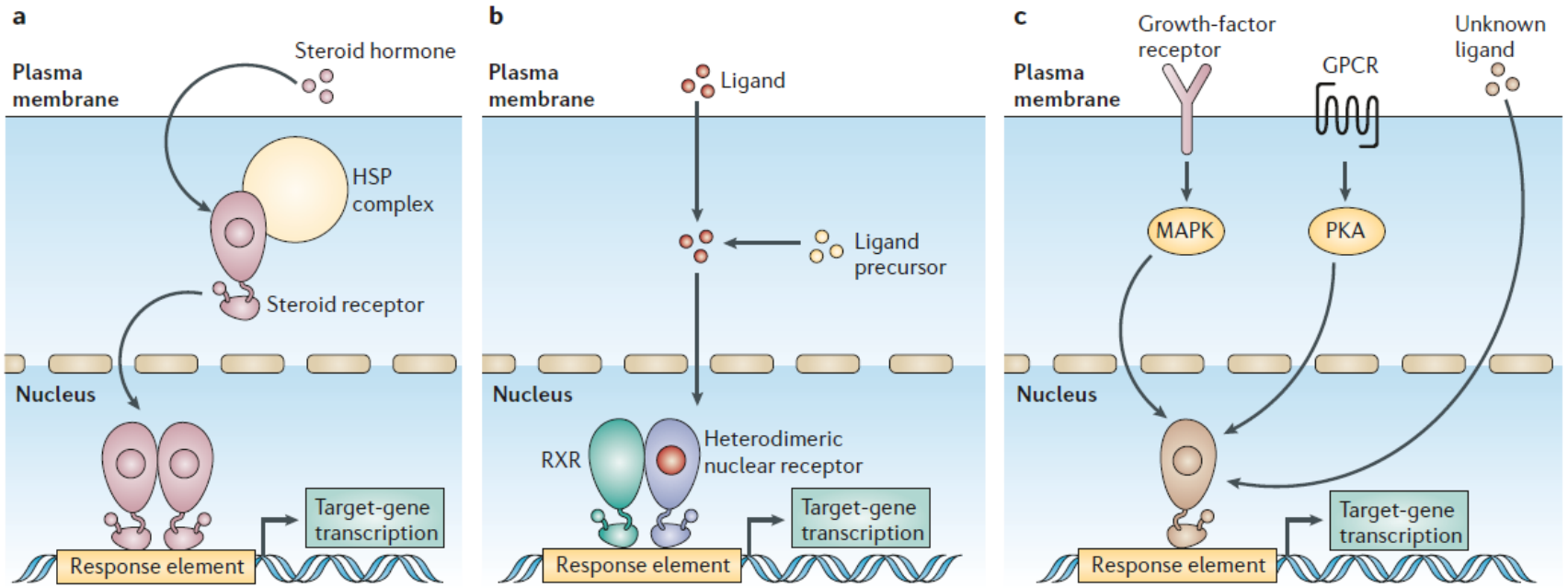
- Estrogen, progesterone, testosterone
- Vitamin D
- Glucocorticoids and mineralocorticoids
- Thyroid hormone

# Domain structure of nuclear receptors



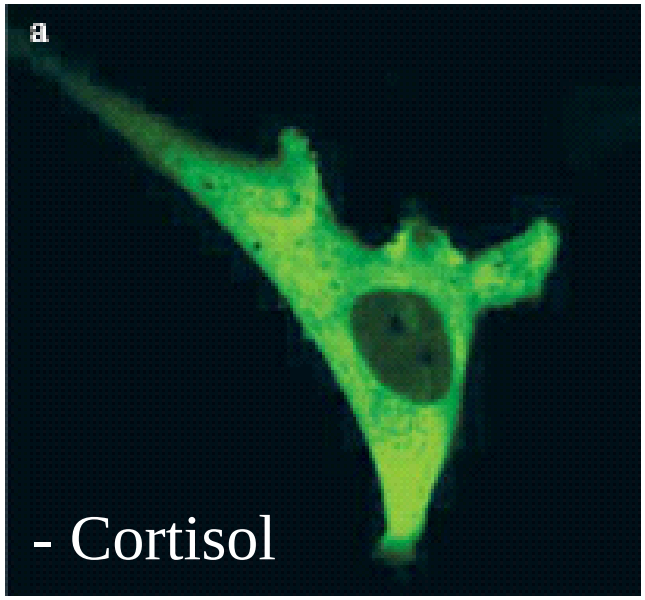

Christopher KG & Sumito O (2006) Combinatorial roles of nuclear receptors in inflammation and immunity
*Nat Rev Immu* 6: 44–55 doi:10.1038/nri1748

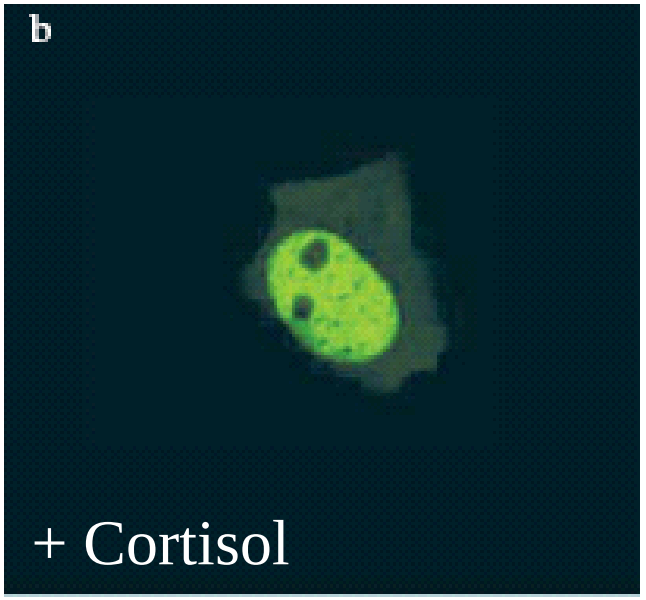
# Nuclear-receptor classes




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

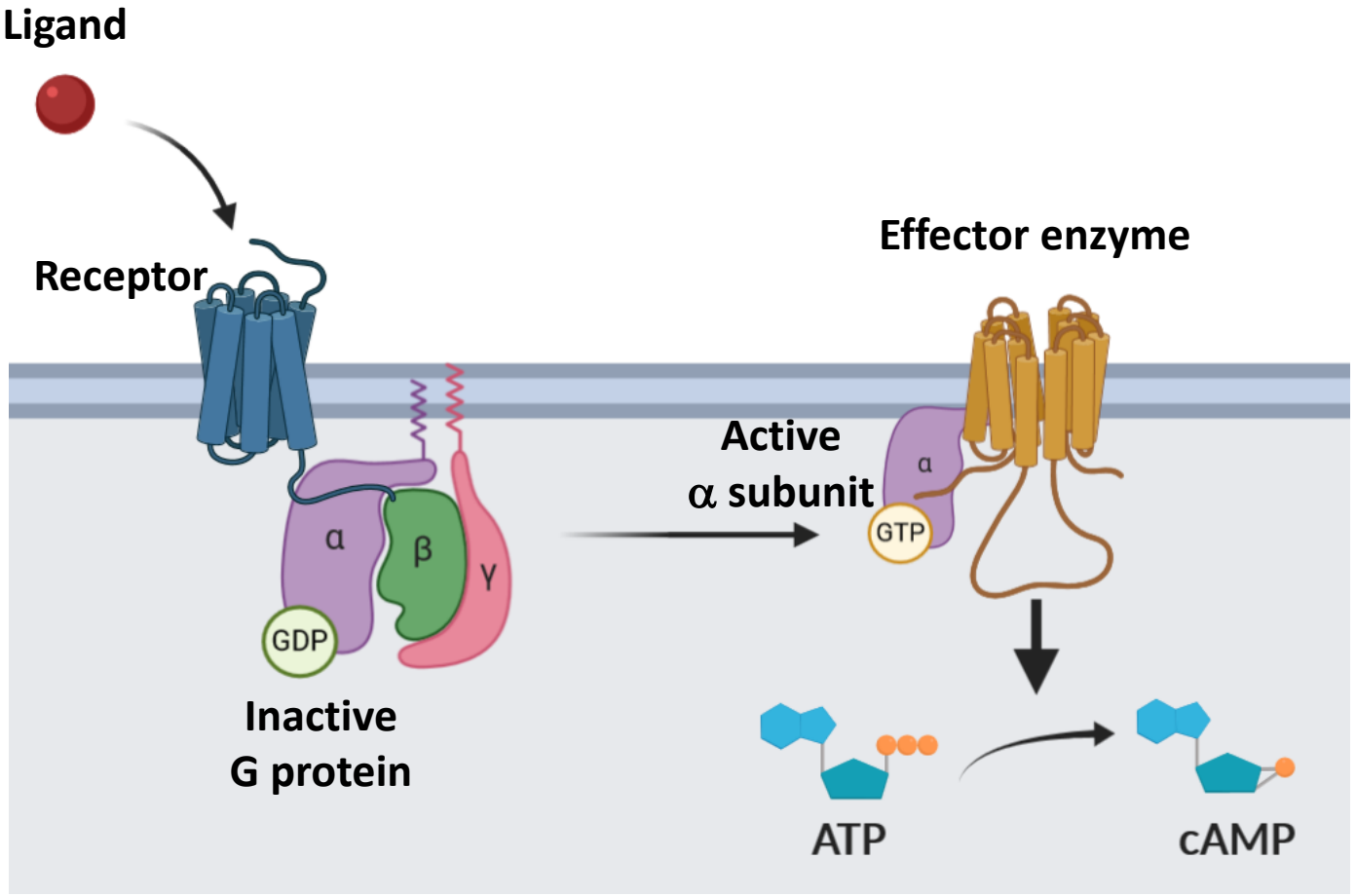
- Glucocorticoids inhibit NF-κB and AP-1 pathway activation preventing expression of inflammatory genes

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

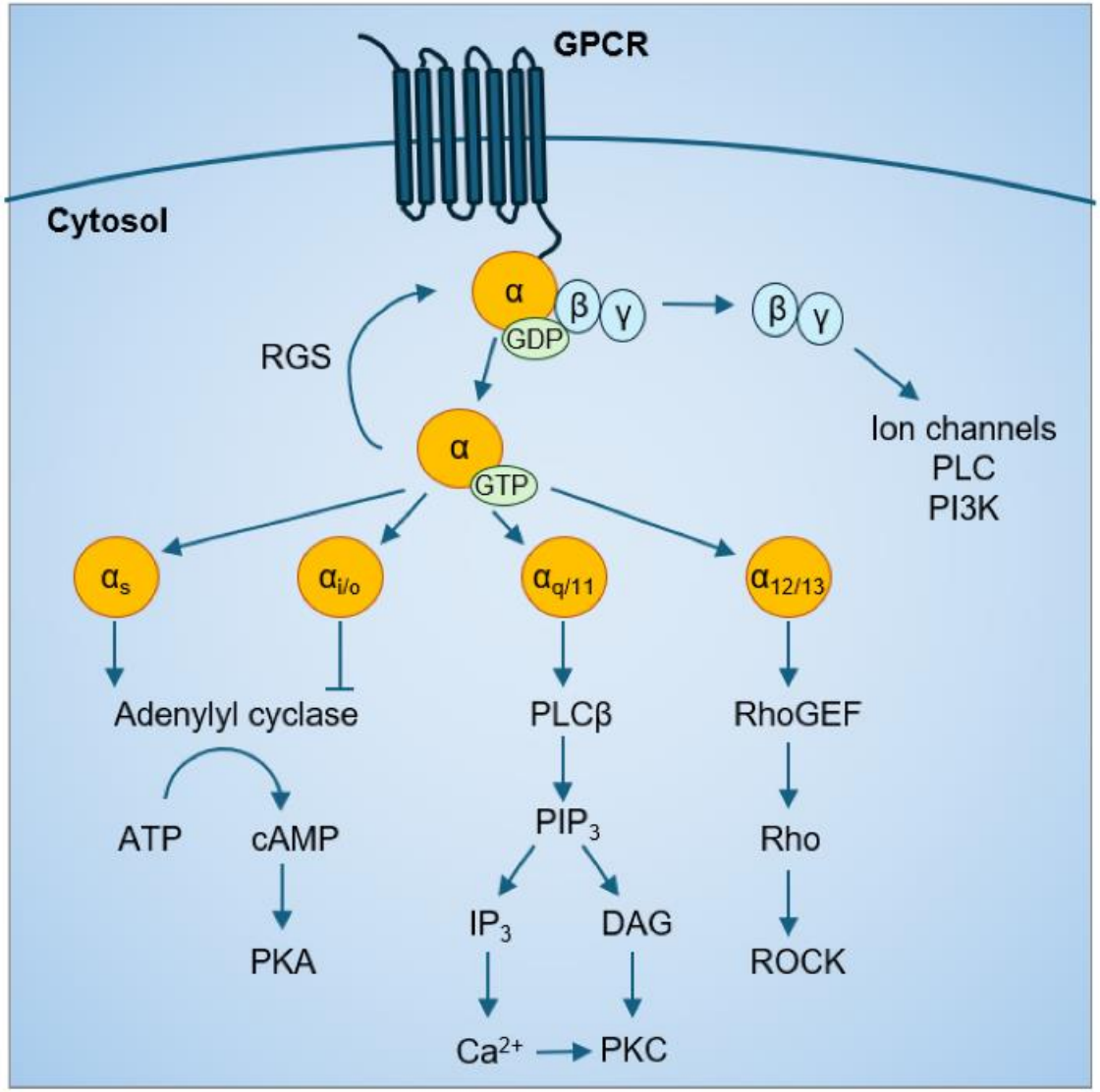
# Surface Receptors

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

# G-protein coupled receptors (GPCR)

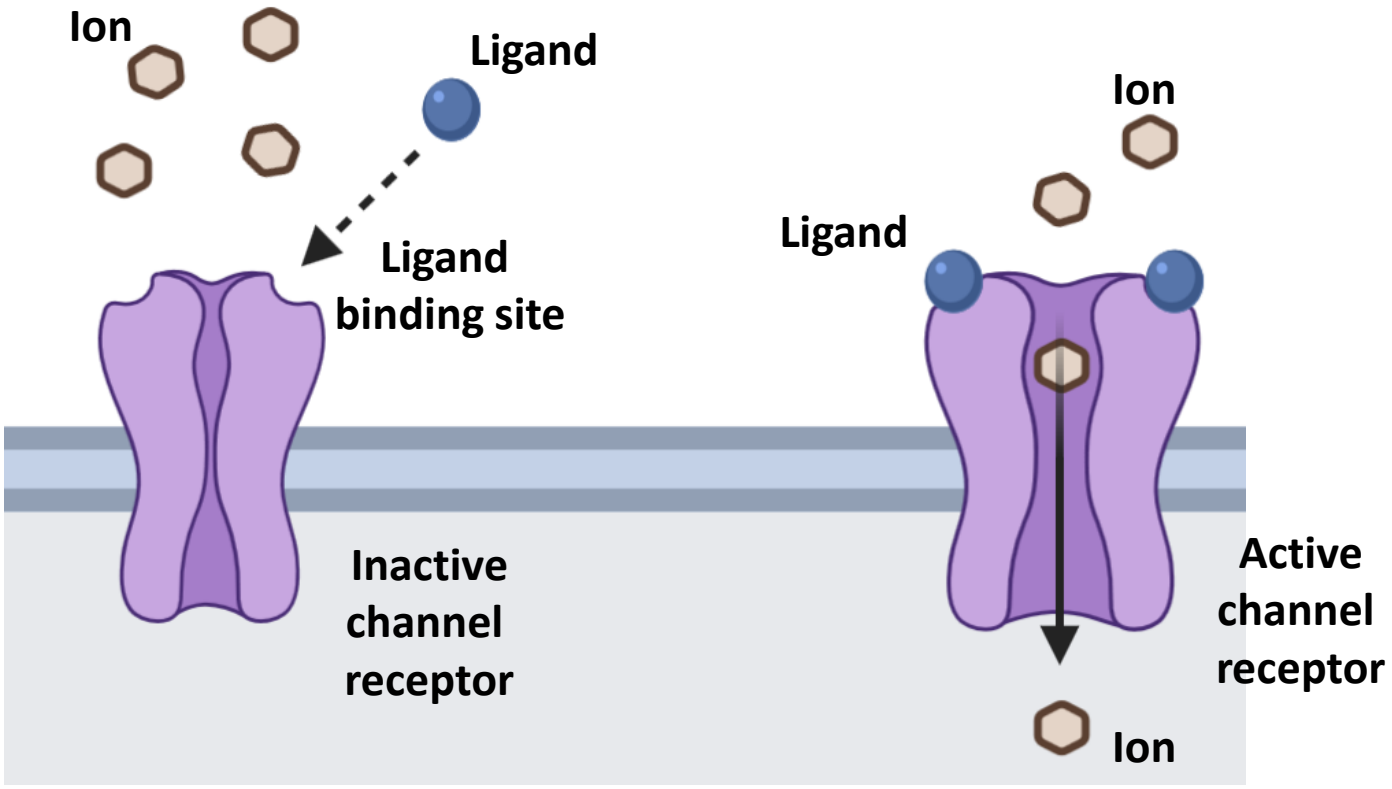


# G-protein coupled receptors (GPCR)

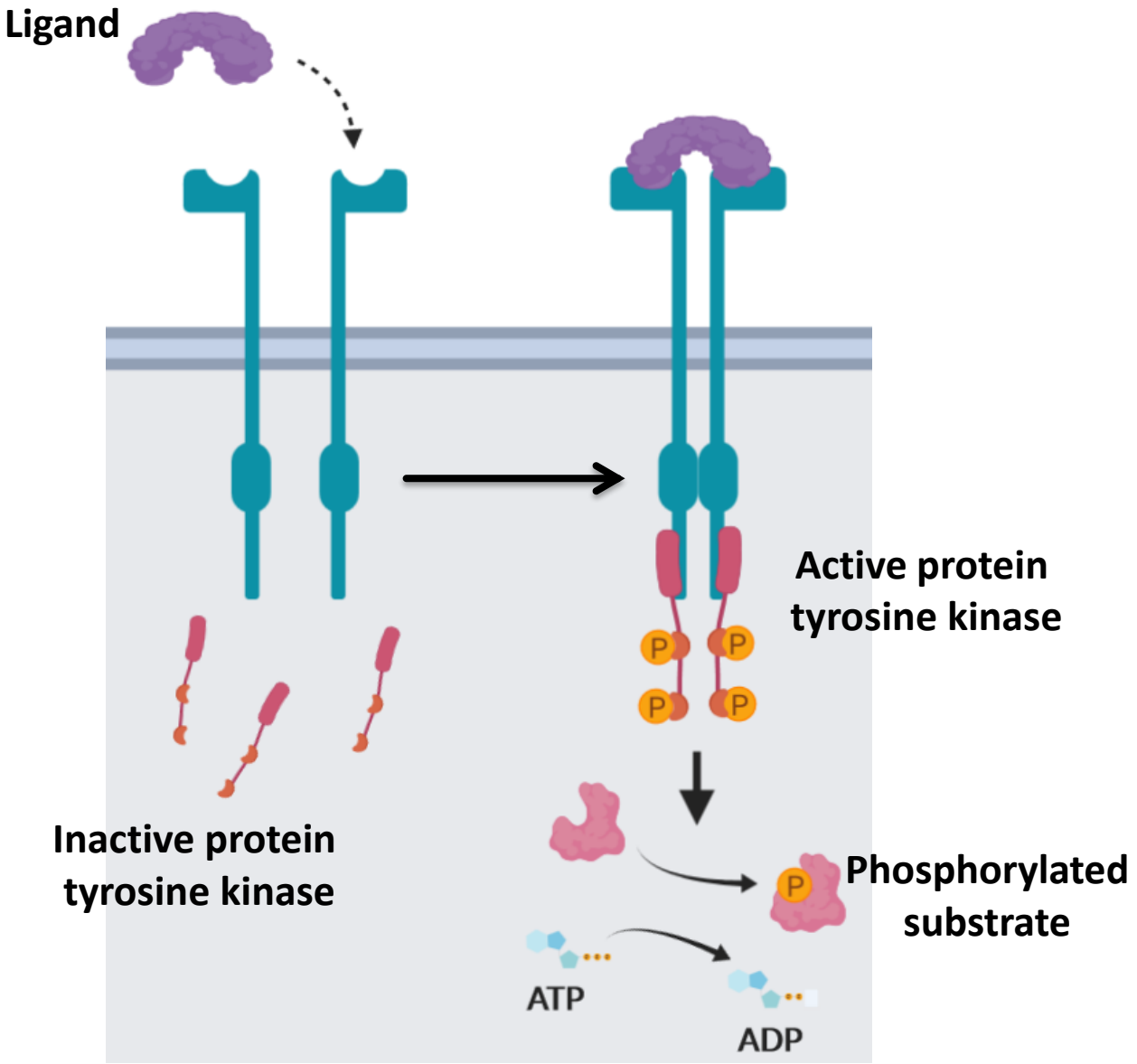



https://doi.org/10.3390/ijms25137162

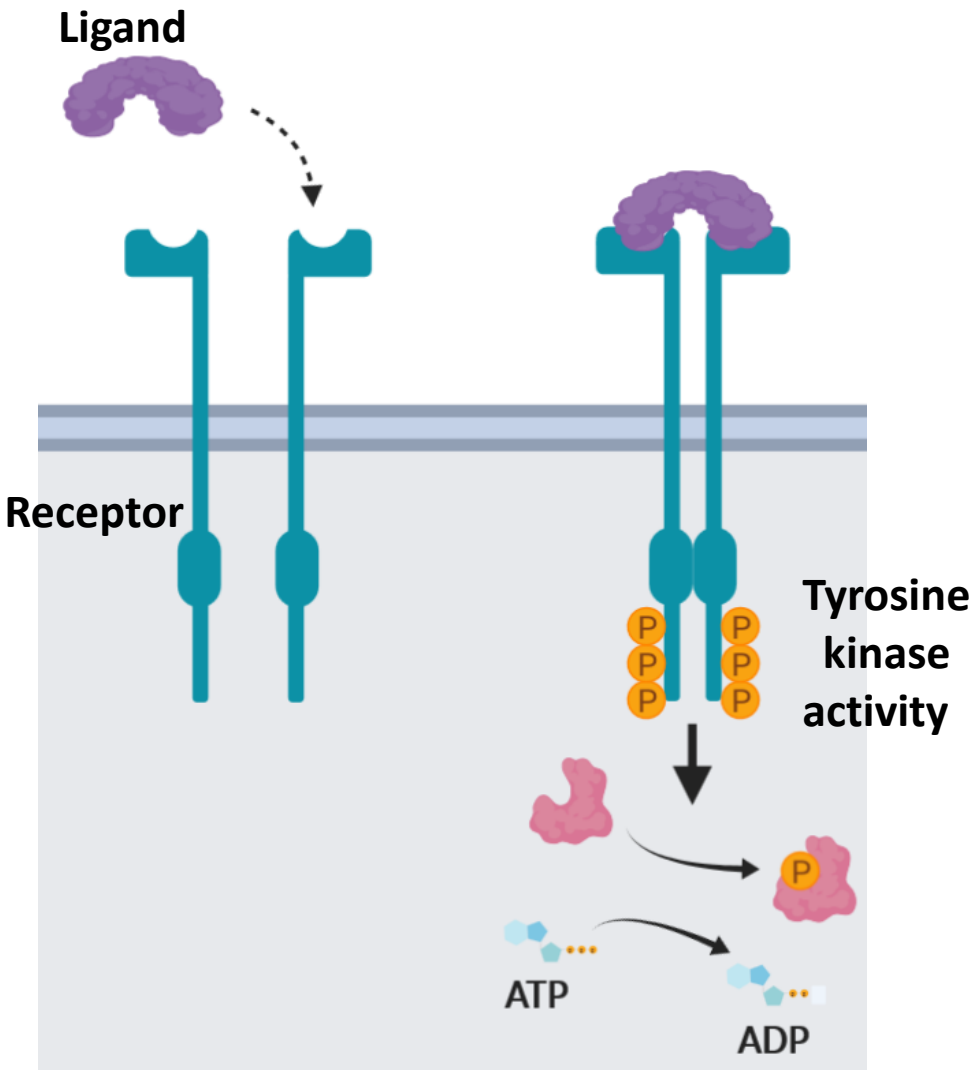
# Ion channel Receptor



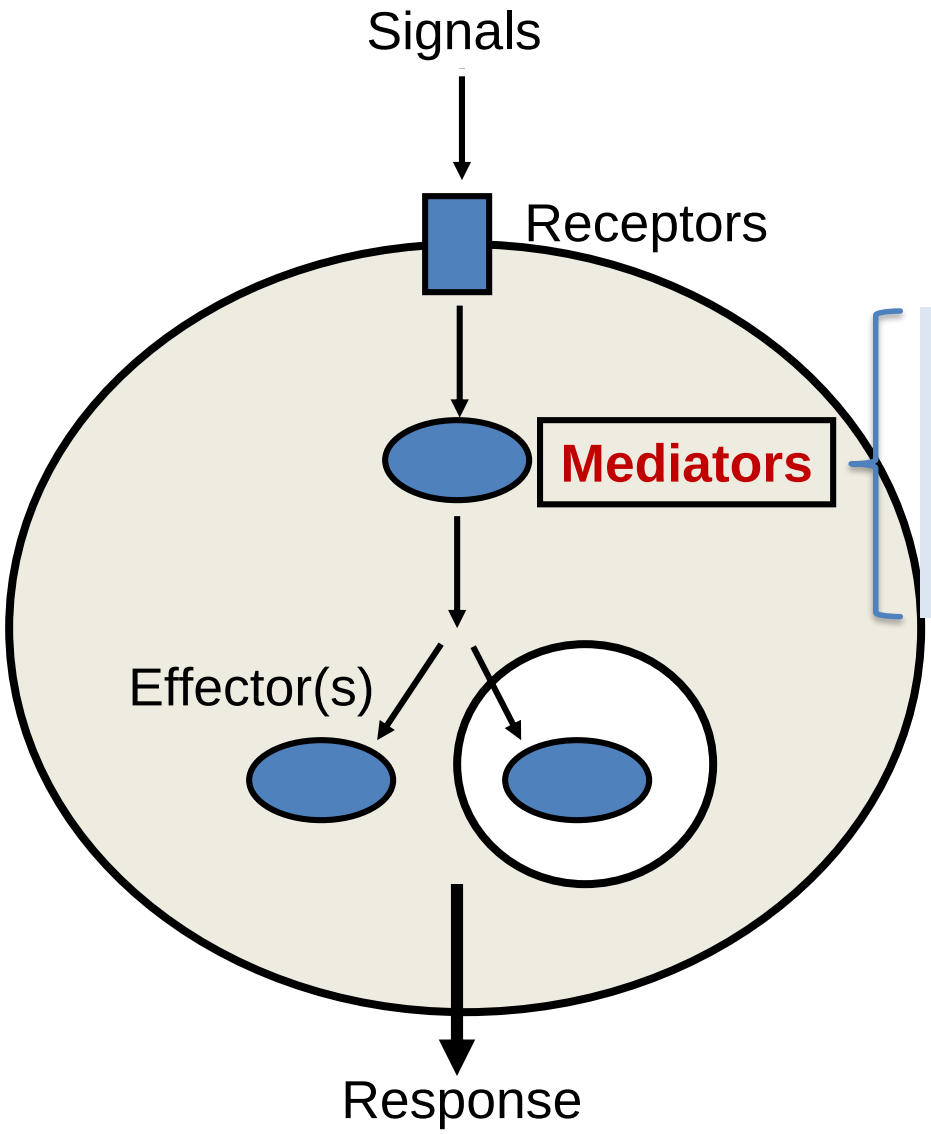
# Tyrosine kinase-linked receptors



# Receptors with intrinsic enzymatic activity

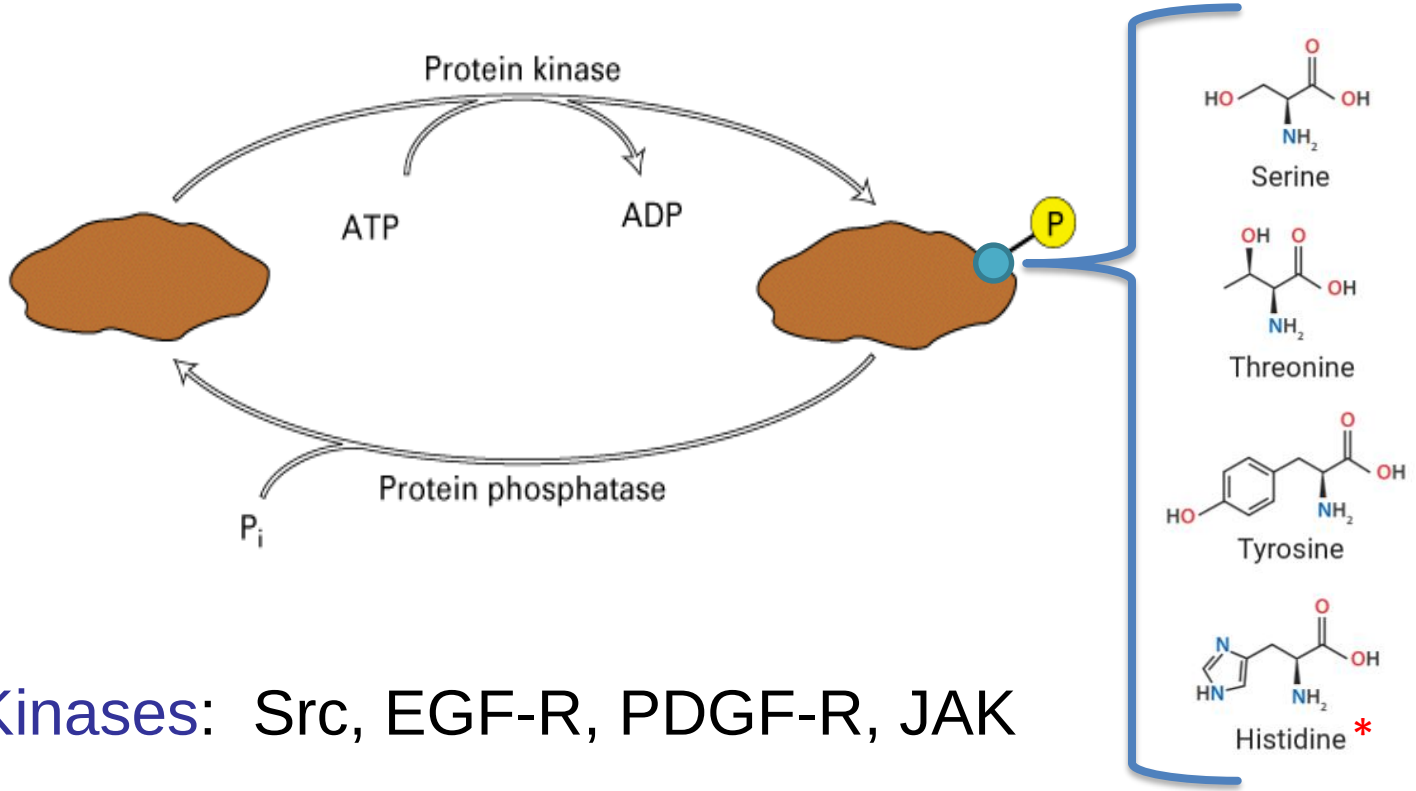


# Mediators




**Kinases & Phosphatases**
Phospholipases
Second messengers
GTPases
Adaptors

# Protein Kinases & Phosphatases



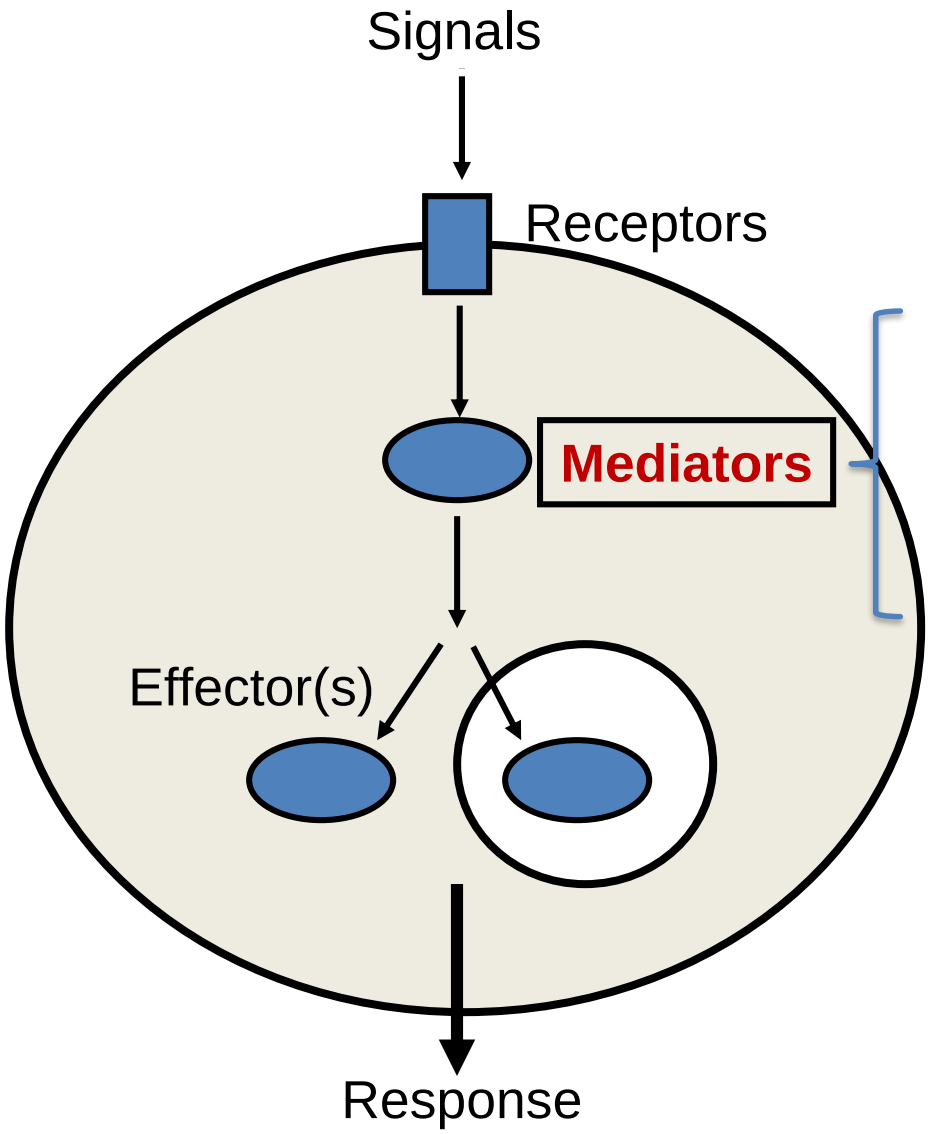

Tyr Kinases: Src, EGF-R, PDGF-R, JAK

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

Dual-specificity kinases: MEK, MKK

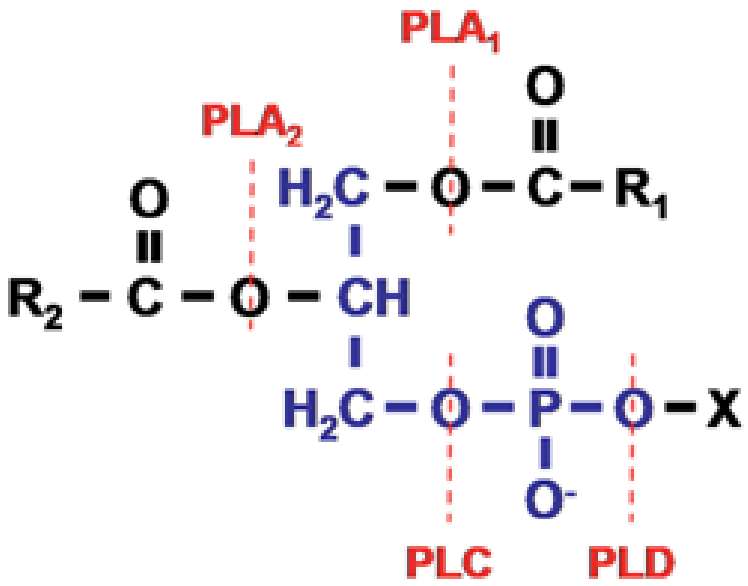
Lipid Kinase: PI3K

# Mediators



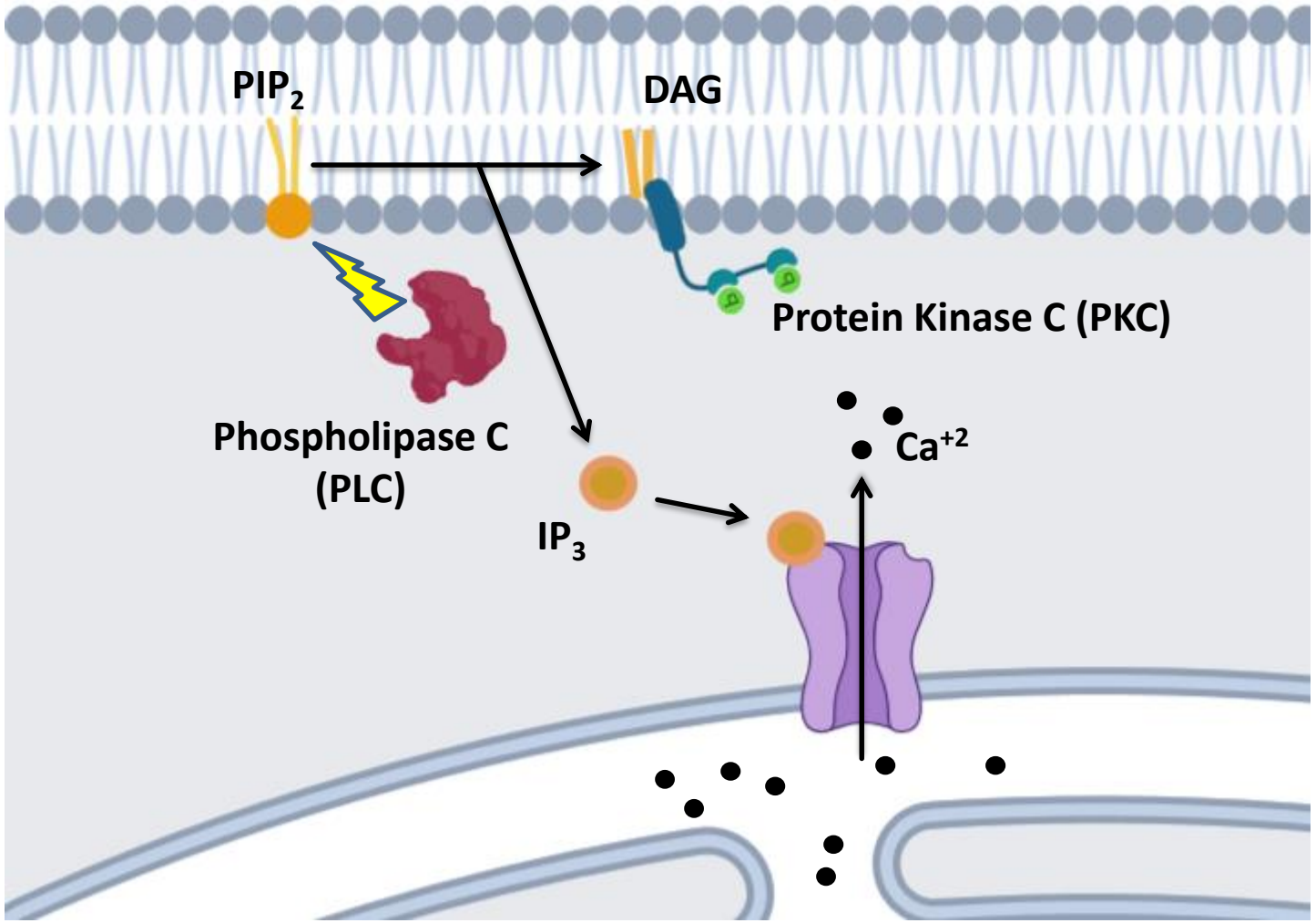

Kinases & Phosphatases
**Phospholipases**
Second messengers
GTPases
Adaptors

# Phospholipases

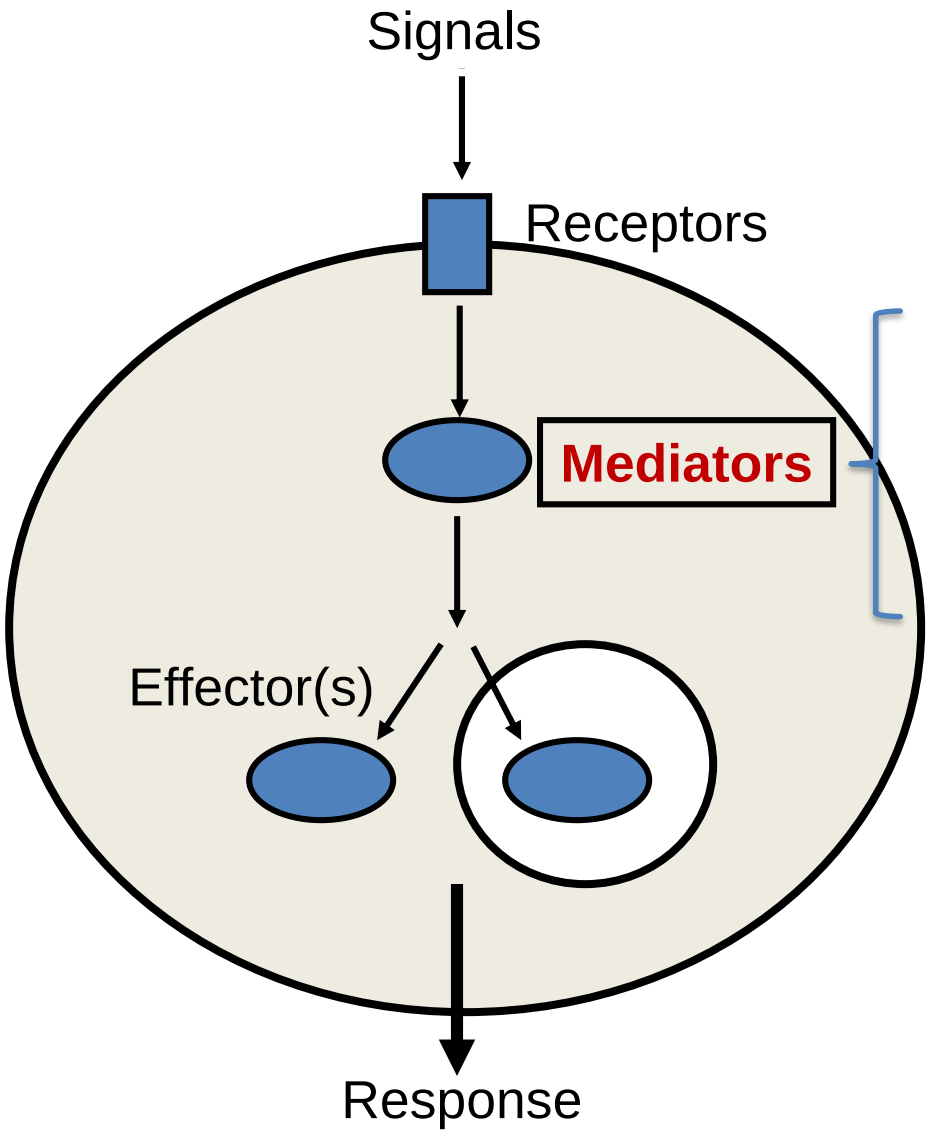



**Hydrolyze phospholipids**

# Phospholipases



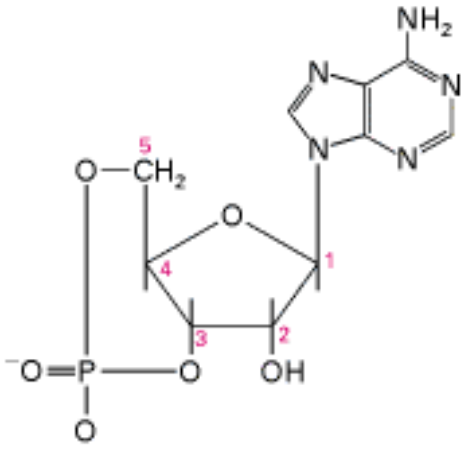
# Mediators



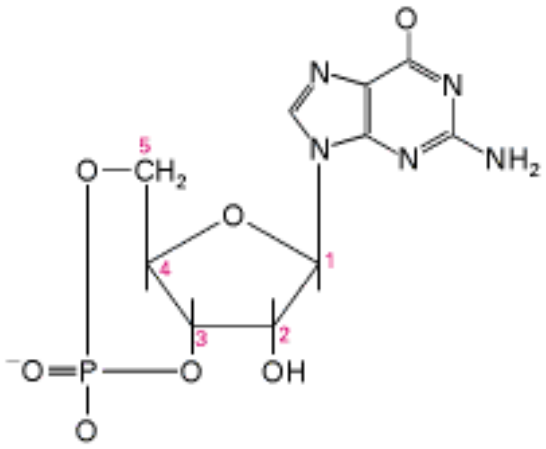

Kinases & Phosphatases
Phospholipases
**Second messengers**
GTPases
Adaptors

# Second Messengers

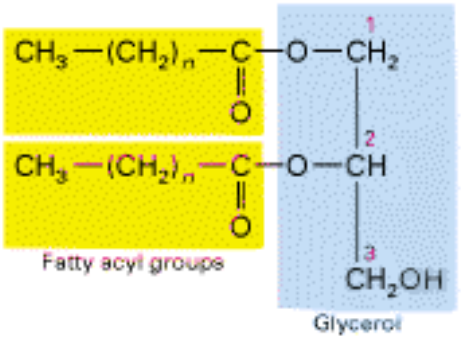
- Cyclic nucleotides
  - Cyclic AMP and cyclic GMP
- Lipid metabolites
  - Diacylglycerol
  - Inositol triphosphate
- Calcium



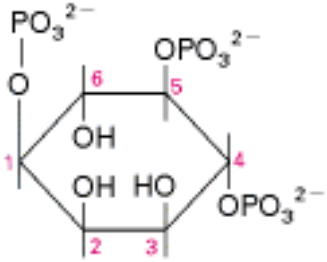

3′,5′-Cyclic AMP
(cAMP)




3′,5′-Cyclic GMP
(cGMP)

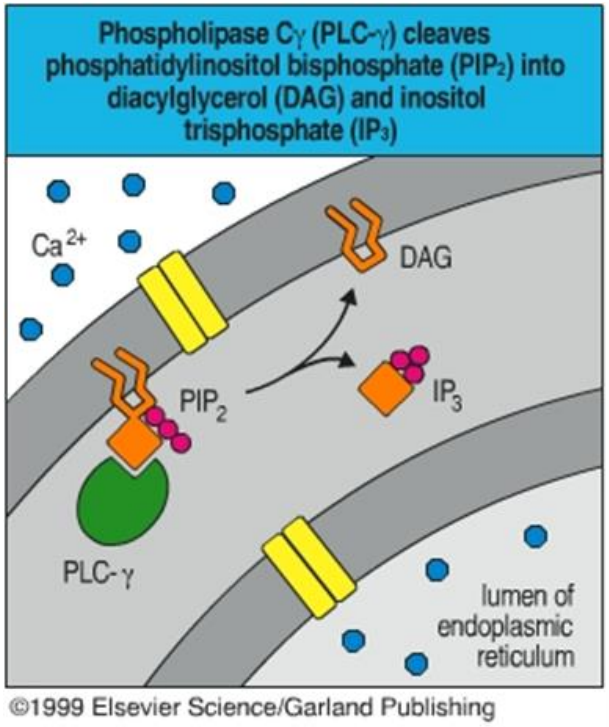


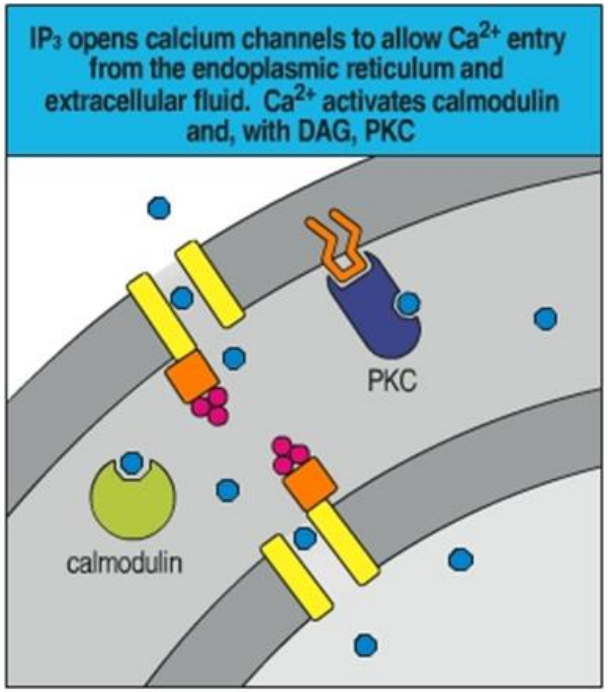

1,2-Diacylglycerol
(DAG)




Inositol
1,4,5-trisphosphate
($IP_3$)

# Second Messengers

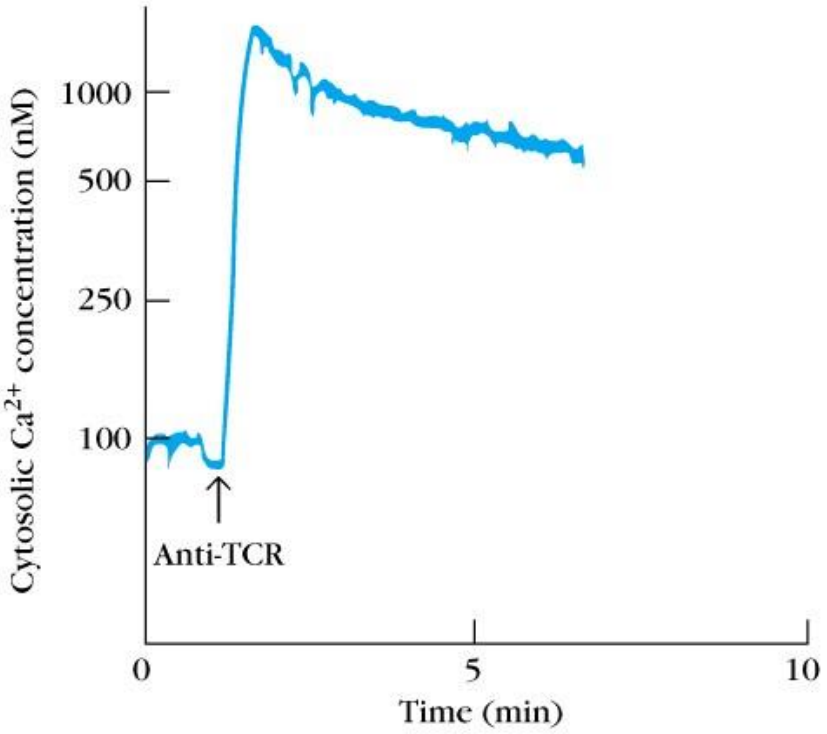


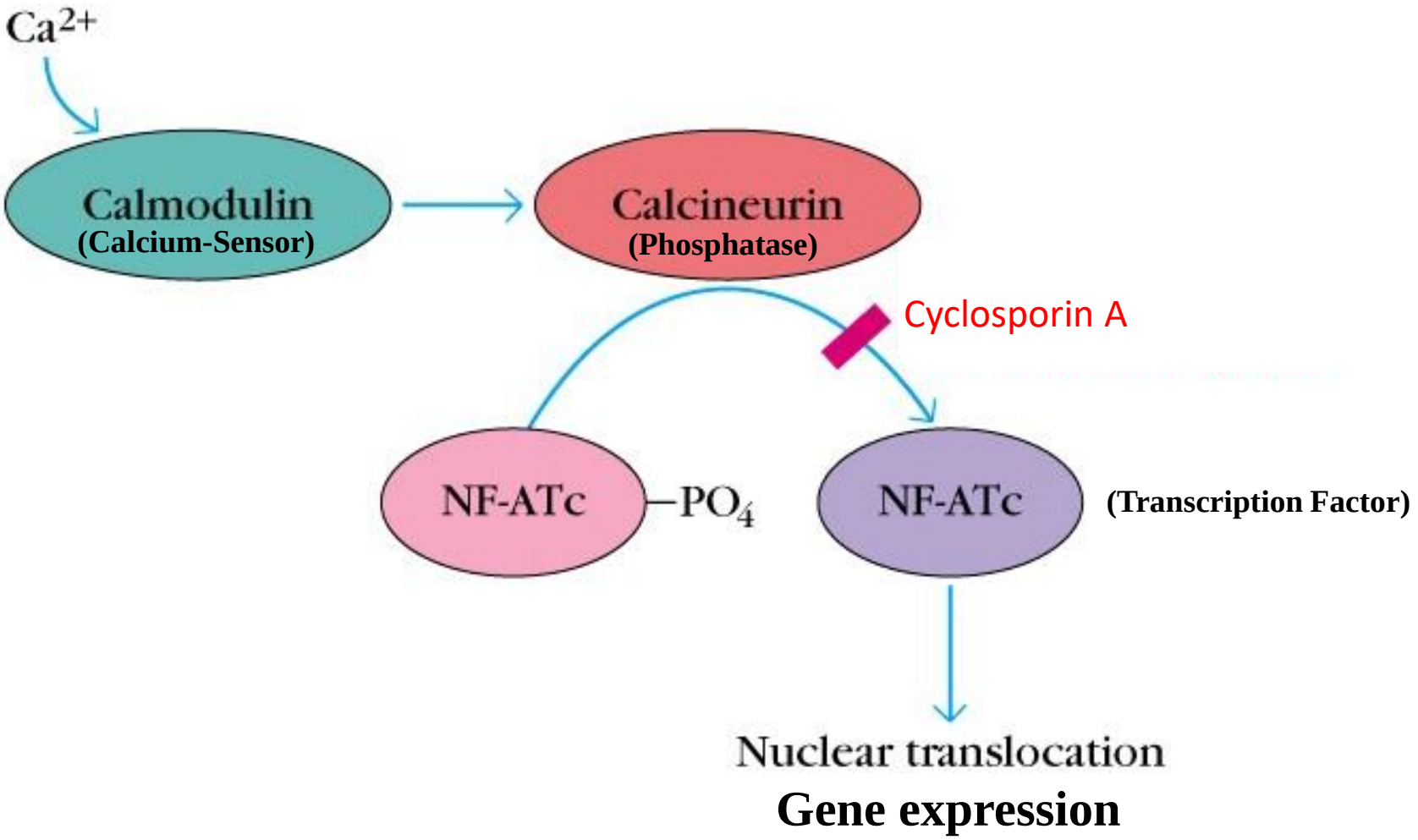
# Calcium

**Cellular calcium concentrations:**

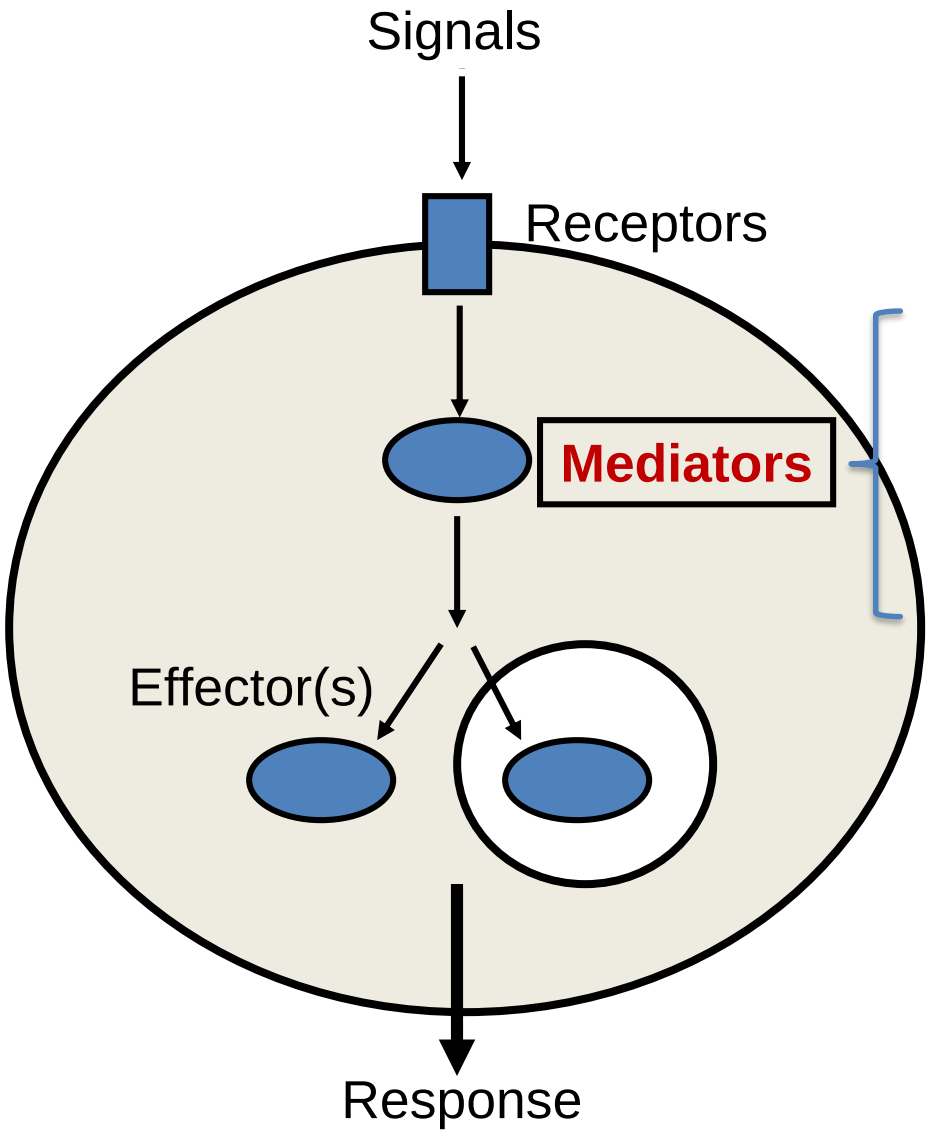
- extracellular: 1-2 mM
- cytosolic: 50 - 100 nM
- intracellular lumenal (ER etc.): 30 - 300 μM



# Calcium

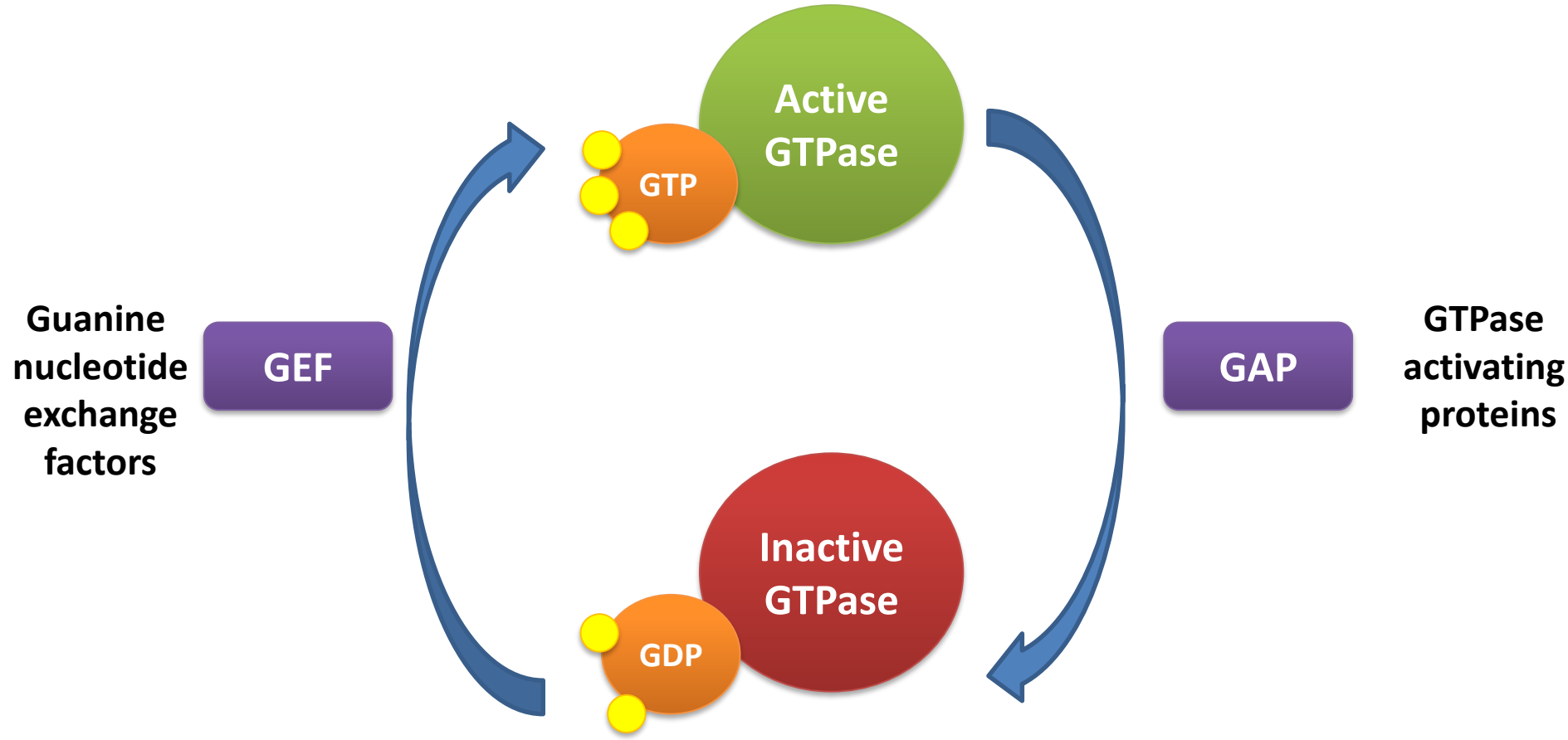

Ca2+
Calmodulin
(Calcium-Sensor)
Calcineurin
(Phosphatase)
Cyclosporin A
NF-ATc
PO4
NF-ATc
(Transcription Factor)
Nuclear translocation
Gene expression

# Mediators




Kinases & Phosphatases
Phospholipases
Second messengers
**GTPases**
Adaptors

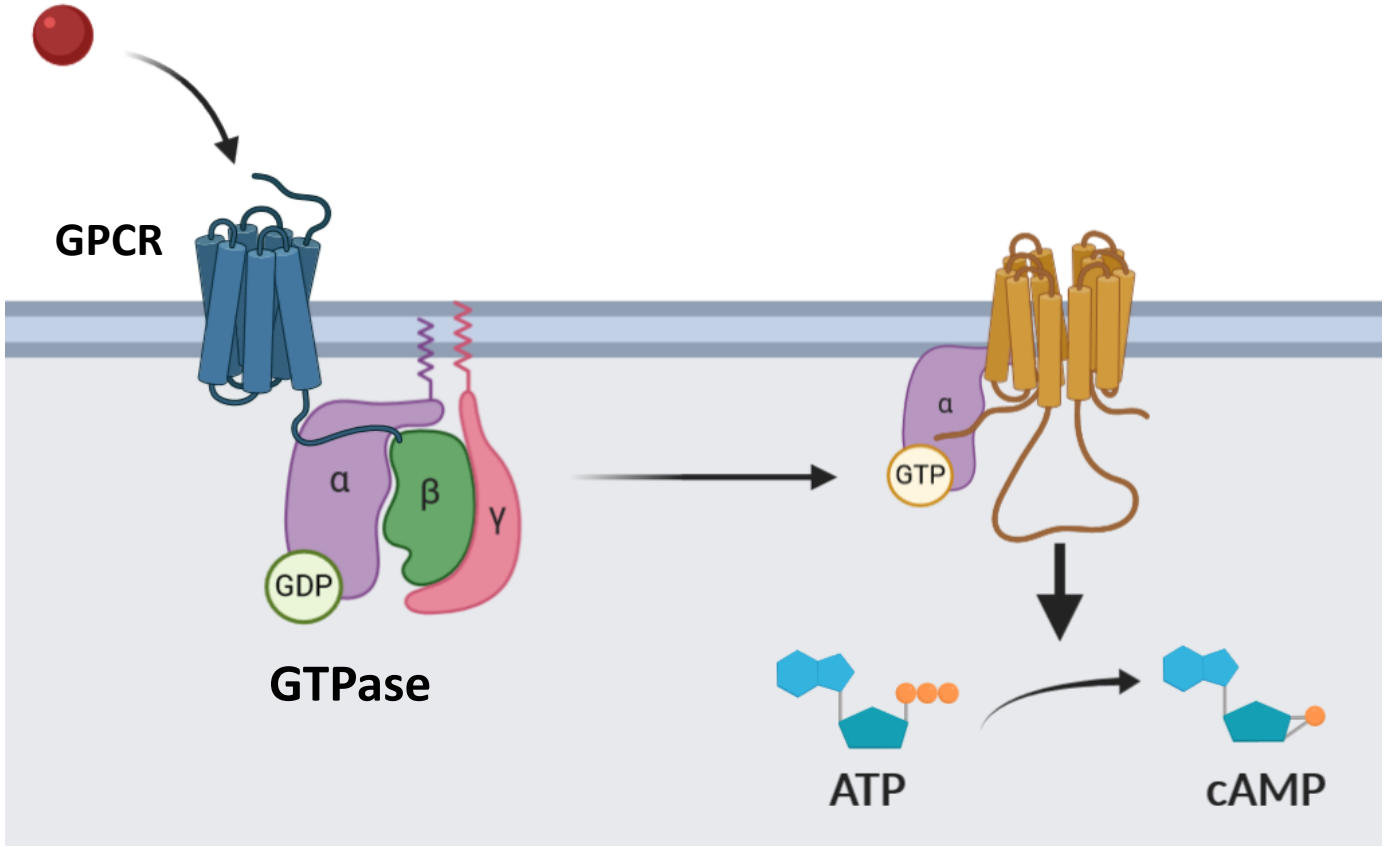
# GTPases


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

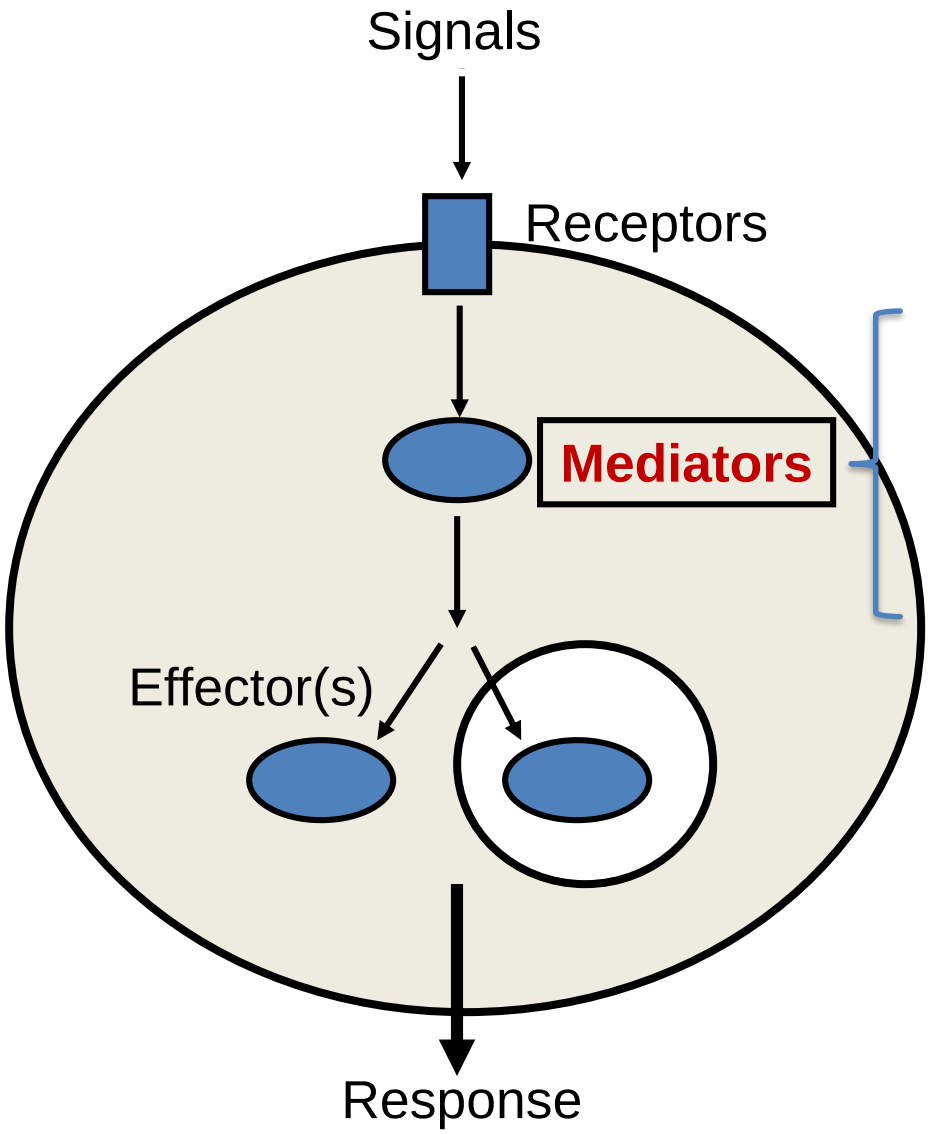
# Small GTPases superfamily

- ***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

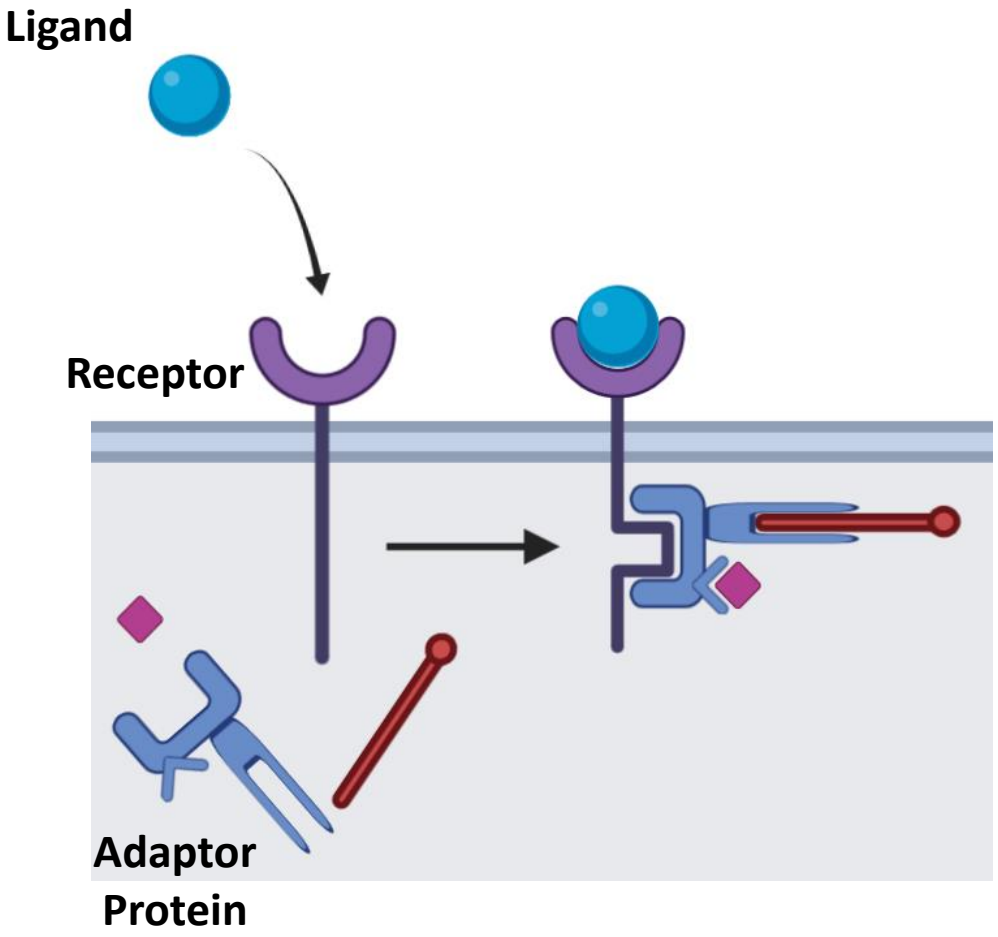


# Mediators



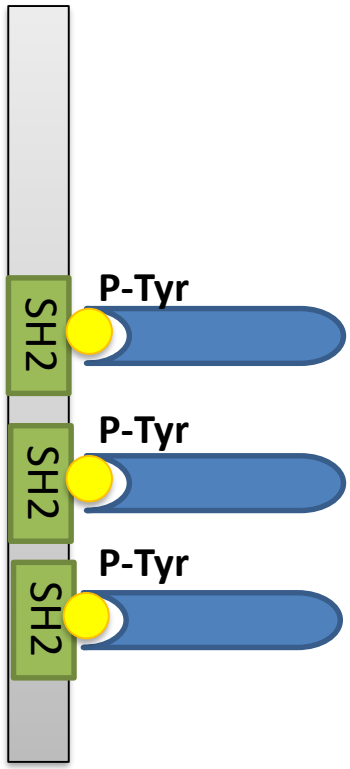

Kinases & Phosphatases
Phospholipases
Second messengers
GTPases
**Adaptors**

# Adaptor proteins



# Adaptor proteins

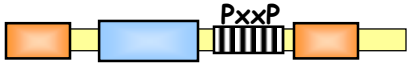
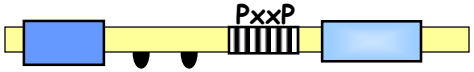
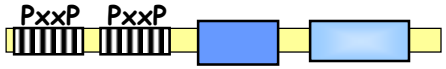
## SH2 Adaptor Proteins




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

# Adaptor proteins

## Cytoplasmic adaptor proteins

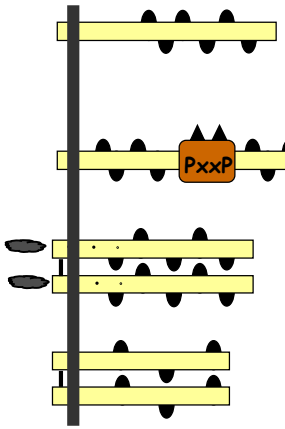
| Name | Structure | MW (kD) | Interaction partners | Expression |
|---|---|---|---|---|
| Grb2 |  | 28 | Sos,LAT,c-Cbl,Shc,SLP-76, Vav,SHP-2,WASP,HPK1 | ubiquitous |
| Grap |  | 28 | Sos,LAT,Shc,SAM68 | B-Cells, T-Cells |
| Gads |   | 40 | LAT,Shc,SLP-76,HPK1 | T-Cells, NK Cells Mast cells. Macrophages, 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 |

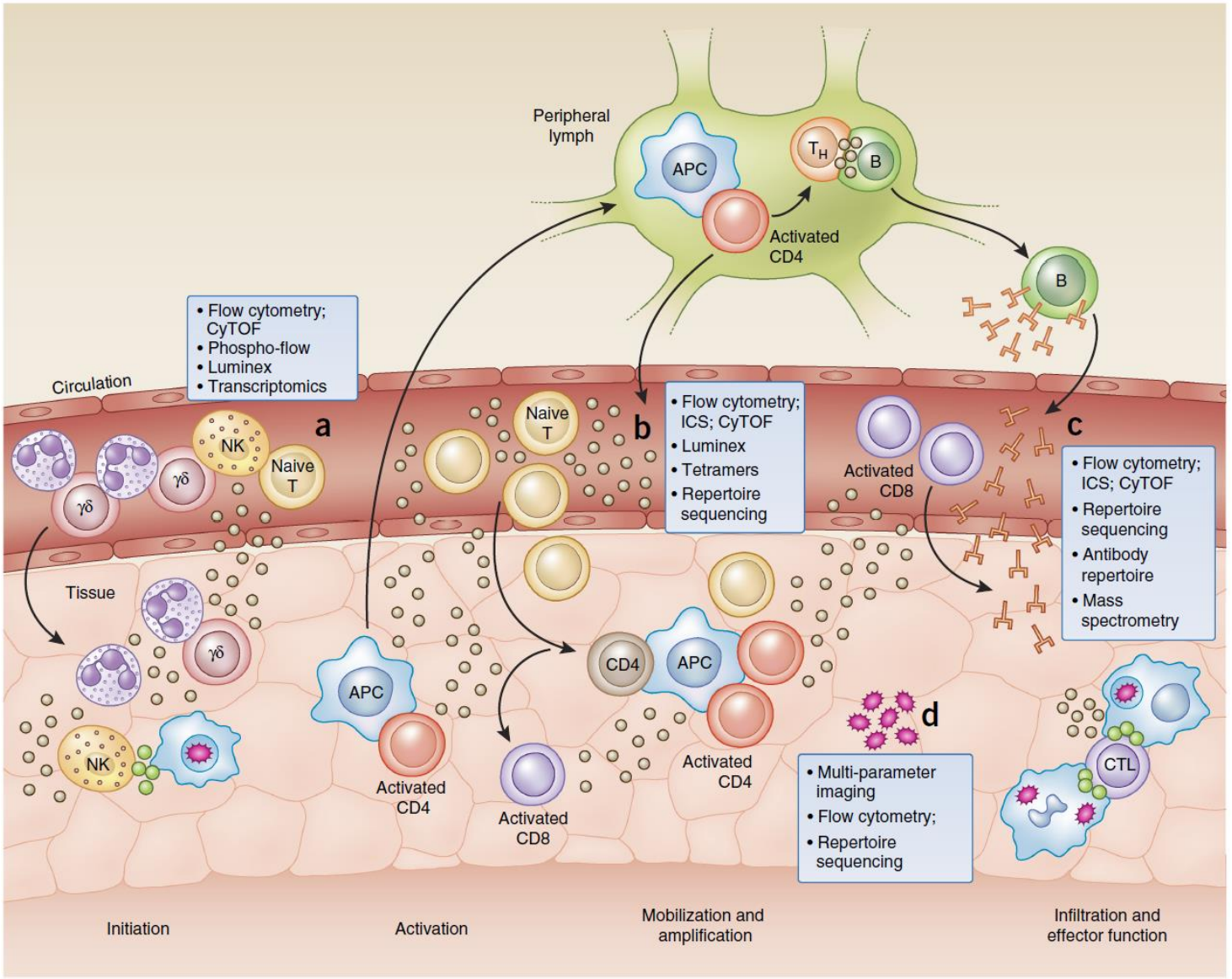


● SH2-Binding Site

PxxP SH3-Binding Site

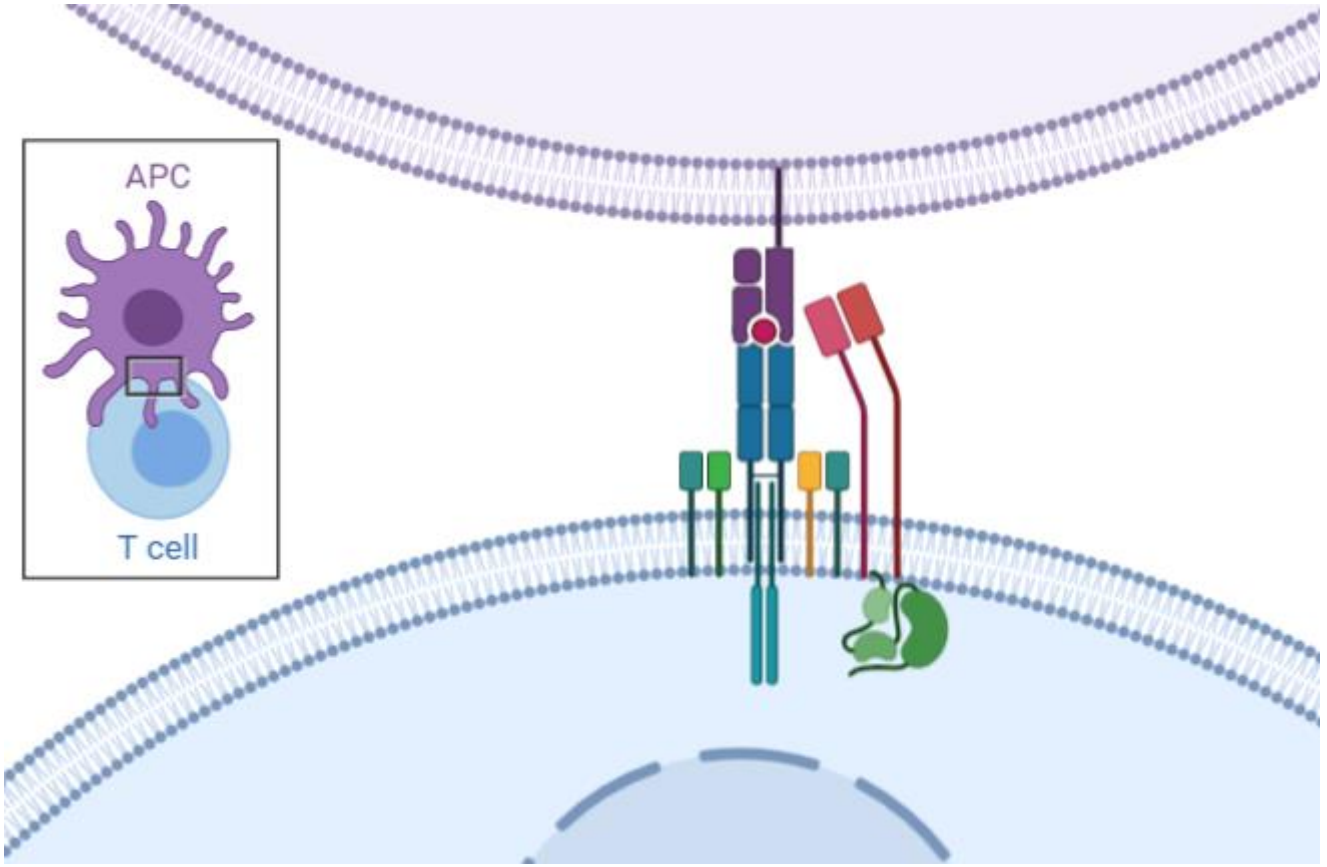
A. Leo and B.Schraven, Current Opinion in Immunology, 2001

# T cell receptor (TCR) signaling



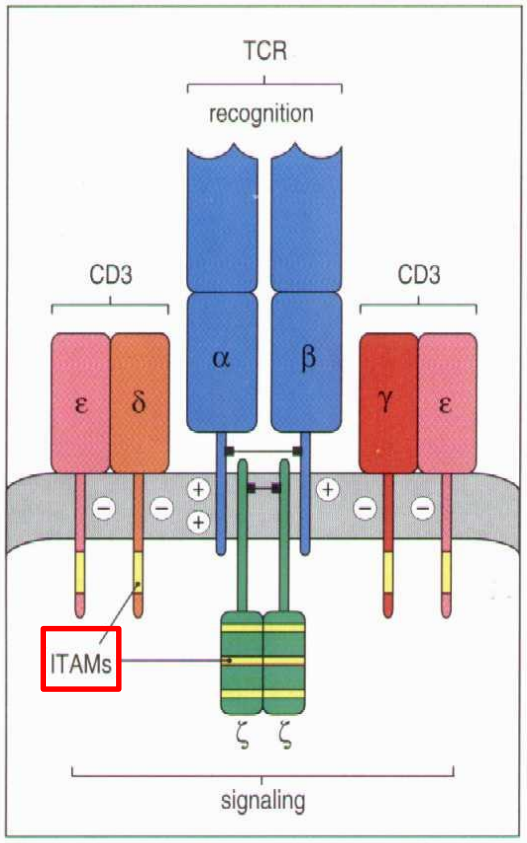

Davis MM, Tato CM, Furman D. Systems immunology: just getting started. *Nat Immunol.*

# Signal transduction induced by T cell receptor engagement



# TCR structure

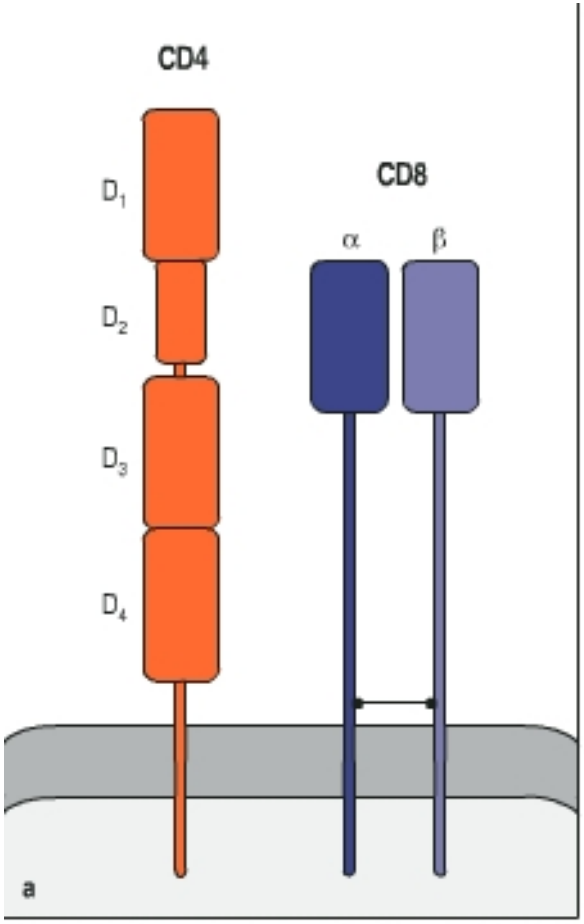
# T cell receptor:CD3 complex




- T cell receptor
  - $\alpha$ and $\beta$ chain heterodimer
  - antigen recognition
- CD3
  - transmembrane proteins with extracellular domains and cytoplasmic tails
    - two $\varepsilon$-chains
    - one $\delta$-chain
    - one $\gamma$-chain
  - transmembrane/cytoplasmic $\zeta$-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

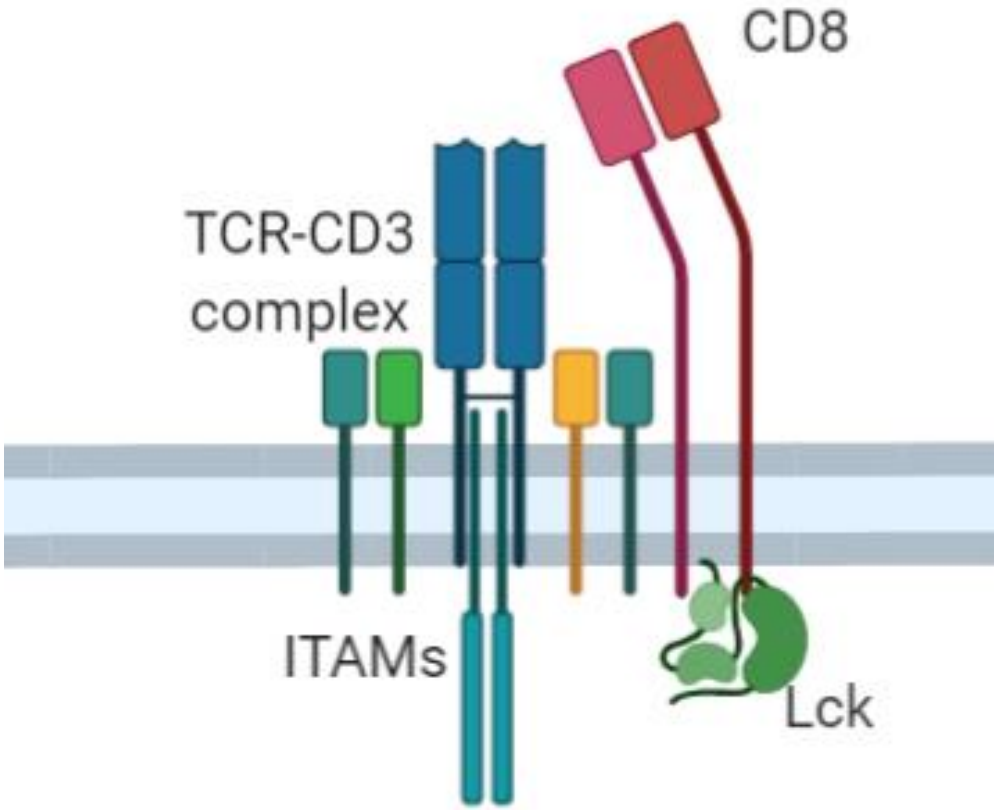
Co-receptors function: increasing efficiency of lymphocyte activation

# Lipid Rafts

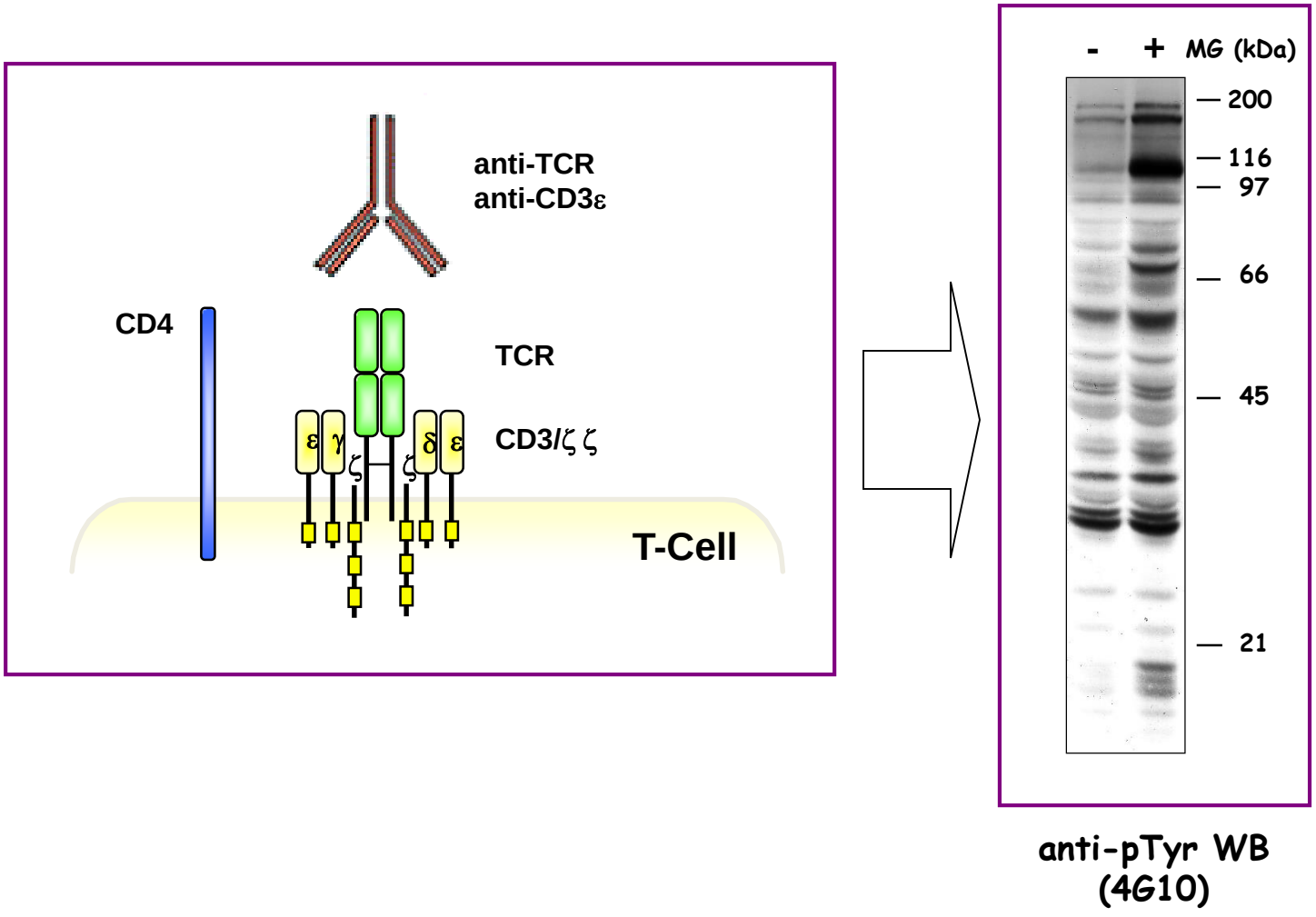
- Membrane compartments enriched with cholesterol, glycosphingolipids and sphingomyelin
- Selectively concentrate membrane proteins with lipid anchor of saturated acyl chains
- Contain lipid modified signal proteins
  - Src kinases (Lck, Fyn)
  - GTPases (Ras proteins, G-proteins)
  - Adaptor proteins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

# TCR signaling

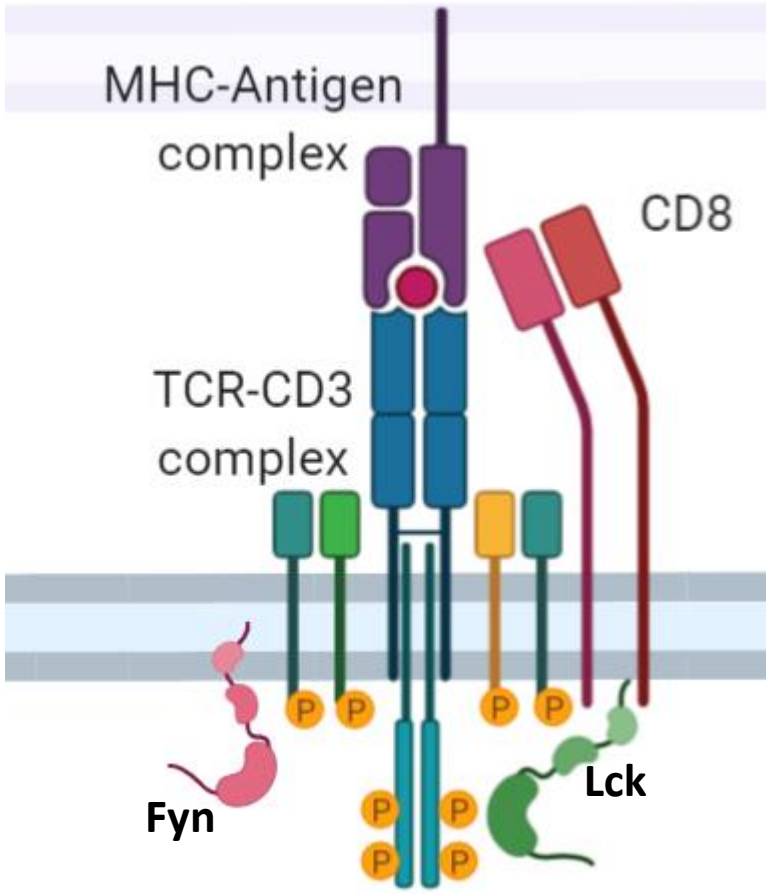
# Inactive TCR



# Induction of a tyrosine phosphorylation wave

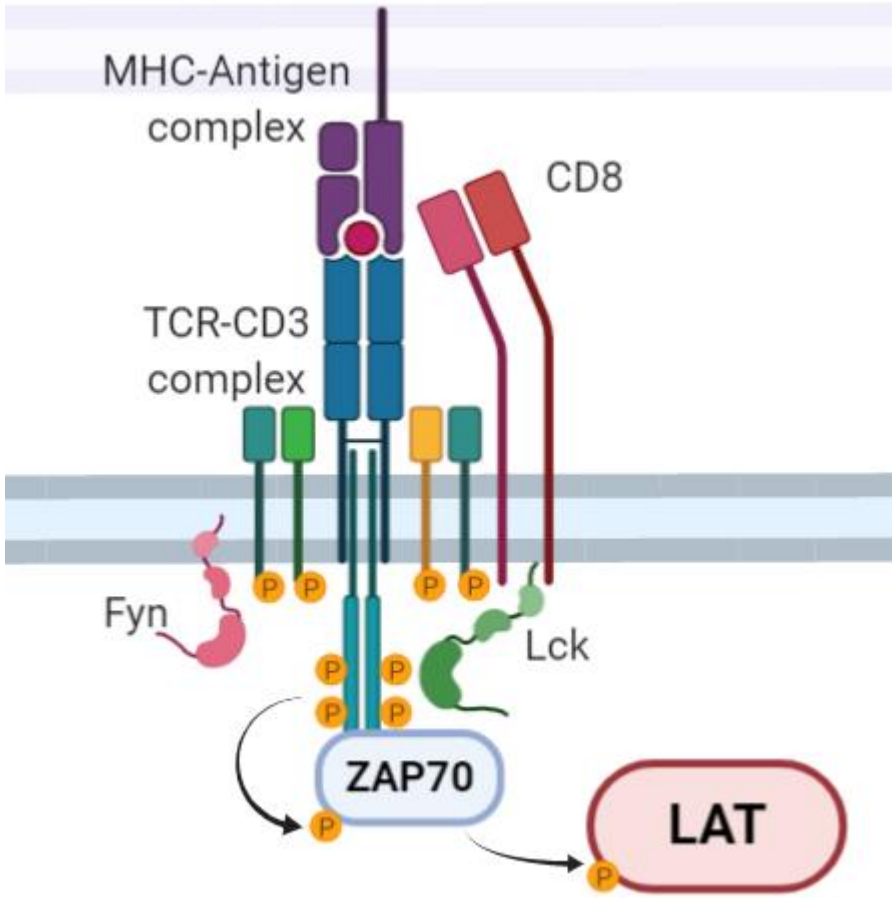


# ITAMs phosphorylation



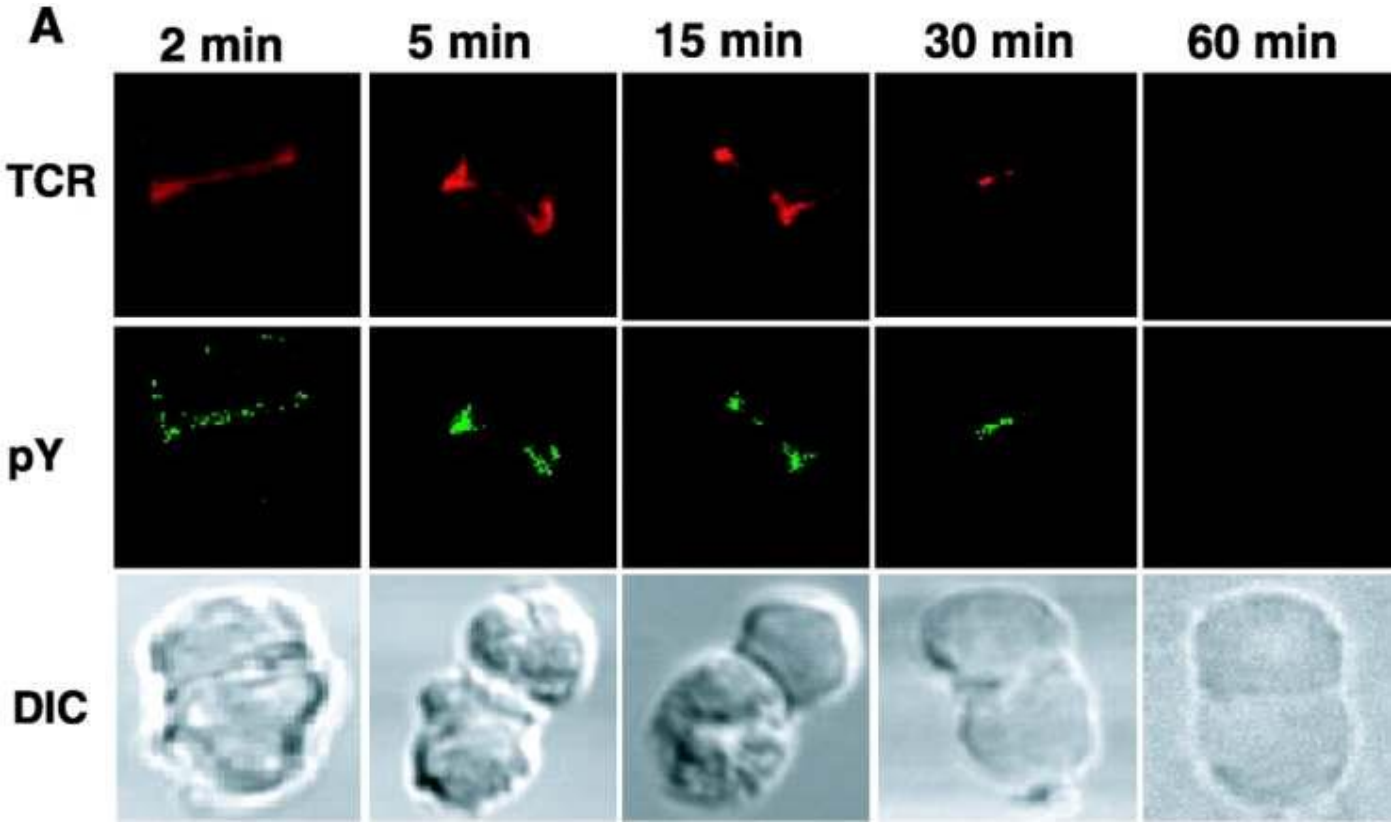

- Recruitment and activation of Lck and Fyn (Src-family tyrosine kinases)
- Lck and Fyn phosphorylate ITAMs of TCR-CD3 complex

# ZAP70 and LAT



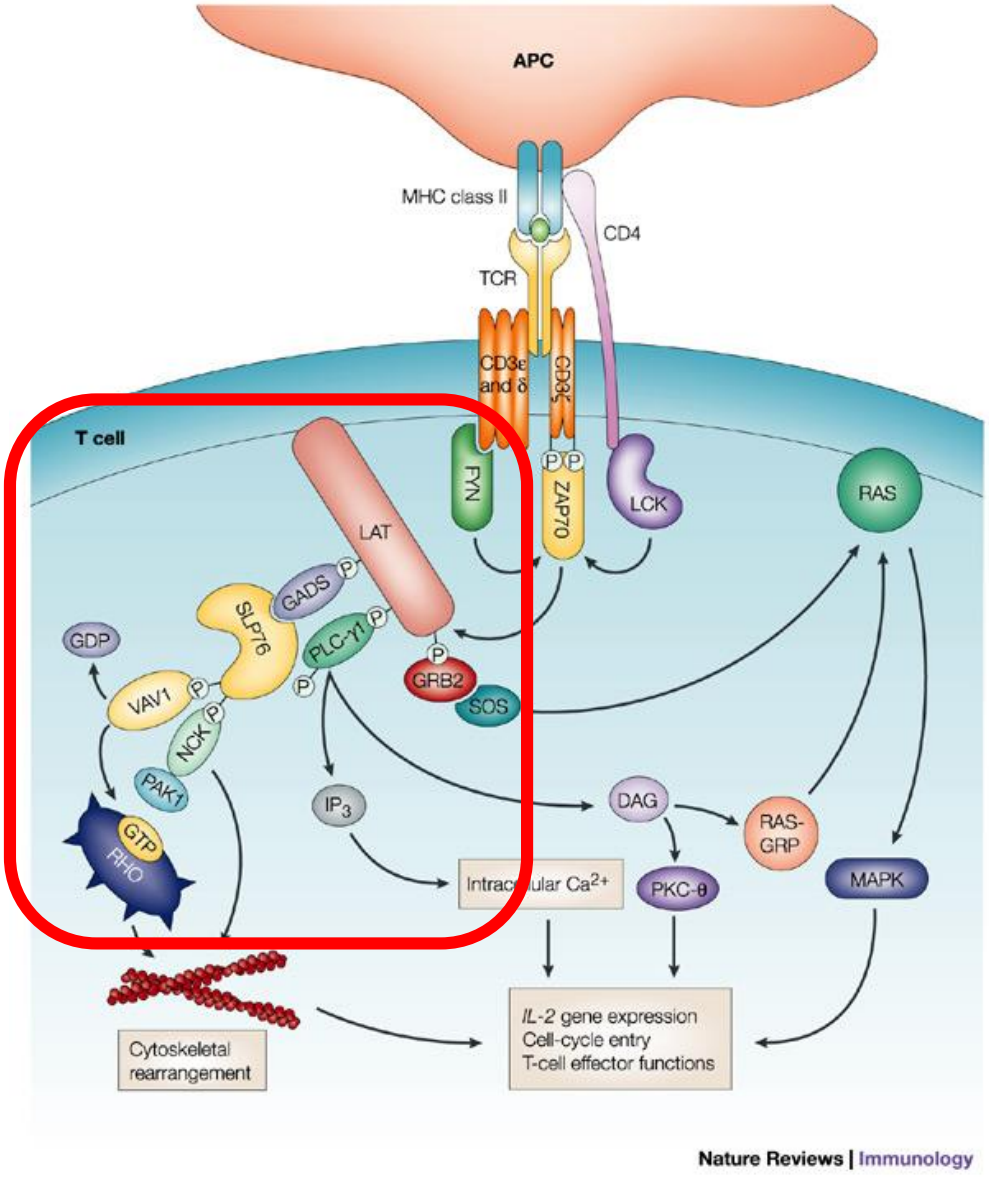

- Recruitment and phosphorylation of ZAP70 (Tyr-kinase)
- Recruitment of and phosphorylation of LAT (adaptor protein)

# Time course of pTyr migration in T cell:APC conjugates




Lee et al, 2002

# LAT complex

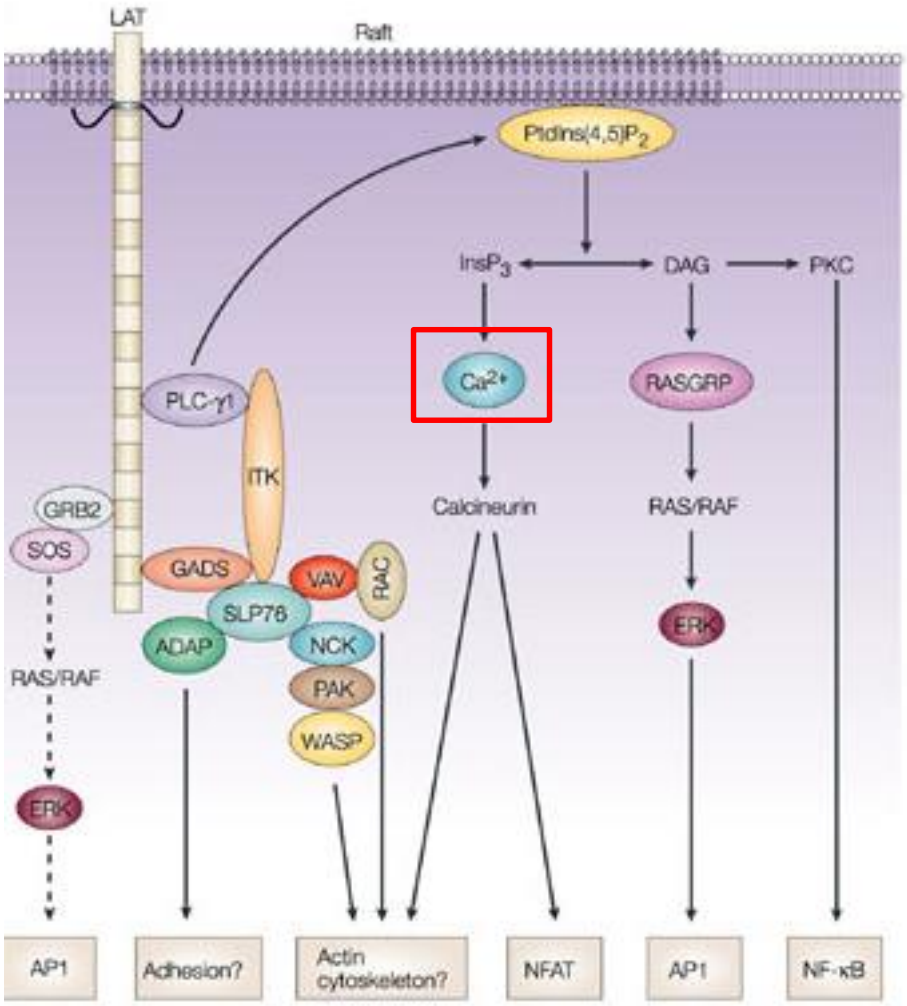


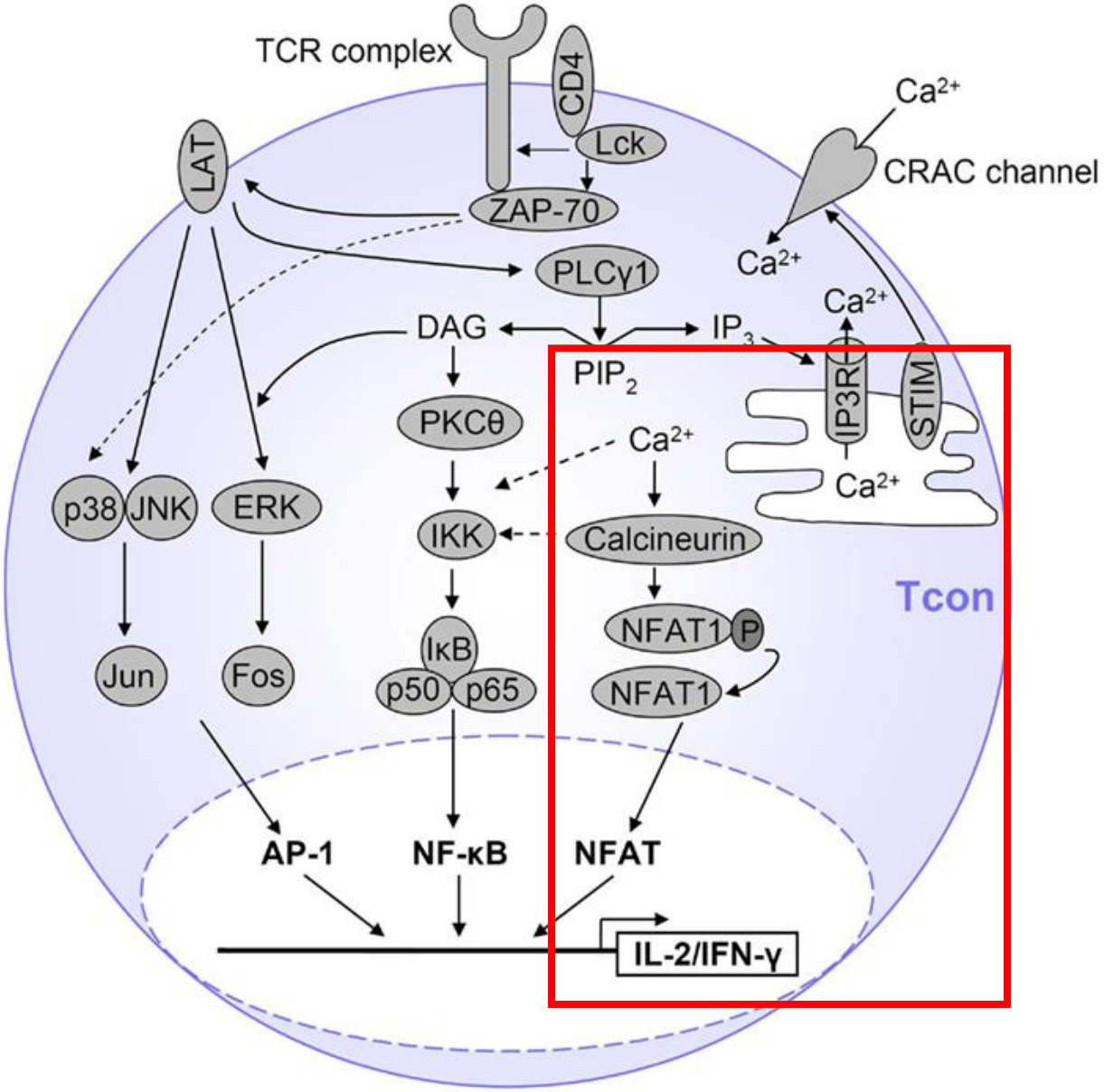

- LAT phosphorylation triggers the formation of multi-protein signaling complexes.

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

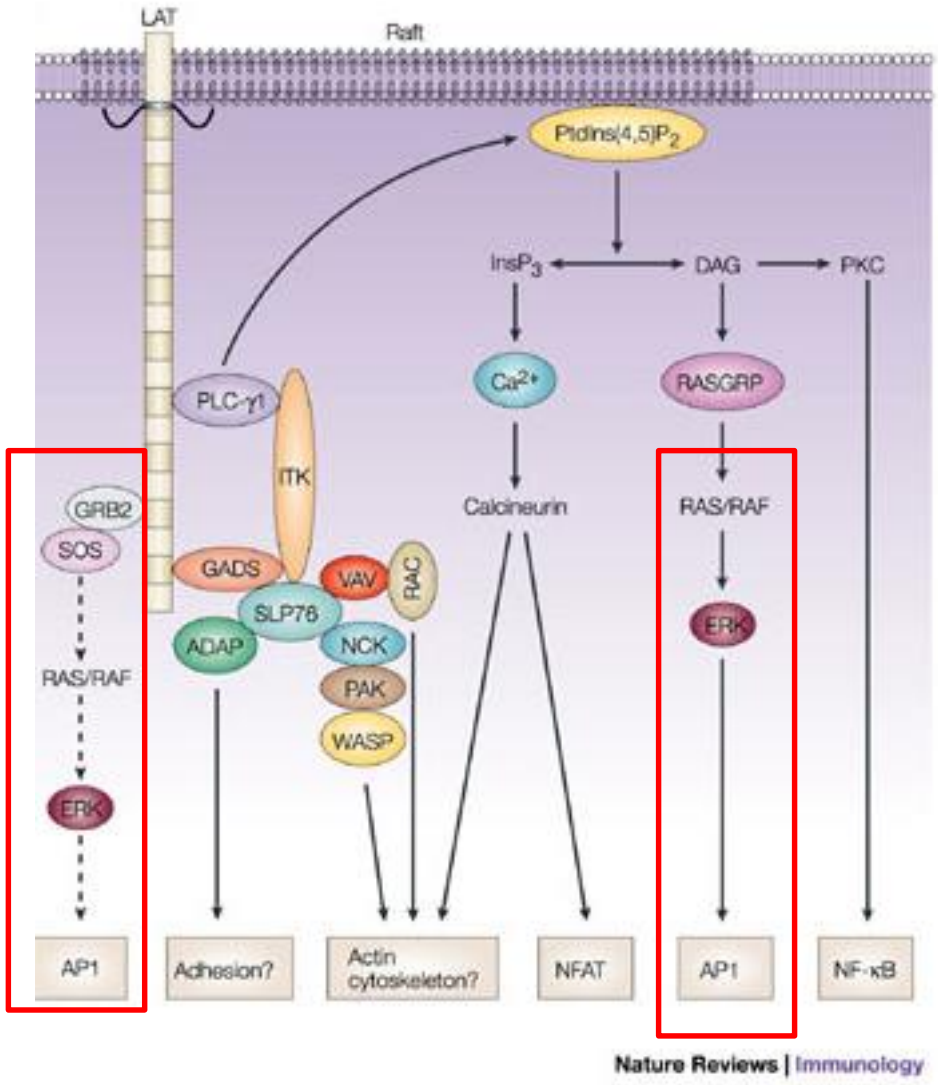
# Calcium-NFAT signaling

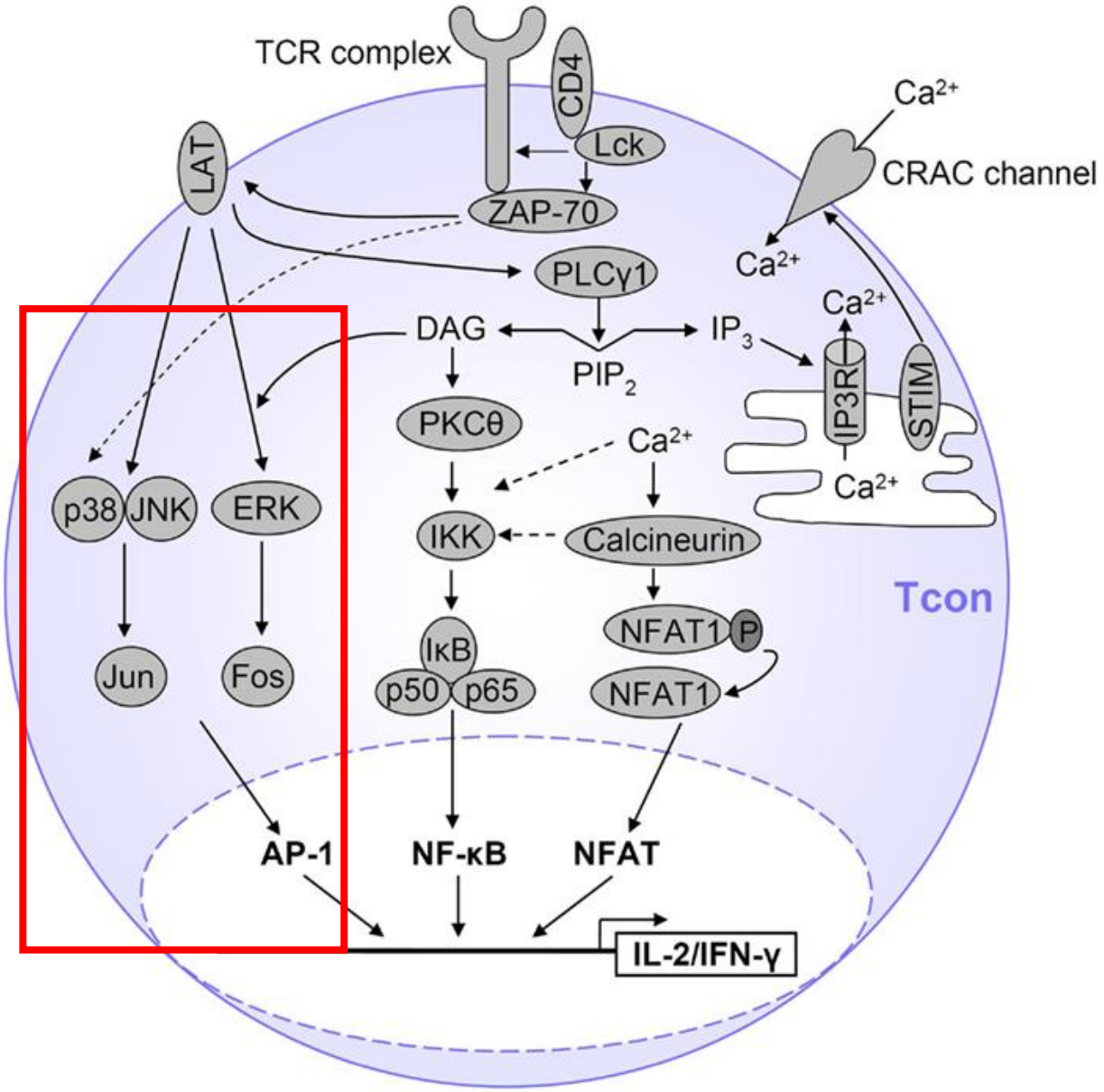





Schmidt A, Oberle N, Krammer PH. *Front Immunol*. 2012

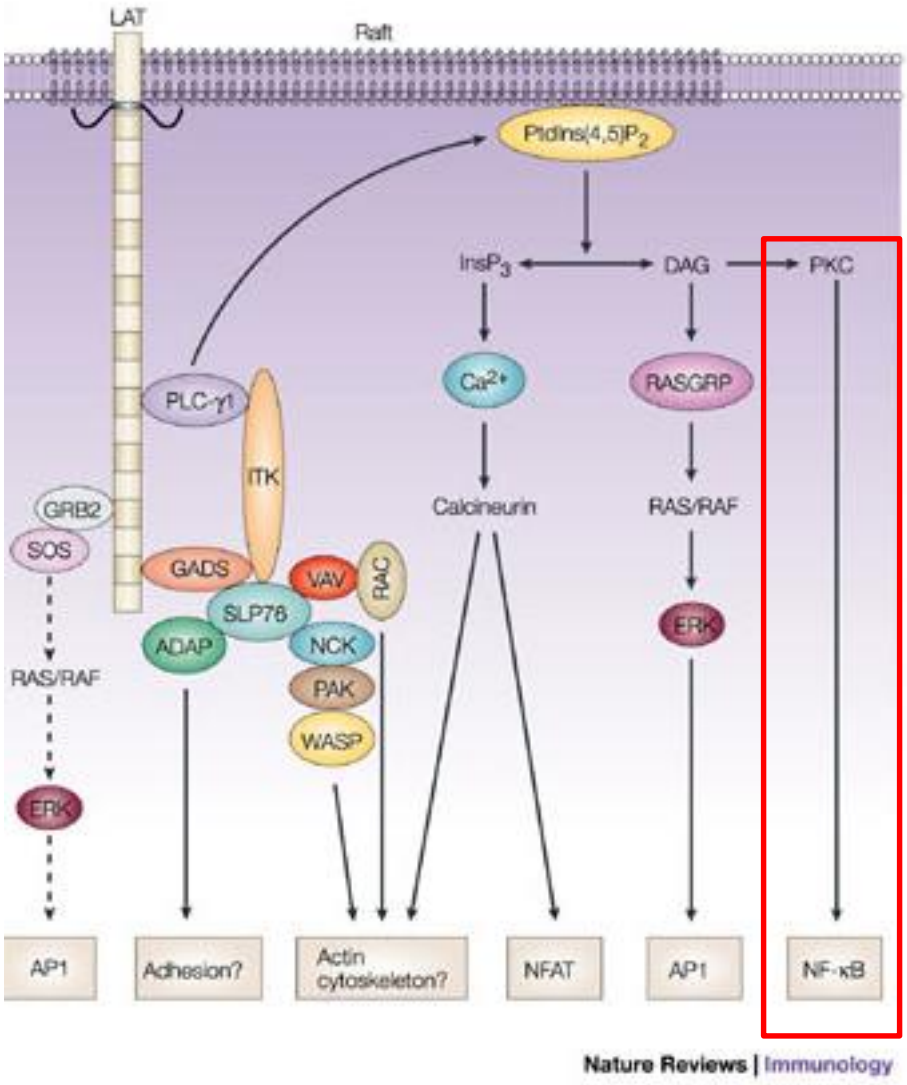
# AP-1 pathway activation

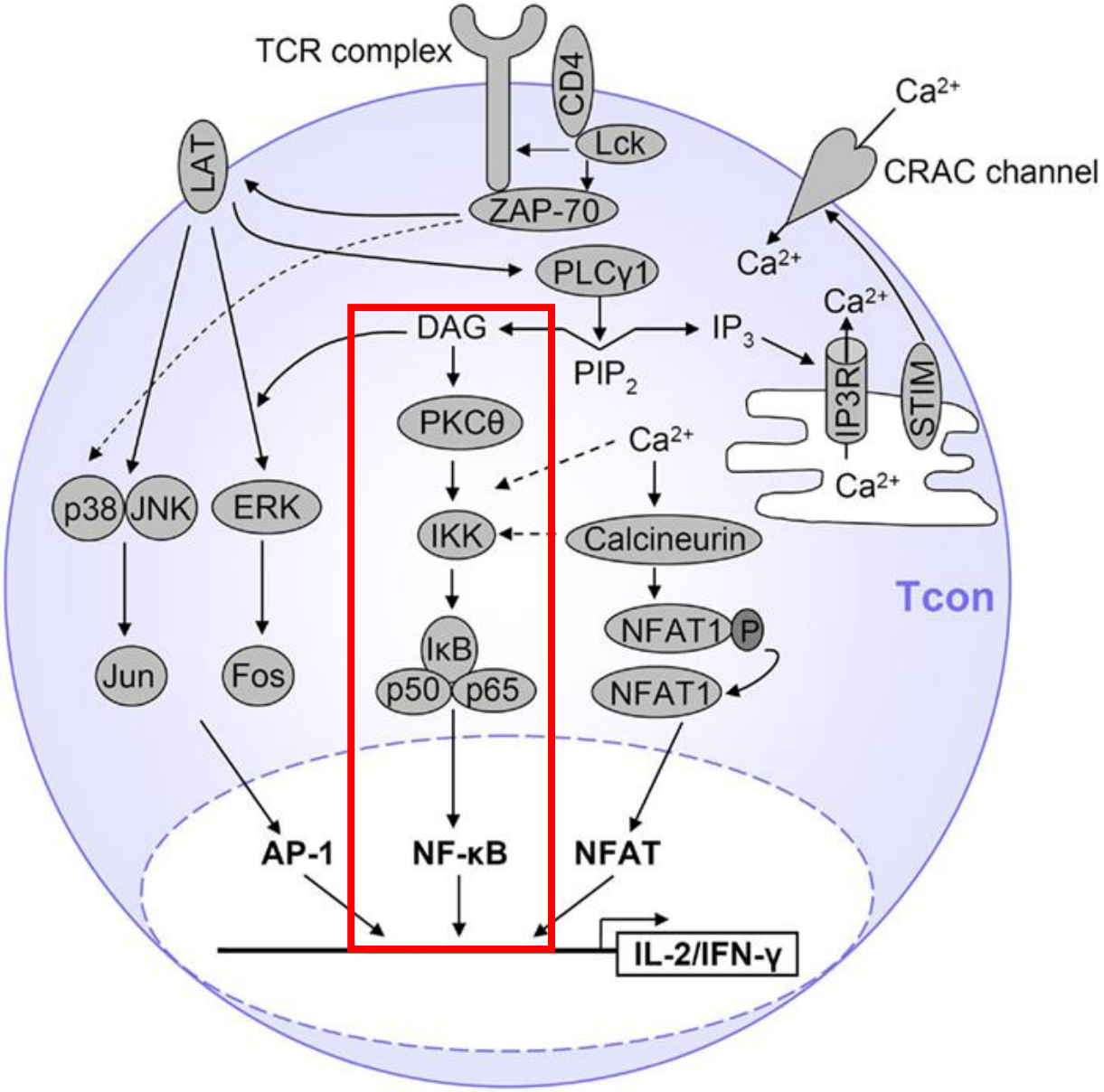





Schmidt A, Oberle N, Krammer PH. *Front Immunol*. 2012

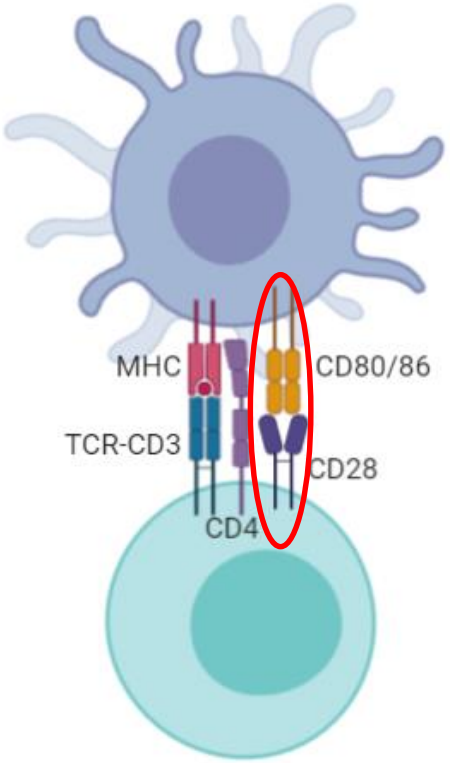
# NF-κB pathway activation

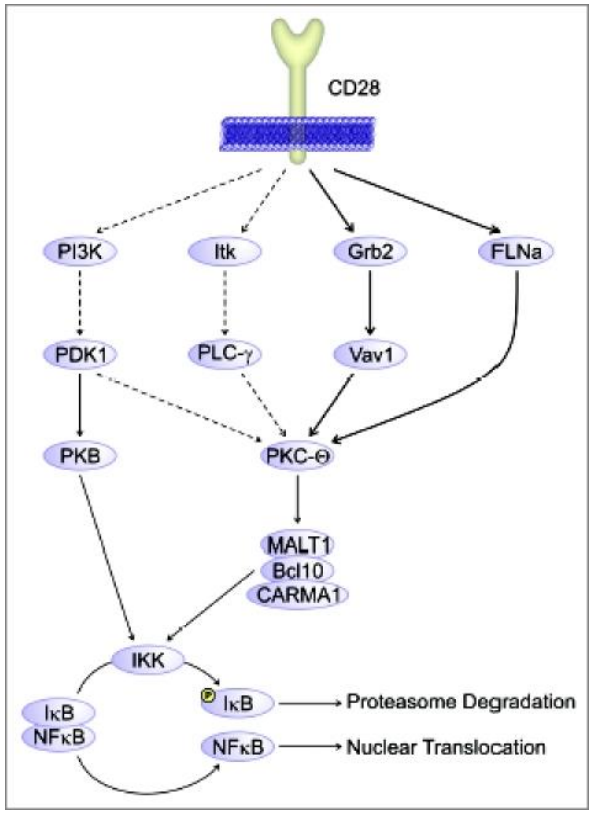





Schmidt A, Oberle N, Krammer PH. *Front Immunol*. 2012

# CD28 costimulation



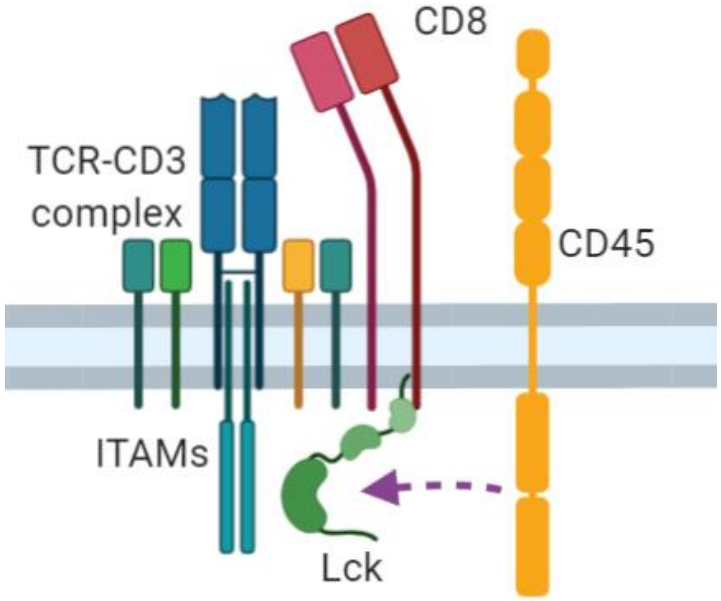




Riha P, Rudd CE. 2010

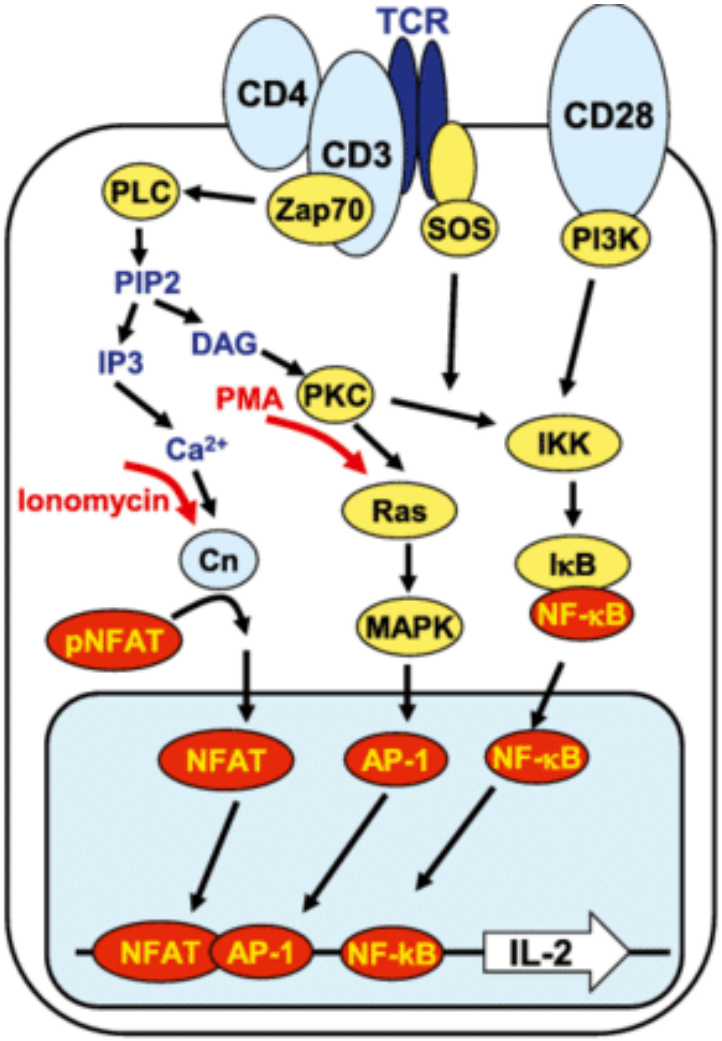
- CD28 stimulation is necessary to induce T cell activation
- CD28 stimulation potentiates TCR signaling and triggers NF-κB activation

# 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

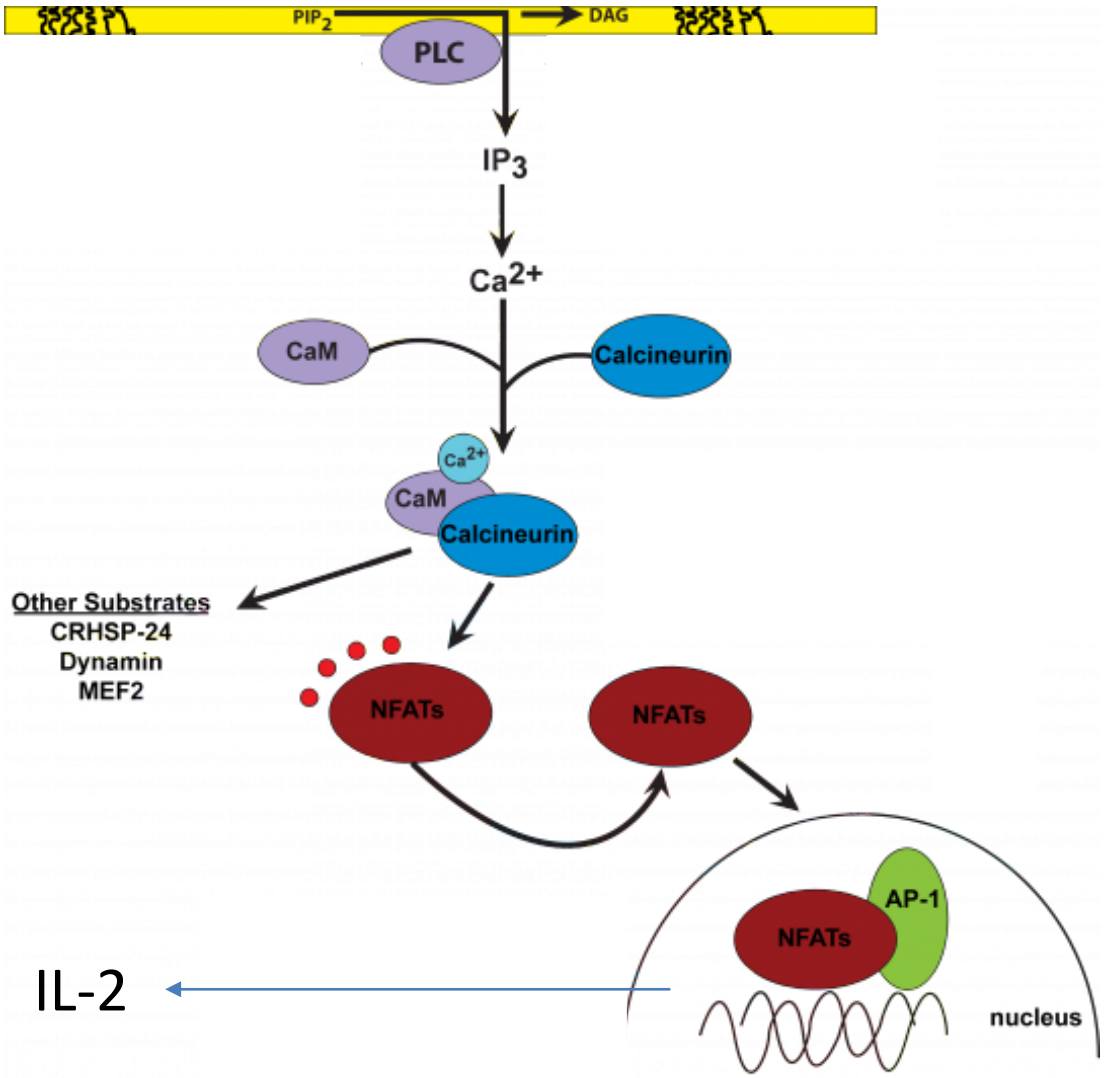


# TCR dysregulation

- 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



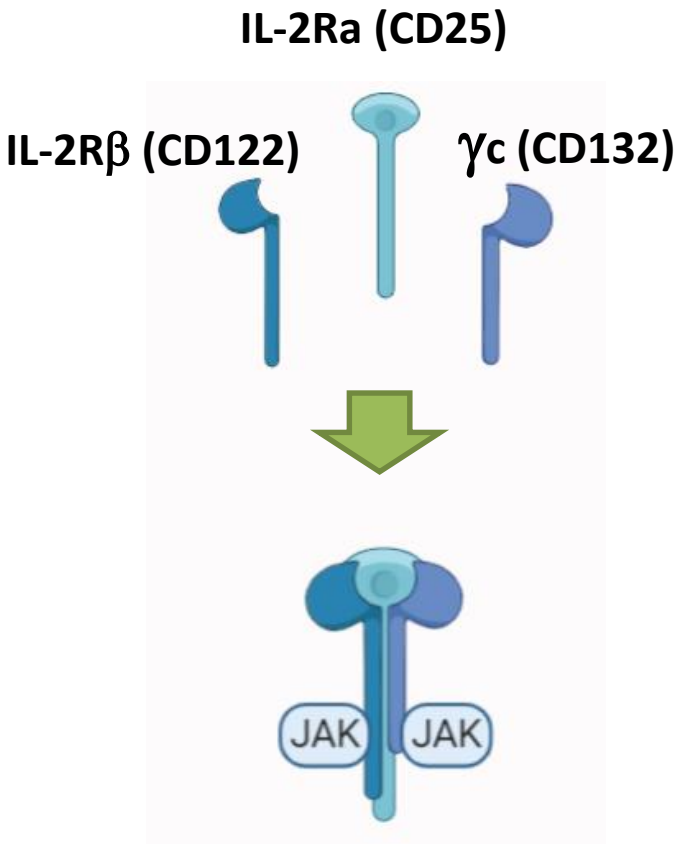
# IL-2R signaling pathway

# IL-2R signaling pathway

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

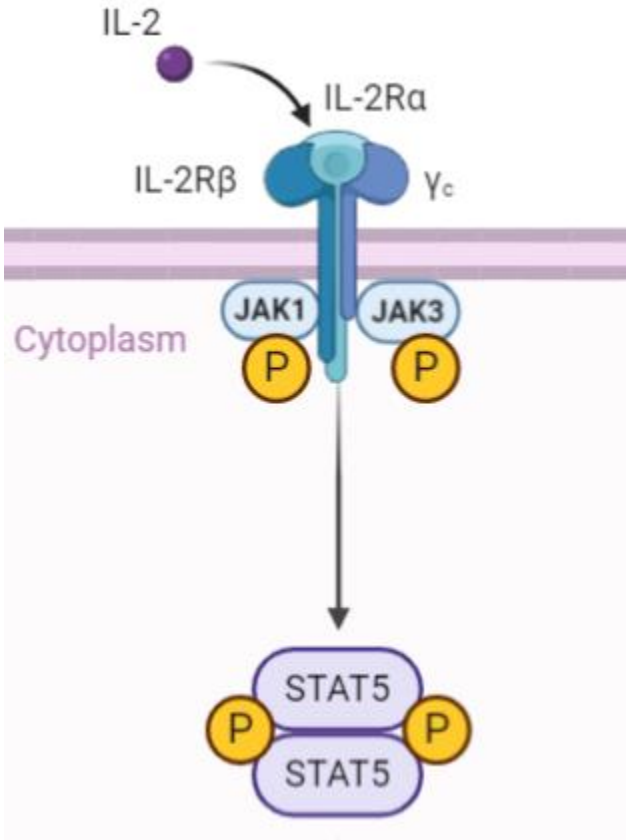
- JAK/STAT signaling pathway:
  - JAK: Janus Kinase (JAK1, JAK2, JAK3, TYK2)
    - Tyrosine kinase activity
    - SH2 domain
  - STAT: Signal Transducer and Activators of Transcription
    - Transcription factor

# IL-2 receptor (IL-2R)




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

# JAK/STAT signaling

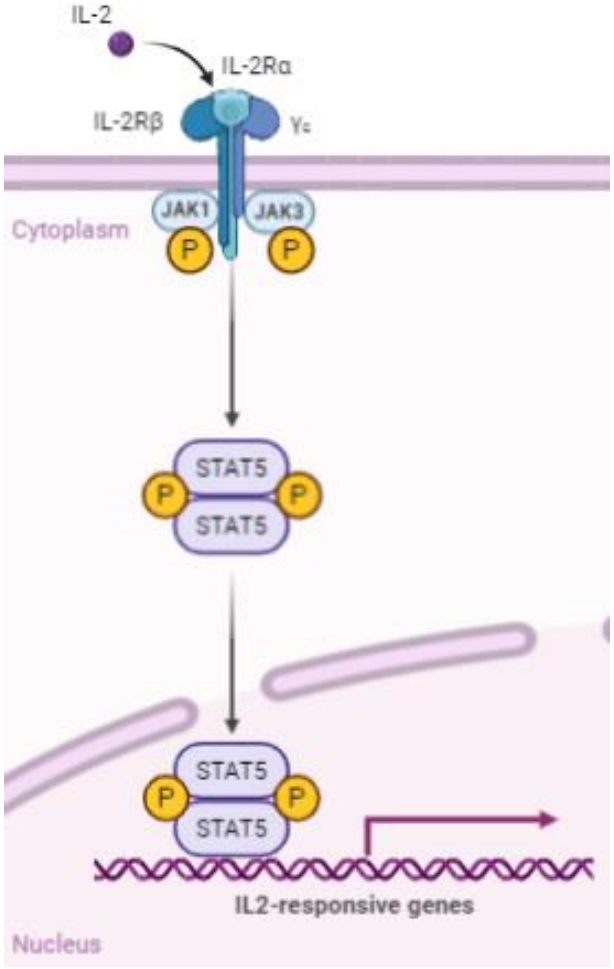



IL-2Rβ chain - JAK1

$\gamma_c$ subunit - JAK3

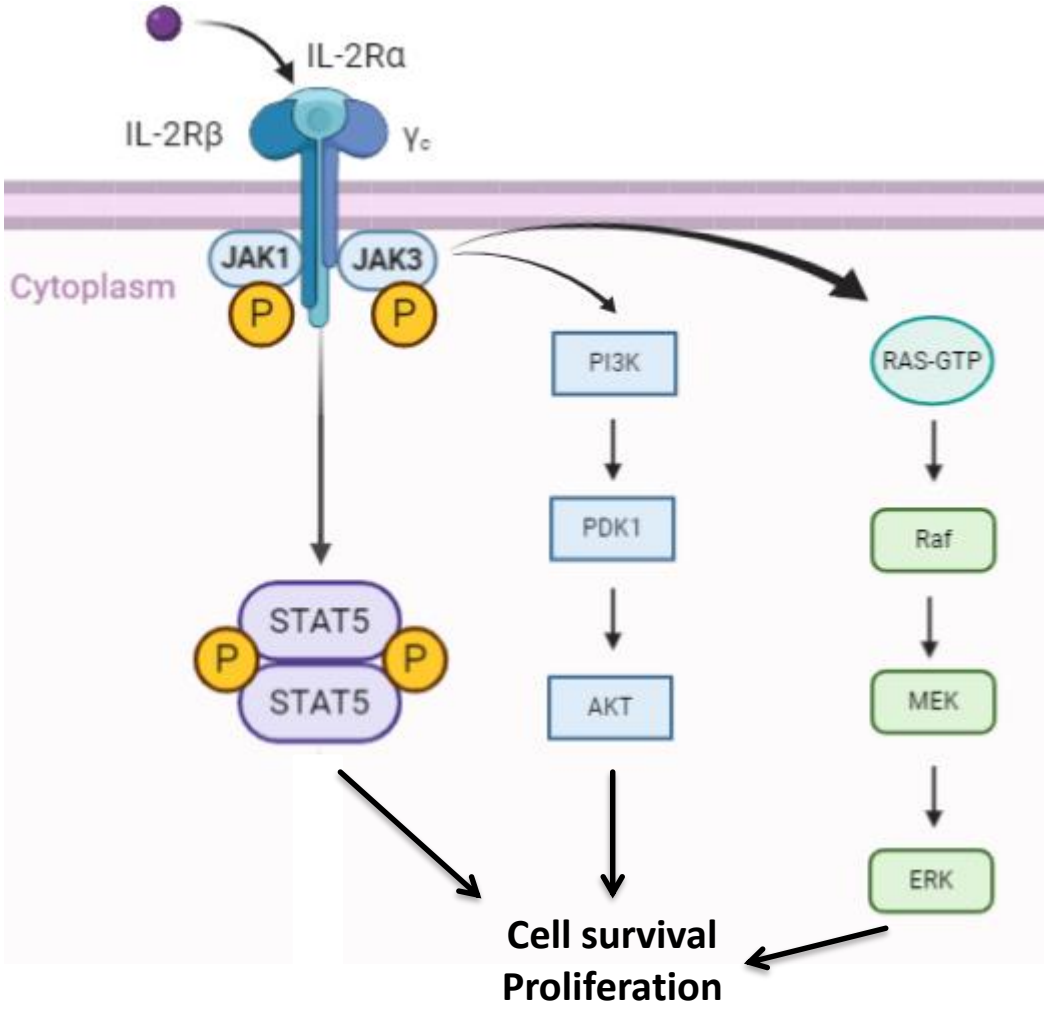
- JAK activation results in IL-2Rβ and γc subunit phosphorylation
- Tyrosine phosphorylation permits the recruitment of STAT5A, STAT5B, and STAT3

# JAK/STAT signaling




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

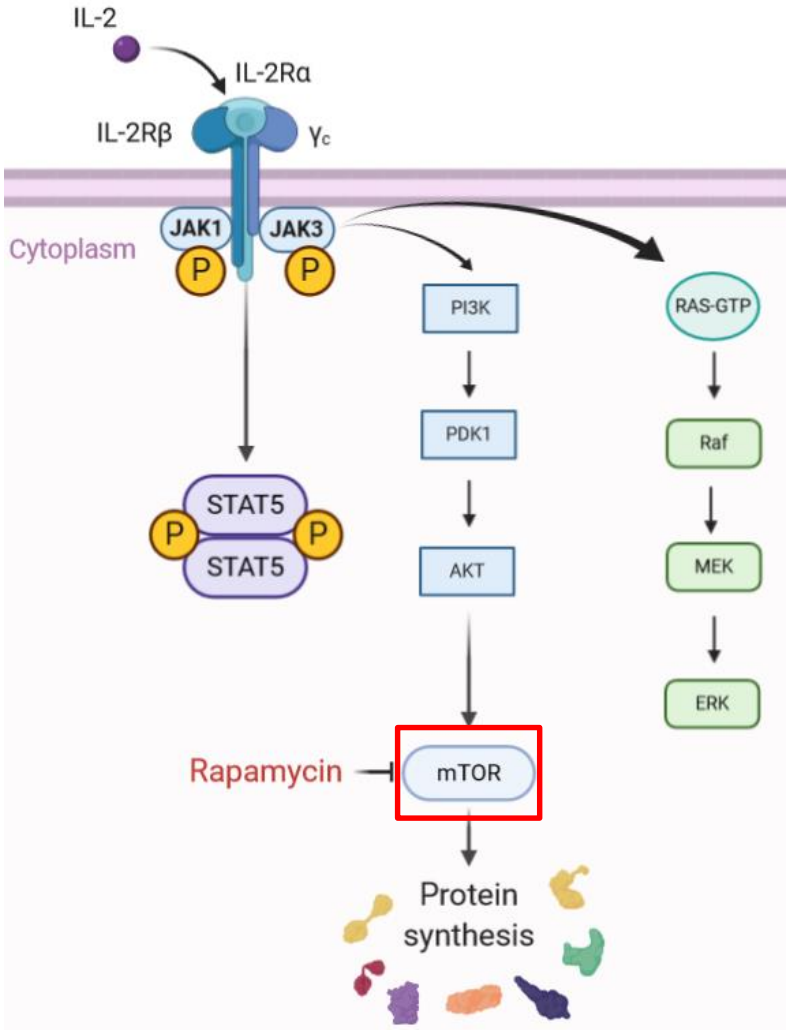
# IL-2R signal transduction




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-2$\alpha$ 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-2R$\beta$ chain mutations: Severe immune dysregulation autoantibodies, hypergammaglobulinemia, bowel inflammation, dermatological abnormalities, lymphadenopathy.

- IL-2R$\gamma$ 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***