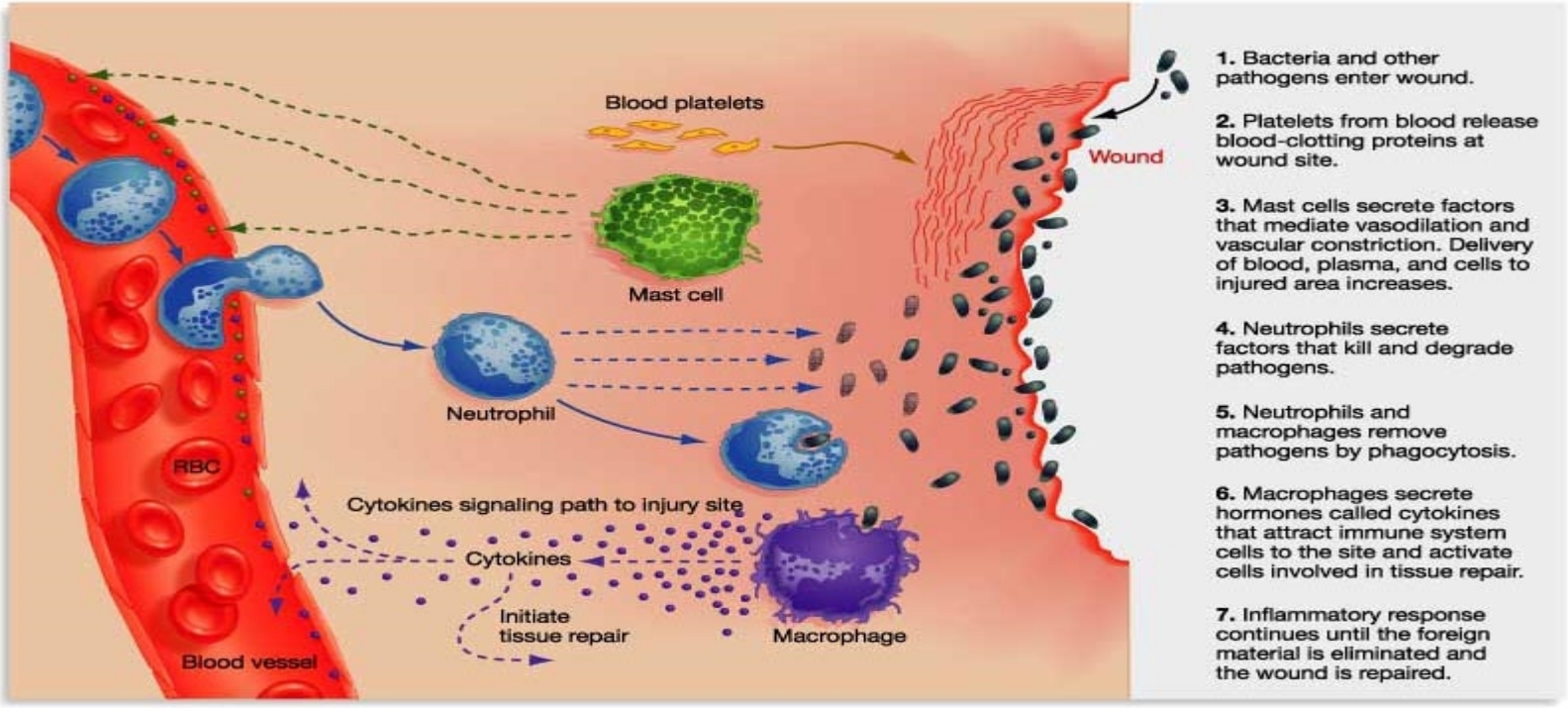
Pathogen Recognition

Christos Tsatsanis

Division of Laboratory Medicine
School of Medicine, University of Crete,
Heraklion, Crete, Greece

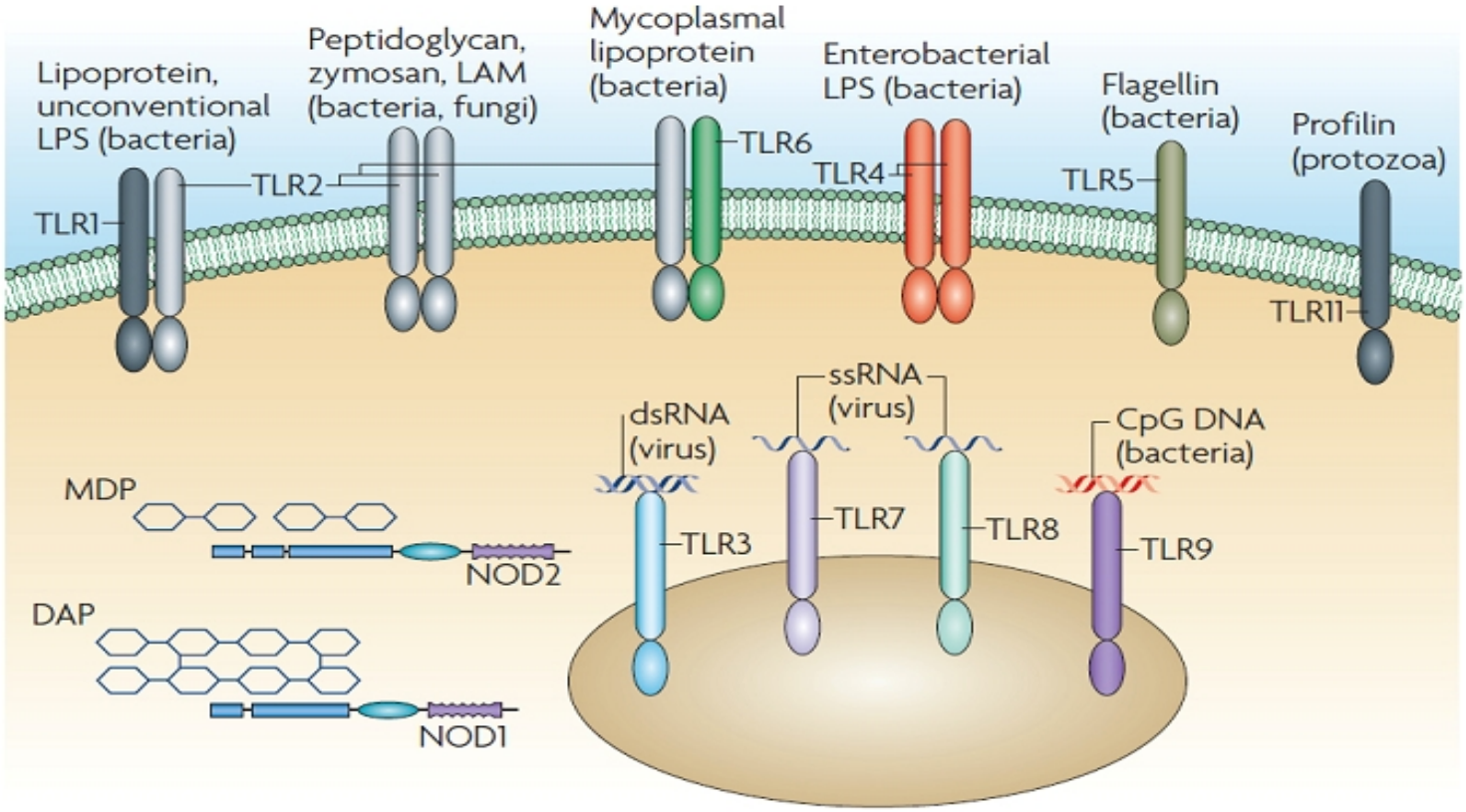
- Mechanisms of pathogen recognition: Cells and receptors
- Pathogen signals: TLR signaling
- Response to pathogens: Inflammatory cytokines and signaling
- T cell receptor signaling, the IL-2 paradigm
- Cytokine signaling and regulation

Exposure to pathogens and initiation of immune responses

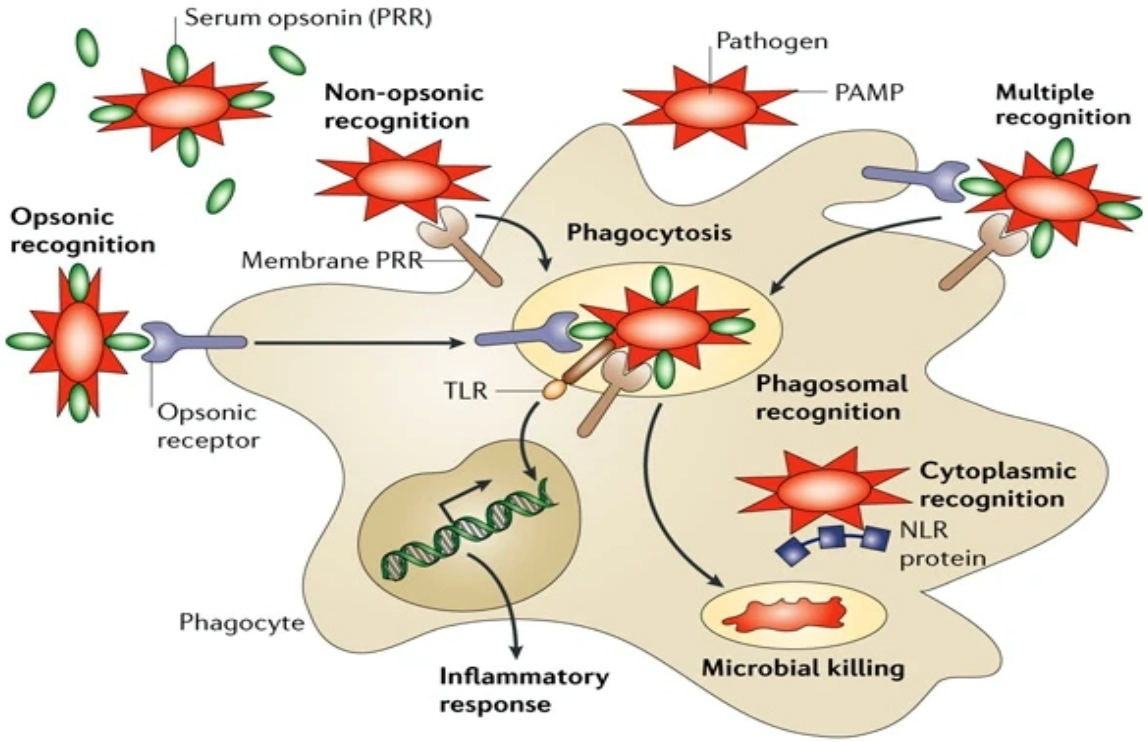


Pathogen-Associated Molecular Patterns: PAMPs
Pattern Recognition Receptors: PRRs

Pathogen recognition: Toll Like Receptors



Opsonization: efficient recognition

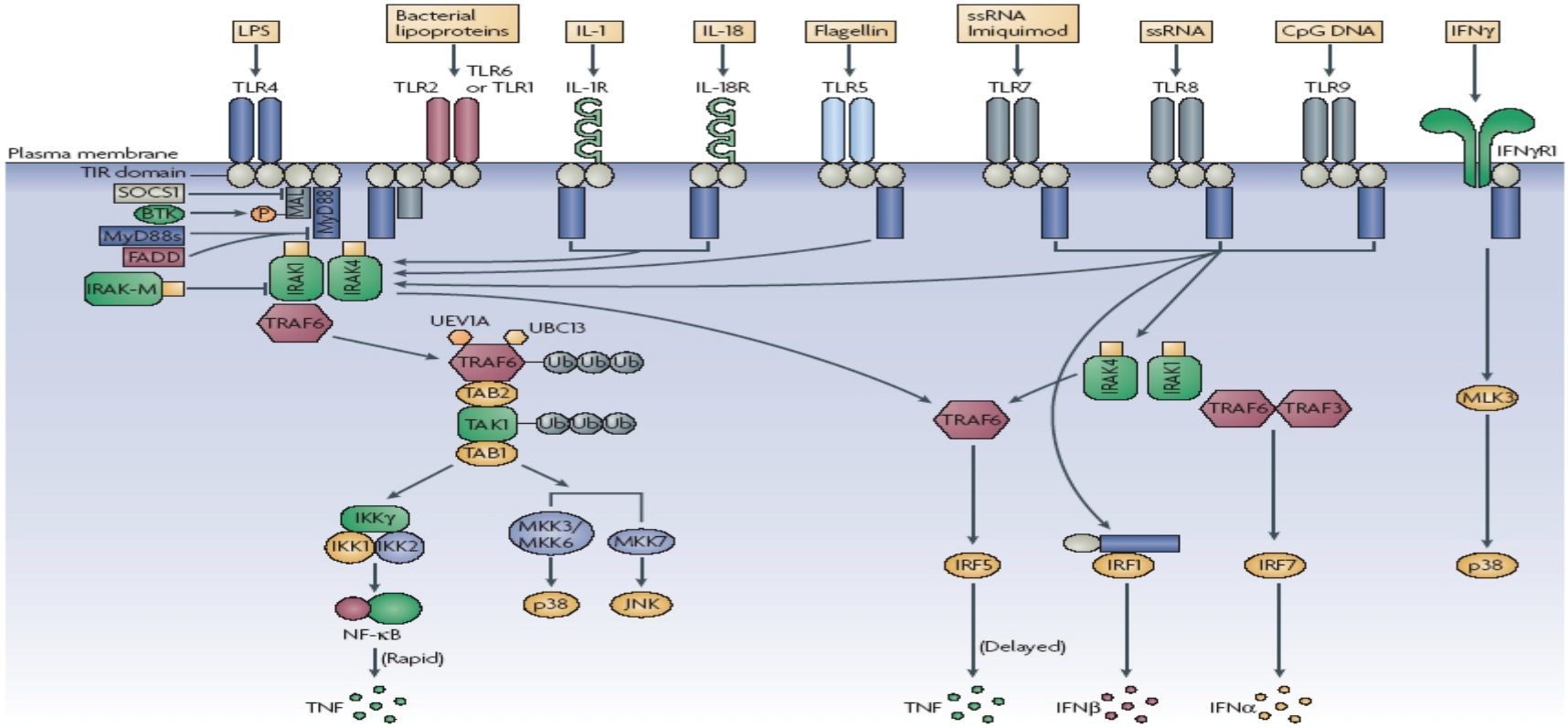


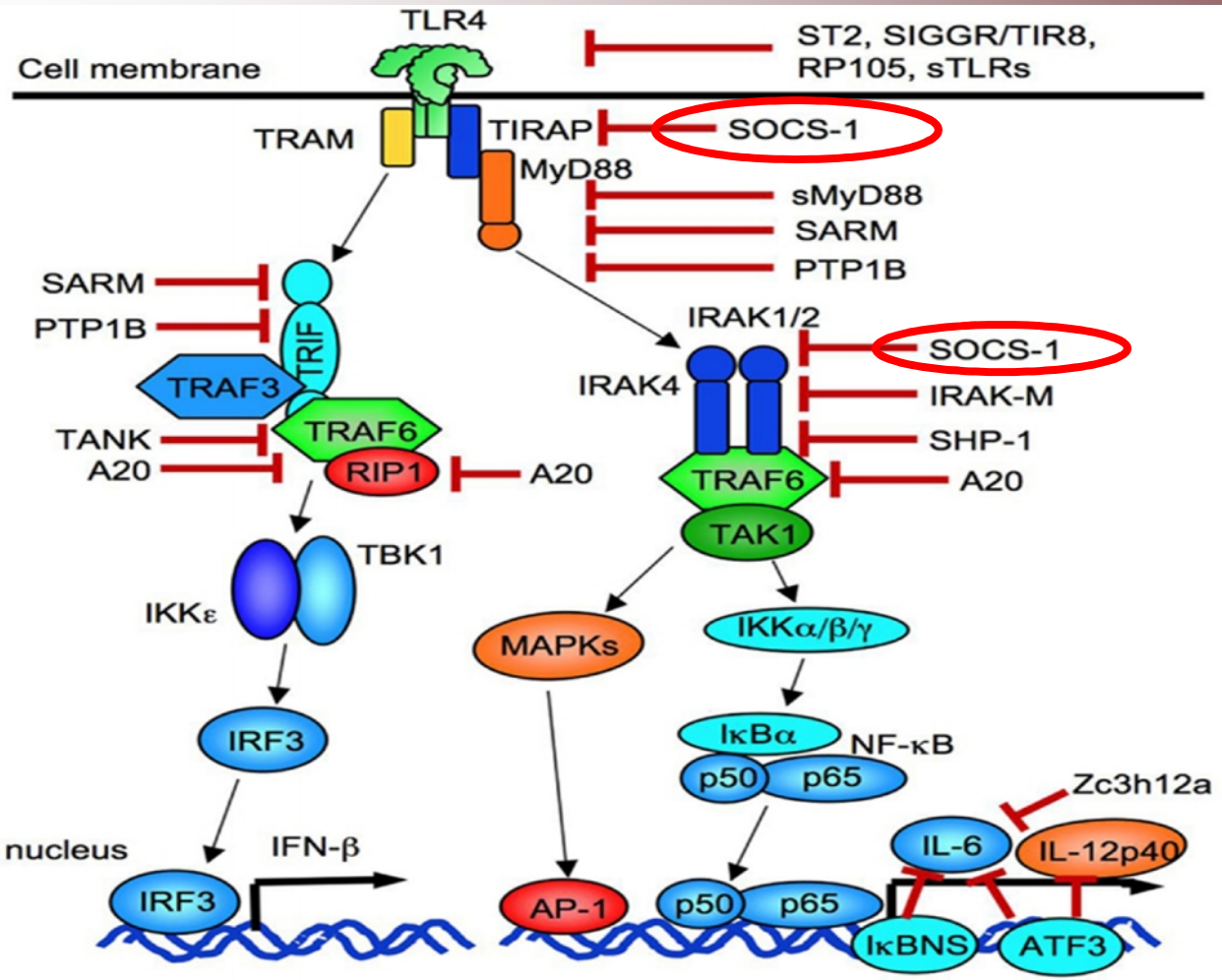
Opsonization

- Antigen is marked with opsonin
- Enhances phagocytosis of an antigen
- Opsonins: Immunoglobulins(i.e. IgG), Complement (i.e. C3b), Fibronectin, fibrinogen, Acute phase proteins (i.e. CRP)

Allows phagocytosis through specialized receptors

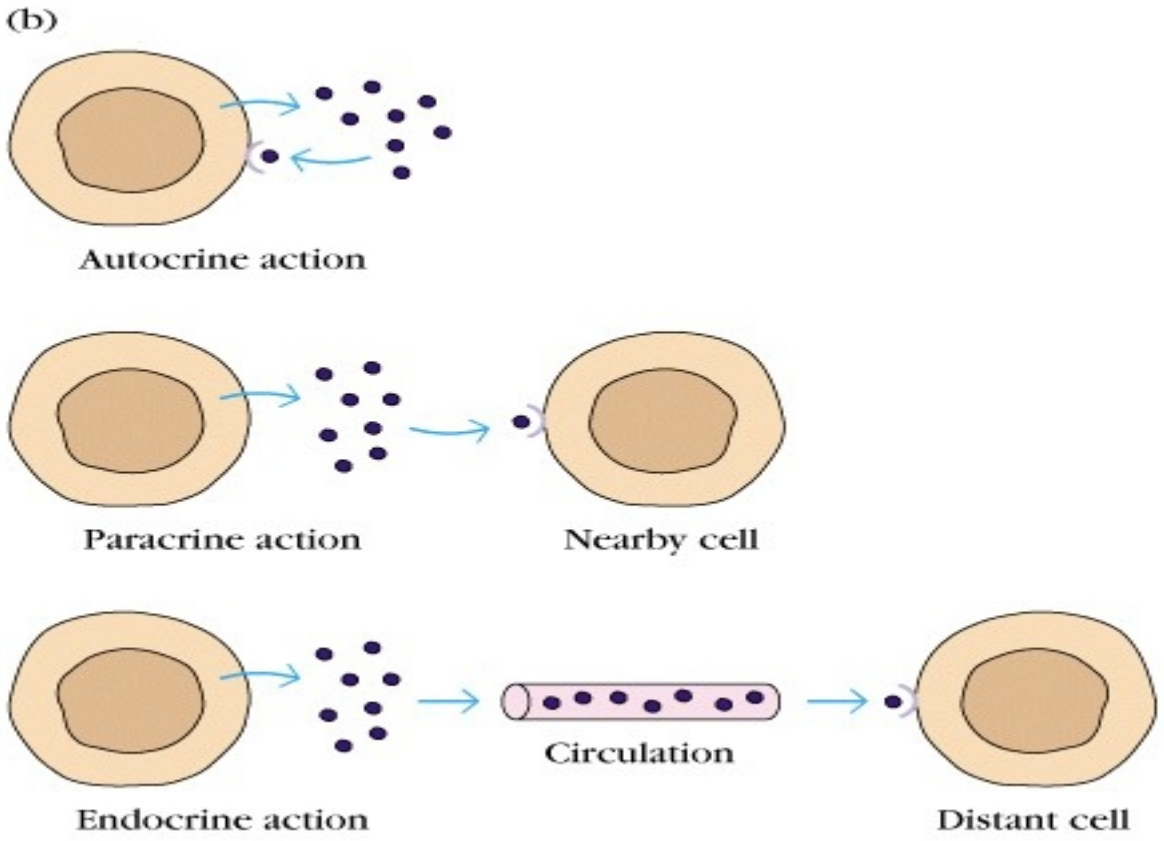
TLR signaling cascades




TLR4
Cell membrane
ST2, SIGGR/TIR8,
RP105, sTLRs
TRAM
TIRAP
SOCS-1
MyD88
sMyD88
SARM
PTP1B
SARM
PTP1B
TRIF
TRAF3
TRAF6
TANK
A20
RIP1
A20
IRAK1/2
SOCS-1
IRAK4
IRAK-M
SHP-1
TRAF6
A20
TAK1
TBK1
IKKε
MAPKs
IKKα/β/γ
IRF3
IκBα
NF-κB
p50
p65
Zc3h12a
nucleus
IFN-β
IL-6
IL-12p40
IRF3
AP-1
p50
p65
IκBNS
ATF3

- Pathogenic signals result in changes in immune cell functions, among which is expression of cytokines and chemokines.

- Cytokines and chemokines orchestrate Immune responses


(b)
Autocrine action
Paracrine action
Nearby cell
Endocrine action
Circulation
Distant cell

Pathogenic cytokines

- TNF, IL-1, IL-6, RANKL
- IL-15, IL-18, IL-17, VEGF, IL-8, MCP-1
- IFNγ
  - increased inflammation: ↑ cytokine production, ↓ IL-10 production, migration arrest
  - decreased tissue destruction: ↓ MMPs, ↓ osteoclastogenesis, suppression of IL-1 responses

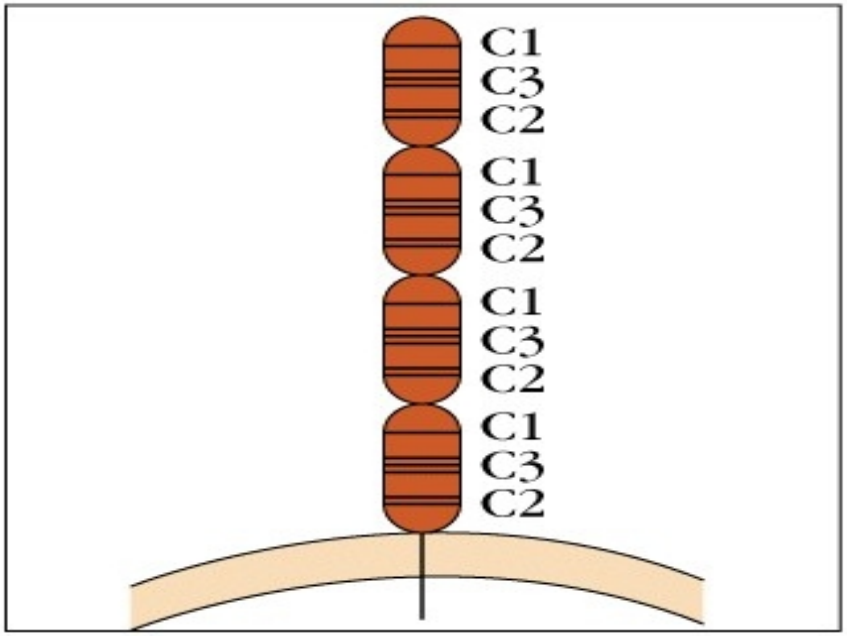
Homeostatic cytokines

- IL-10: inhibits TNF, IL-1 and IL-6 production
- IL-1RA: antagonizes IL-1
- TGFβ: inhibits cytokine production; dual role on T cells (↓Th1, ↑Th17)
- corticosteroids: inhibit cytokine production
- type I IFNs (IFNα/β)
  - inhibit synoviocyte proliferation
  - promote cytokine and chemokine production
- IL-27
  - inhibits cytokine production and Th1 and Th17
  - promotes cytokine production and Th1

Cytokine signaling

- Cytokines signal via distinct receptors, some with common structures and downstream signaling effectors
- The cytokine milieu and the type of receptor expressed in the different cell types determines the phenotype/response

(d) TNF receptors



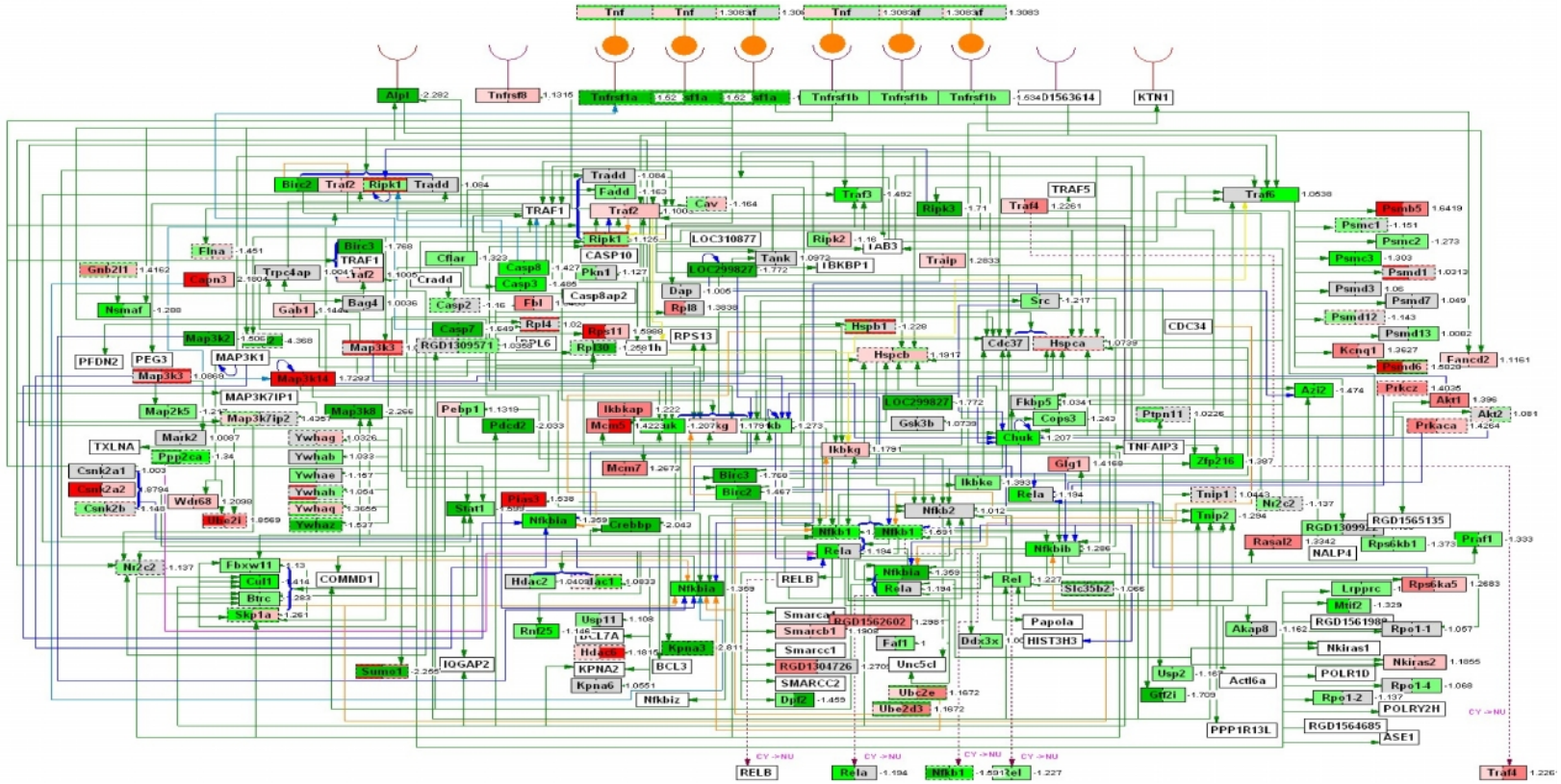
TNF-α
TNF-β
CD40
Nerve growth factor (NGF)
FAS

TNF receptor family transduce the signals following their trimerization

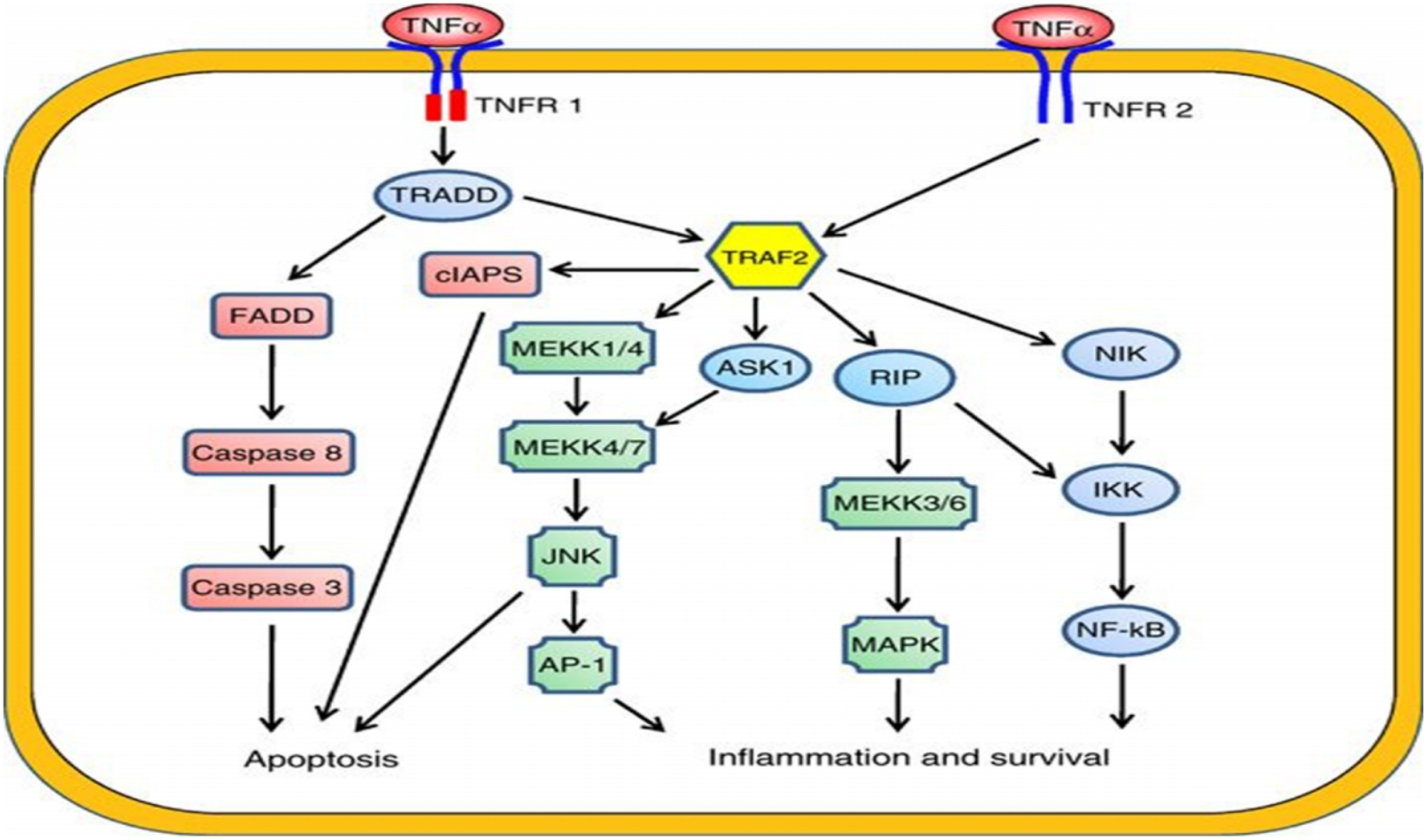
TNF receptors

- TNFR1 mainly initiates signals to promote apoptosis but also contributes to cell activation
- TNFR2 initiates activation signals and strongly activates NFkB without activating the apoptotic cascade

TNFα signaling pathways

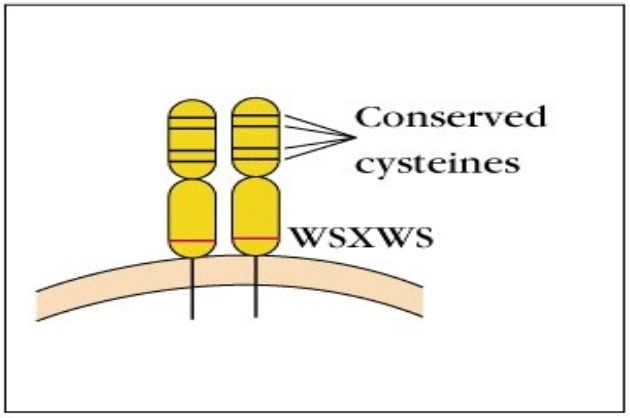


TNFR signaling

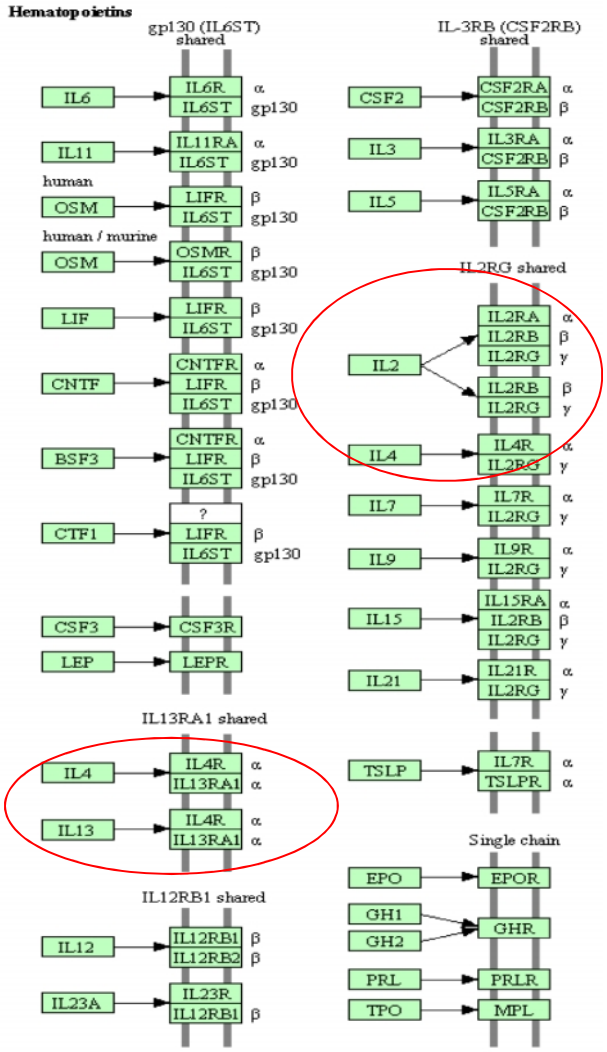


Class I and Class II cytokine receptors mediate signals via the Jak/STAT pathway

Class I cytokine receptors

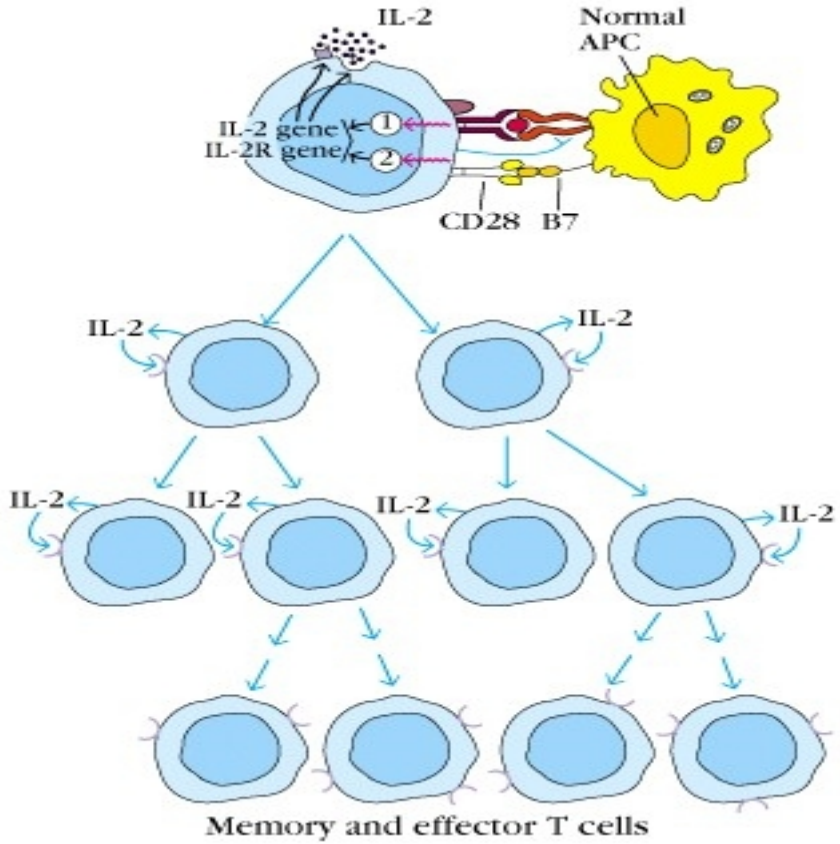


IL-2	IL-13
IL-3	IL-15
IL-4	GM-CSF
IL-5	G-CSF
IL-6	OSM
IL-7	LIF
IL-9	CNTF
IL-11	Growth hormone
IL-12	Prolactin

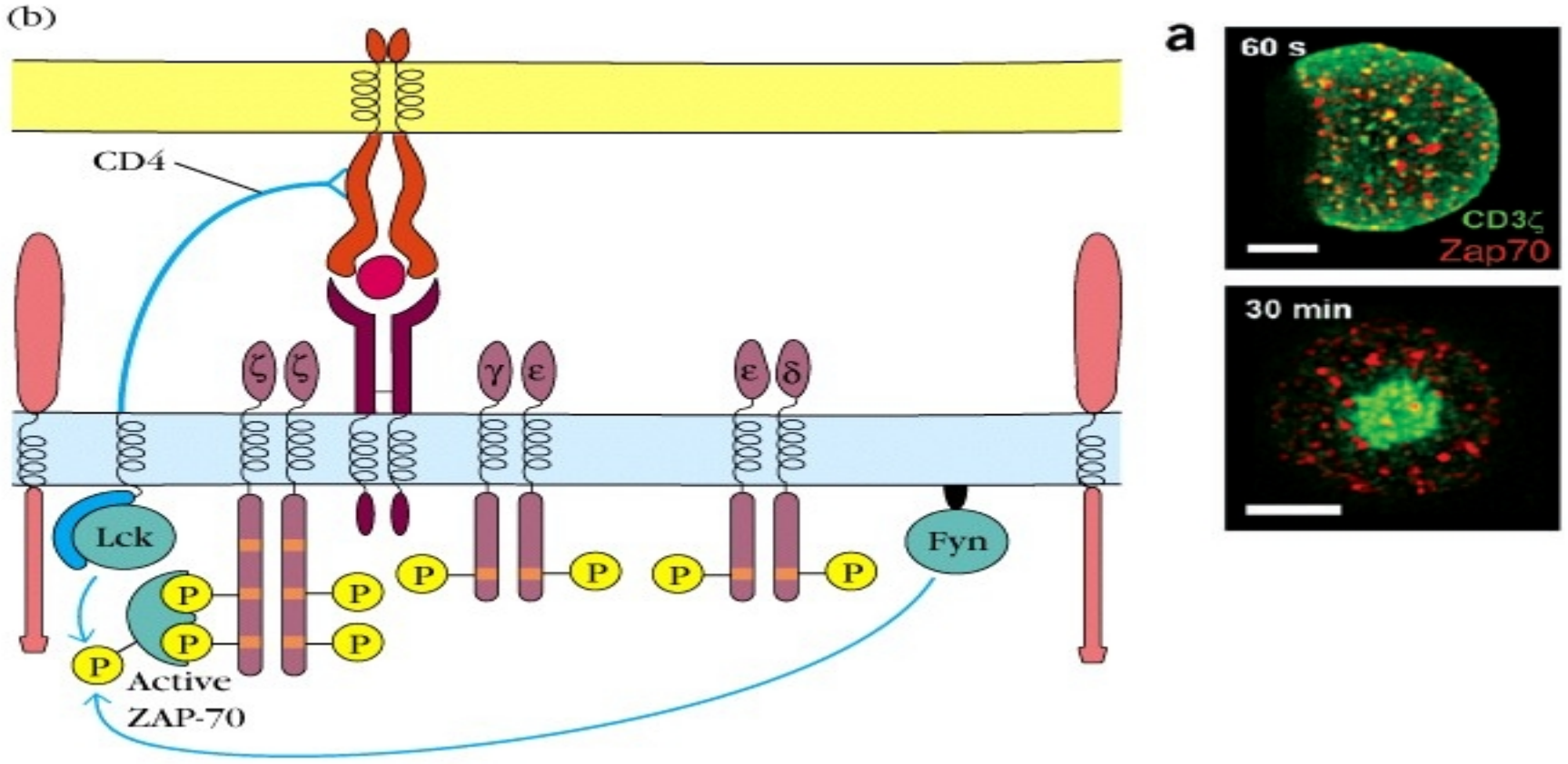


IL-2 signaling controls T-cell activation

- Engagement of TCR results in induction of IL-2 secretion and IL-2R upregulation
- IL-2 induces T-cell proliferation
- T-cell activation requires TCR plus co-stimulatory signals

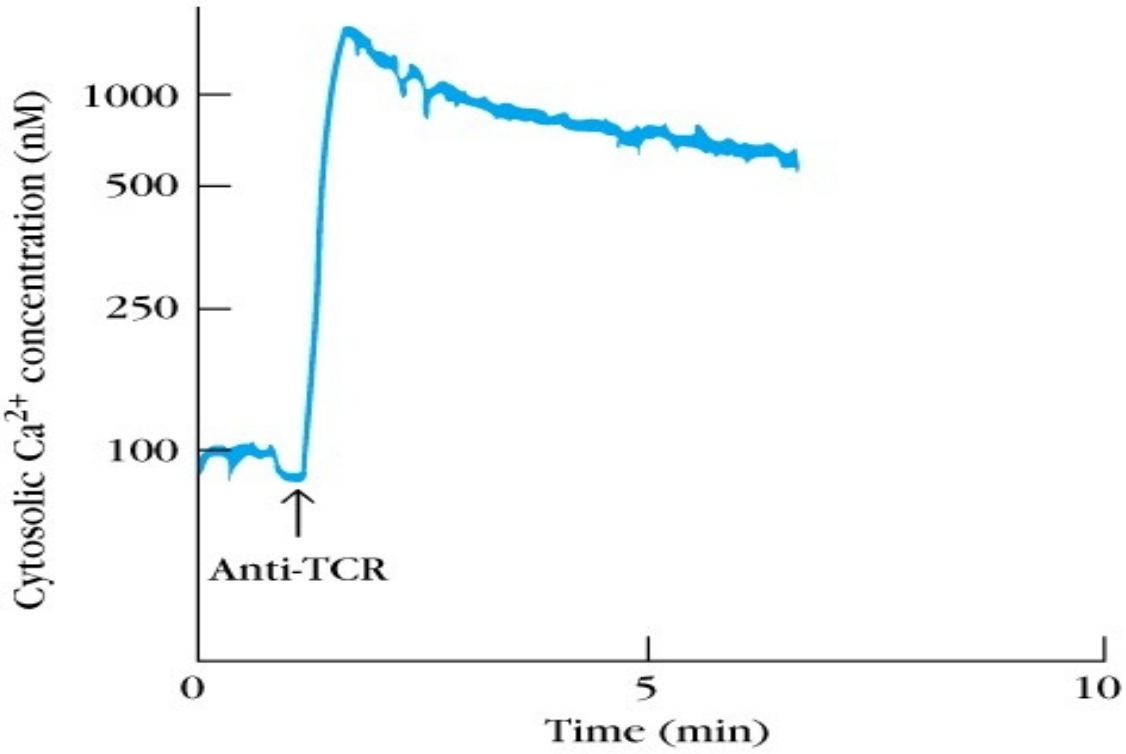

IL-2
Normal APC
IL-2 gene
IL-2R gene
1
2
CD28
B7
IL-2
IL-2
IL-2
IL-2
IL-2
IL-2
Memory and effector T cells

TCR-mediated T-cell activation



TCR signals are mediated by phosphorylation and de-phosphorylation events

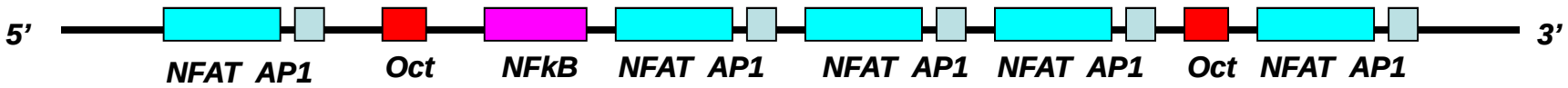
- Engagement of TCR by a peptide-presenting MHC of an antigen-presenting cell (APC) activates the tyrosine kinase Lck, which in turn phosphorylates ZAP70 and the intracellular ITAM motifs of TCR.



A few seconds following TCR engagement Ca++ influxes and is also released from intracellular stores

Induction of IL-2 expression

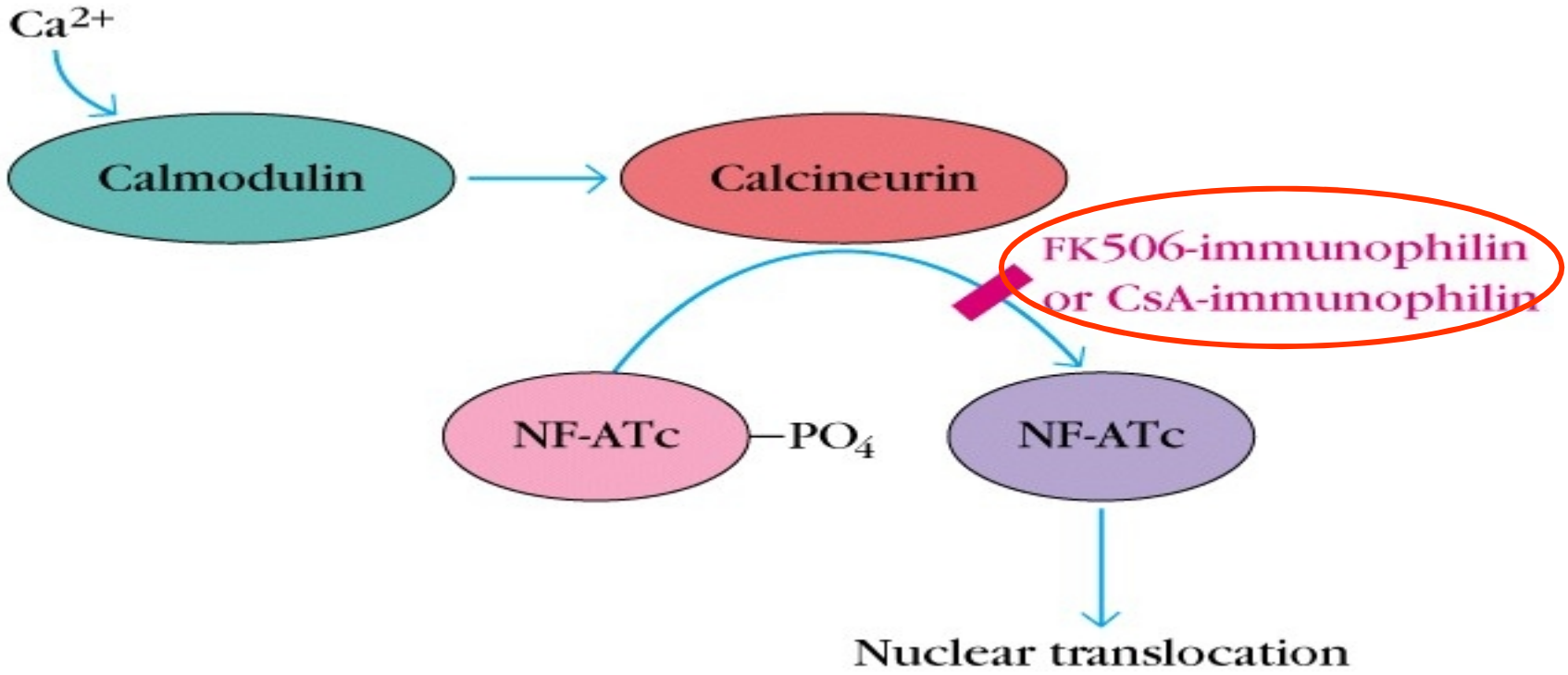
- TCR signals induce activation of key transcription factors that bind to the promoter of IL-2 gene
- These include: NFkB, NFAT, AP1, Oct1 etc



IL-2 promoter

Ca++ signaling in TCR activation

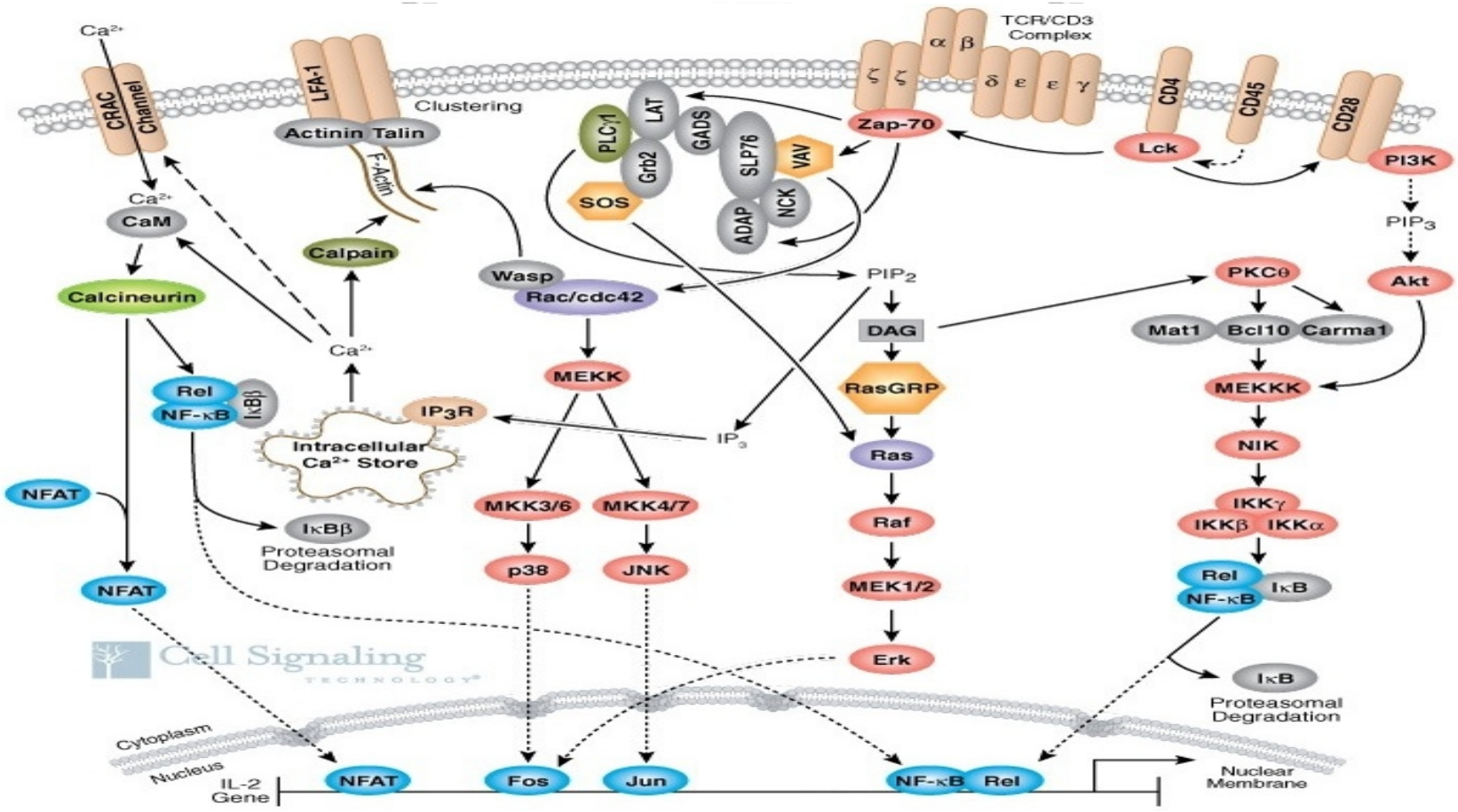
- A few seconds following TCR engagement Ca++ influxes and is also released from intracellular stores.
- Ca++ is an important signaling molecule activating calmodulin and the serine phophatase calcineurin, which in turn, dephosphorylates and activates the transcription factor NFAT.
- Dephosphorylated NFAT enters the nucleus and activates genes including this of IL-2



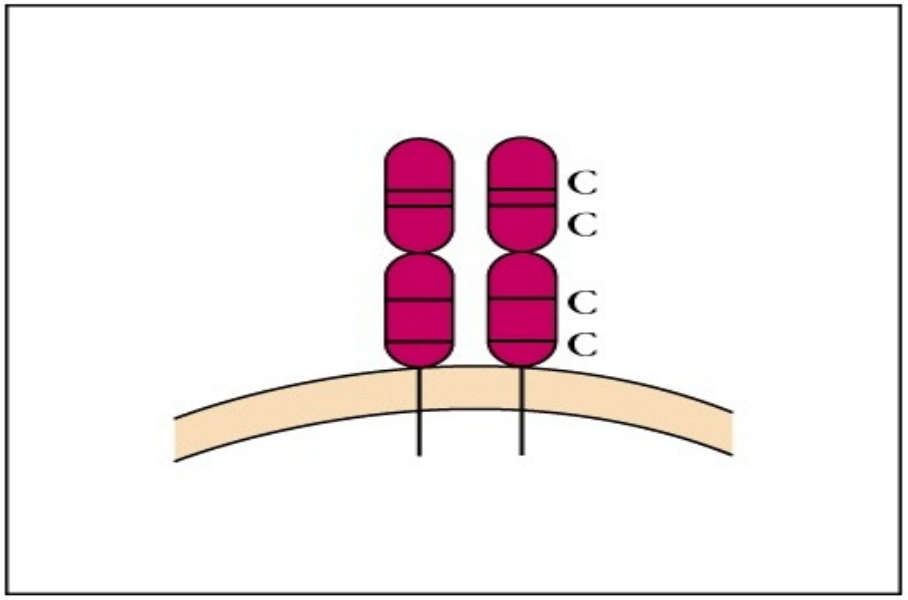
Therapeutic interventions

- ***Cyclosporin and Tacrolimus (FK506) target NFAT activation***

Co-stimulation results in additive activation of downstream molecules: Positive co-stimulatory signals



Class II (Interferon family) receptors

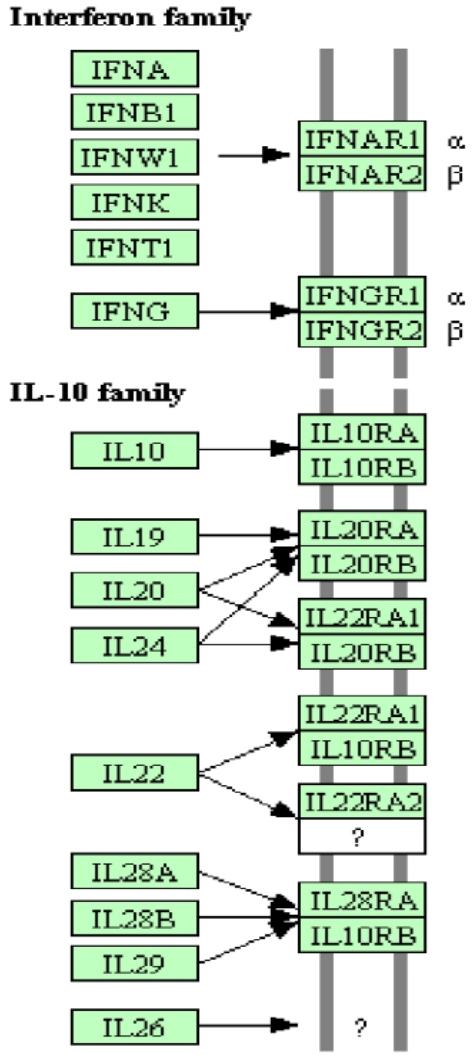


IFN-α

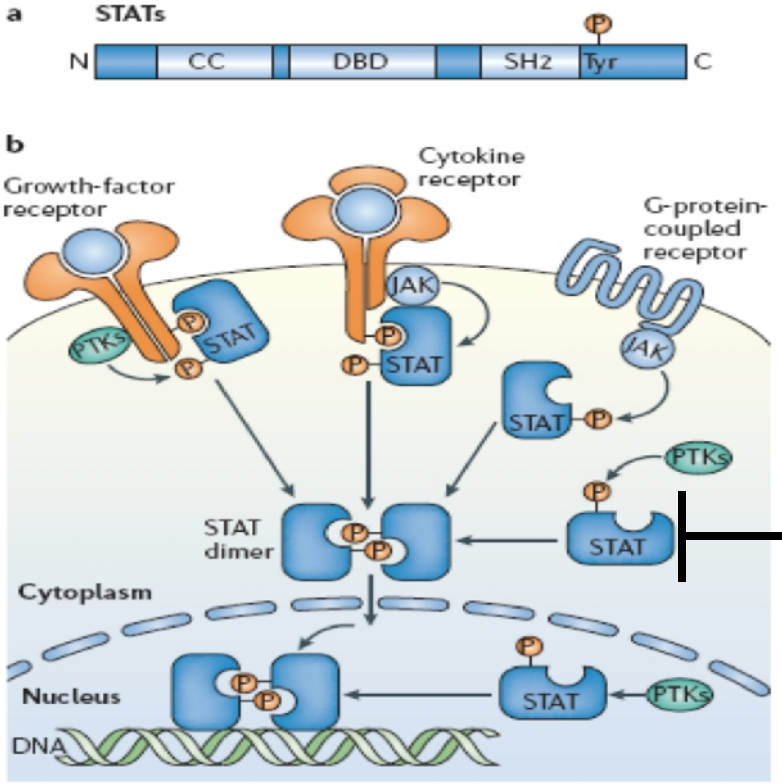
IFN-β

IFN-γ

IL-10

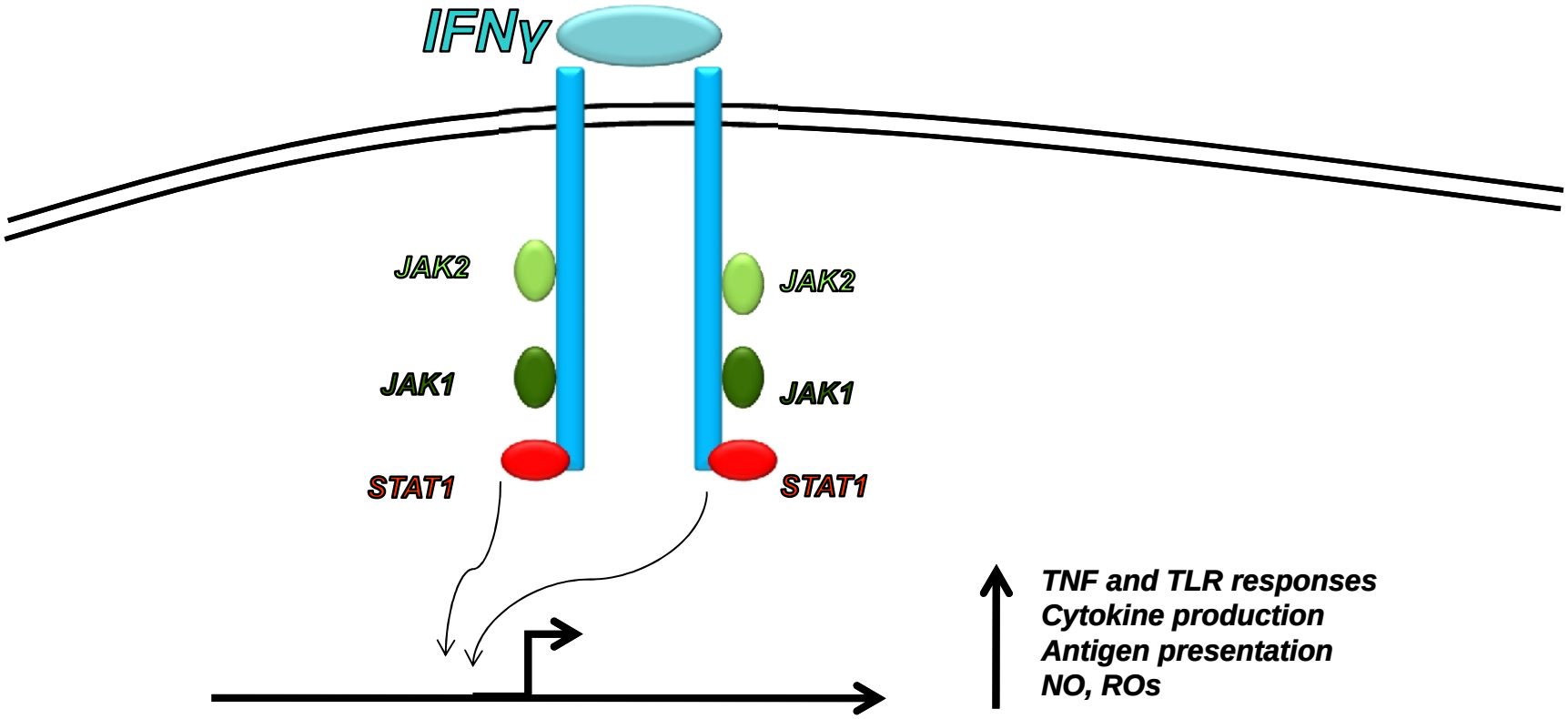


STATs are activated via tyrosine phosphorylation

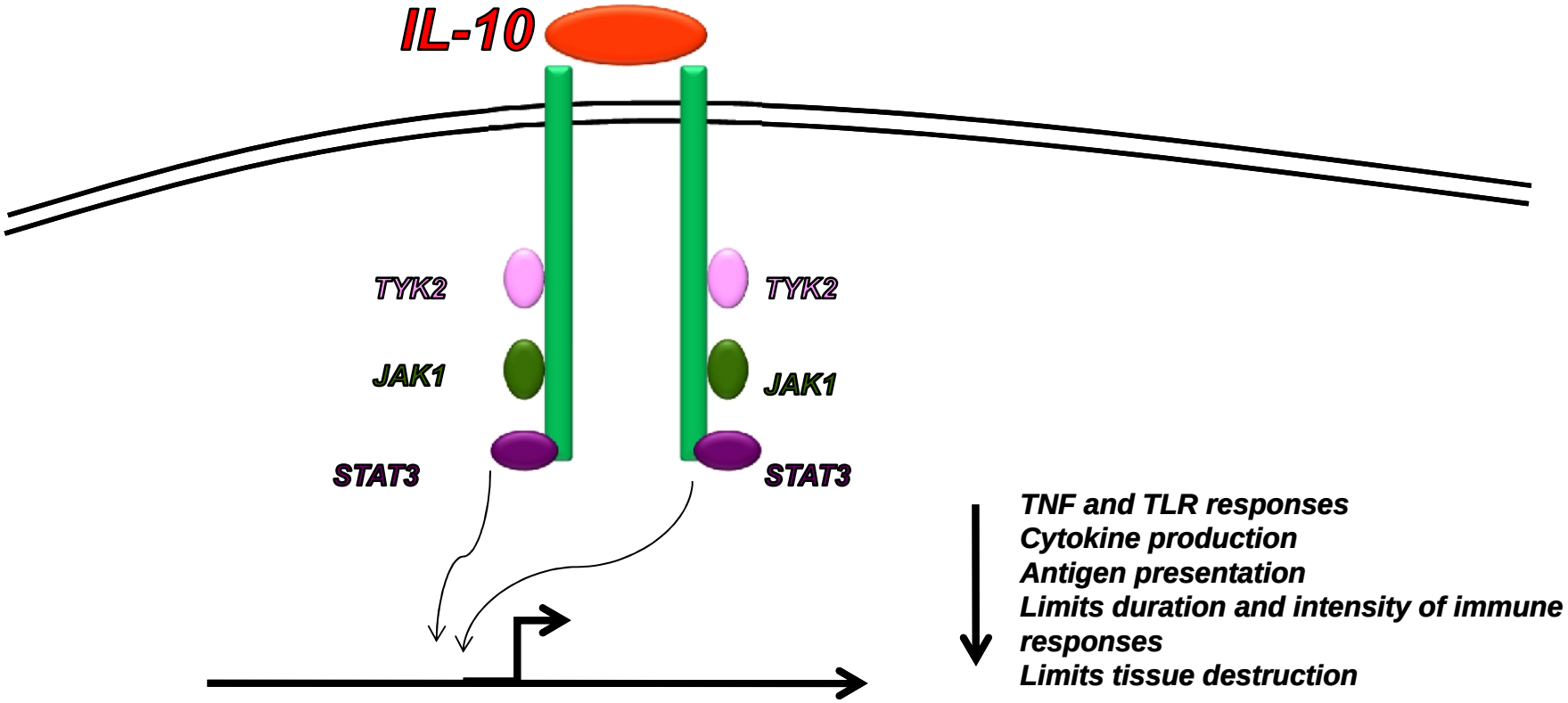


SHP phosphatases

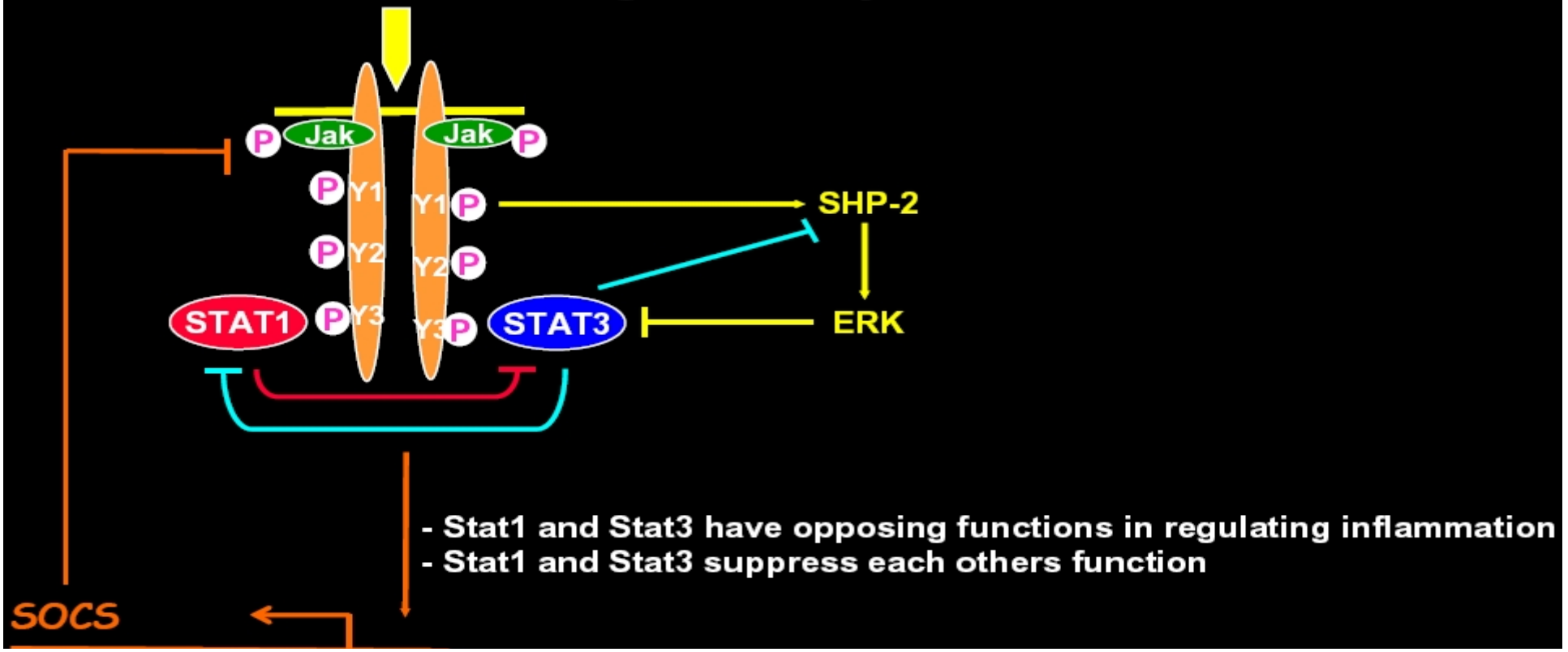
STAT1 mediates the pro-inflammatory effect of IFNγ



STAT3 mediates the anti-inflammatory effects of IL-10

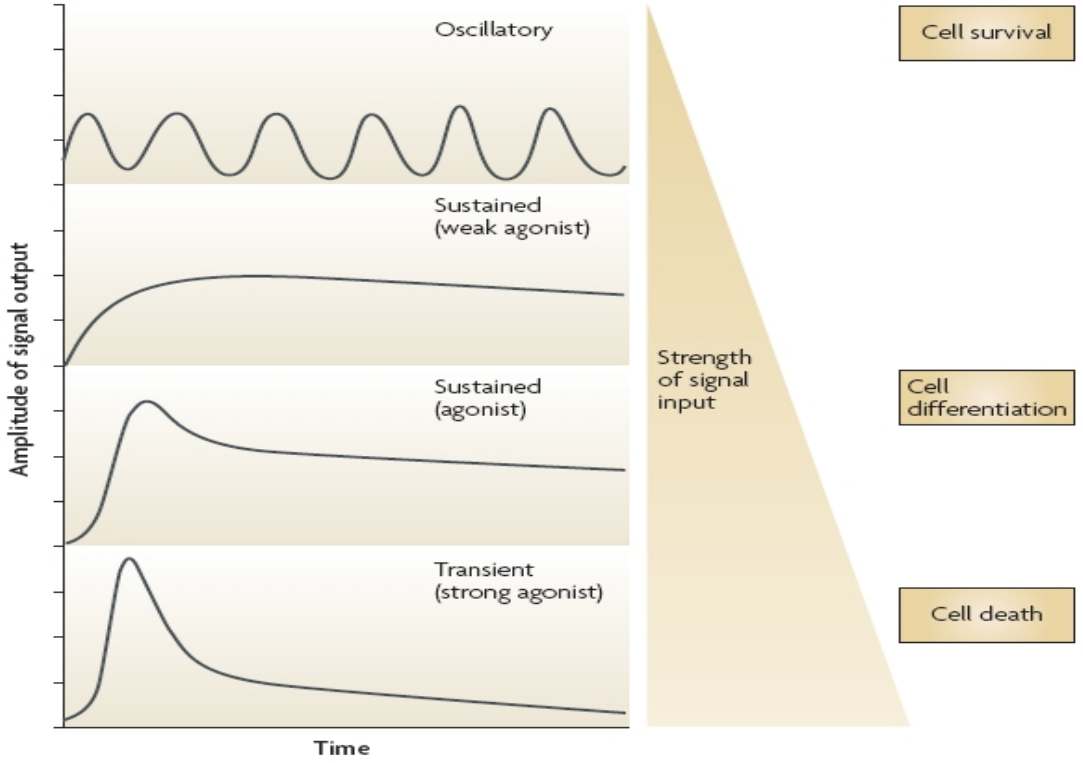


Intracellular signaling molecules regulate cytokine signaling

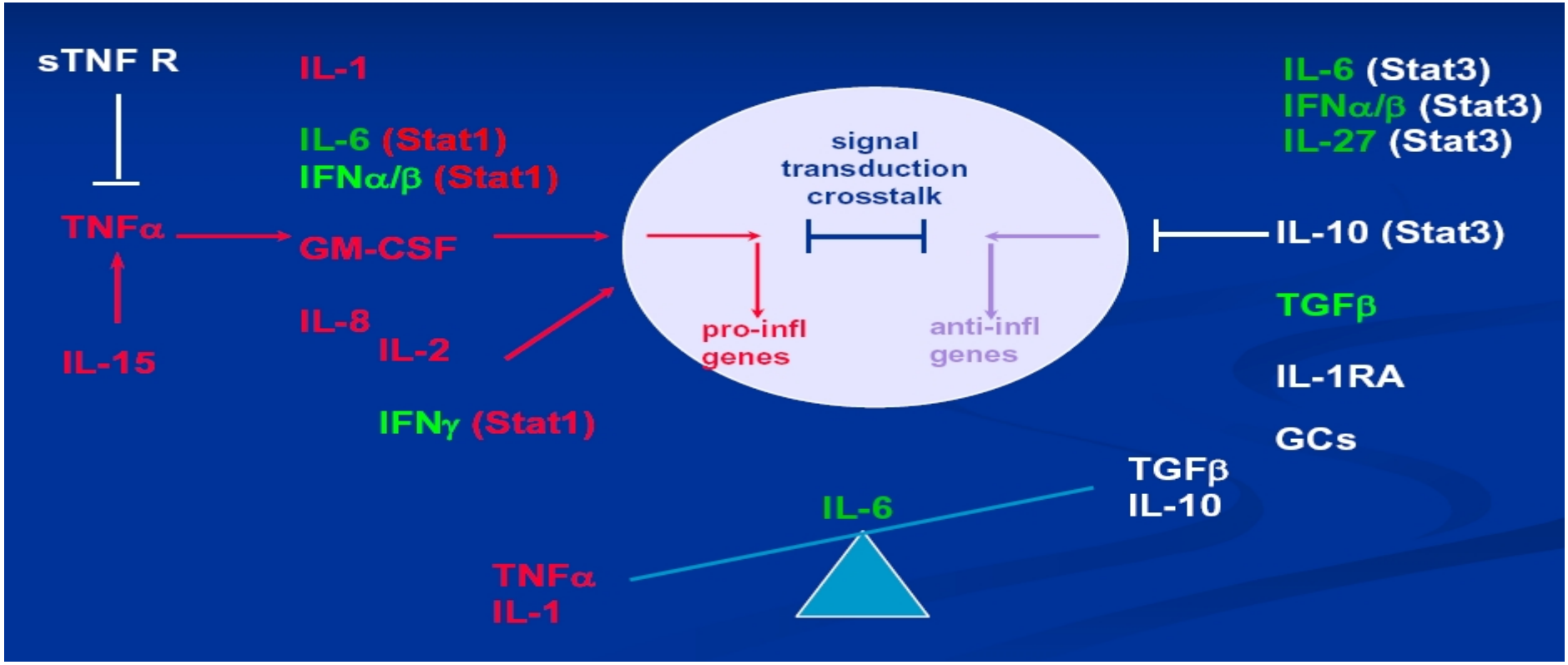


- Cytokine expression pattern and levels determine the fate of the inflammatory response
- Intracellular signaling molecules regulate cytokine action

Dependence of signal amplitude and kinetics on stimuli strength and consequences on biological outcomes

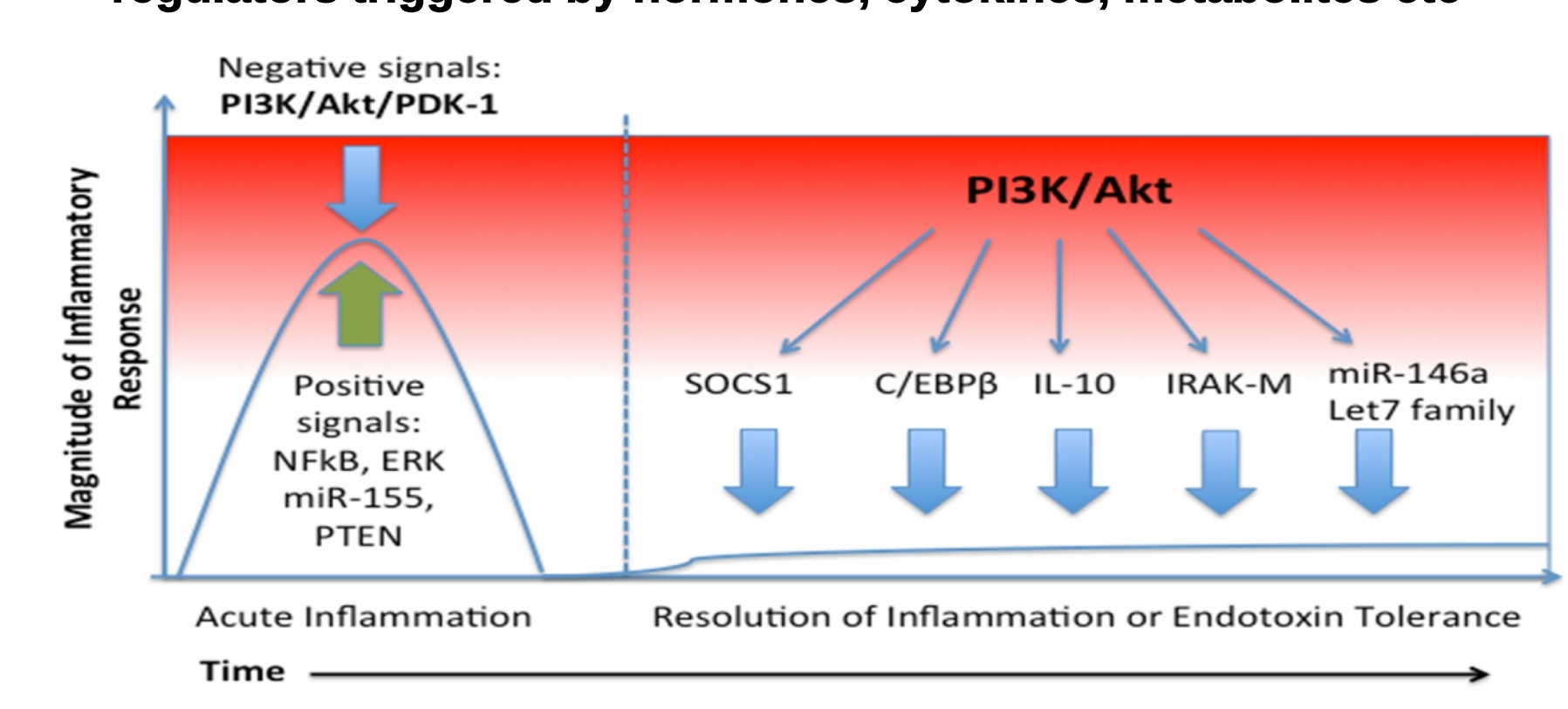


Signal integration determines the outcome in the complex inflammatory environment: The balance between cytokine action determines the severity of inflammation



Pathogenic and cytokine signal integration: the example of sepsis

Control of responses to pathogens: Positive and negative regulators triggered by hormones, cytokines, metabolites etc



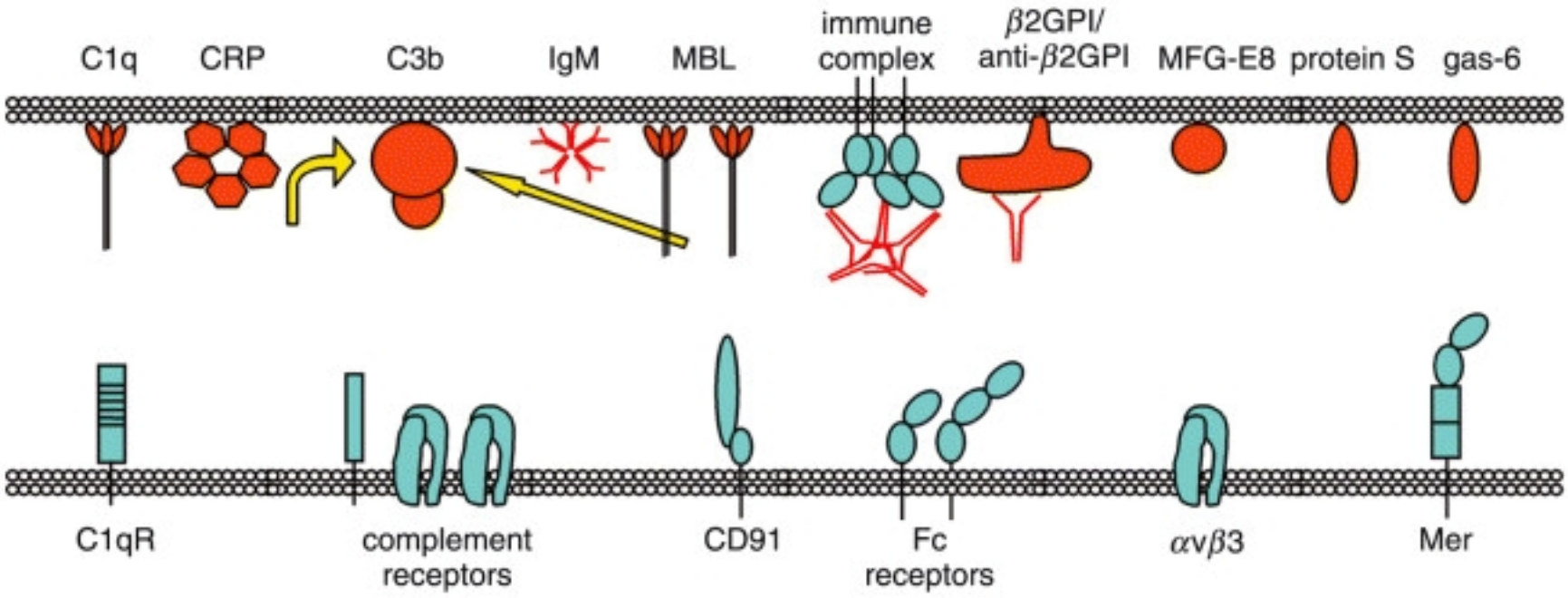
Vergadi et al, J Immunol. 2017; 198:1006-1014

Conclusions

- Pathogens are recognized through receptors and transmit signals in immune cells.
- Signal integration results in cytokine production and cell activation (i.e. phagocytosis).
- Cytokine and pathogen-receptor signaling is regulated in a dynamic manner during immune responses
  - It is augmented or suppressed
  - Cytokine signals crosstalk with signals from pathogen receptors, hormones, adipokines etc, and their balance determines the phenotype
  - Cytokines have different effects on the same cell depending on the timing and state of activation

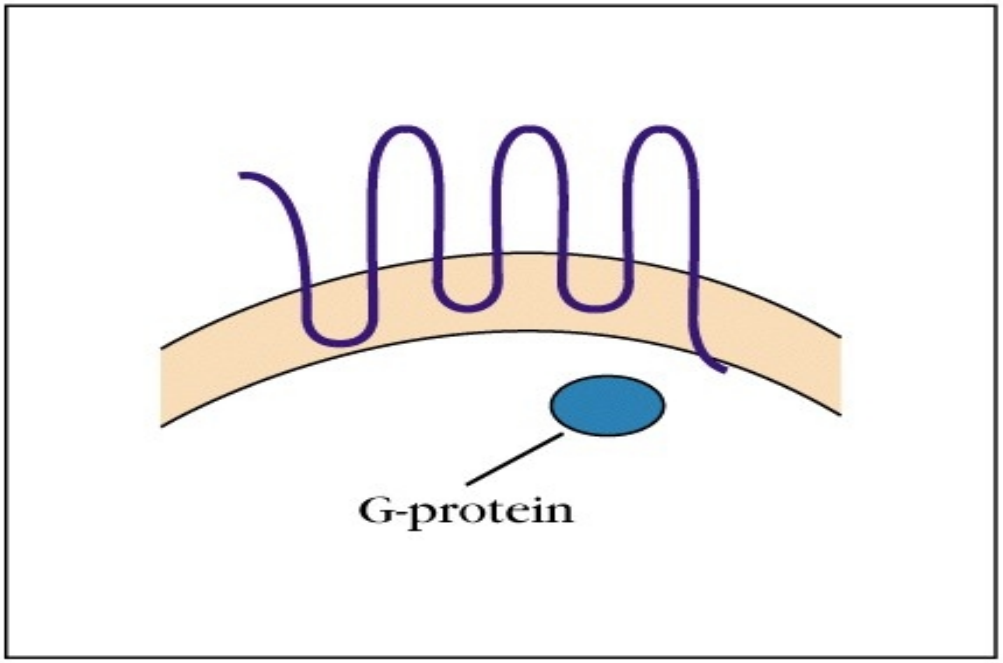
Regulation of cytokine signaling and function will impact disease progression: new therapeutic approaches that modulate cytokine signaling

Receptors recognizing opsonized molecules



Chemokine signaling

(e) Chemokine receptors



IL-8

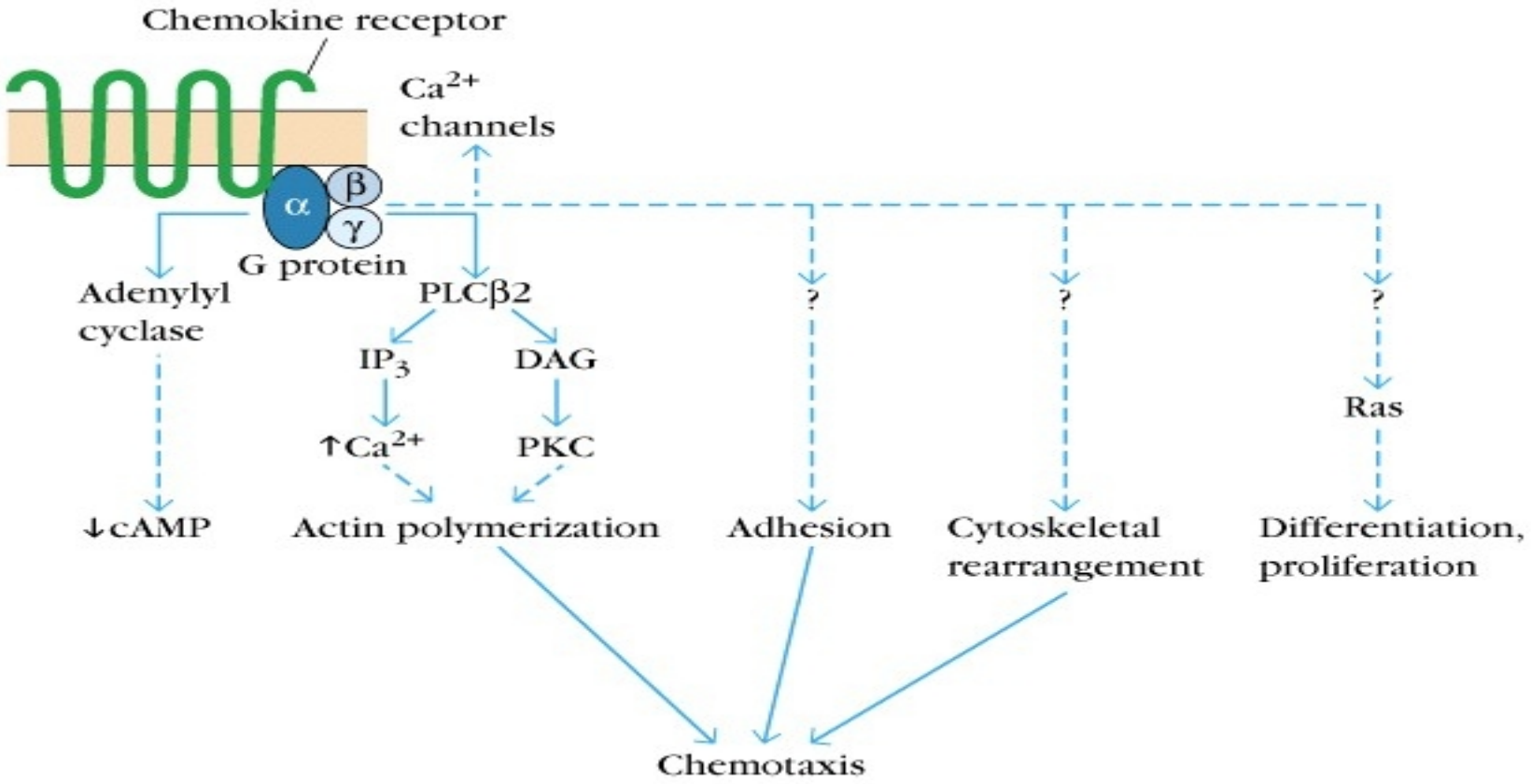
RANTES

MIP-1

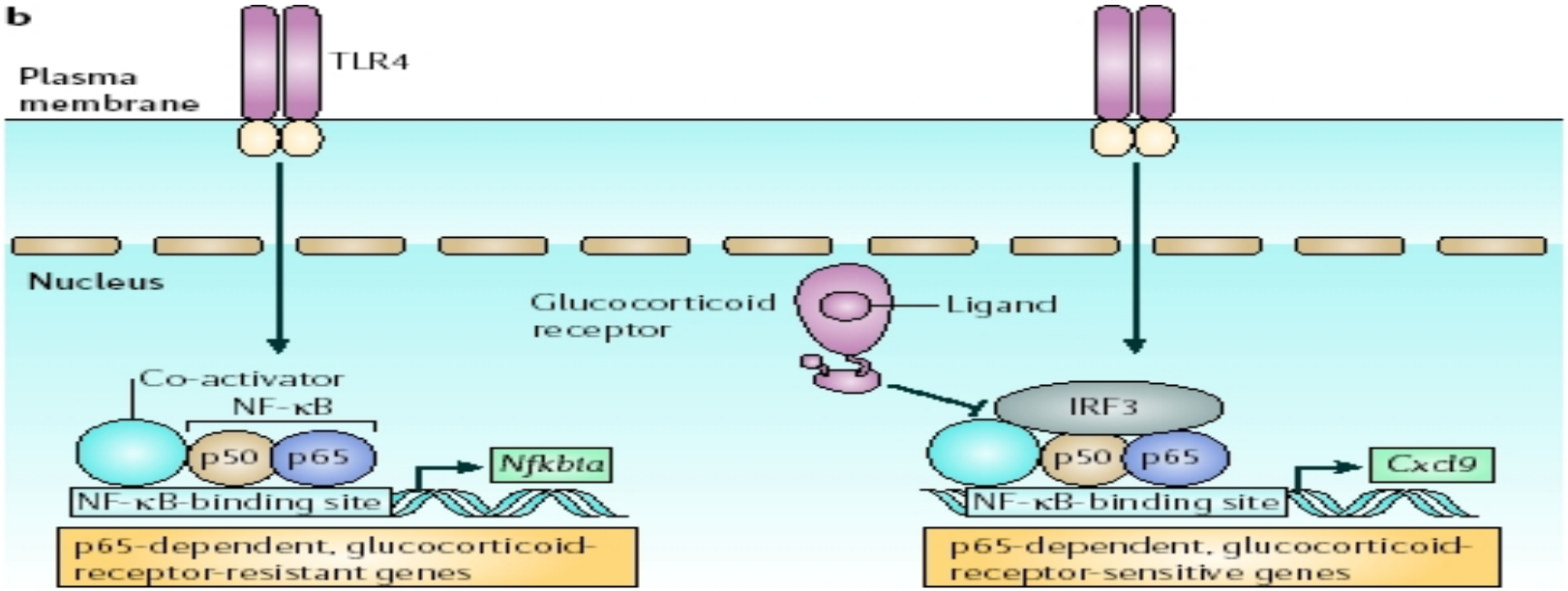
PF4

MCAF

NAP-2

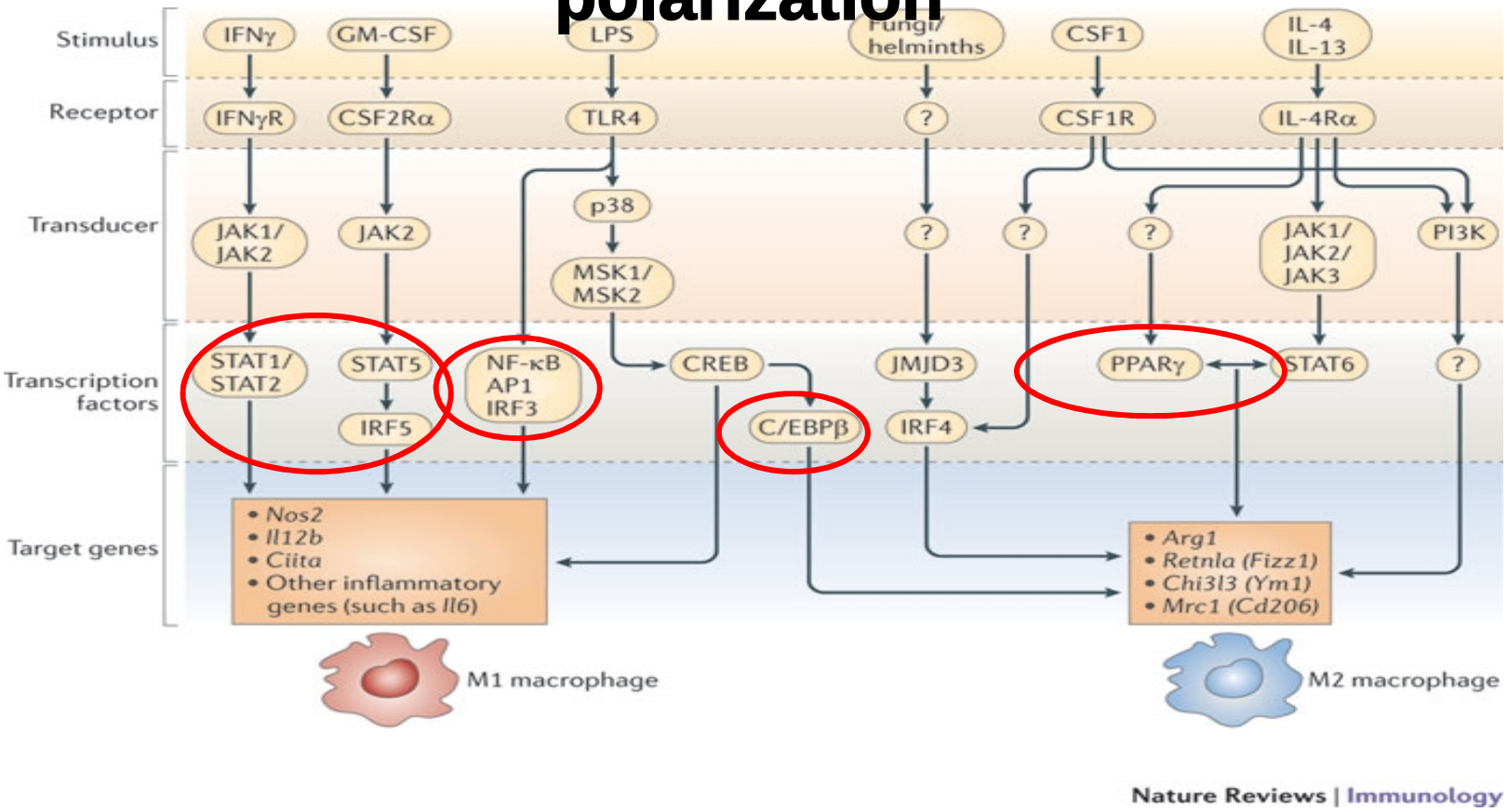

Chemokine receptor
Ca2+ channels
α
β
γ
G protein
Adenylyl cyclase
PLCβ2
IP3
DAG
↑Ca2+
PKC
?
?
?
Ras
↓cAMP
Actin polymerization
Adhesion
Cytoskeletal rearrangement
Differentiation, proliferation
Chemotaxis

Inhibition by glucocorticoid receptors

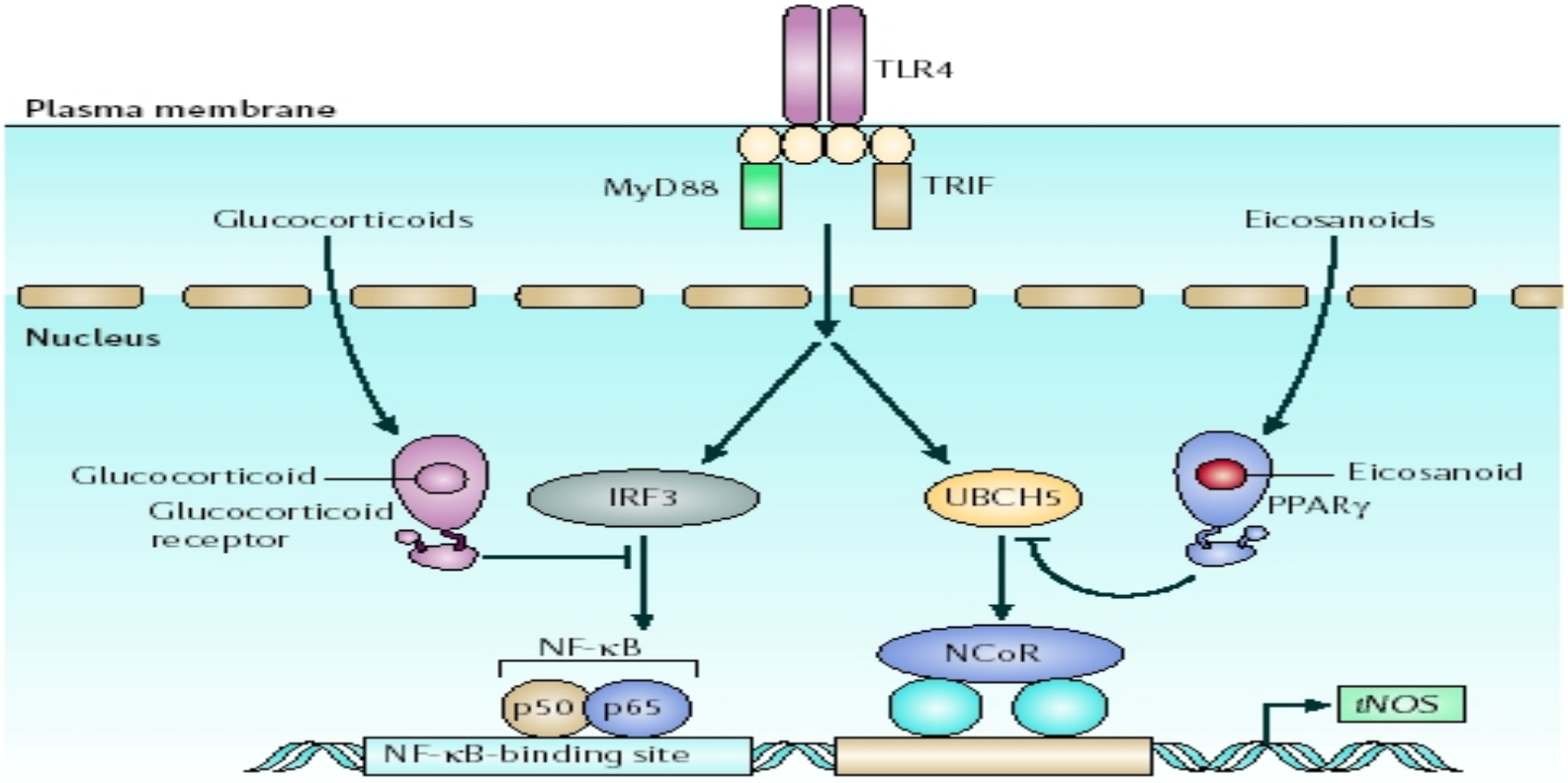


Nat Rev Immunol. 2005 5:446

Signaling mechanisms controlling macrophage polarization

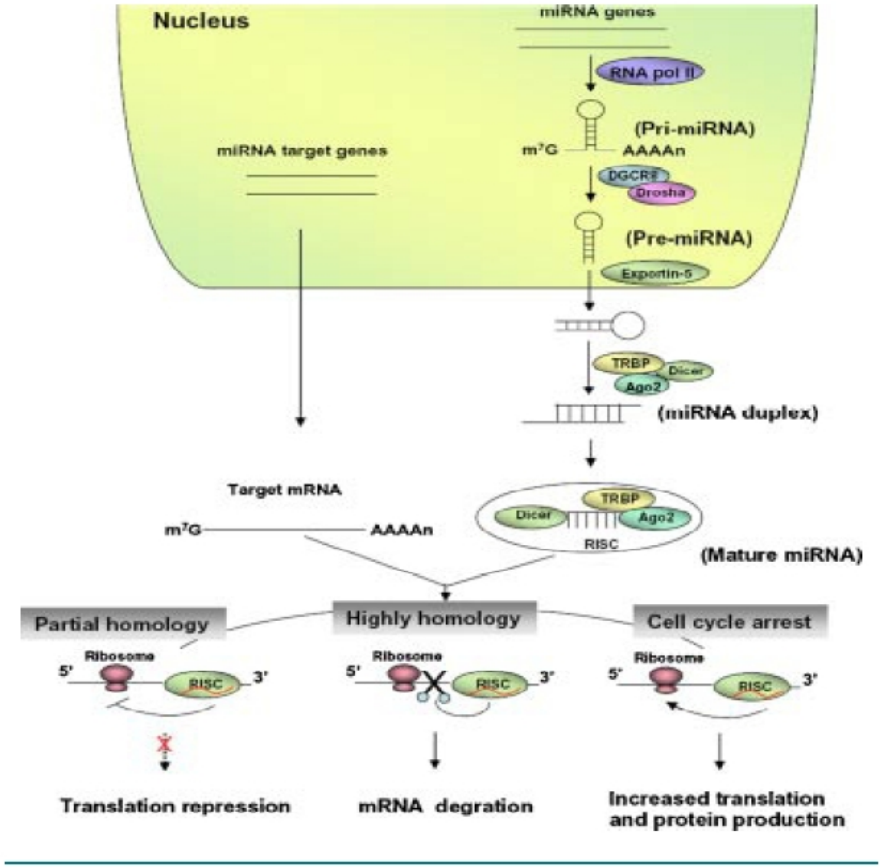


Inhibition of inflammatory signals by PPARγ and glucocorticoids



miRNAs in TLR signaling as regulators of inflammatory responses

MicroRNAs: novel players in the regulation of immunity



J. Cell. Physiol. 2009, 218: 467–472

Endogenous, non protein coding, small RNAs

Exhibit tissue specific or developmental stage specific expression

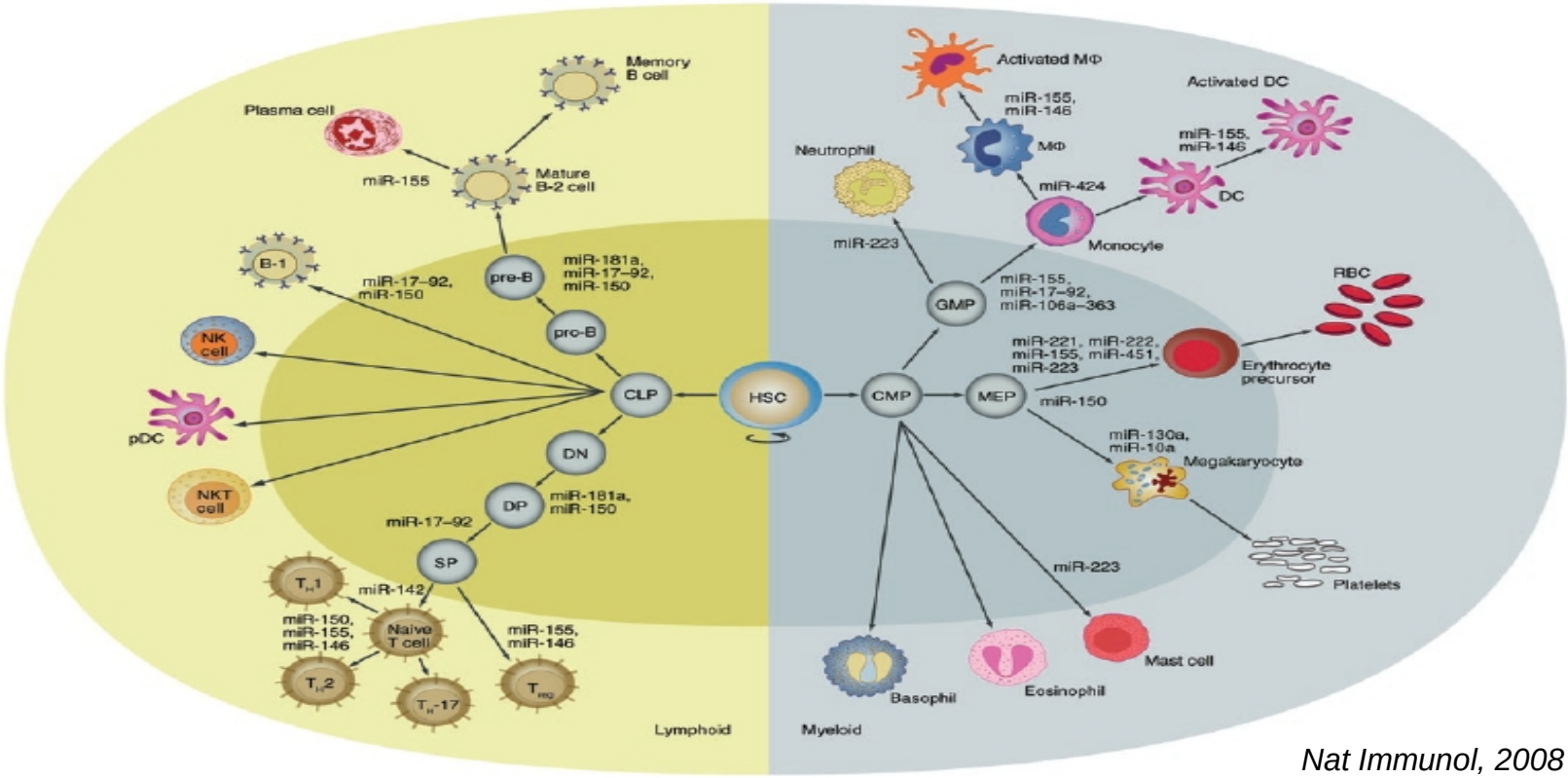
Regulate translation and stability of mRNAs

Role

in hematopoiesis

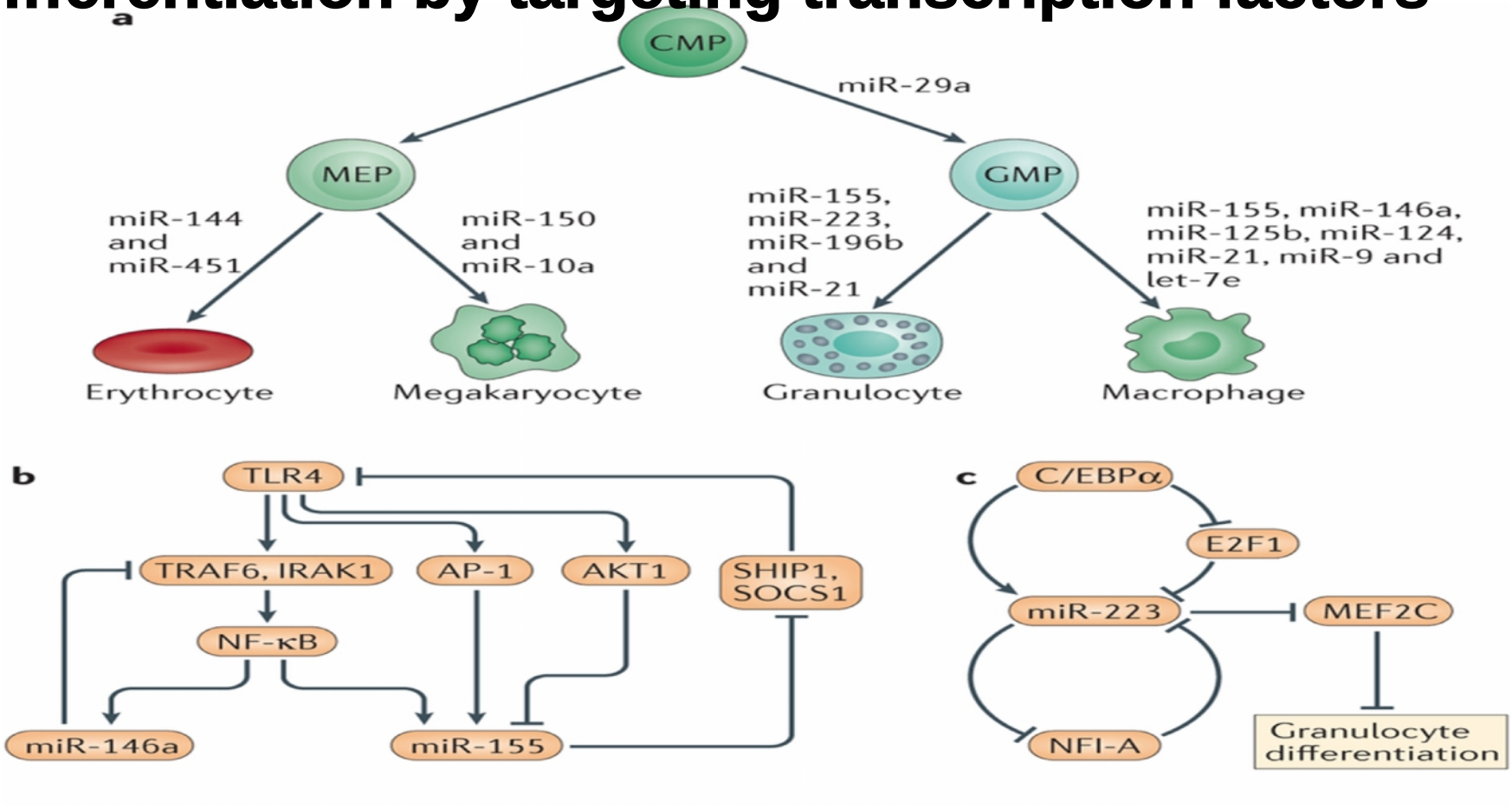
In control of cell survival /proliferation

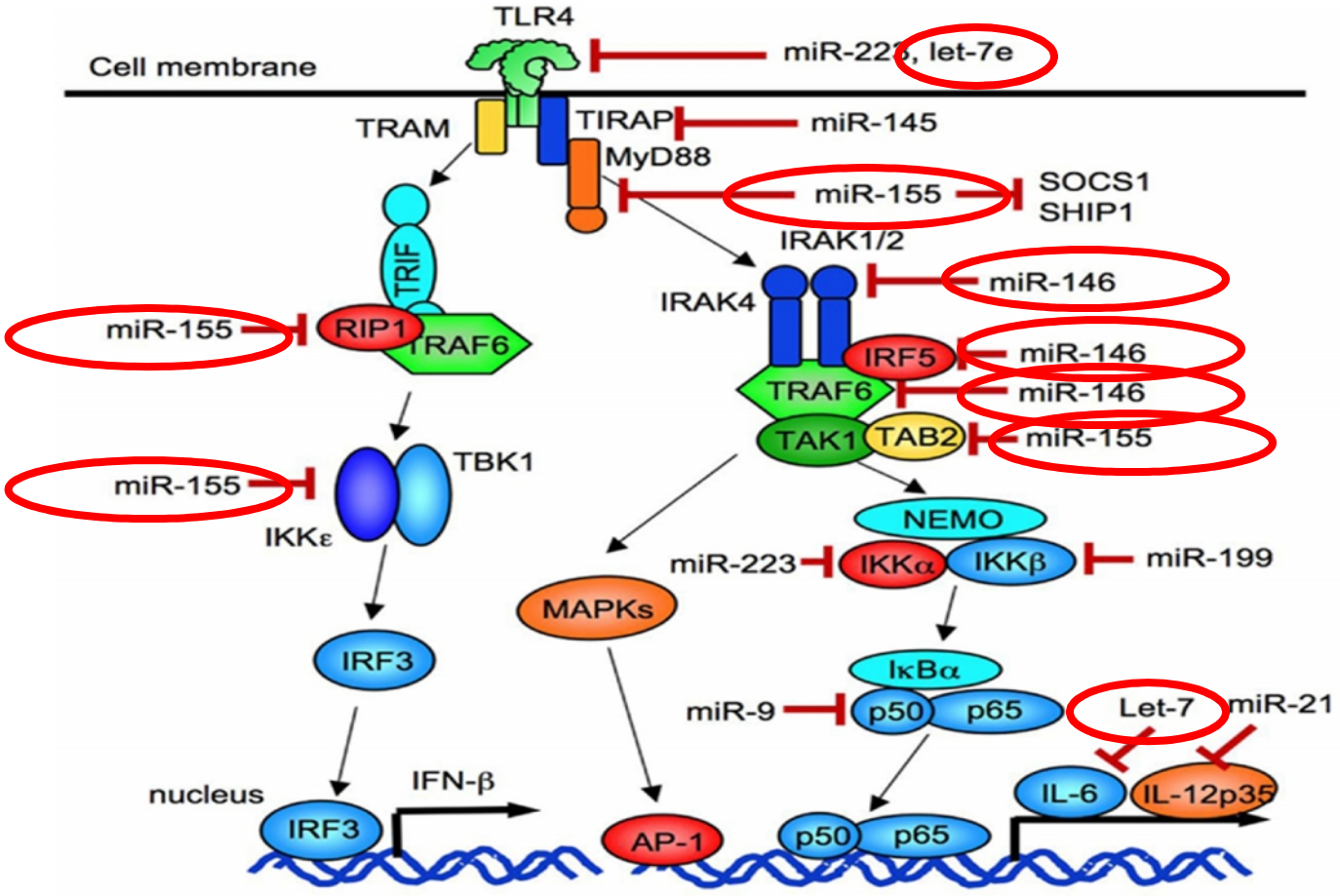
miRNAs in hematopoietic cells



Nat Immunol, 2008

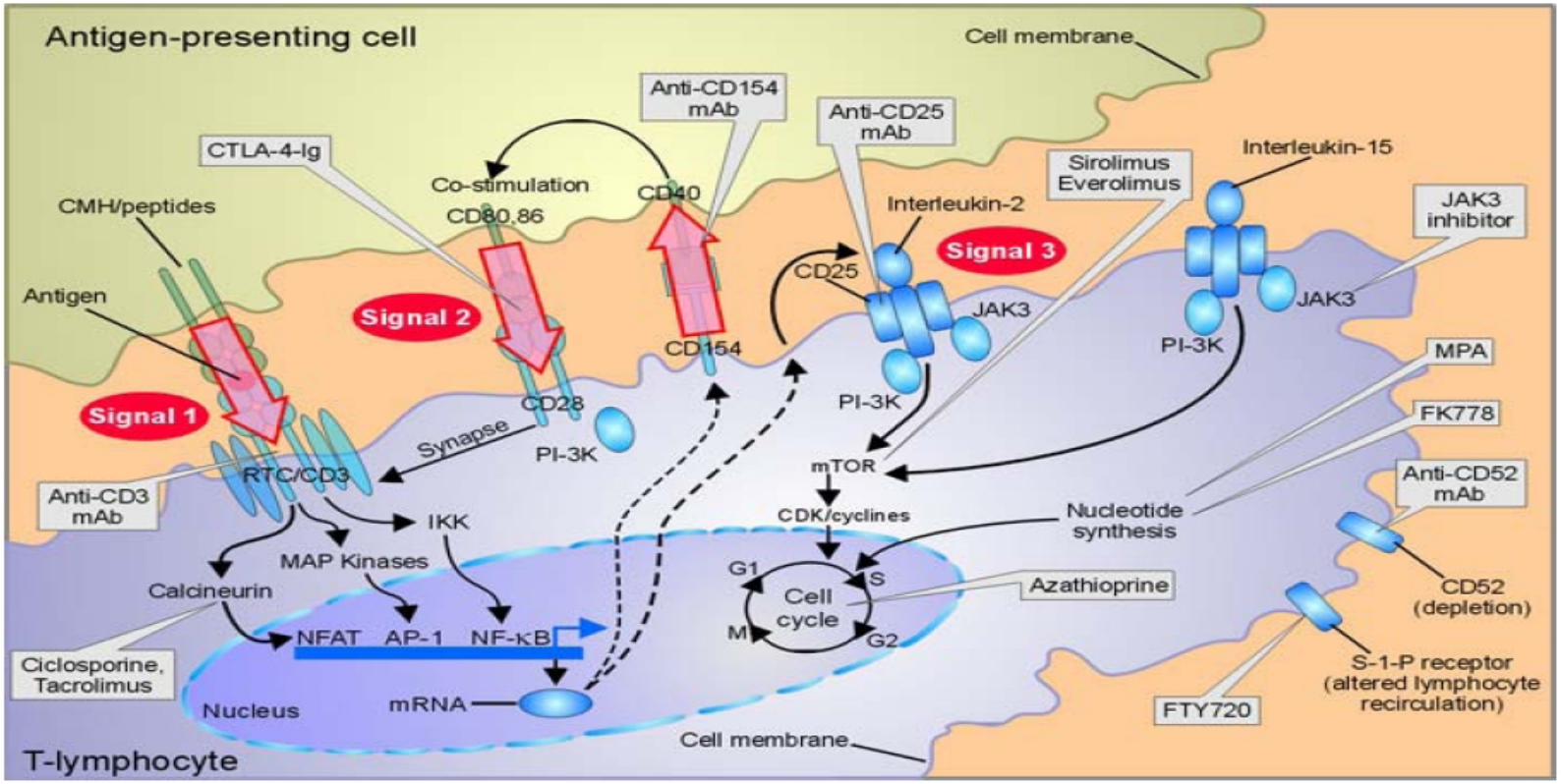
miRNAs control macrophage lineage cell differentiation by targeting transcription factors



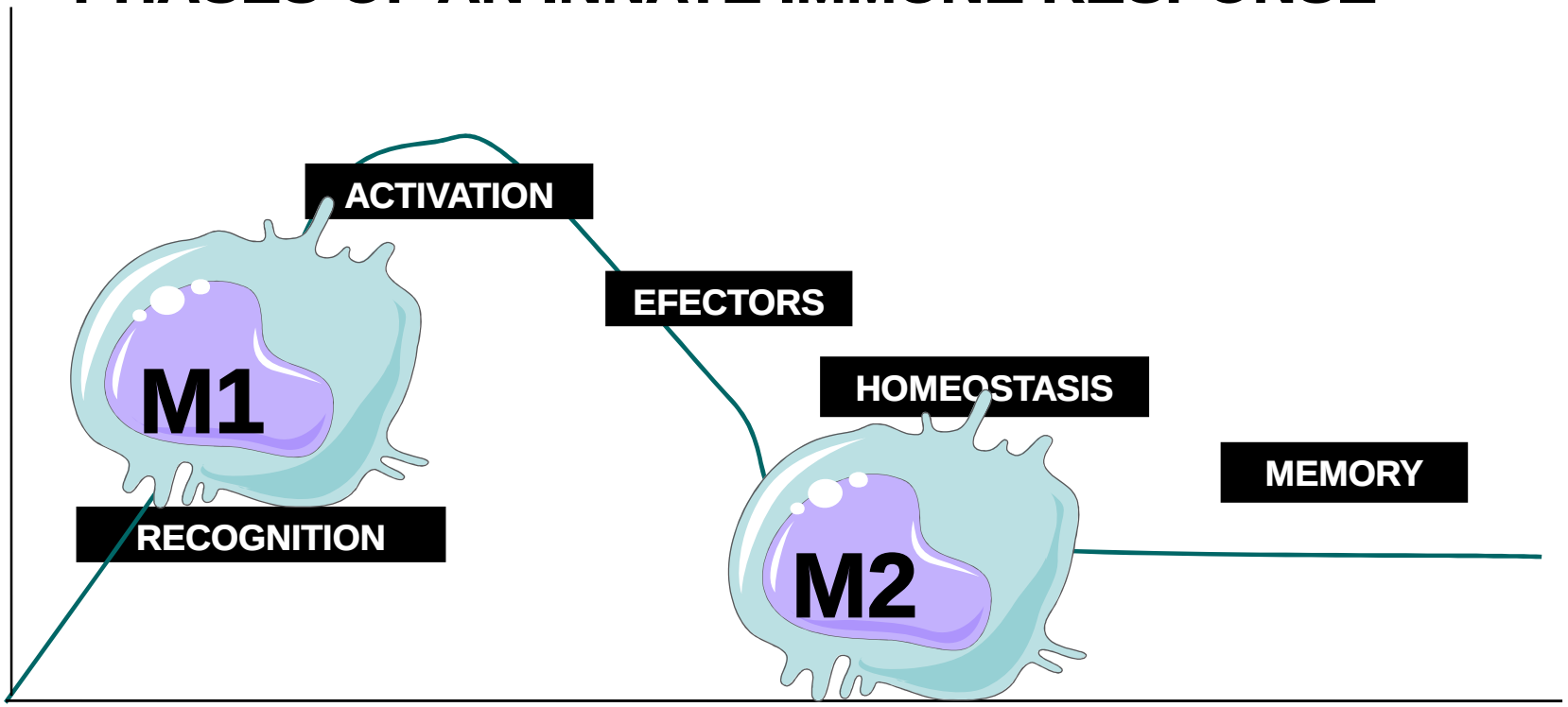


Front. Physiol., 2012

Therapies targeting T-cell activation signals



PHASES OF AN INNATE IMMUNE RESPONSE



Jak and Stat interaction with different cytokine receptors

Cytokine receptor	JAK	STAT
IFN-γ	JAK1 and JAK2	Stat1
IFN-α/β	JAK1 and Tyk-2	Stat2
IL-2	JAK1 and JAK3	Stat5
IL-3	JAK2	Stat5
IL-4	JAK1 and JAK3	Stat6
IL-6	JAK1 (and sometimes others)	Stat3
IL-10	JAK1 and Tyk-2*	Stat3
IL-12	JAK2 and Tyk-2*	Stat4

TCR activation signals- overview

- Activation signals are mediated via several kinases including ZAP-70, PKC, Raf, MAPKs, JNK
- They lead to activation of transcription factors such as NFAT, NFkB, AP1
- They induce transcription of cytokines and other genes involved in activation or fate (i.e. that contribute to proliferation and/or Th1/Th2 polarization)
- Co-stimulatory signals use the same pathways

Recognition of pathogens: Fungi