Main inflammatory cytokines and intracellular signaling pathways

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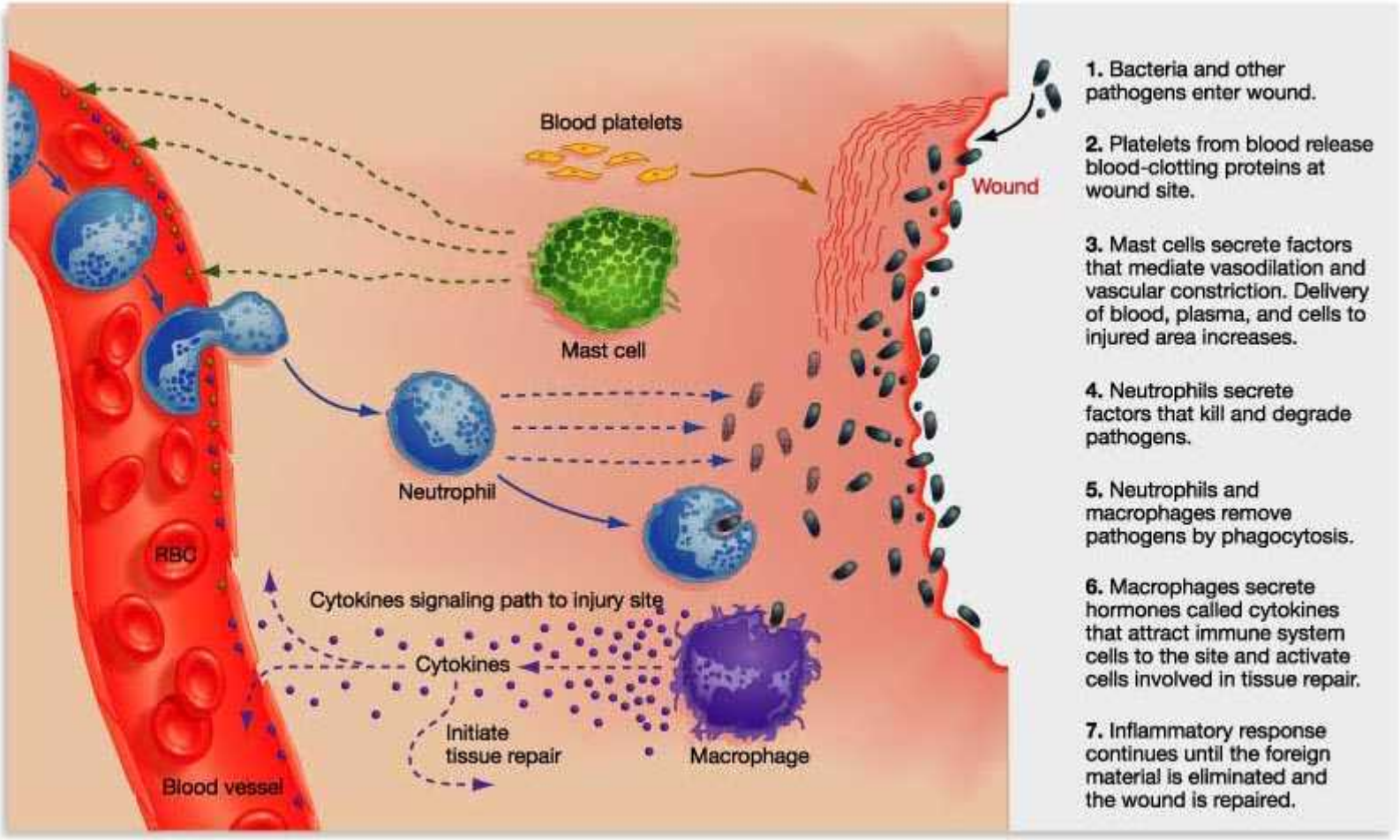
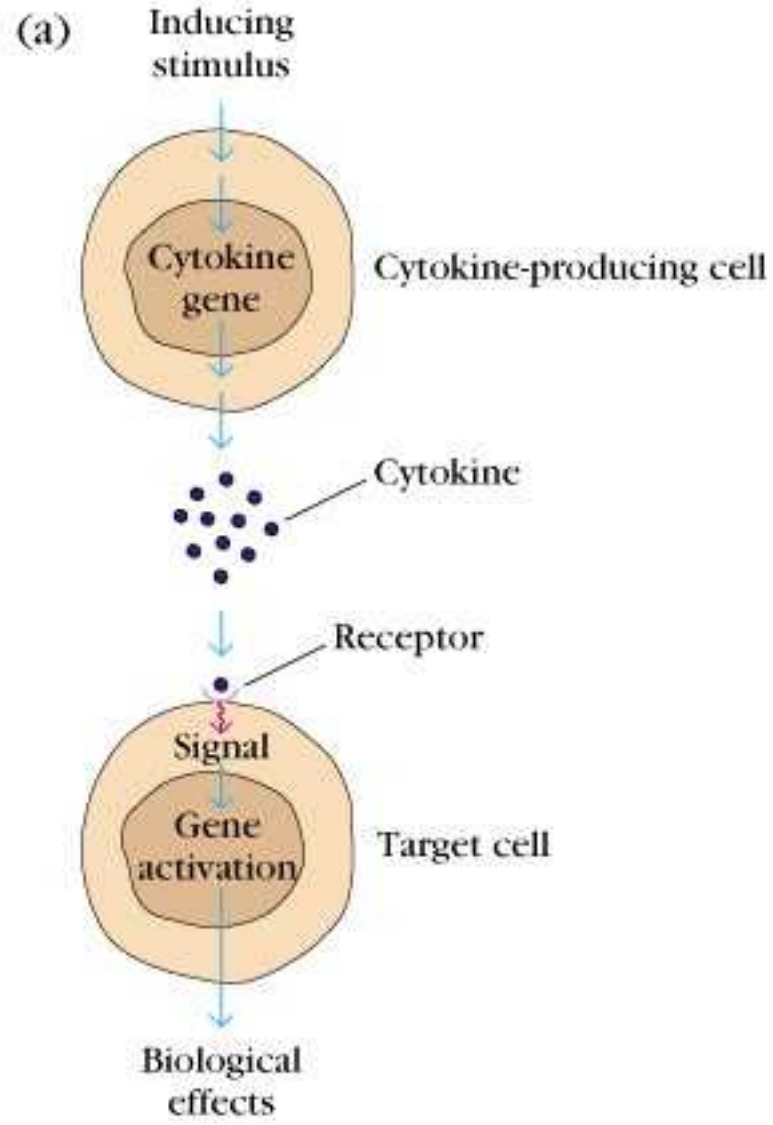
- Inflammatory cytokines and signaling
- T cell receptor signaling, the IL-2 paradigm
- Cytokine signaling and regulation
- Pathogen signals TLRs

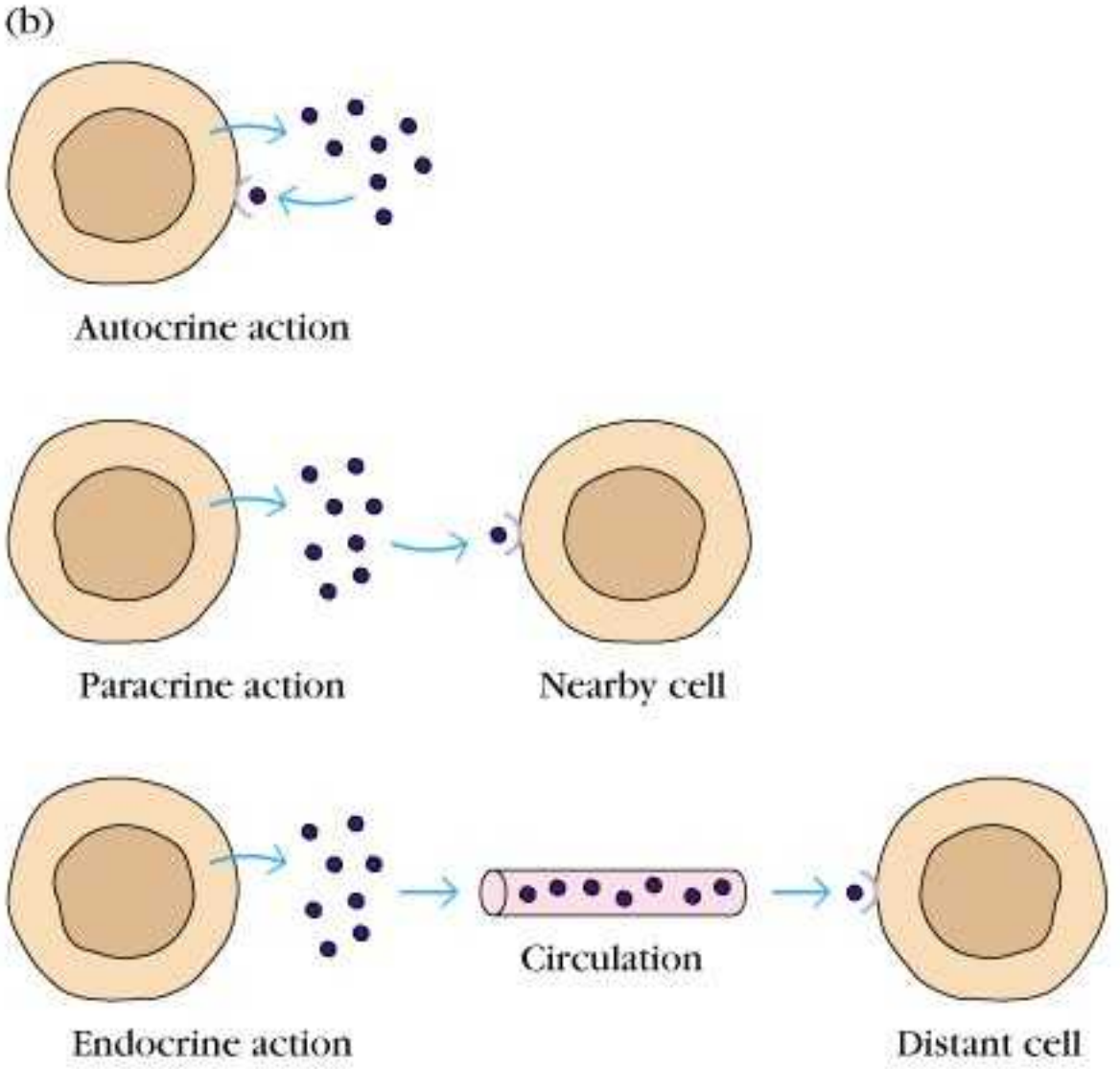
- The role of miRNAs in inflammation

Inflammation



- Cytokines and their signals that regulate and modulate the responses
- Tissue damage or pathogen signals activate inflammatory responses


(a)
Inducing stimulus
Cytokine gene
Cytokine-producing cell
Cytokine
Receptor
Signal
Gene activation
Target cell
Biological effects


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

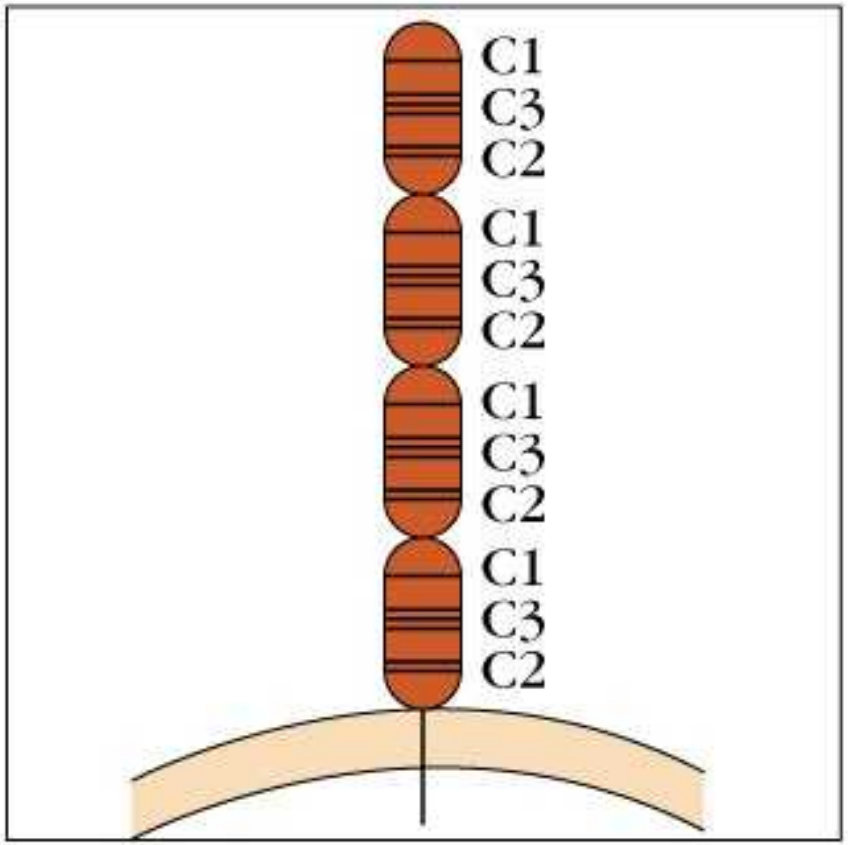
- IL-10: inhibits TNF, IL-1 and IL-6 production
- IL-1RA: antagonizes IL-1
- TGFβ: inhibits cytokine production; dual role on T cells (↓Th1, ↑Th17)
- corticosteroids: inhibit cytokine production
- type I IFNs (IFNα/β)
  - inhibit synoviocyte proliferation
  - promote cytokine and chemokine production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

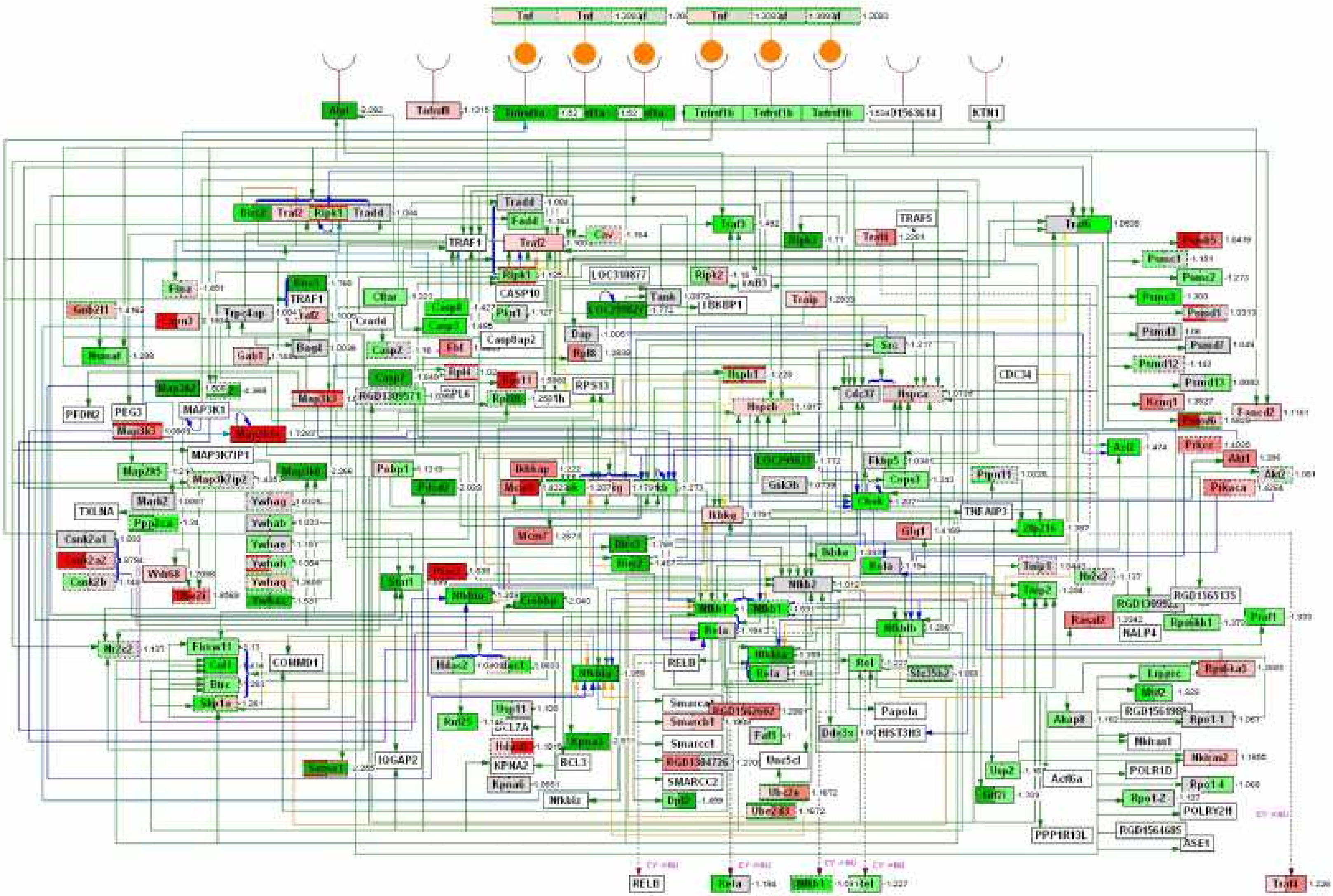
FAS

TNF receptors transduce the signals following their trimerization

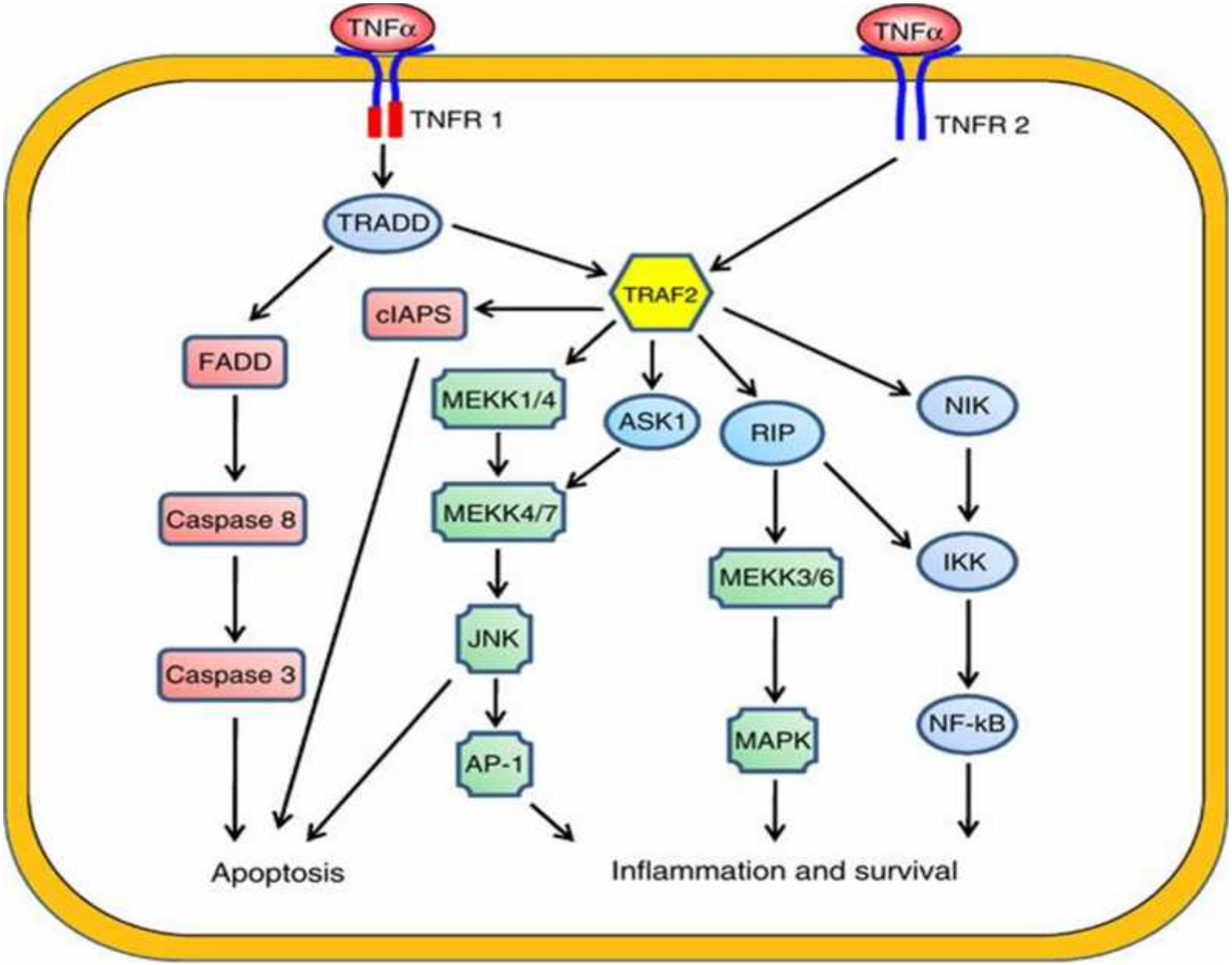
TNF receptors

- 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

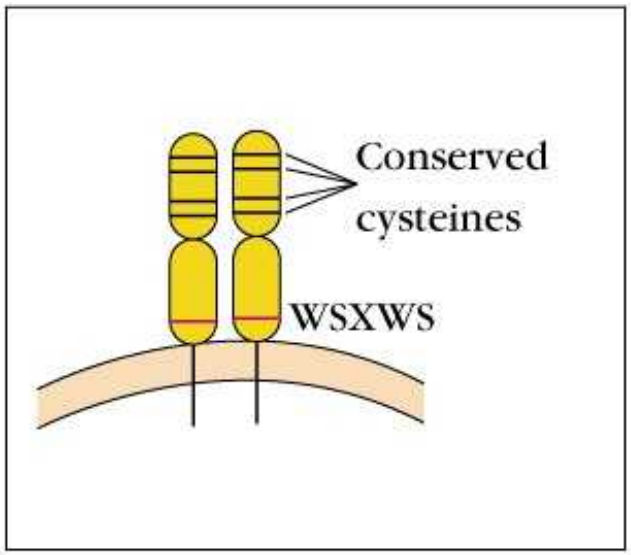


TNFR signaling

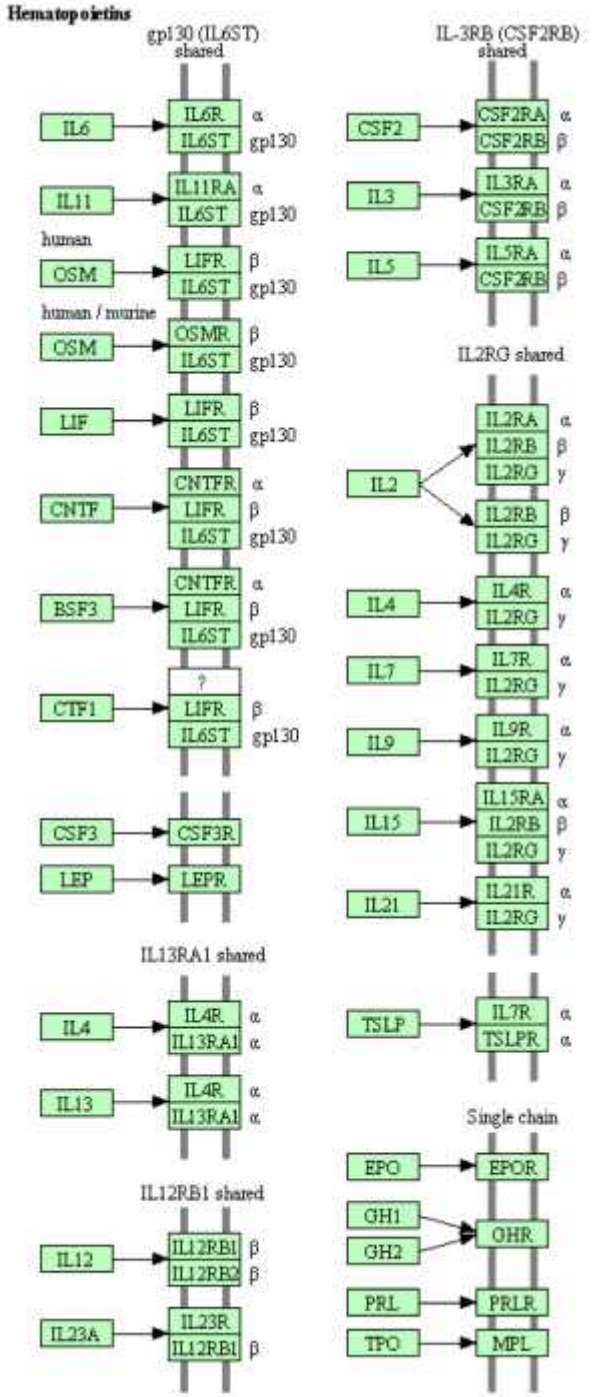


Class I and Class II cytokine receptors mediate signals via the Jak/STAT pathway

Class I cytokine receptors

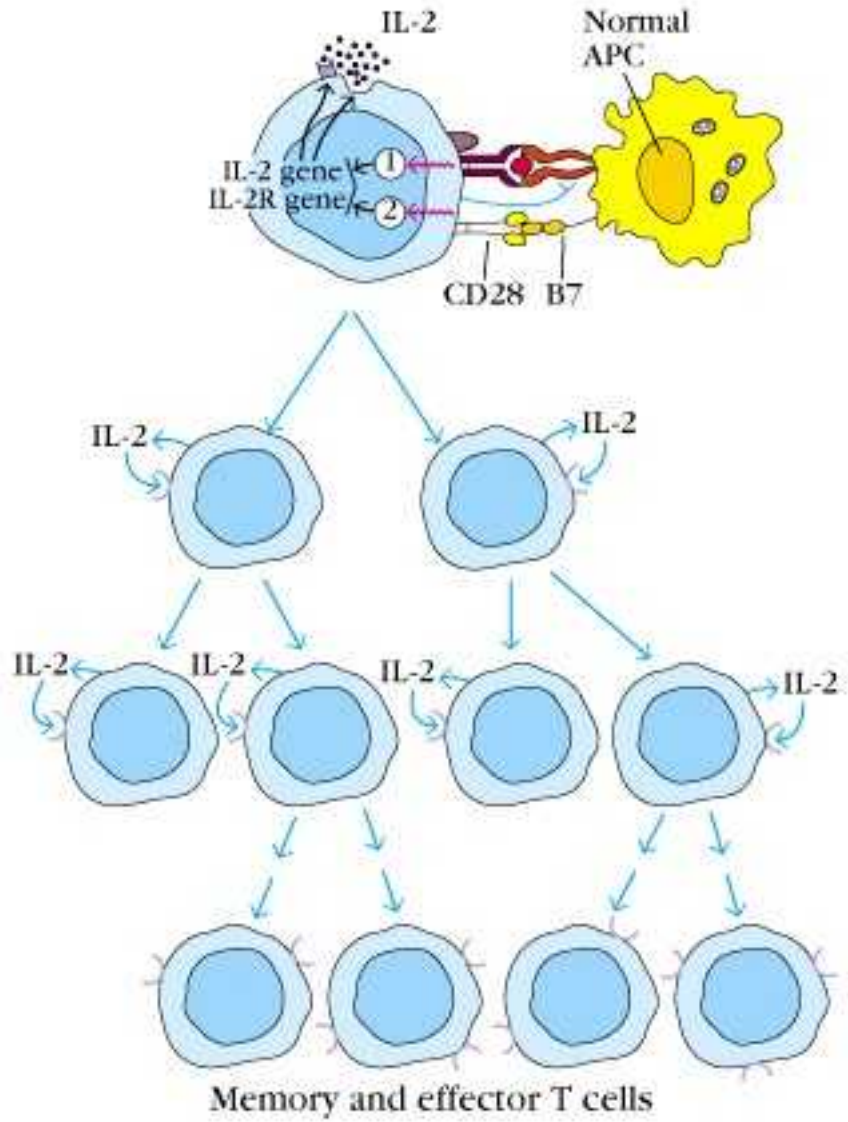


IL-2	IL-13
IL-3	IL-15
IL-4	GM-CSF
IL-5	G-CSF
IL-6	OSM
IL-7	LIF
IL-9	CNTF
IL-11	Growth hormone
IL-12	Prolactin

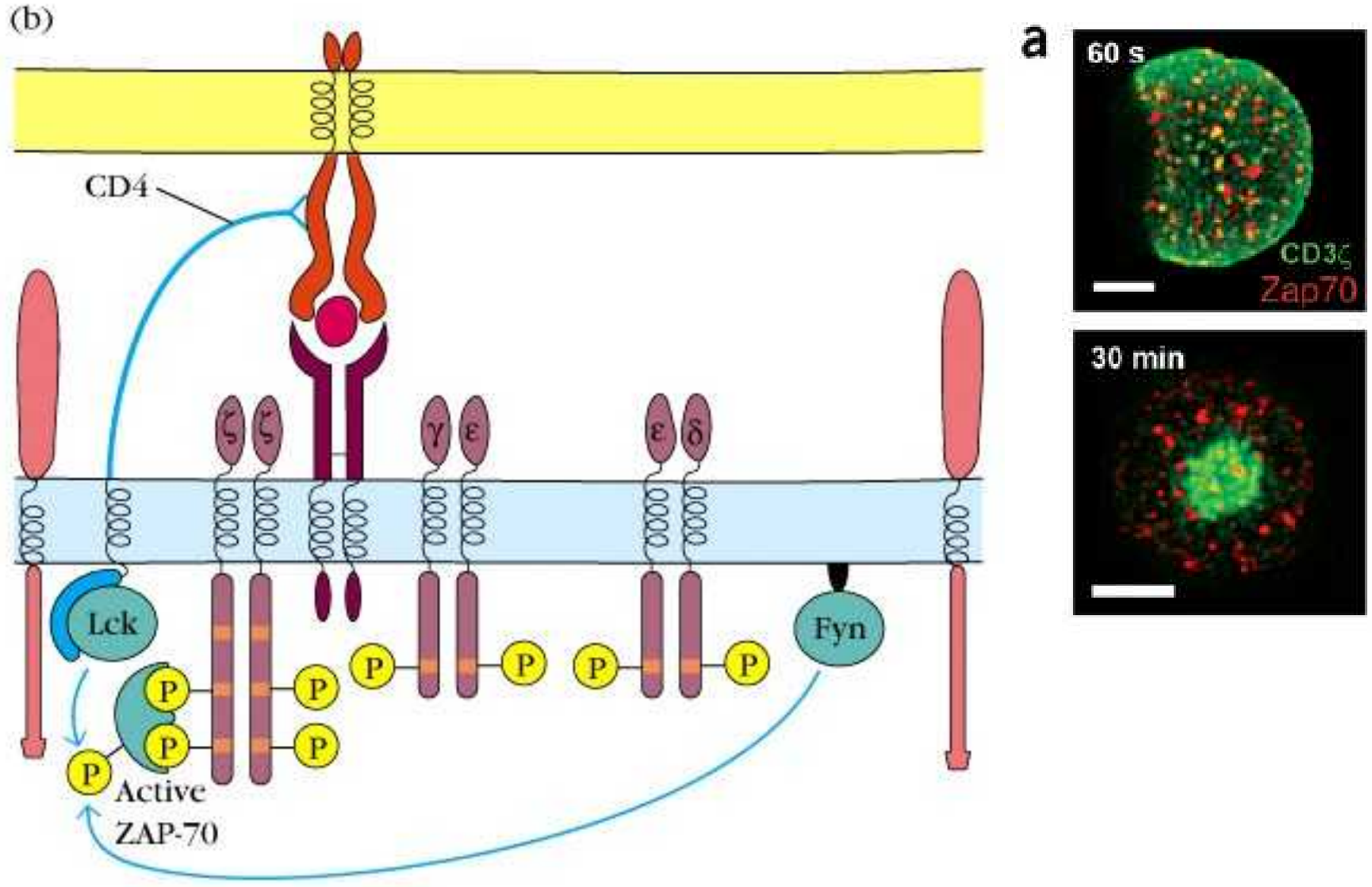


IL-2 signaling controls T-cell activation

- 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

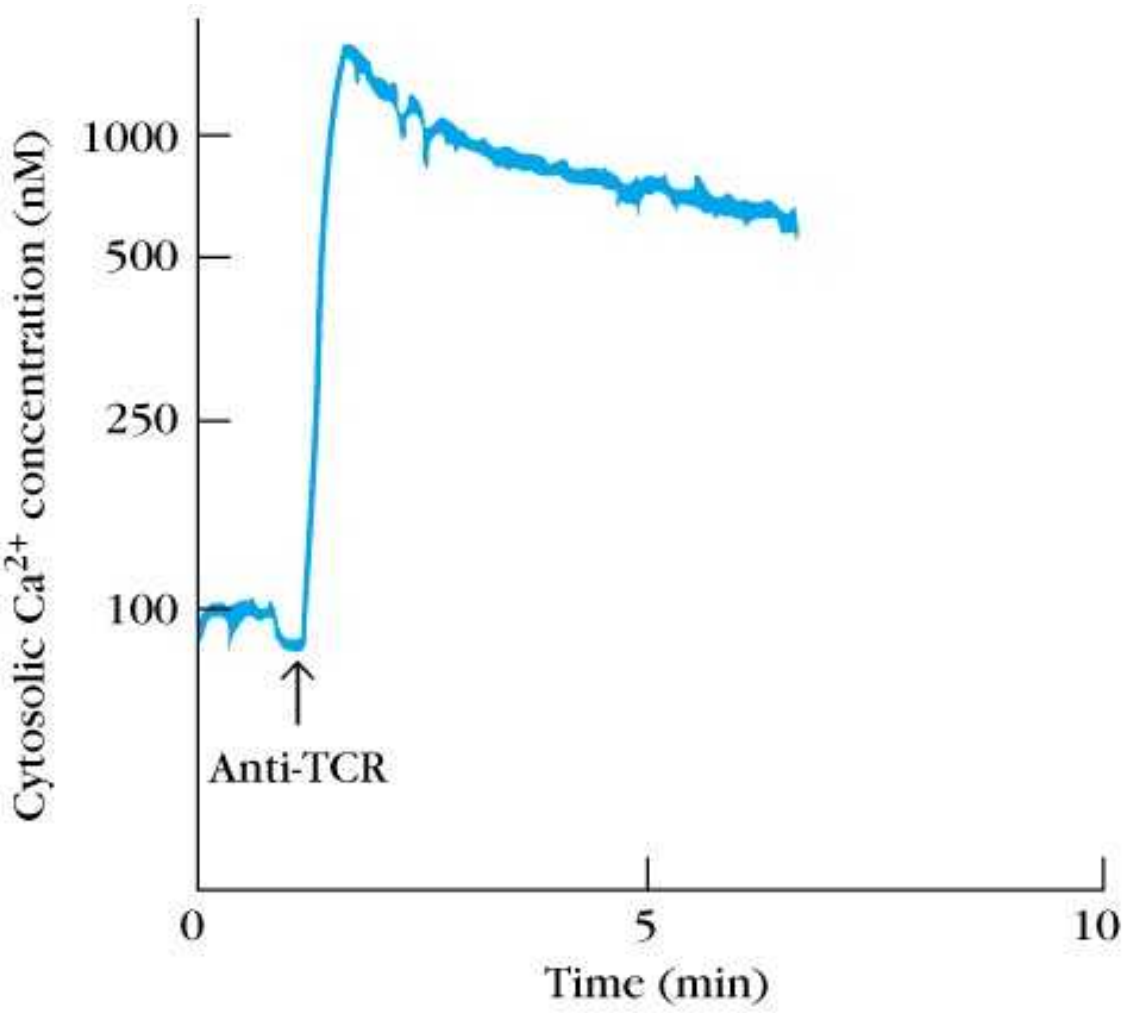

IL-2
Normal
APC
IL-2 gene
IL-2R gene
1
2
CD28
B7
IL-2
IL-2
IL-2
IL-2
IL-2
IL-2
Memory and effector T cells

TCR-mediated T-cell activation



TCR signals are mediated by phosphorylation and de-phosphorylation events

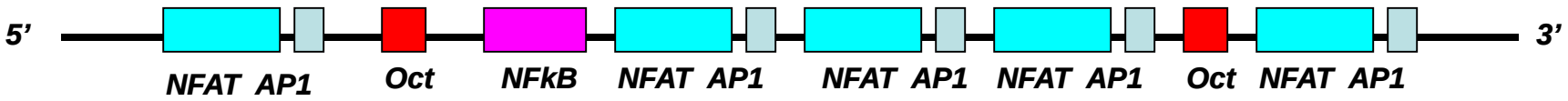
- Engagement of TCR by a peptide-presenting MHC of an antigen-presenting cell (APC) activates the tyrosine kinase Lck, which in turn phosphorylates ZAP70 and the intracellular ITAM motifs of TCR.



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

Induction of IL-2 expression

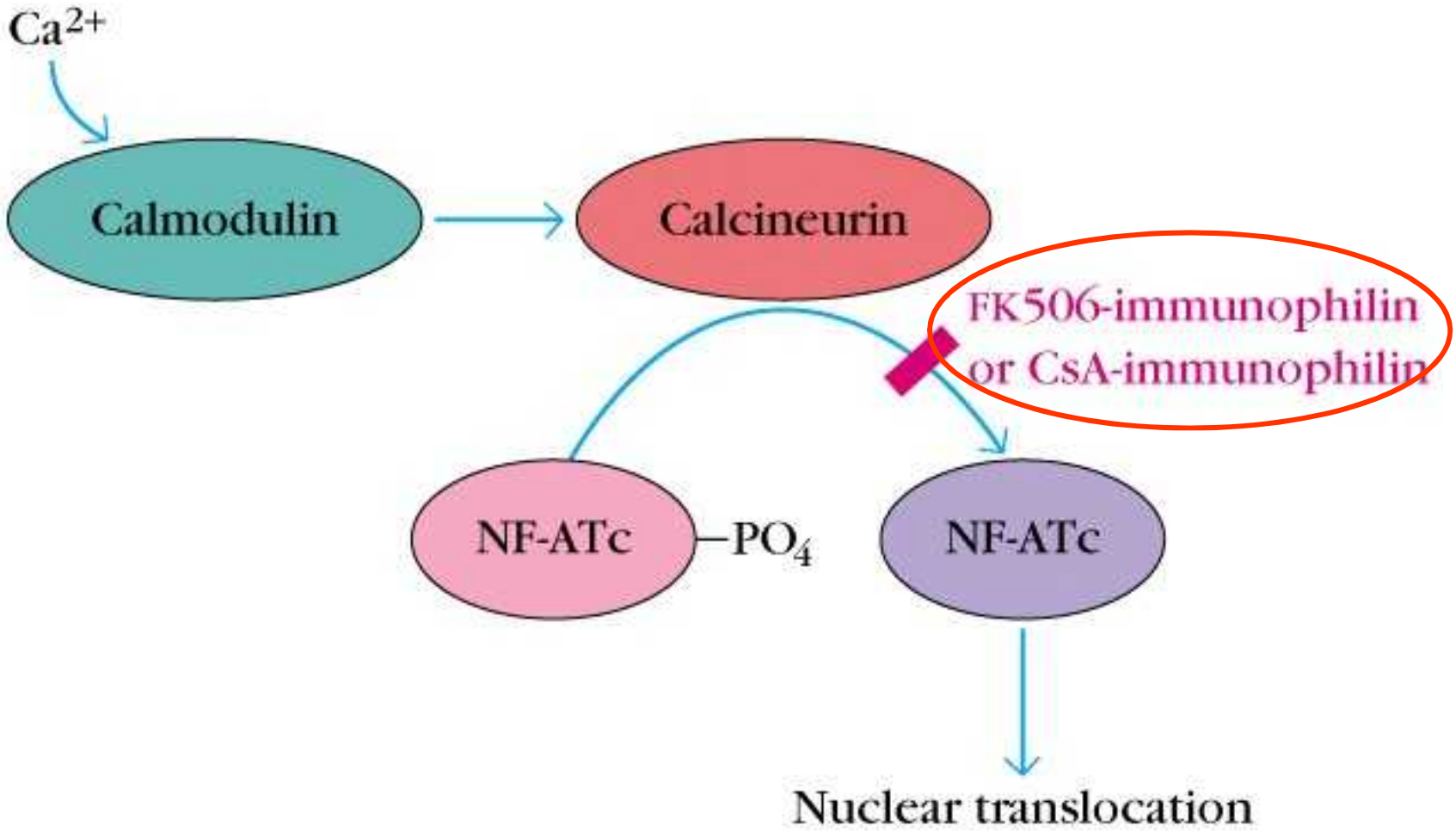
- TCR signals induce activation of key transcription factors that bind to the promoter of IL-2 gene
- These include: NFkB, NFAT, AP1, Oct1 etc



IL-2 promoter

Ca++ signaling in TCR activation

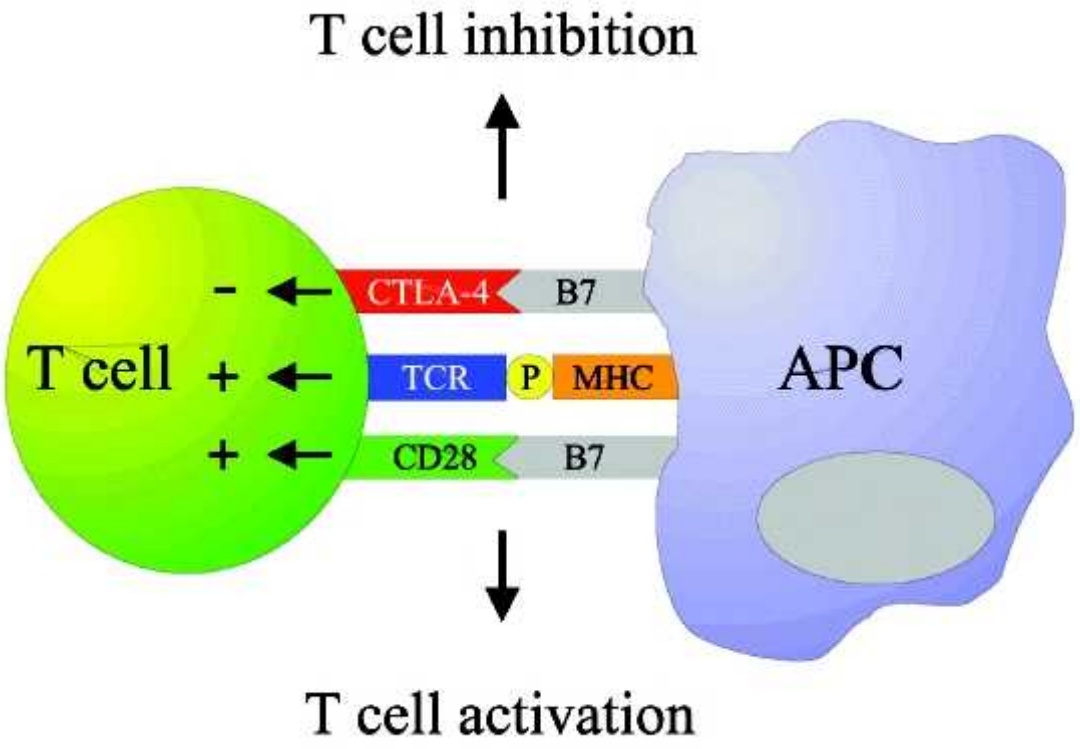
- A few seconds following TCR engagement Ca++ influxes and is also released from intracellular stores.
- Ca++ is an important signaling molecule activating calmodulin and the serine phophatase calcineurin, which in turn, dephosphorylates and activates the transcription factor NFAT.
- Dephosphorylated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



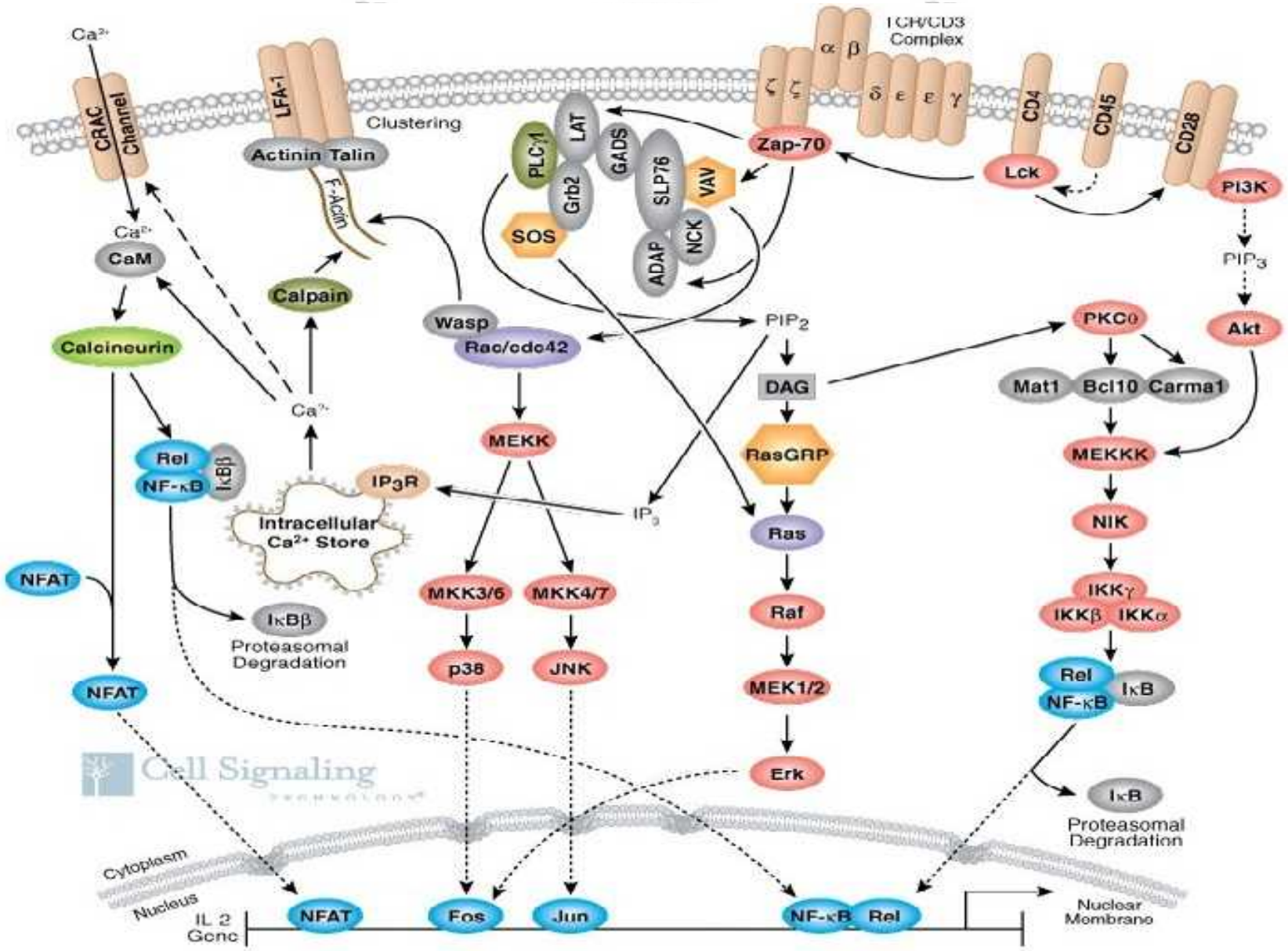
Therapeutic interventions

- ***Cyclosporin and FK506 target NFAT activation***

Lymphocytes require two signals to get activated



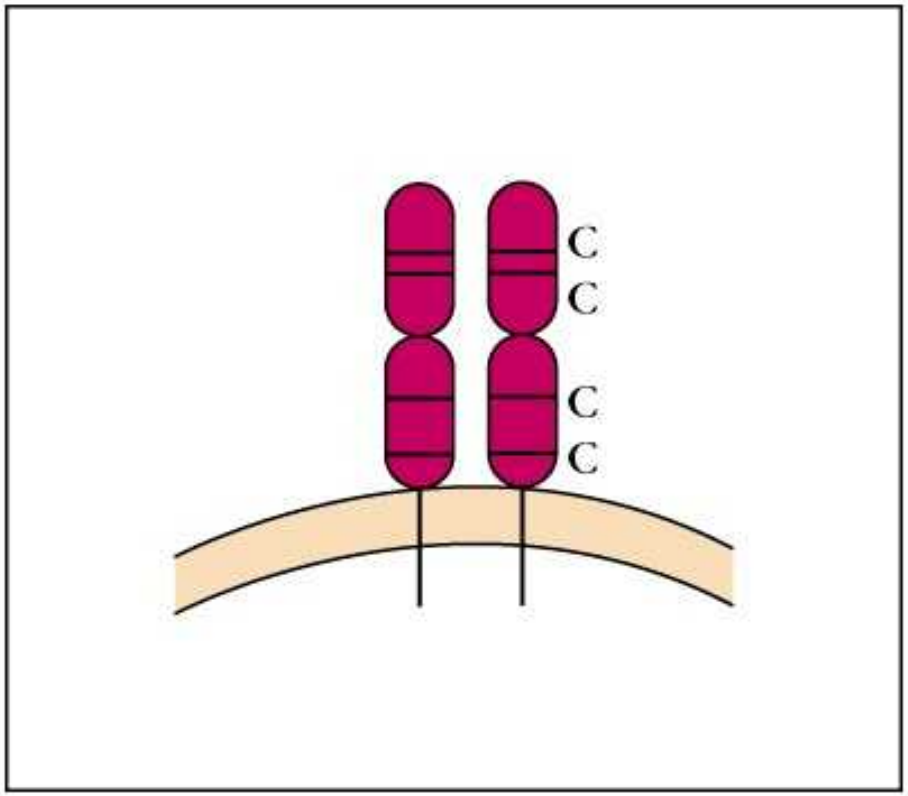
Co-stimulation results in additive activation of downstream molecules: Positive co-stimulatory



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 to boost the effect- they also utilize the PI3K-Akt pathway

Class II (Interferon family) receptors

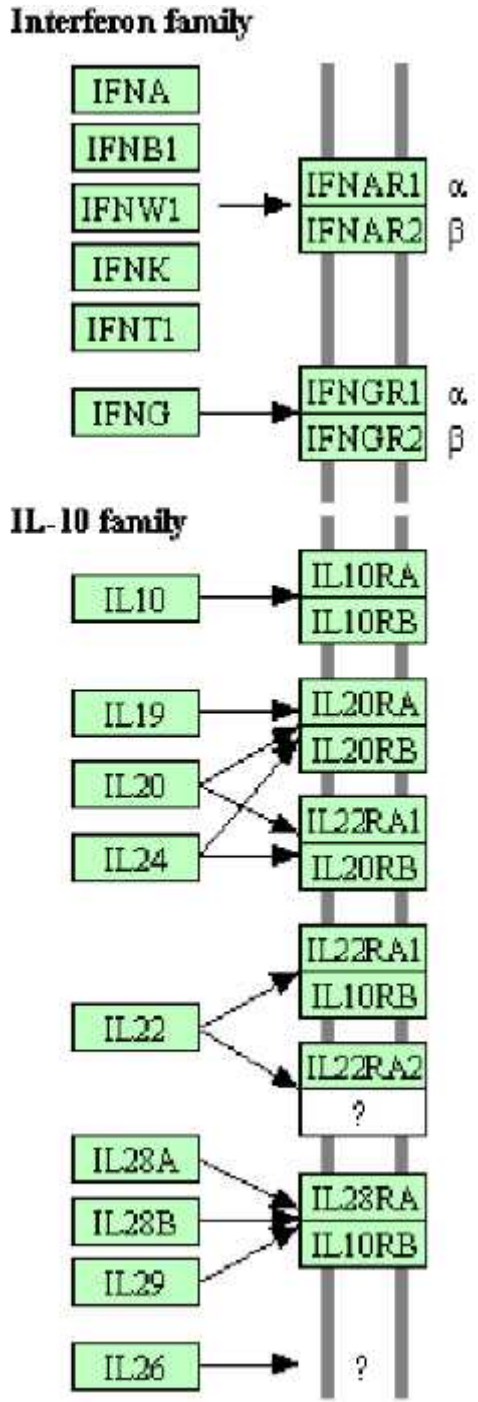


IFN-α

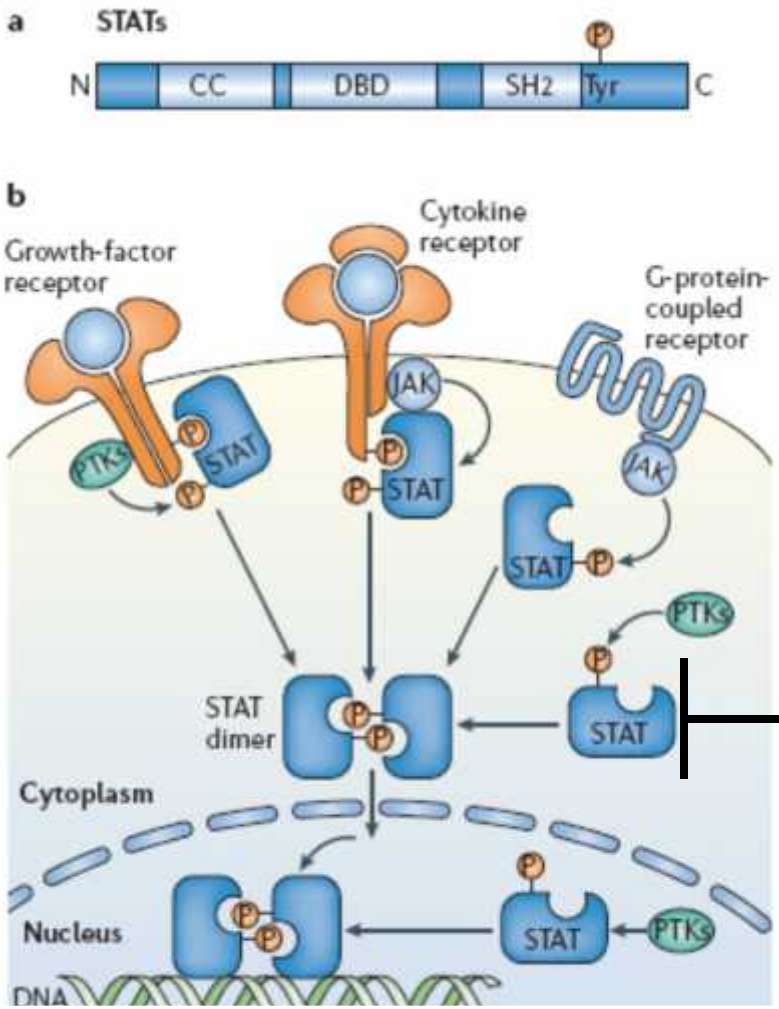
IFN-β

IFN-γ

IL-10



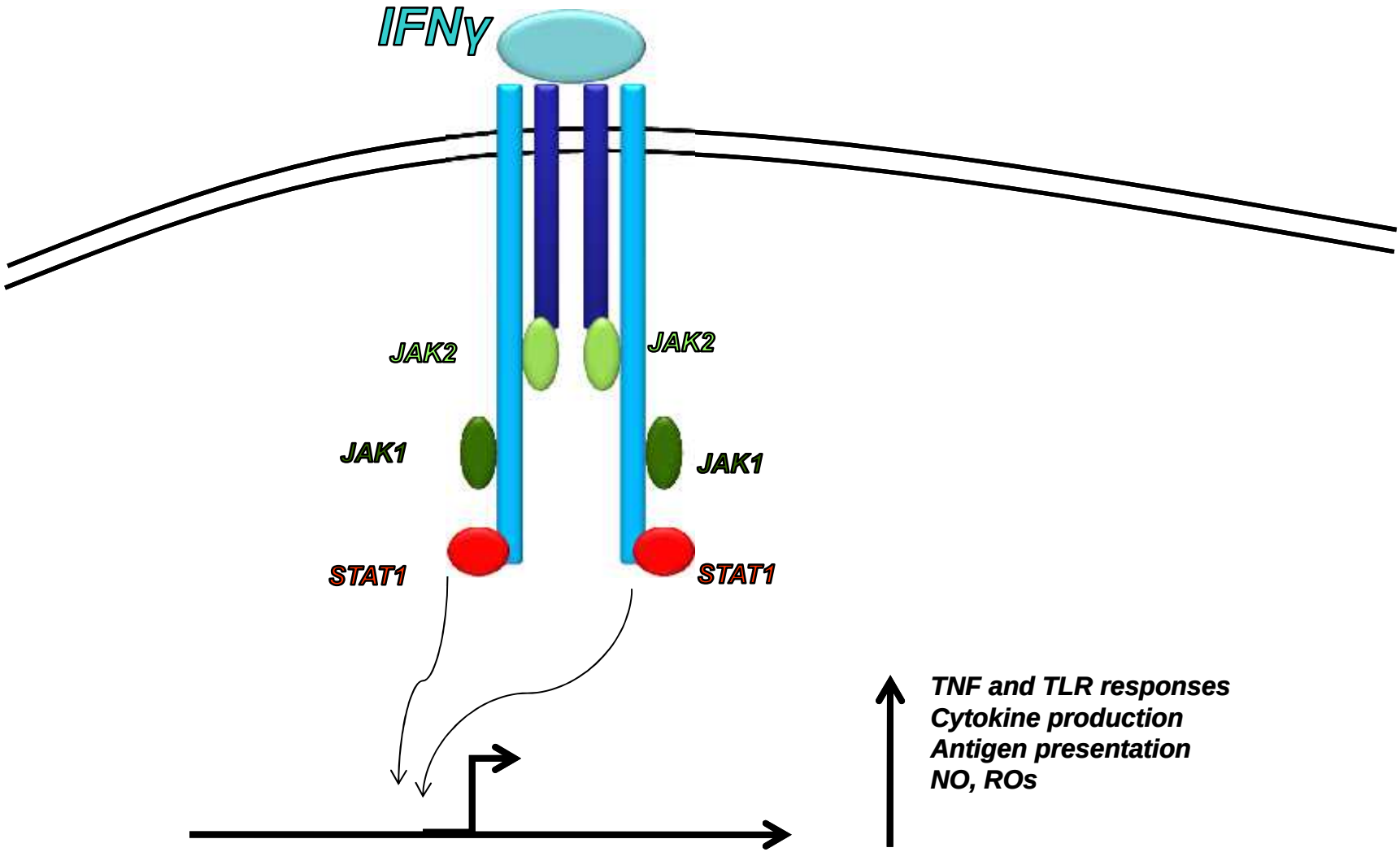
STATs are activated via tyrosine phosphorylation



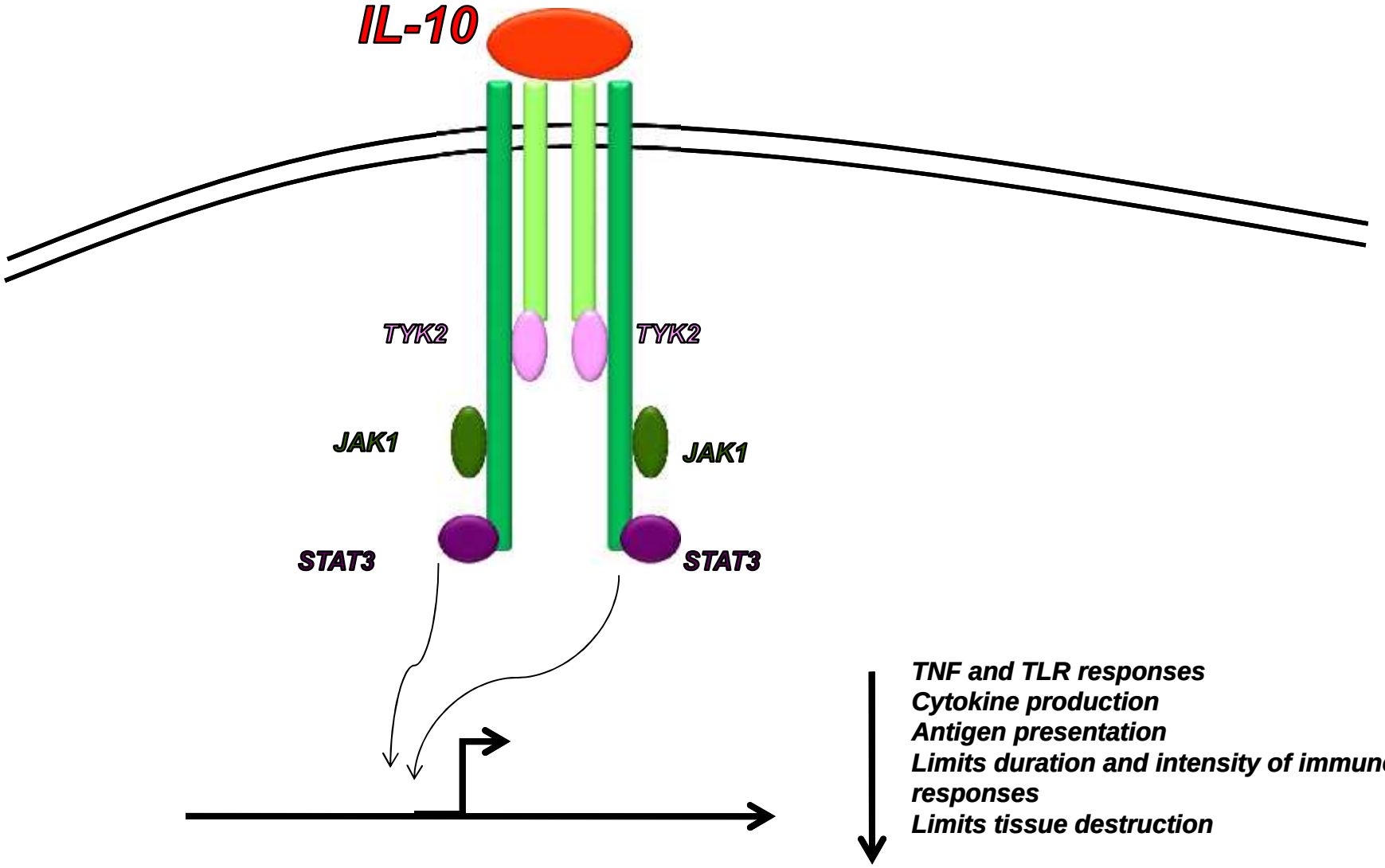
SHP phosphatases

Adapted from Nat. Rev. Immunol 2008

STAT1 mediates the pro-inflammatory effect of IFNγ

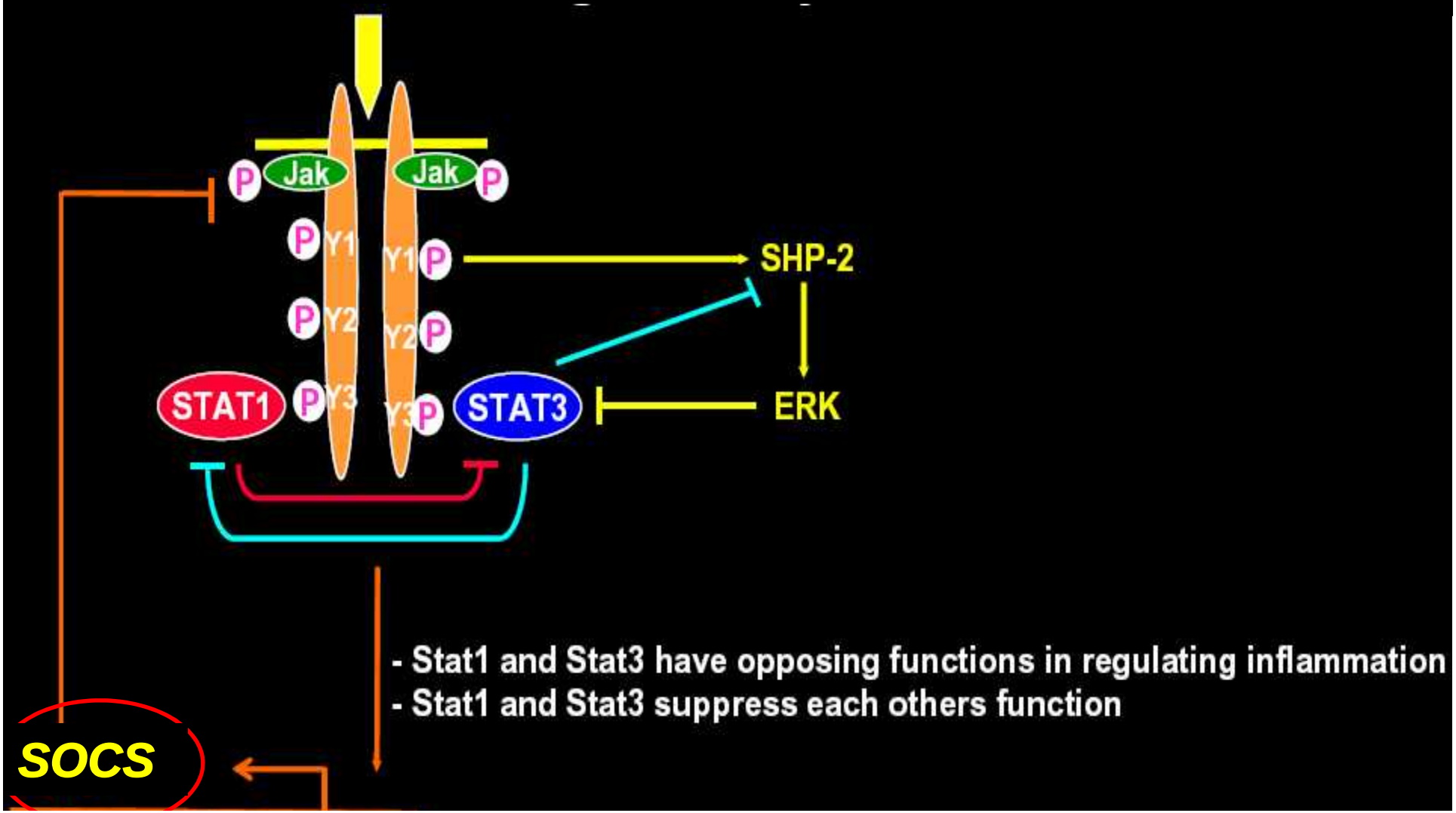


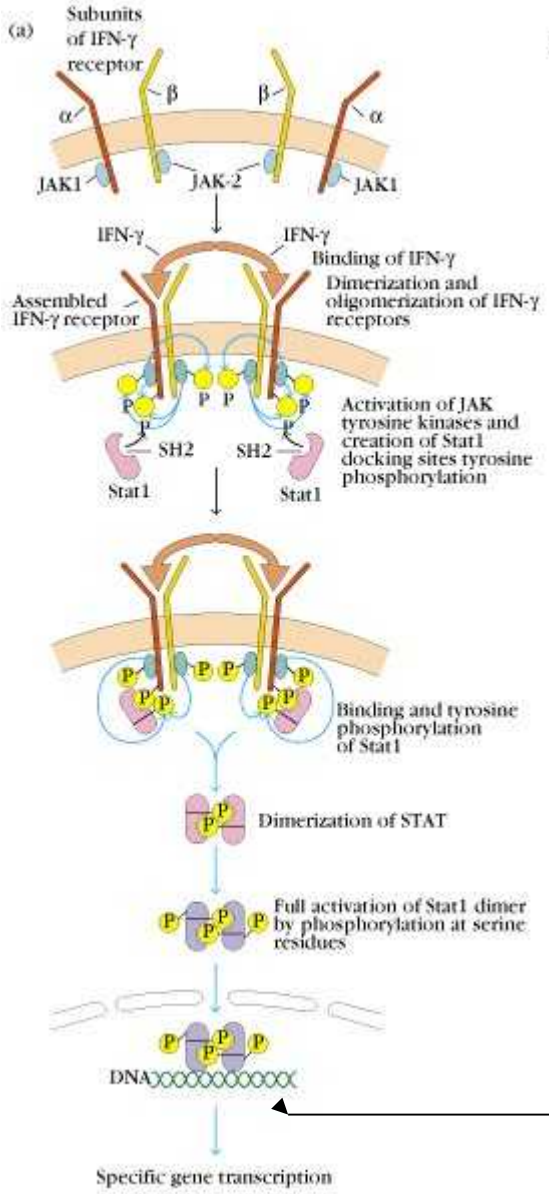
STAT3 mediates the anti-inflammatory effects of IL-10

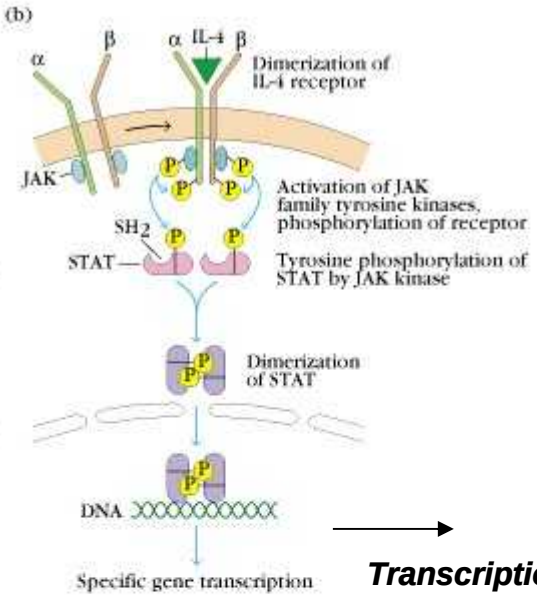


TNF and TLR responses
Cytokine production
Antigen presentation
Limits duration and intensity of immune responses
Limits tissue destruction

Intracellular signaling molecules regulate cytokine signaling







Transcription of GATA3 (specific for Th2 response)

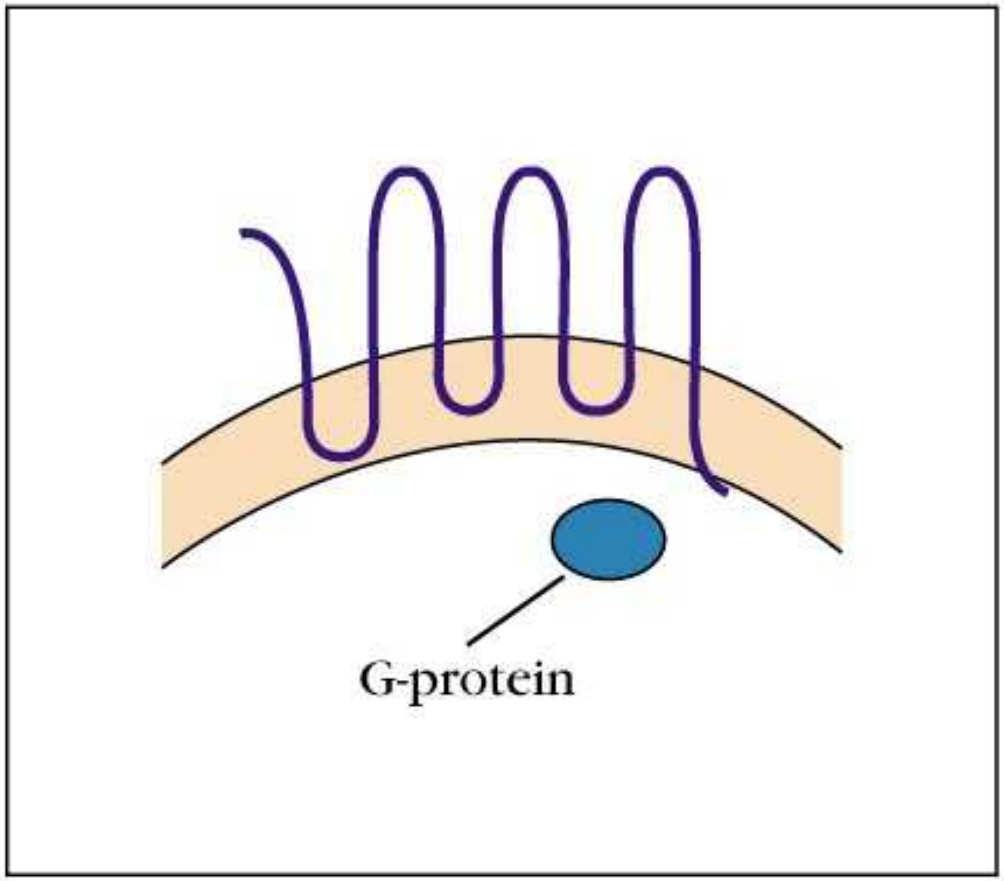
Transcription of T-bet (specific for Th1 response)

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

Chemokine signaling

(e) Chemokine receptors



IL-8

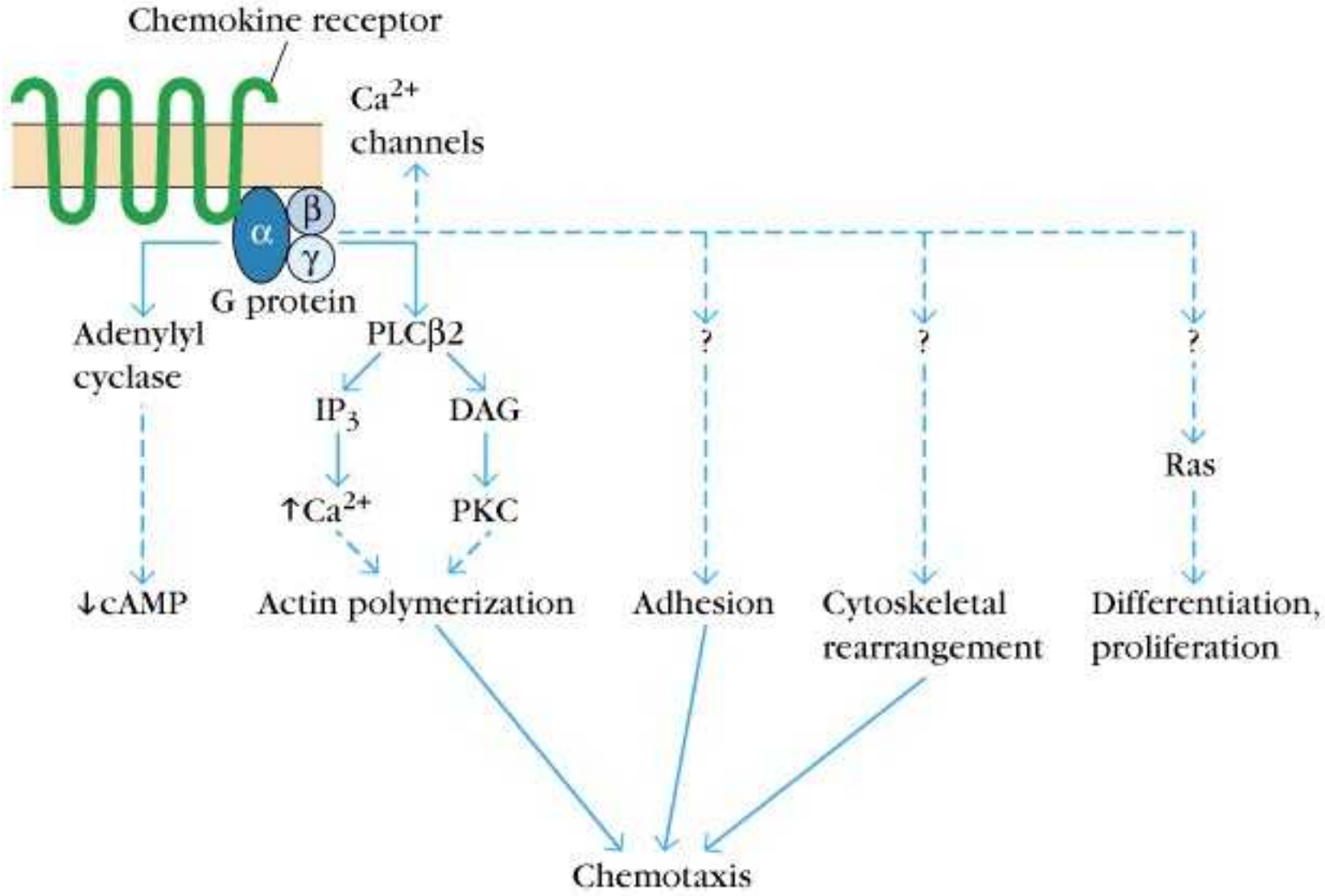
RANTES

MIP-1

PF4

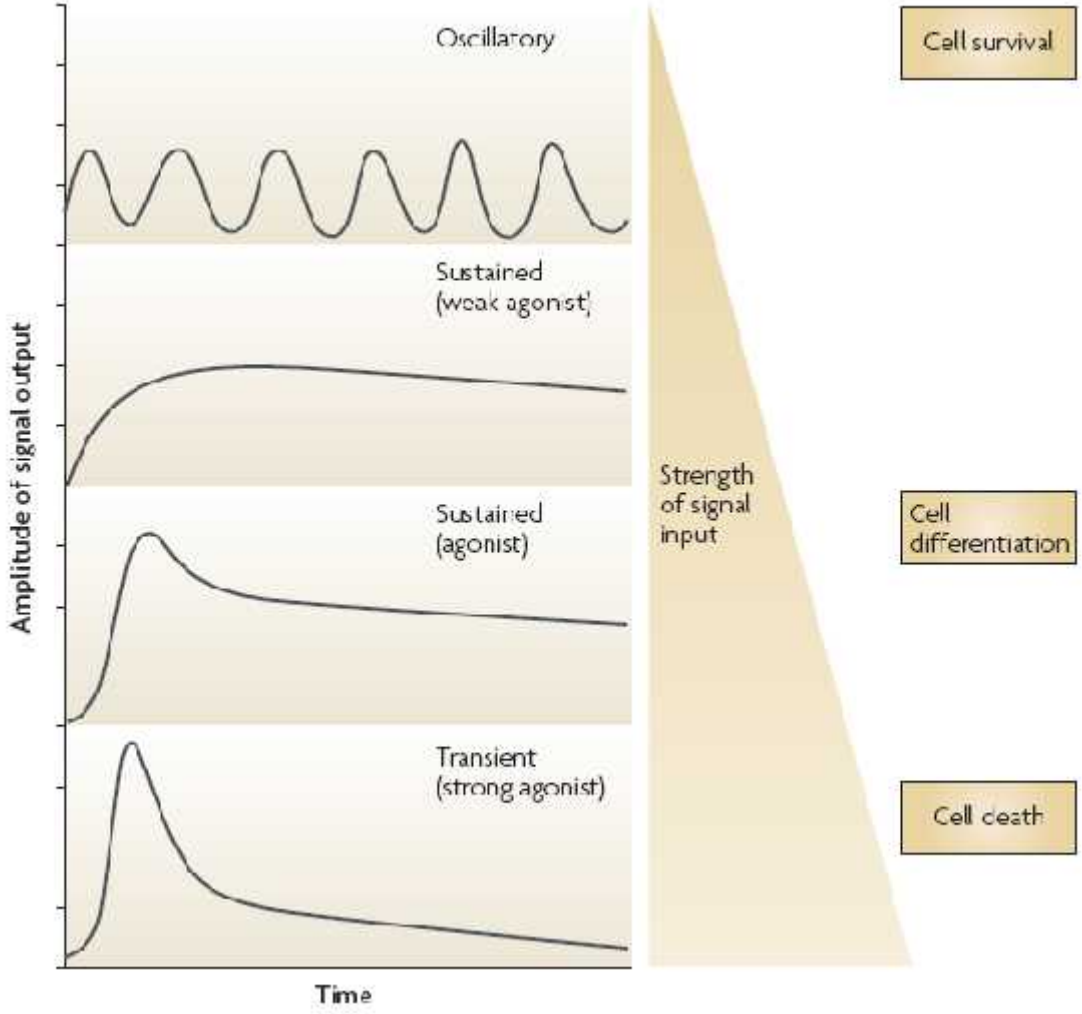
MCAF

NAP-2

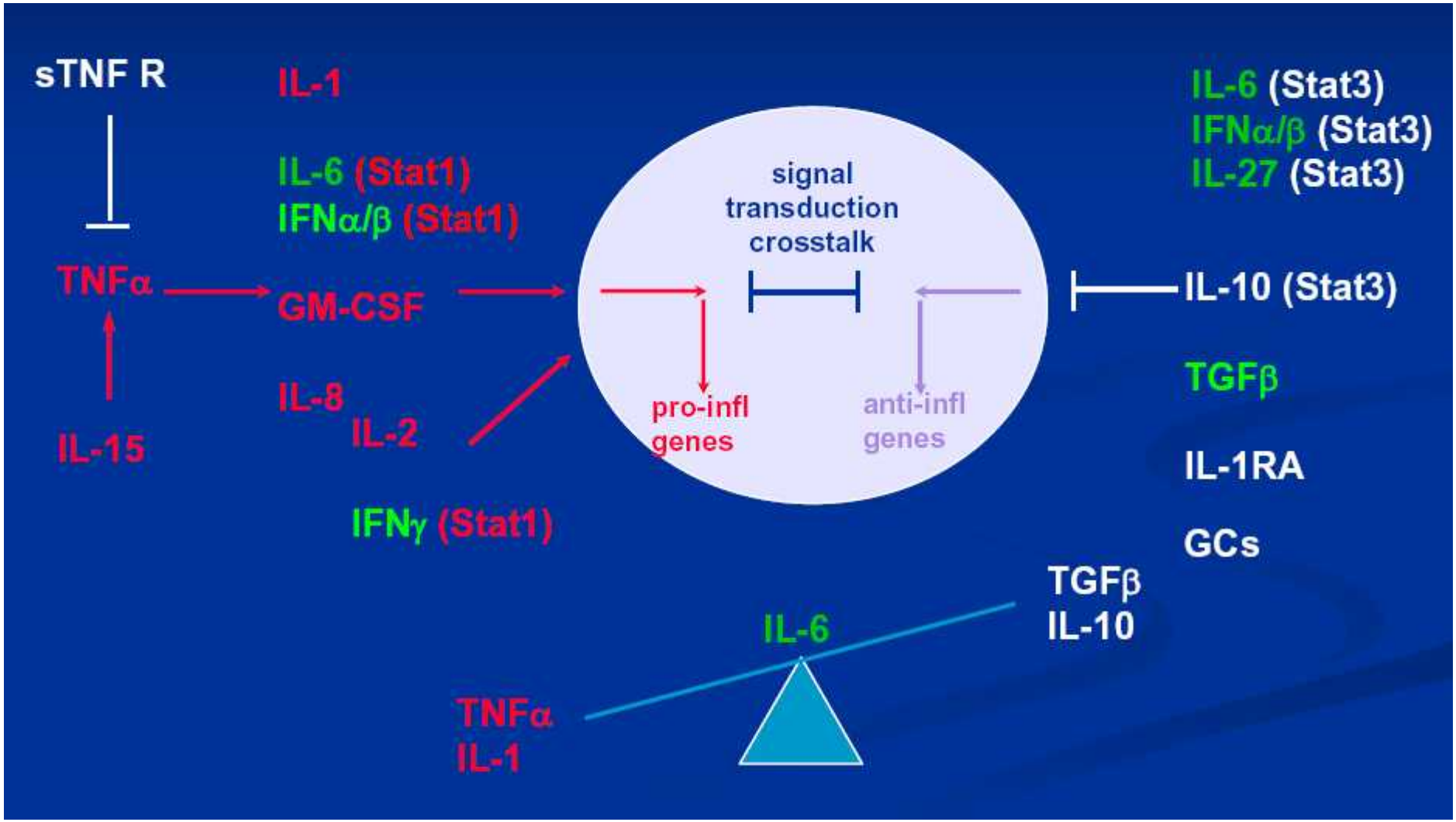

Chemokine receptor
Ca2+
channels
α
β
γ
G protein
Adenylyl
cyclase
PLCβ2
IP3
DAG
↑Ca2+
PKC
?
?
?
Ras
↓cAMP
Actin polymerization
Adhesion
Cytoskeletal
rearrangement
Differentiation,
proliferation
Chemotaxis

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

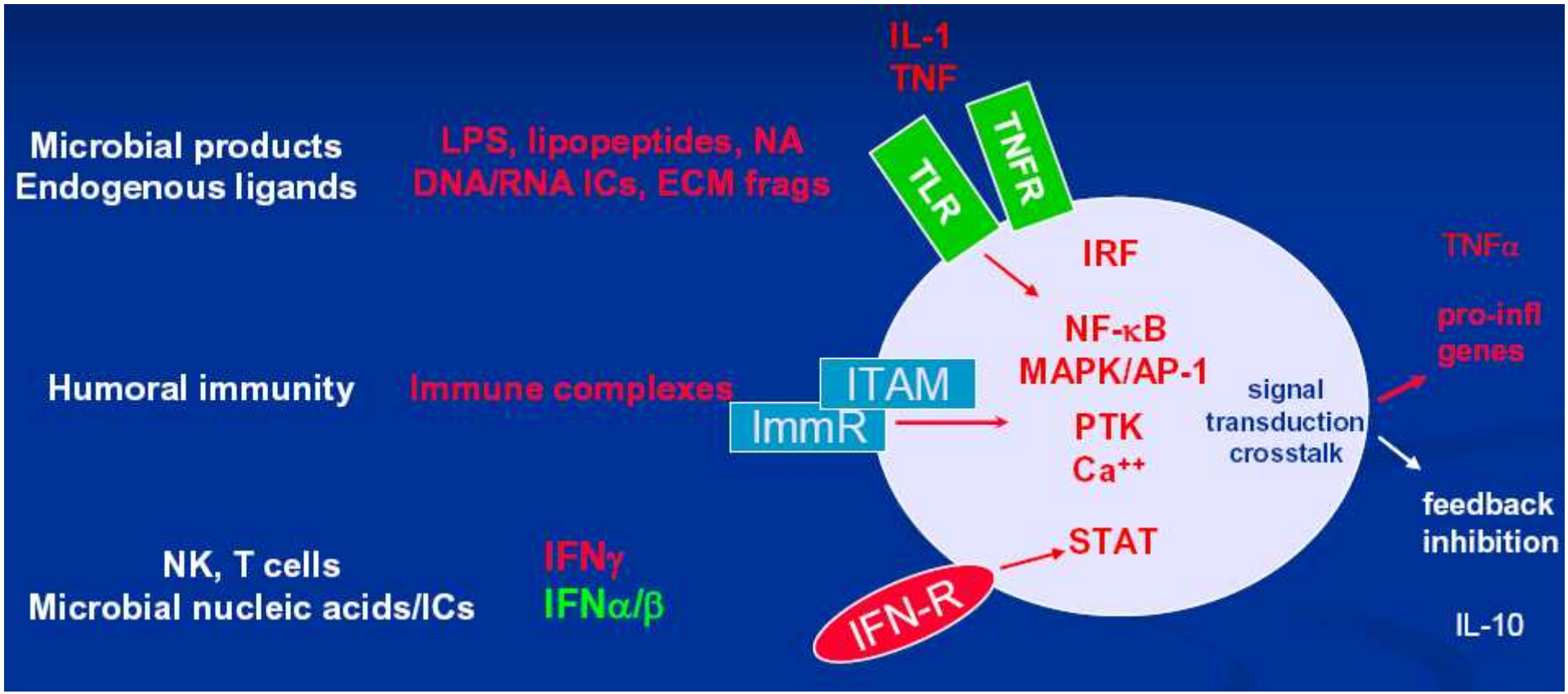
Dependence of signal amplitude and kinetics on stimuli strength and consequences on biological outcomes



The balance between cytokine action determines the severity of inflammation

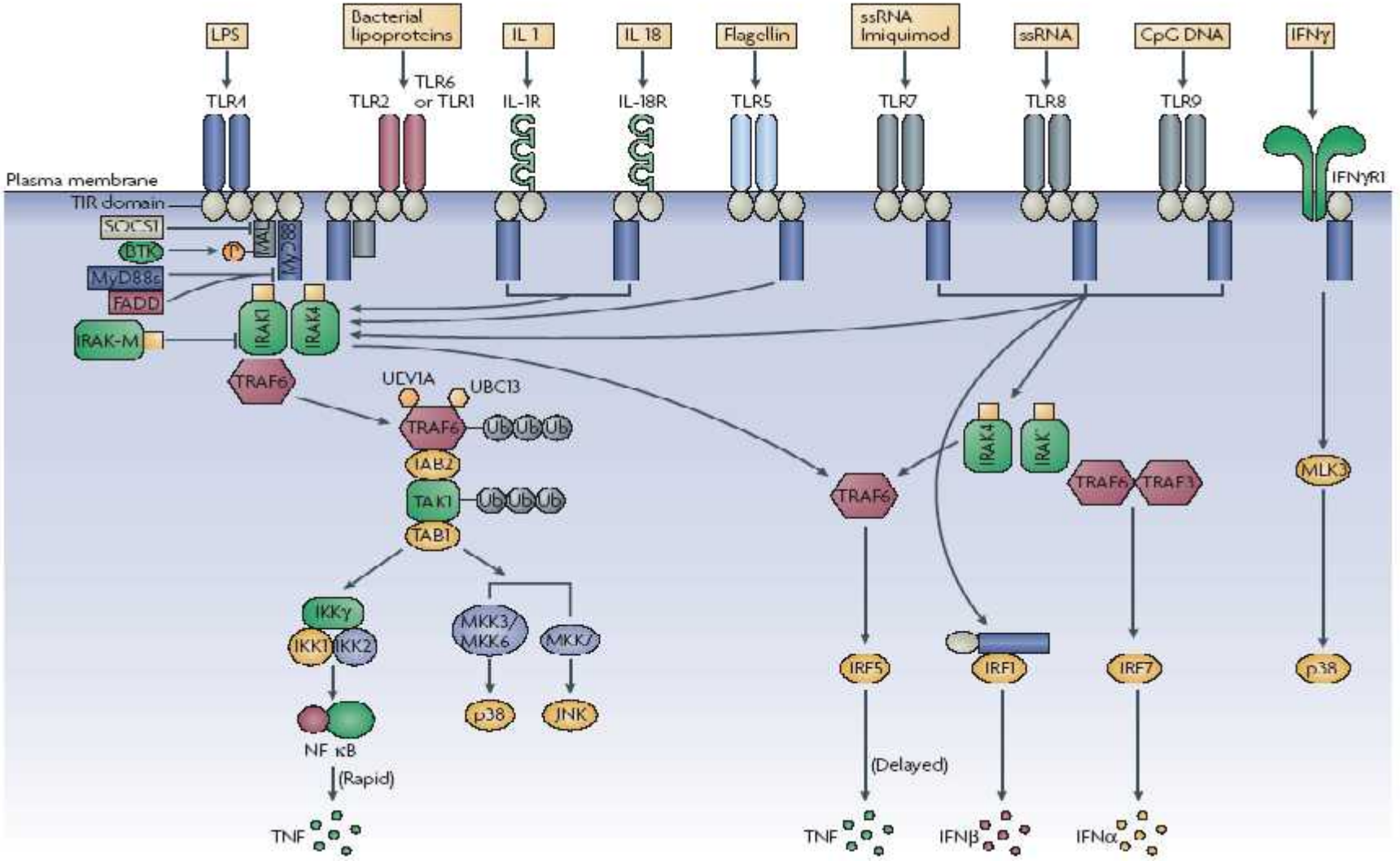


Signal integration determines the outcome in the complex inflammatory environment

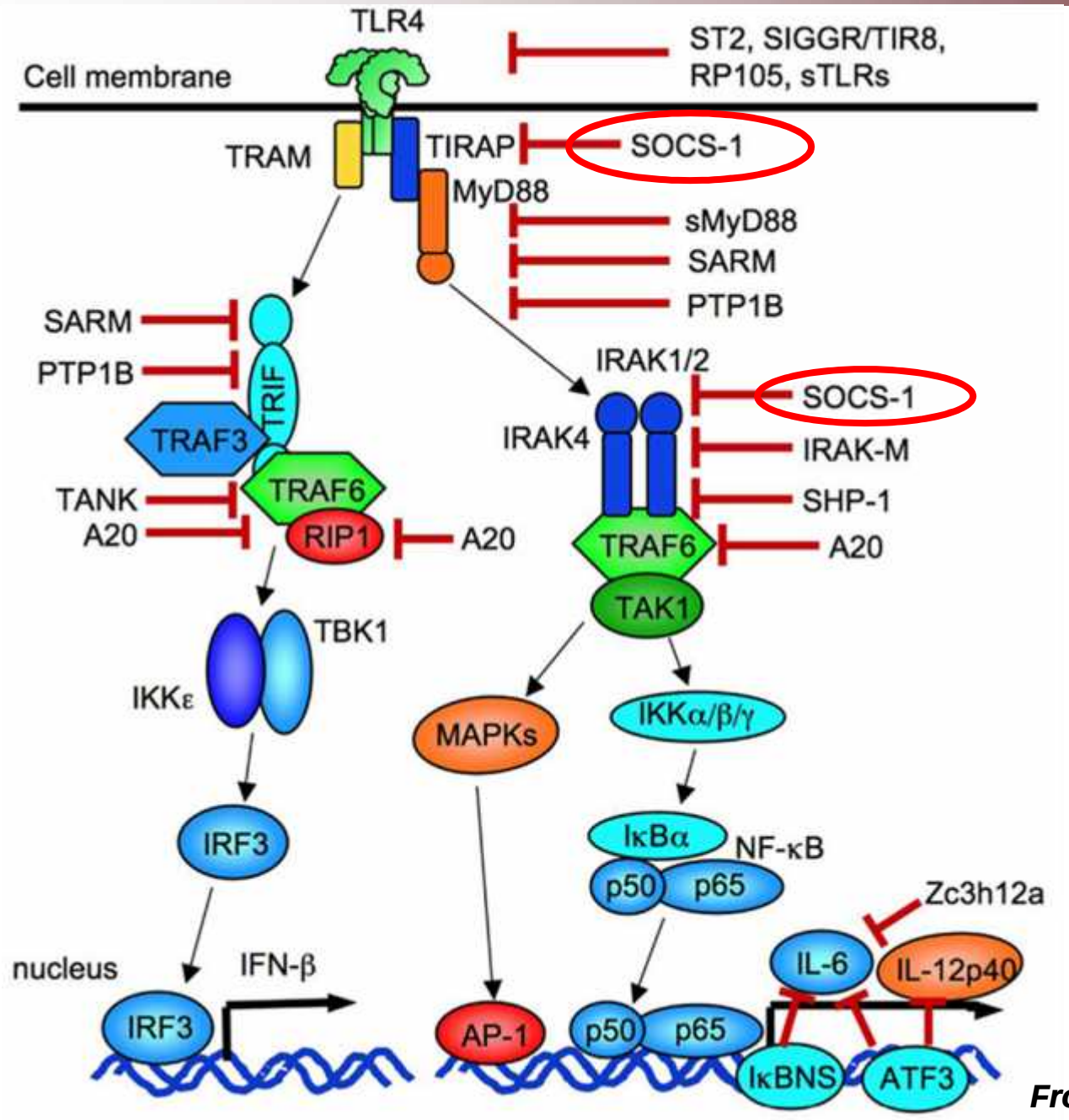


Pathogen-initiated signals

TLR signaling cascades

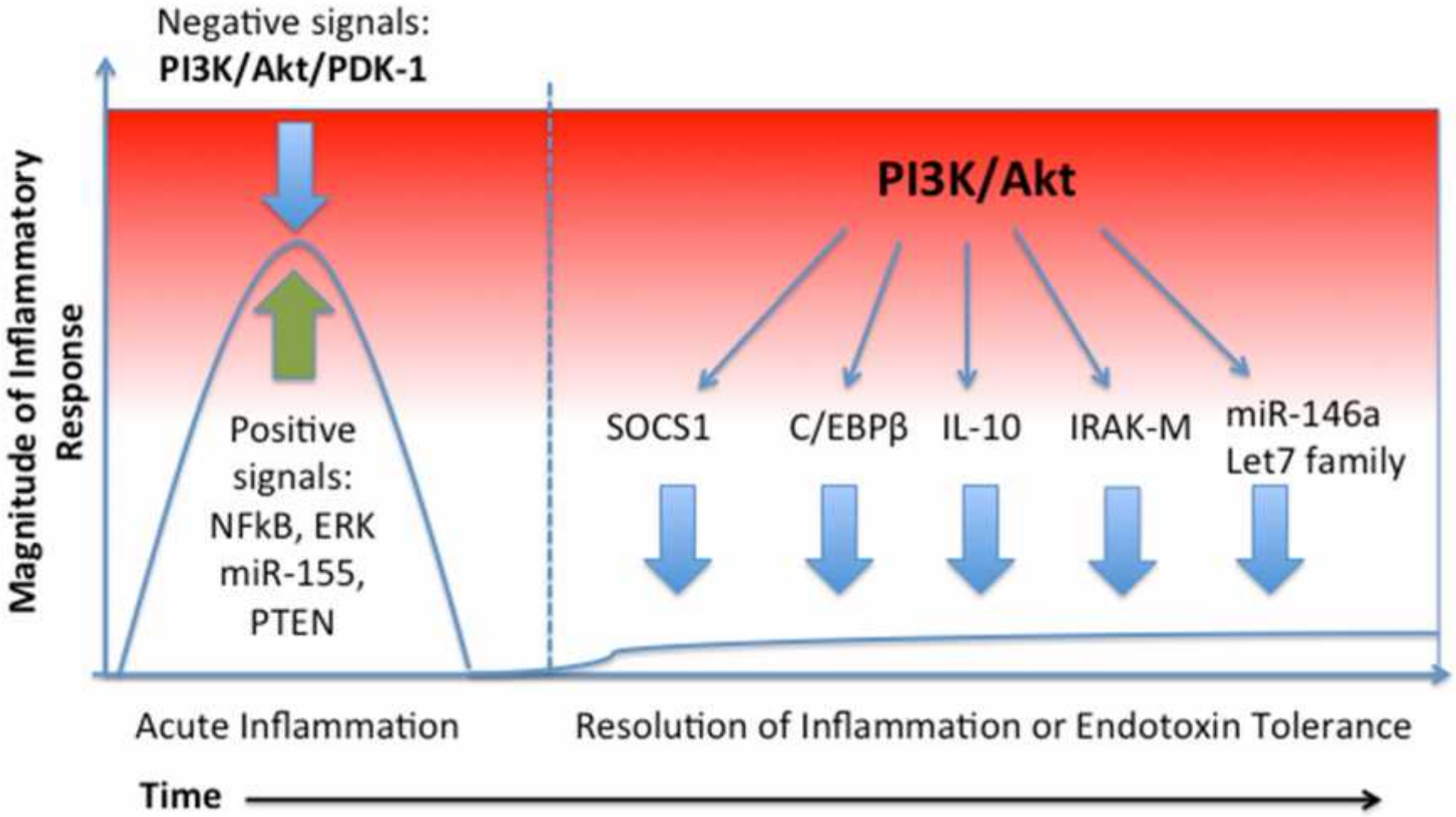


Nat. Rev. Immunol 2007 7:353



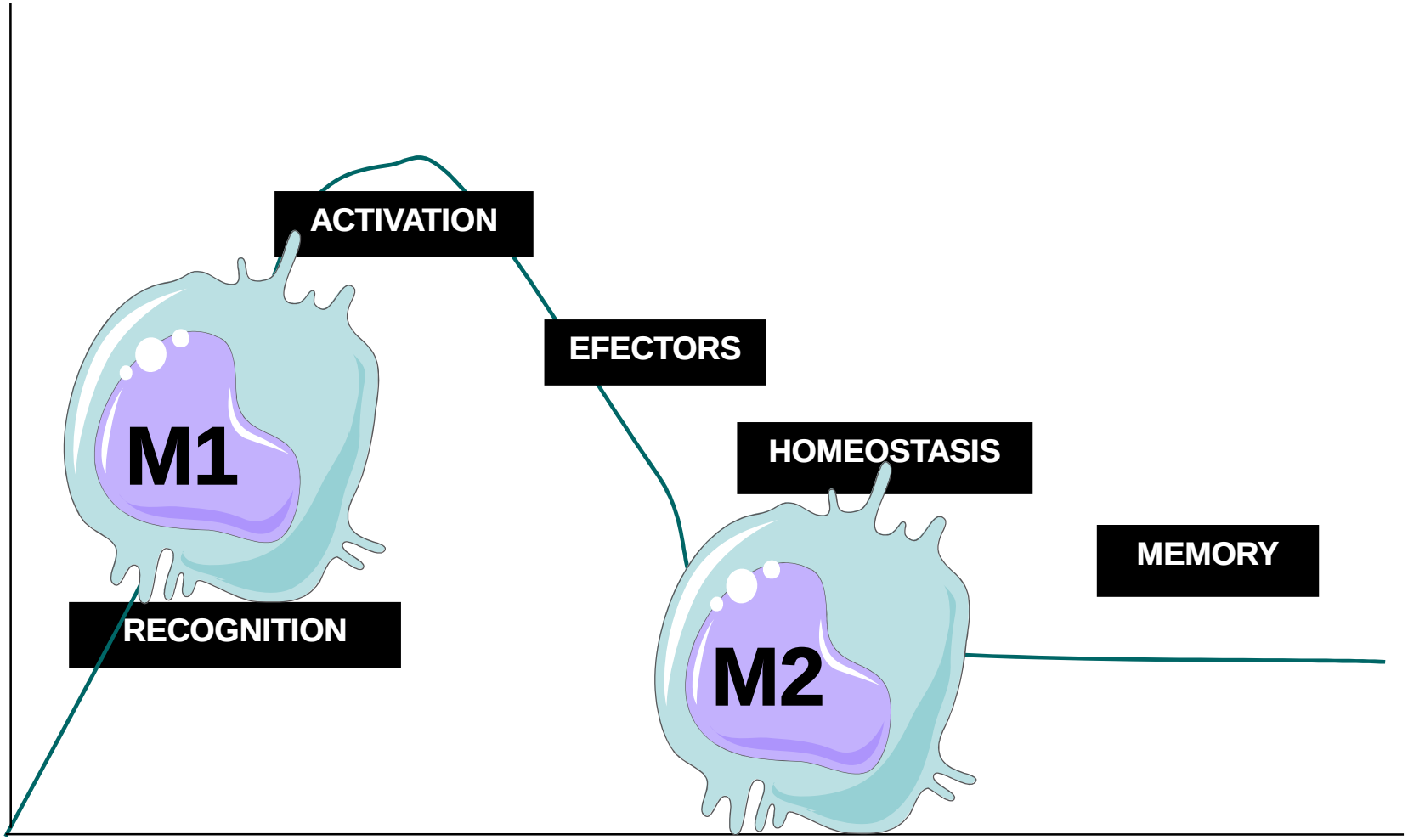
Front. Physiol., 22 May 2012

Control of TLR responses to pathogens: Positive and negative regulators

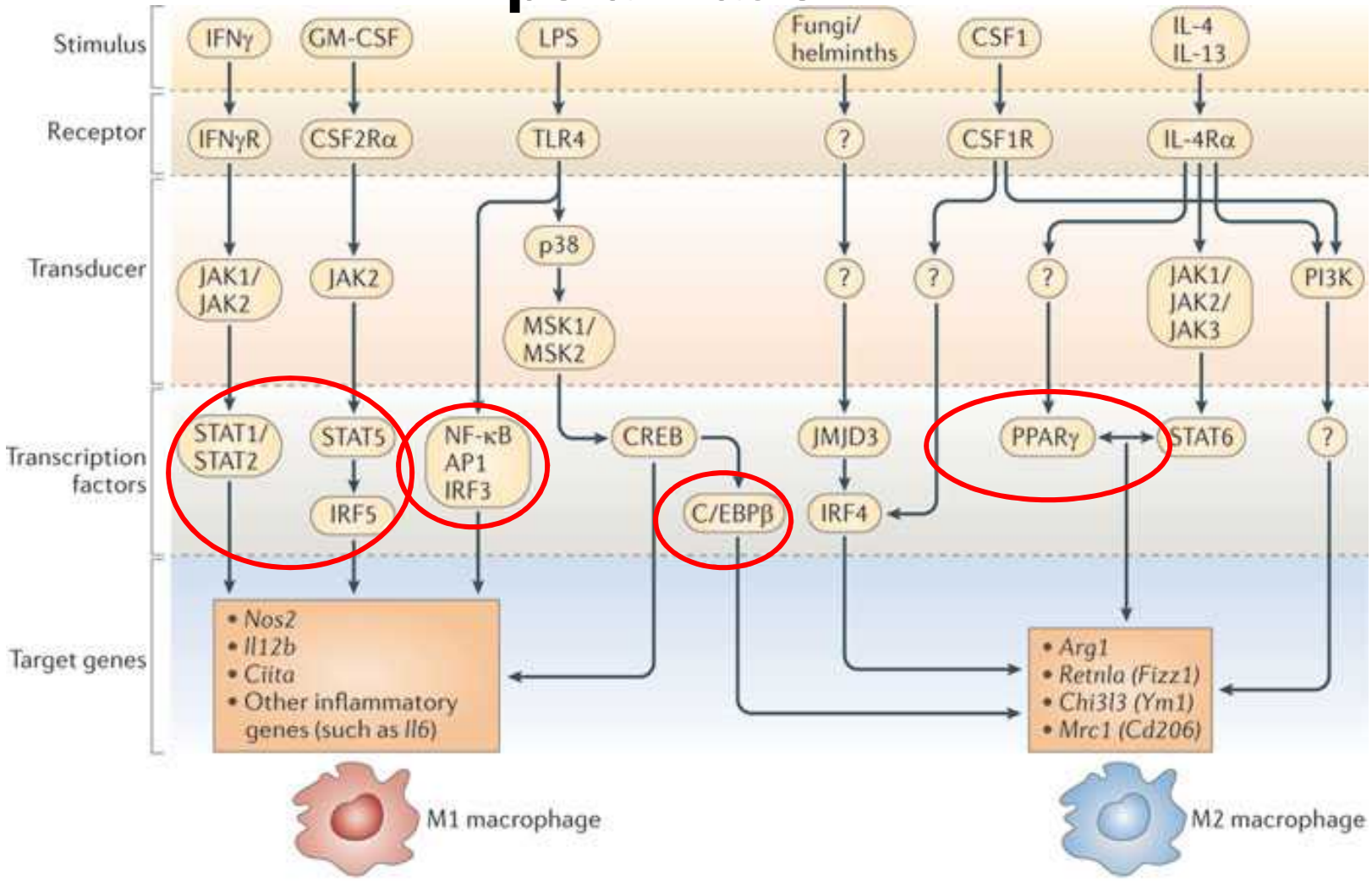


J Immunol. 2017 Feb 1;198(3):1006-1014

PHASES OF AN INNATE IMMUNE RESPONSE

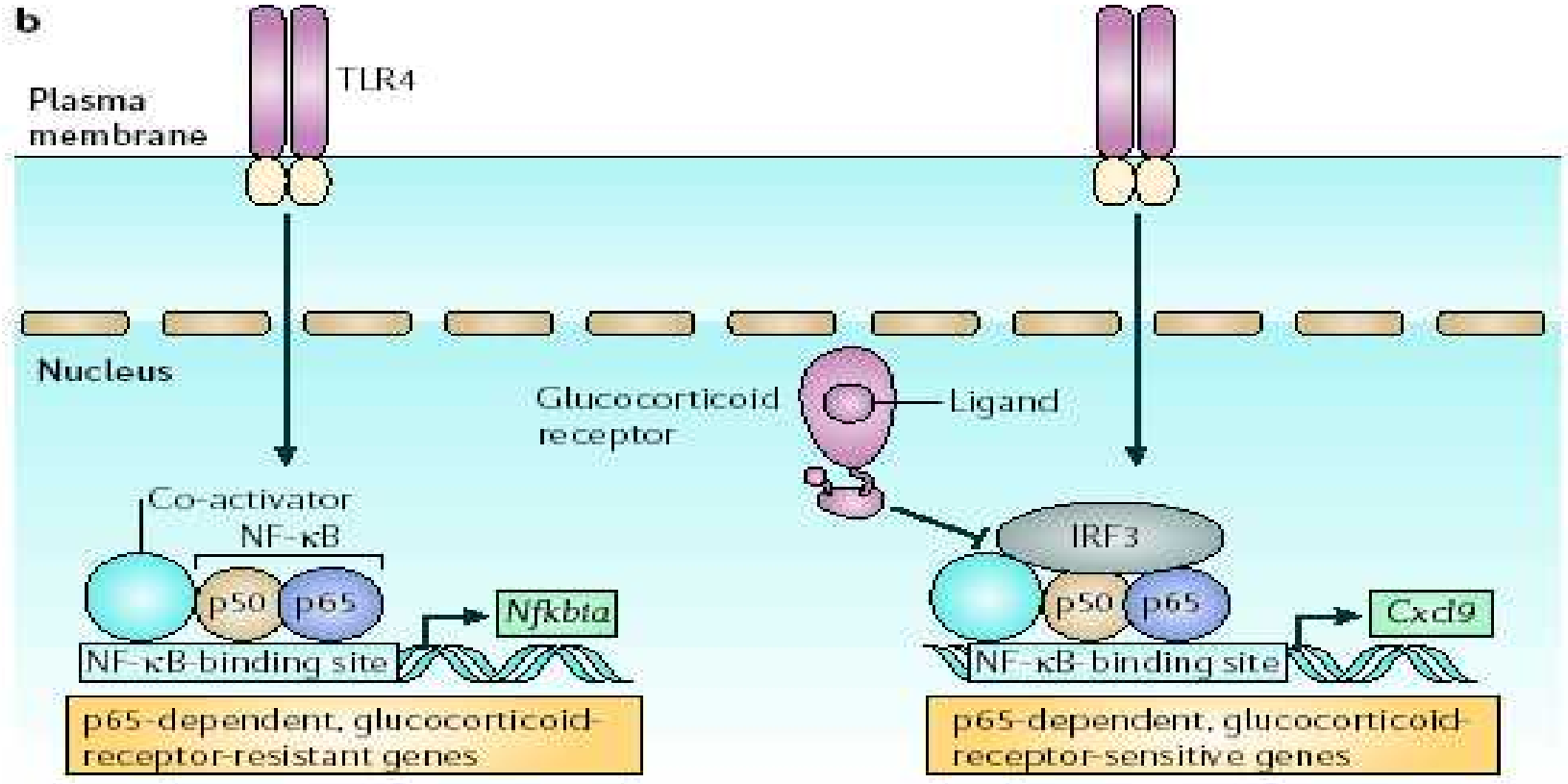


Signaling mechanisms controlling macrophage polarization



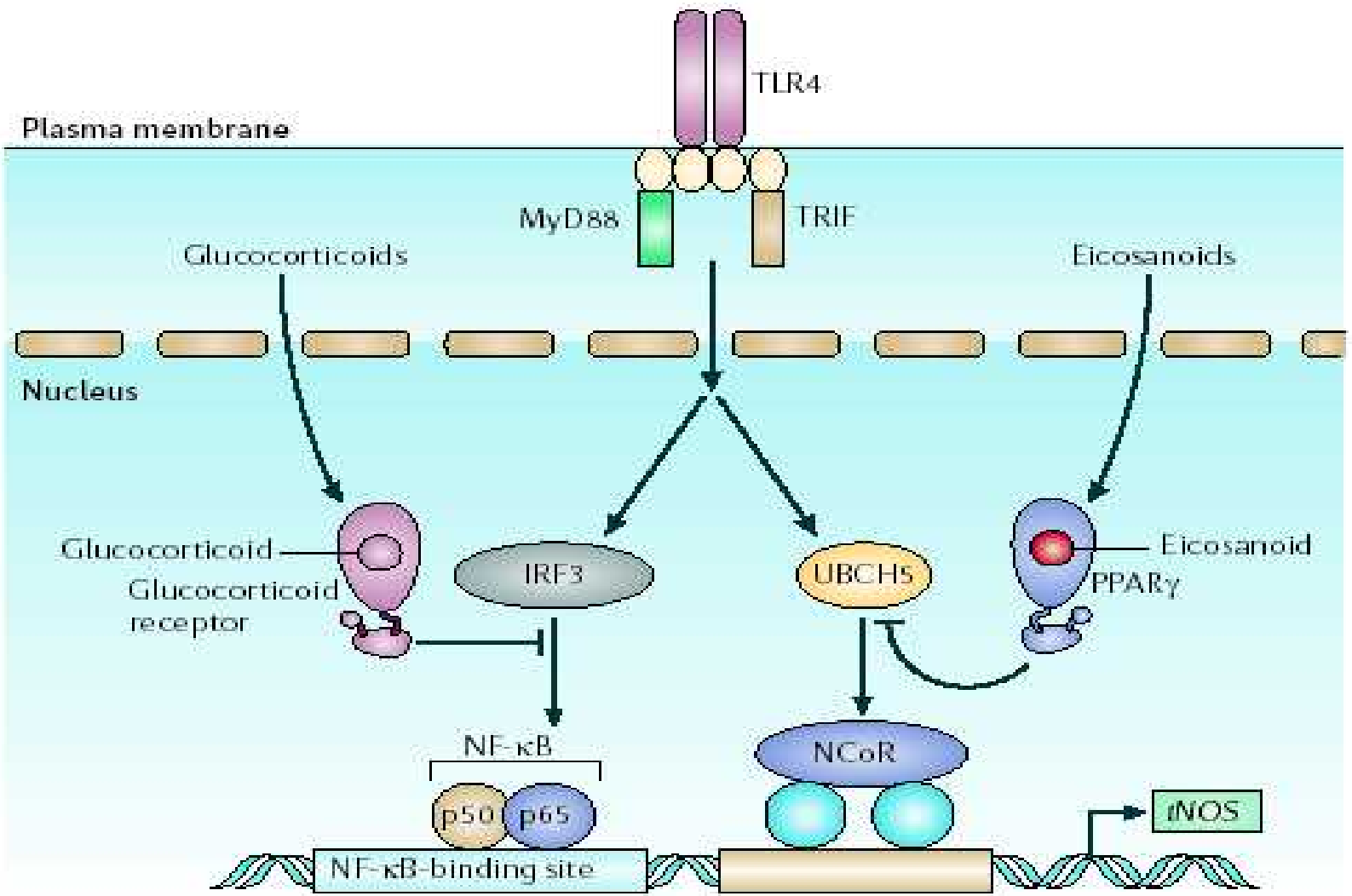
Nature Reviews | Immunology

Inhibition by glucocorticoid receptors



Nat Rev Immunol. 2005 5:446

Inhibition of inflammatory signals by PPARγ and glucocorticoids



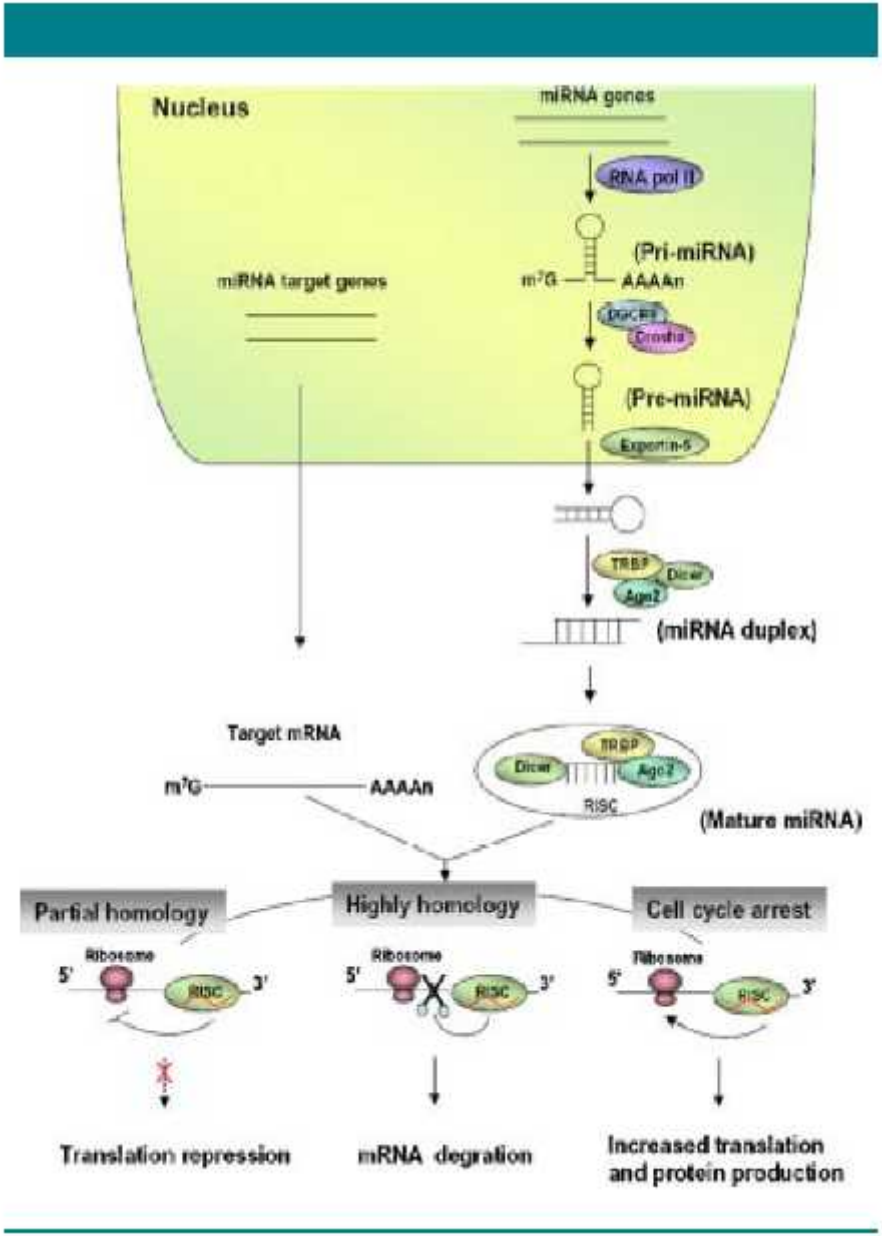
Conclusions

- Cytokine signaling is regulated in a dynamic manner during immune responses
  - Cytokine signaling is augmented or suppressed
  - Cytokine signaling crosstalks with pathogen receptor signaling, hormones, adipokines etc
  - Cytokines have different effects on the same cell depending on the timing and state of activation
  - Balance between cytokine signaling pathways that synergize or antagonize determines the phenotype

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

Thank you

miRNAs in TLR signaling as regulators of inflammatory responses



J. Cell. Physiol. 2009, 218: 467–472

MicroRNAs: novel players in the regulation of immunity

Endogenous, non protein coding, small RNAs

Exhibit tissue specific or developmental stage specific expression

Regulate translation and stability of mRNAs

Role

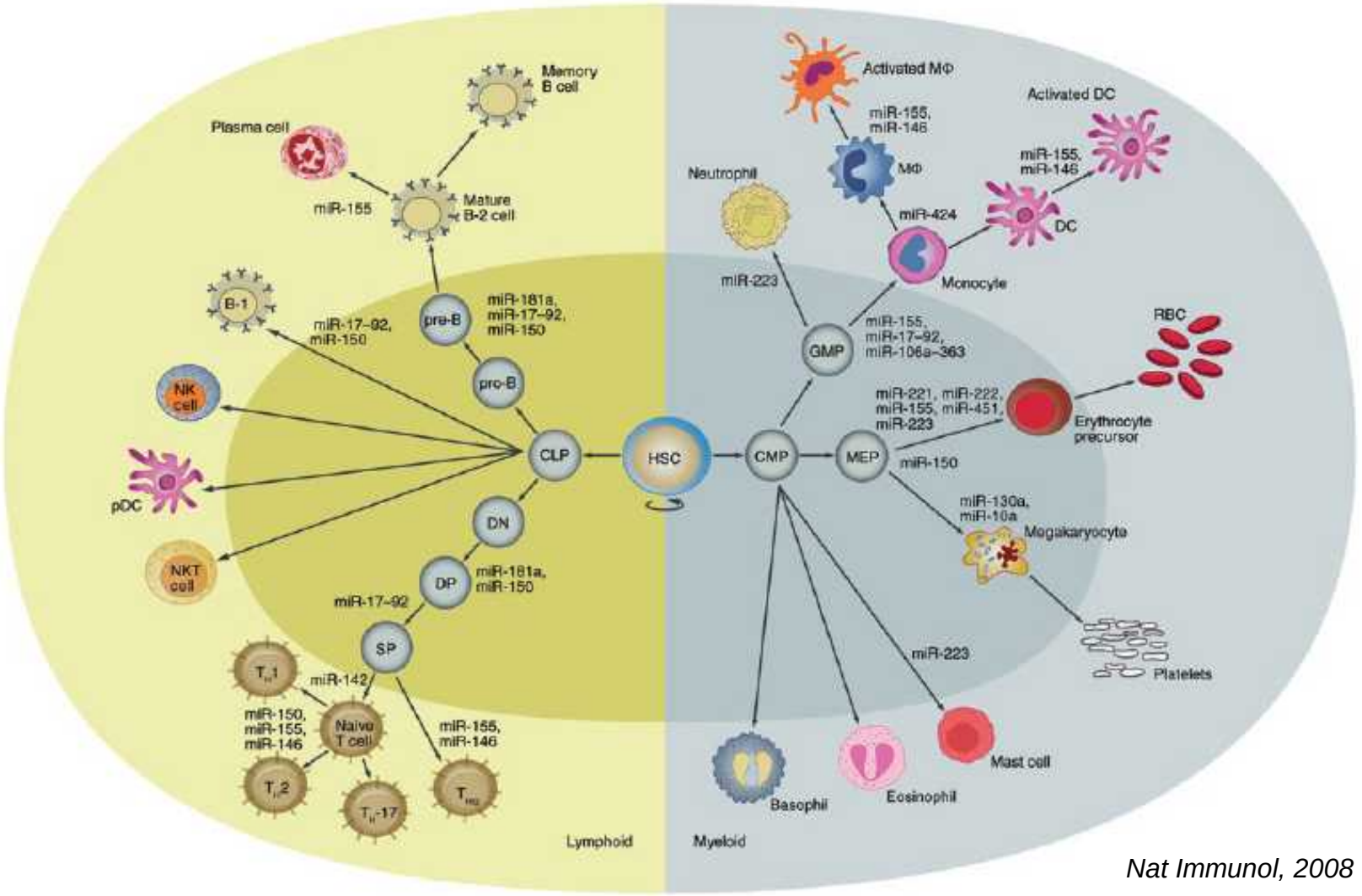
in hematopoiesis

In control of cell survival /proliferation

In chromatin regulation

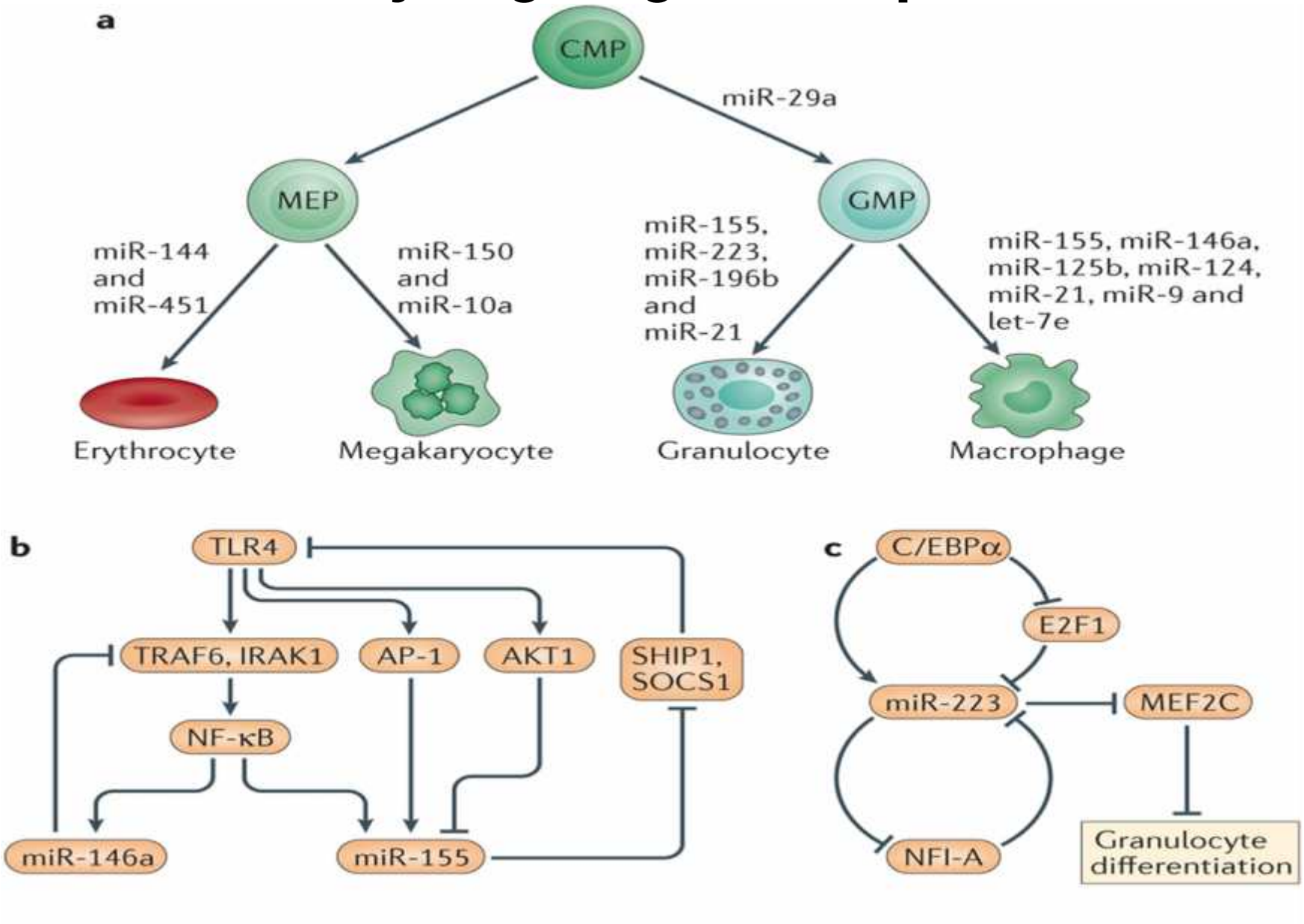
In immunity

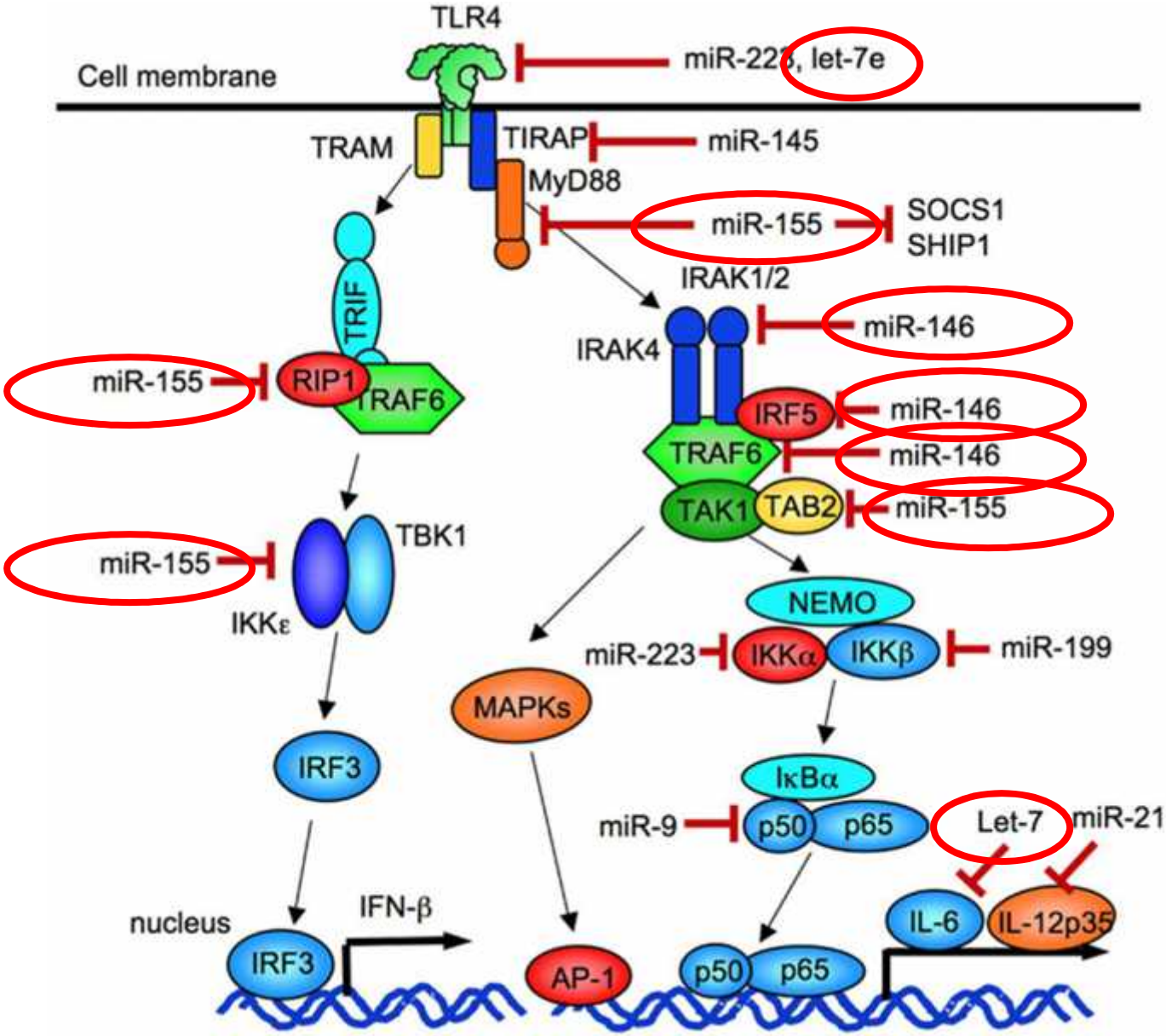
miRNAs in hematopoietic cells



Nat Immunol, 2008

miRNAs control macrophage lineage cell differentiation by targeting transcription factors





Front. Physiol., 2012

Therapies targeting T-cell activation signals