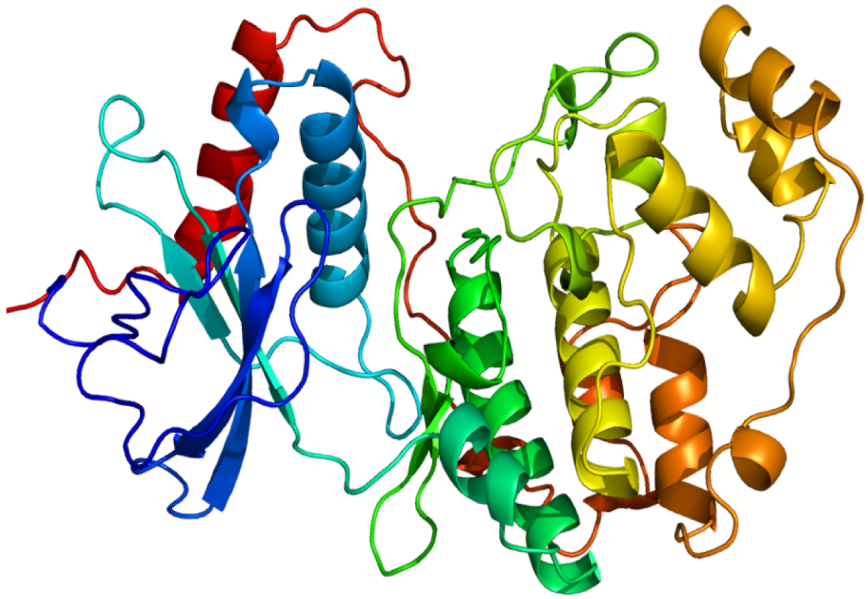
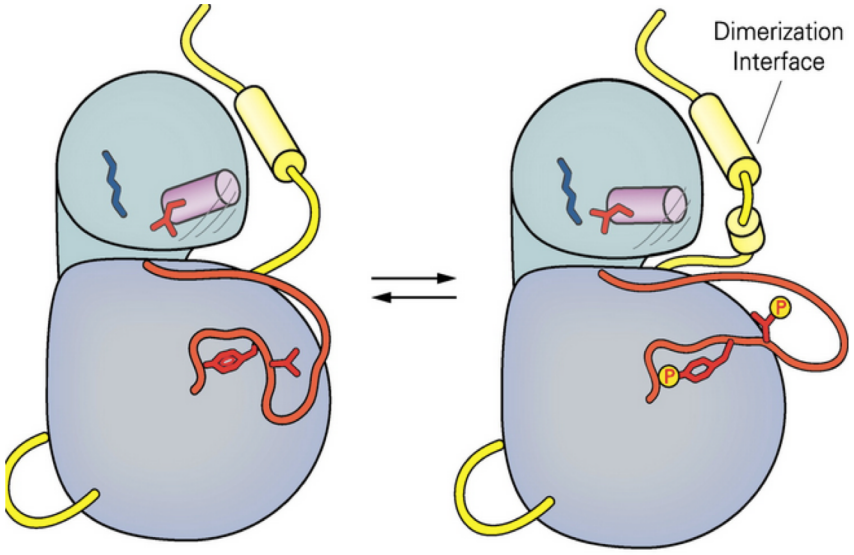
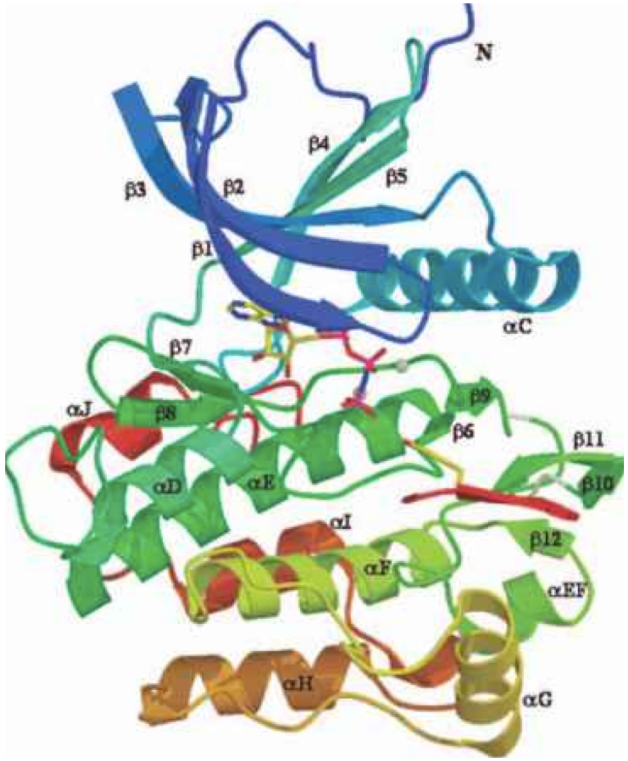


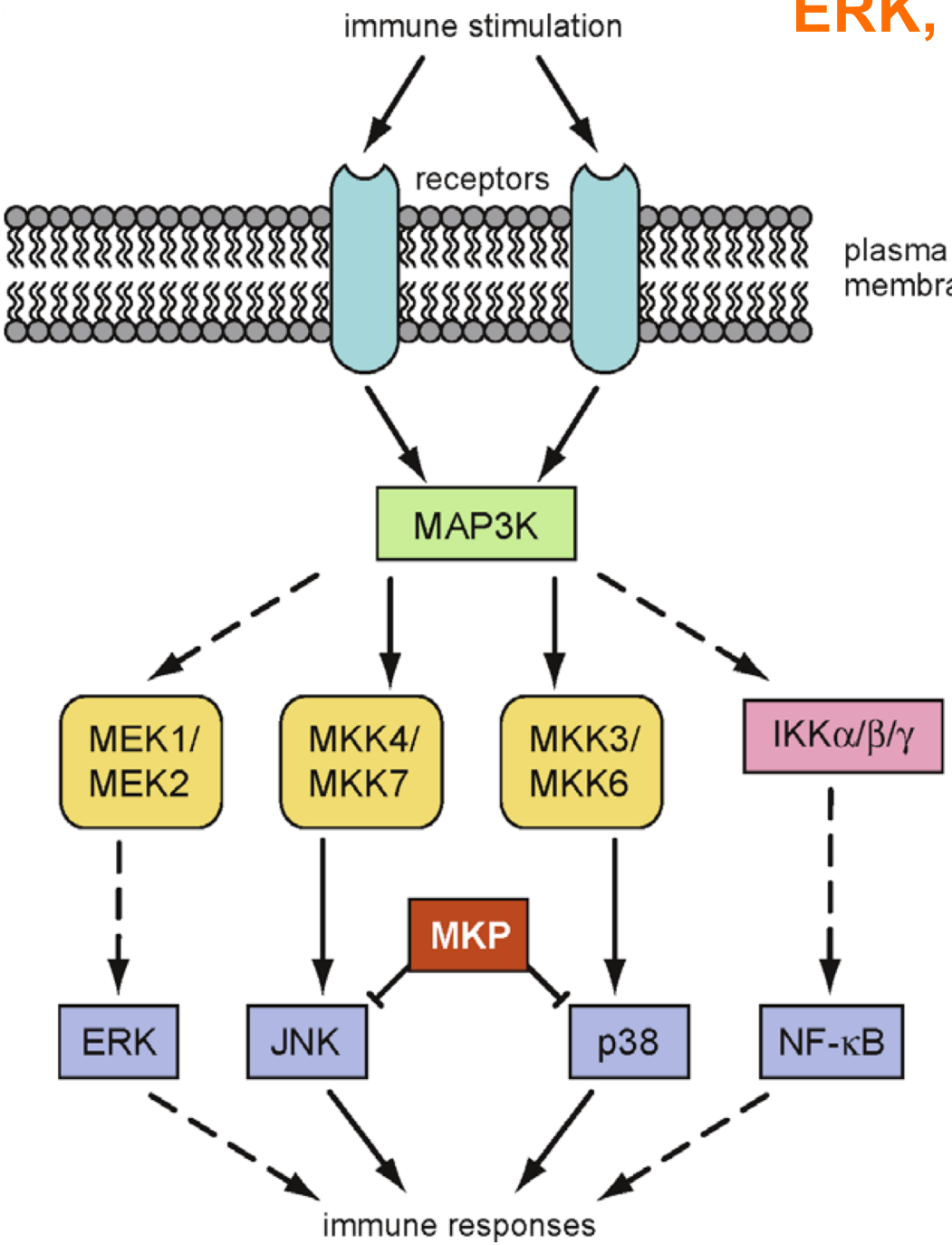


Inactive MAP Kinase Active MAP Kinase

Le MAP chinasi nella trasduzione dei segnali



Mitogen-activated protein Kinases (MAPK): ERK, JNK, p38



La via di segnalazione delle MAP chinasi si riferisce ad una cascata di **proteine** (**serina/treonina chinasi**) altamente conservate nell' evoluzione con un ruolo fondamentale di regolazione della crescita e del differenziamento cellulare.

Questa via è importante anche per la trasduzione del segnale nell'attivazione delle risposte immuni.

RAS oncoproteins

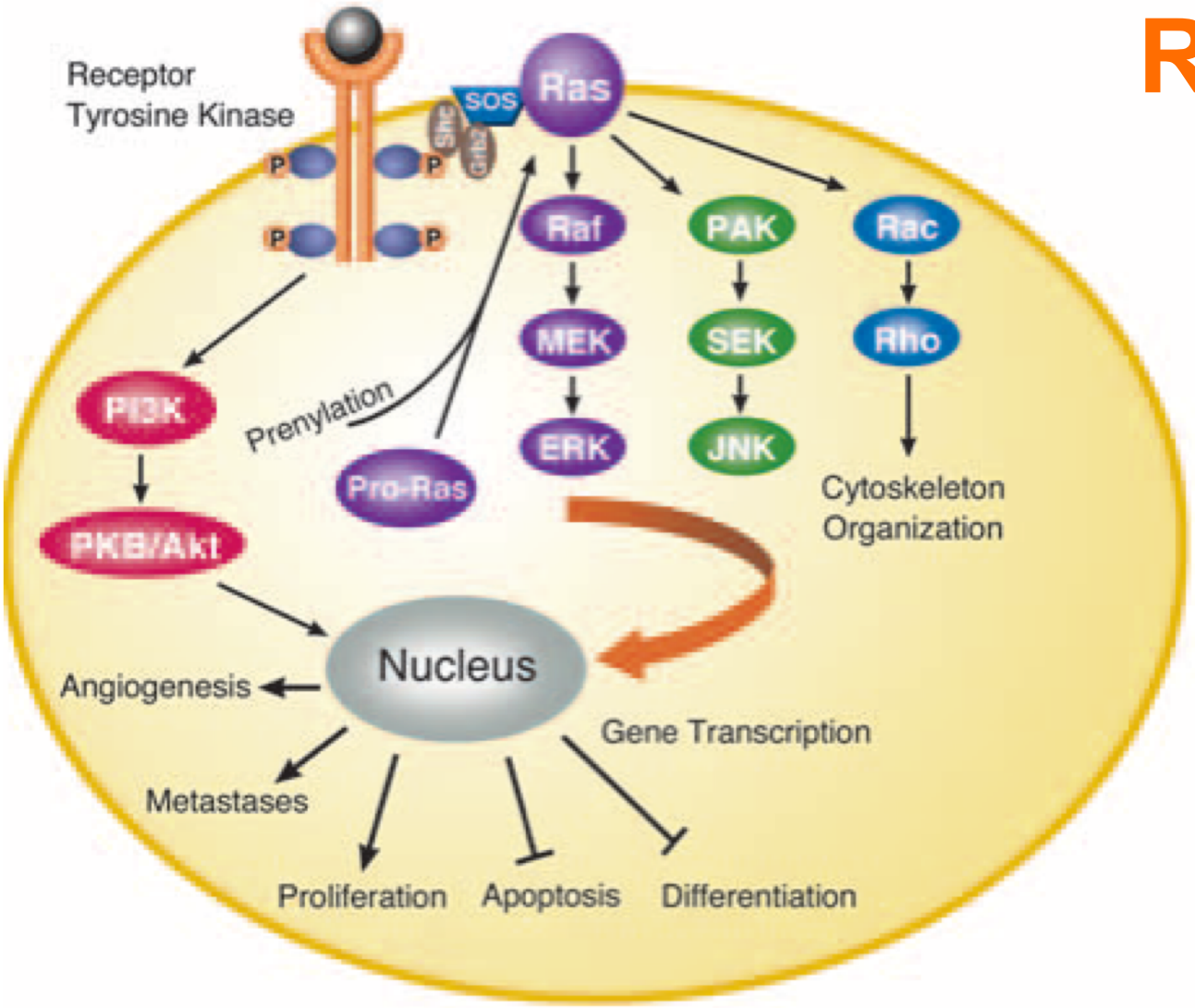
- **1964-1967:** initial evidence for Ras involvement in cancer from the discovery of transforming retroviruses, ***Harvey*** and ***Kirsten sarcoma viruses***, which contained **H-ras** and **K-ras** cellular derived oncogenes.

- **1982**: identification of ***N-ras***, homologous to v-*ras* > mutated in the 30% of human tumors. RAS genes were the first human oncogenes to be identified.

GTPase Superfamily

Ras è il prototipo di una superfamiglia di small GTPasi che trasmettono segnali **proliferativi,** di **sopravvivenza** e **differenziamento** attraverso MAPK, PI3K ed altri pathways.

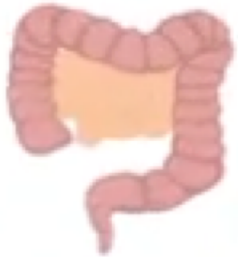
Ras protein

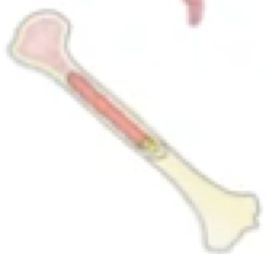


- **Cell survival**
- **Cell growth**
- **Cell differentiation**
- **Cell migration**

Three *ras* proto-oncogenes encode a 21-kD protein, called $p21^{ras}$ or Ras: **H-Ras; N-Ras; K-Ras** (K-Ras 4A and 4B, spliced forms) that are localized to the inner surface of the cell membrane.

HRAS KRAS NRAS





30% OF HUMAN CANCERS

95% of Pancreatic Cancer- KRAS

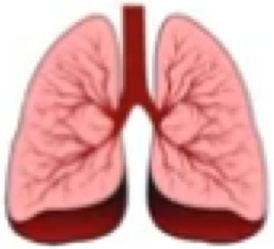
45% of Colorectal Cancer- KRAS

35% of Lung Cancer- KRAS

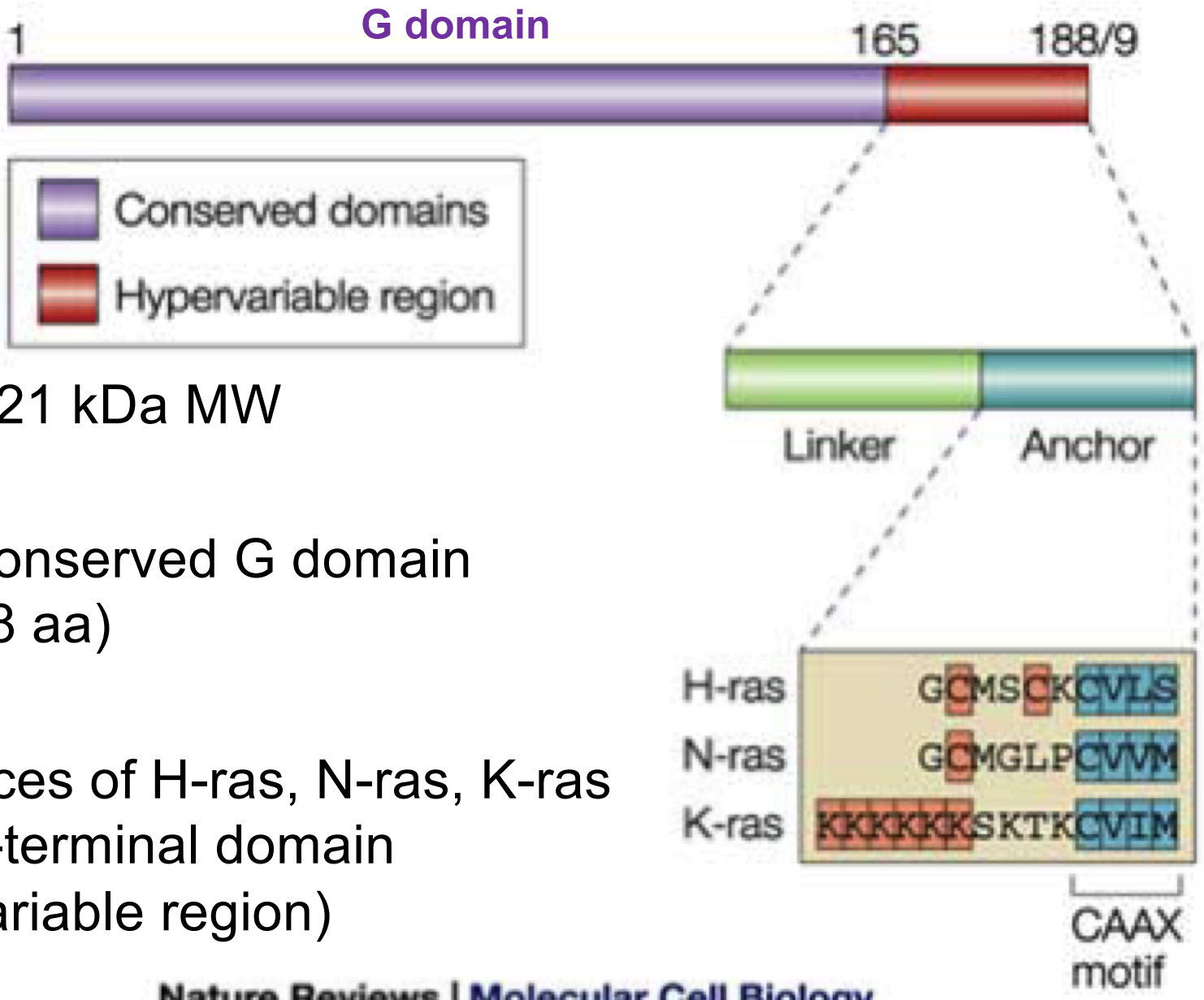
15% of Acute Myeloid Leukemia- NRAS

15% of Melanoma- NRAS

10% of Bladder Cancer- HRAS



Struttura di RAS

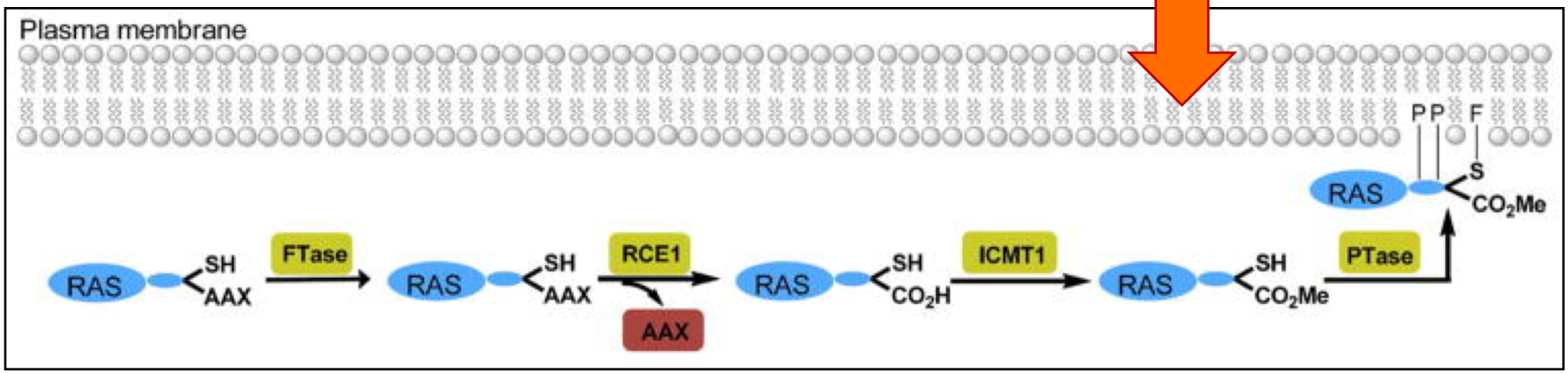


- 189 aa; 21 kDa MW
- Highly conserved G domain (165-168 aa)
- Differences of H-ras, N-ras, K-ras in the C-terminal domain (hypervariable region)

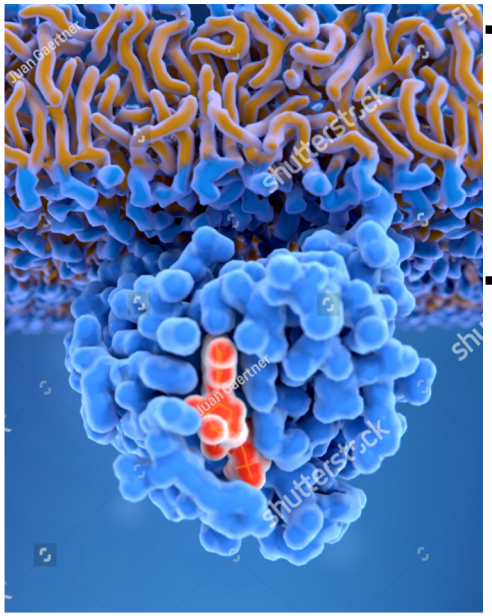
Ras lipidation in cell signalling: crucial step

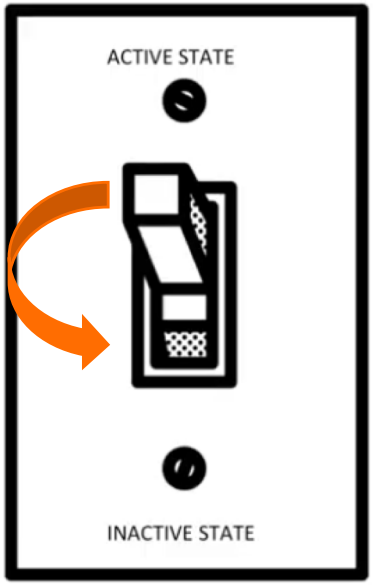
Synthesis as inactive cytosolic pro-proteins

Series of post-translational modifications at the carboxyl-terminus



1. The **thiol group of the terminal Cys** (CAAX: C=Cys A=aliphatic amino acid X= any aa) is **farnesylated** by **farnesyltransferase** (**Ftase**). This adds a 15-carbon hydrophobic farnesyl isoprenyl tail to the carboxyl-terminus of Ras.
2. **RCE1** (**protease Ras-converting enzyme**) **cleaves AAX** and the **Cys is methylated by ICMT.**
3. **Palmitoyl transferase (PTase)** induces the **palmitoylation of Ras** > stable interaction with the plasma membrane.

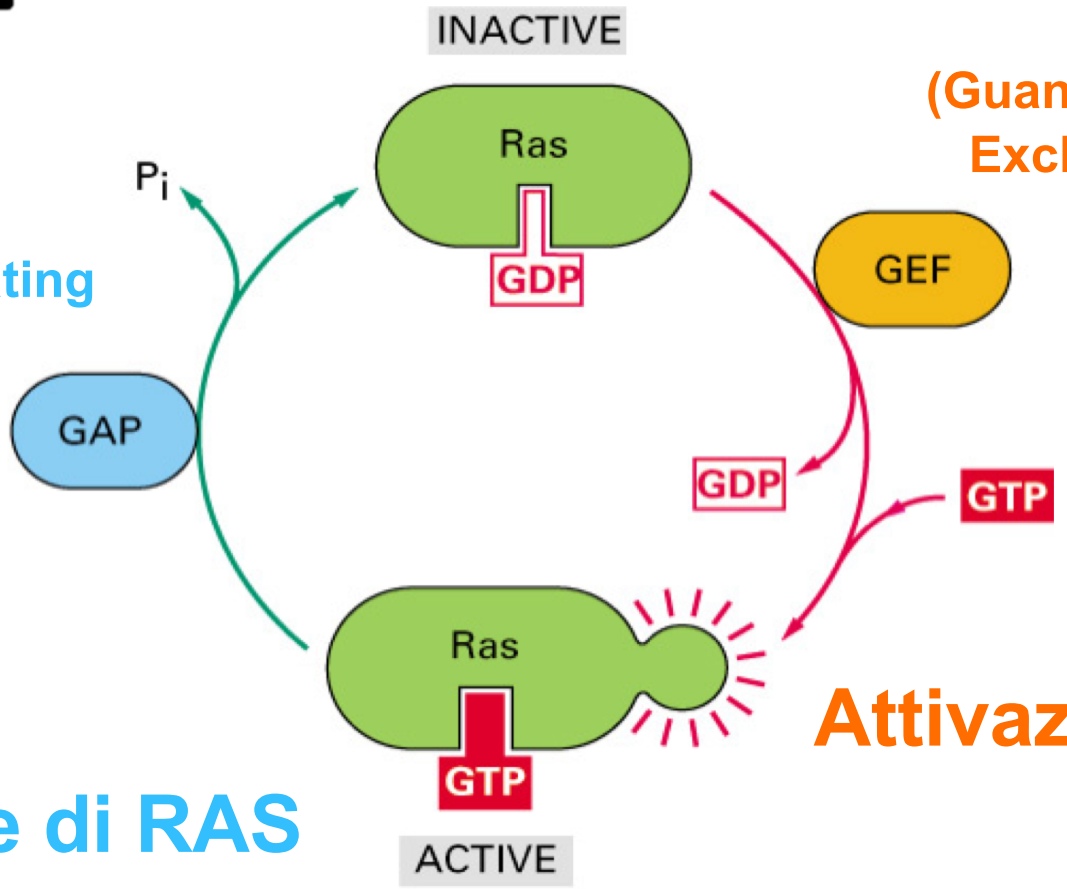




Attivazione di RAS

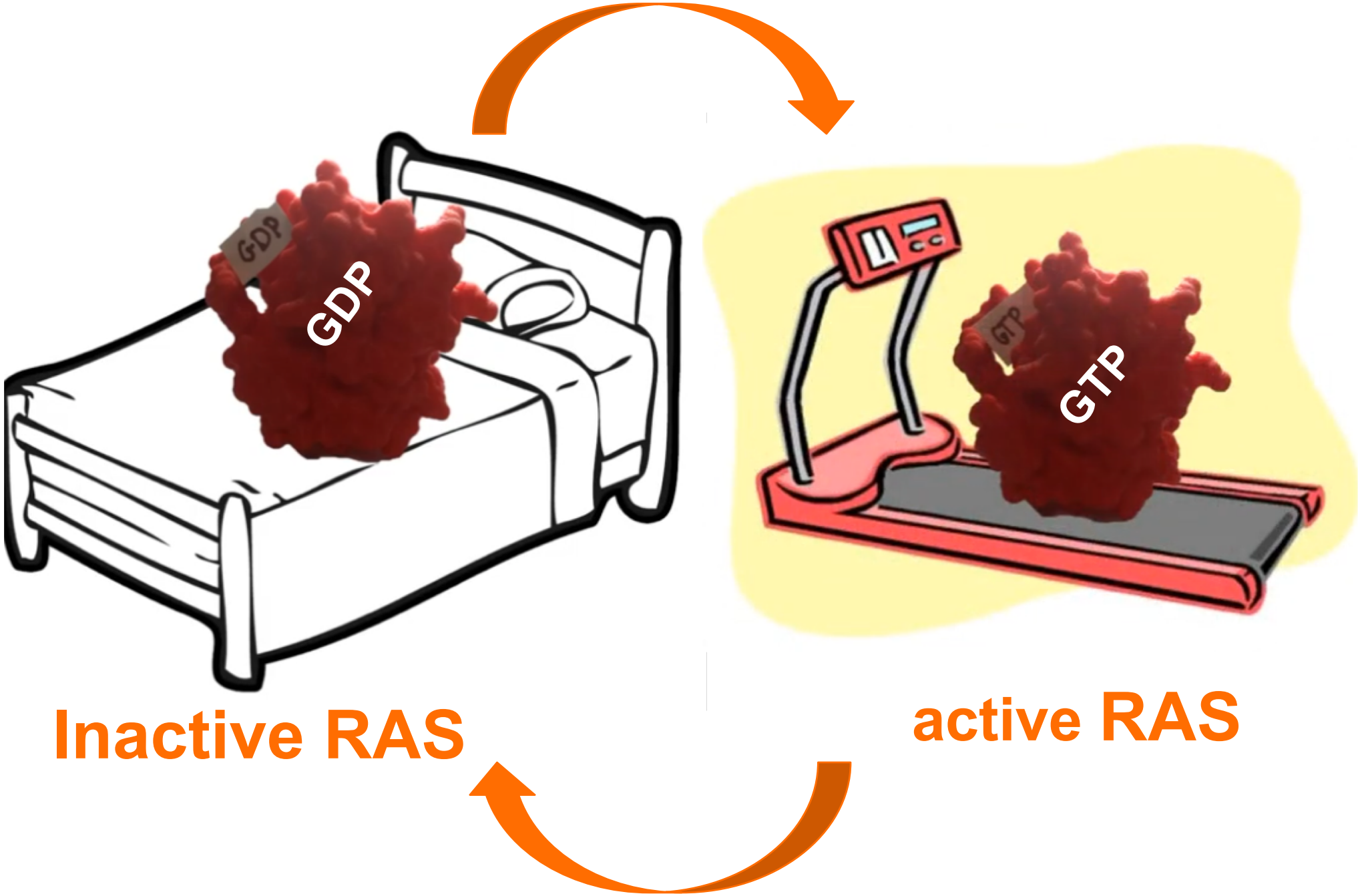
GEF
(Guanine Nucleotide Exchange Factor)

GAP
(GTPase-Activating Protein)



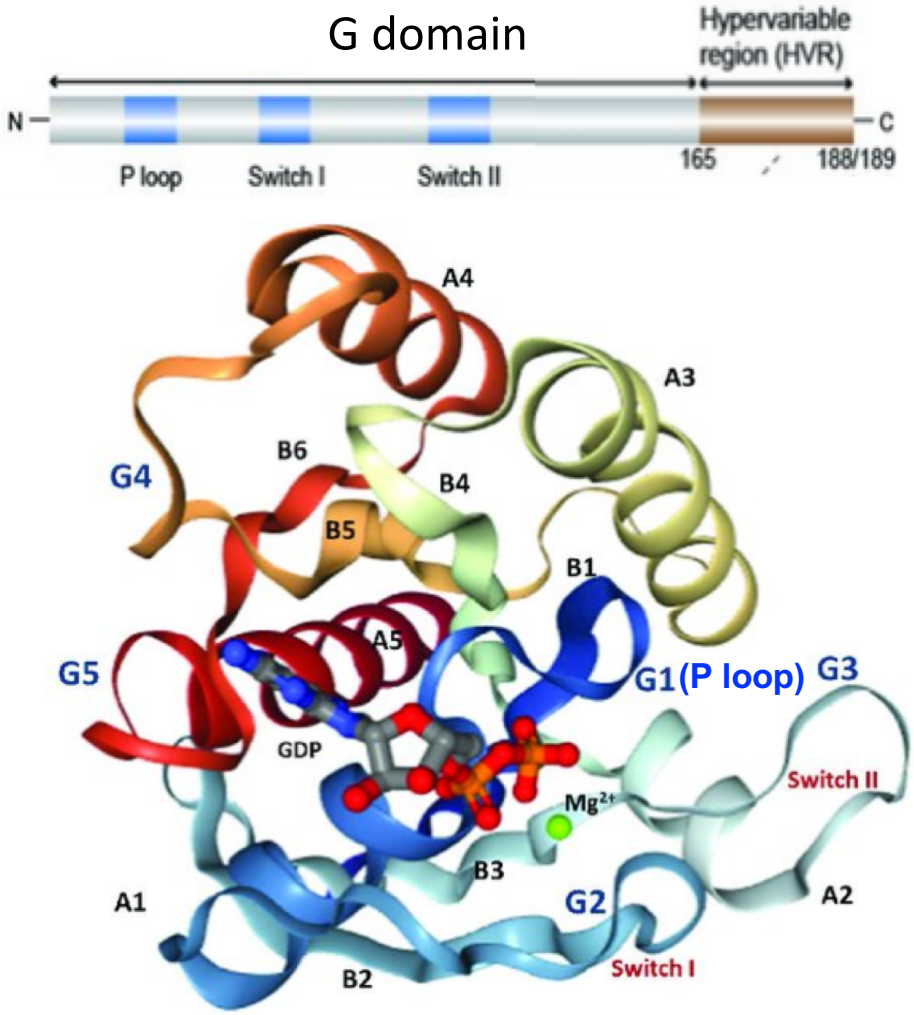
Inattivazione di RAS

Attivazione di RAS


GDP
GDP
GTP
GTP
Inactive RAS
active RAS

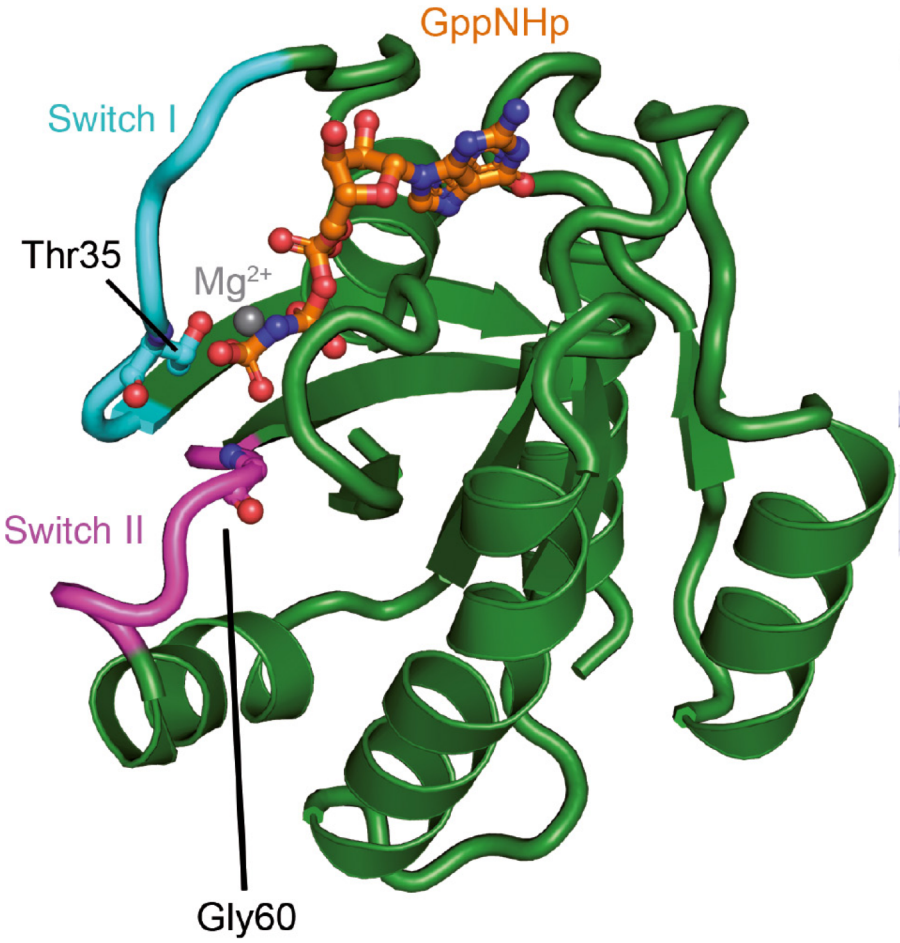
Structure of RAS

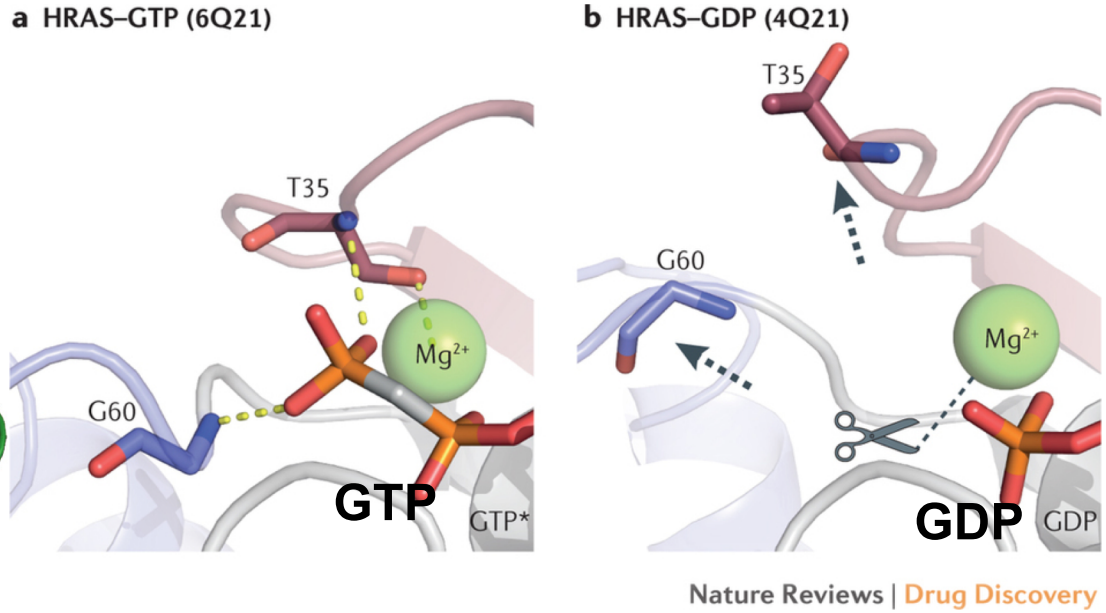
- **The G domain** (first 166-168 aa) consists of **5 α-helices** and **6 stranded β-sheets**.
- Crucial domains: the phosphate-binding loop (**P-loop**, residues 10–17), **switch I** (residues 30–38), **switch II** (residues 60–76)
- The structural changes in GTP-bound and GDP-bound RAS are confined to **Switch I** and **Switch II.**
- **GTP state**: **Thr35** and **Gly60** make hydrogen bonds with the γ-phosphate, holding the switch I and switch II in the active conformation.
- Removal of the γ-phosphate group allows these regions to relax and adopt an inactive conformation.
- The most frequent sites of oncogenic mutations in RAS are residues G12V or G13V in the P-loop, and residue Q61R in switch II.

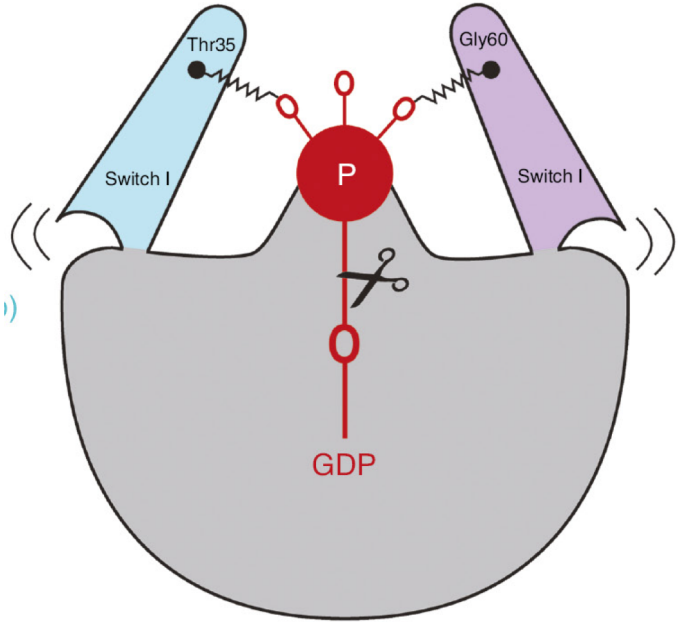


Structural analysis of Ras. The crystal structures of Ras GDP Mg^{2+} complex (PDB 4q21) is showed (upper). This structure contains **five α-helices** (A1-A5), **six β-strands** (B1-B6), and **five polypeptide loops** (G1-G5) and the position relationship among various parts is displayed (below)

Structure of RAS



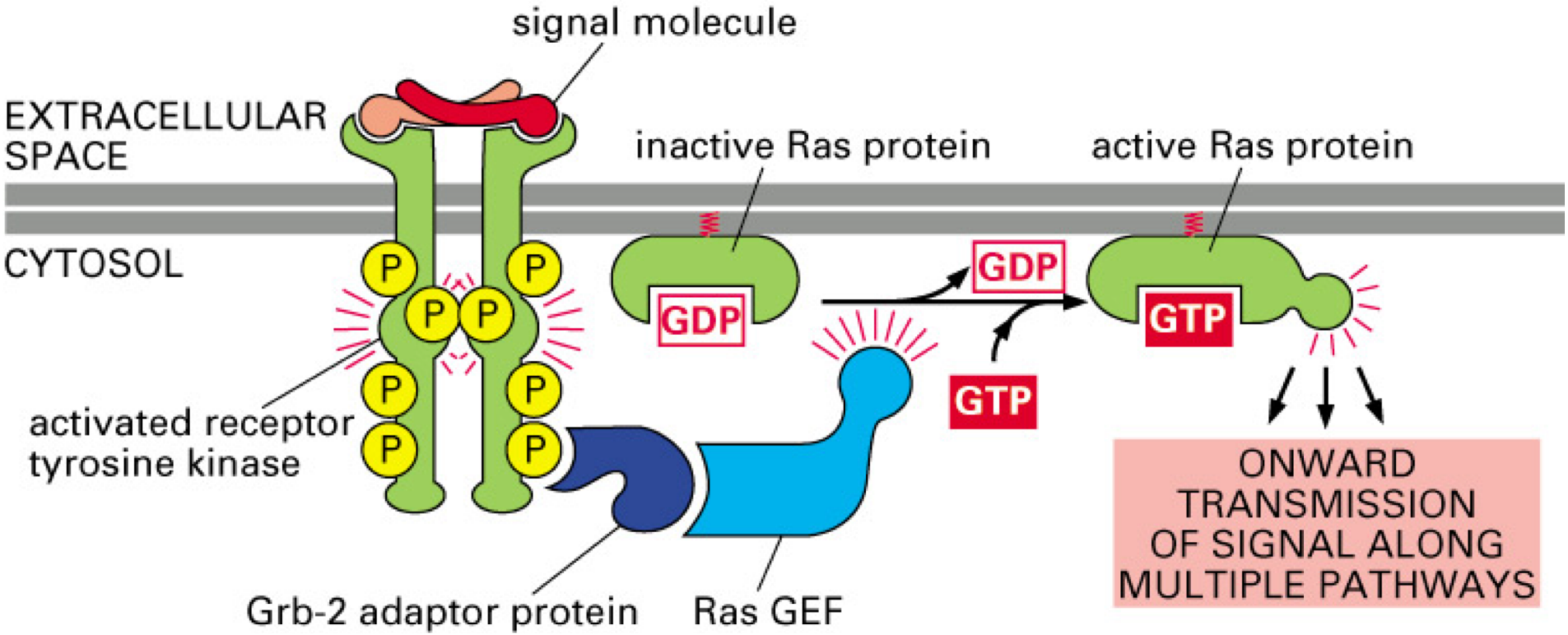




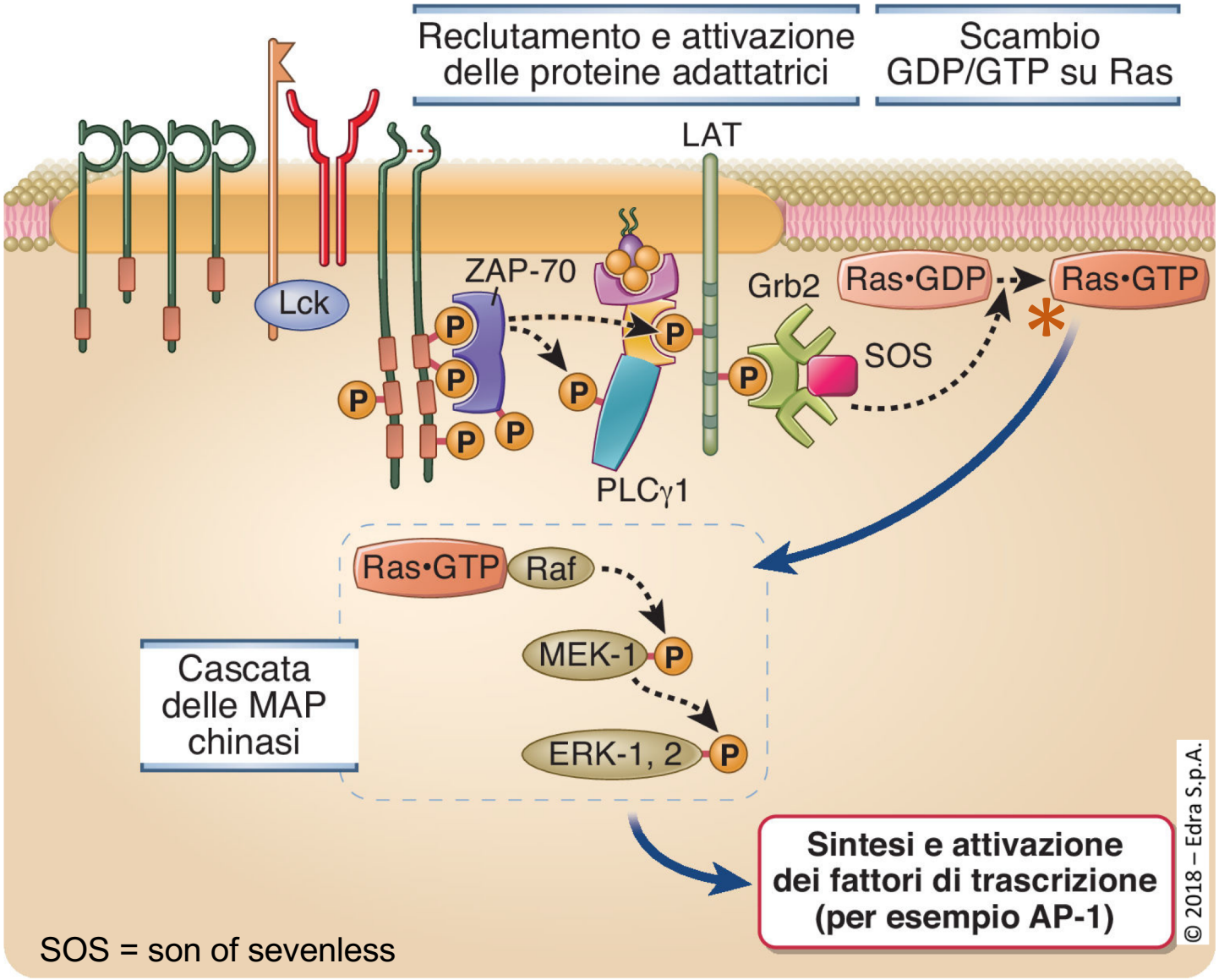
- **GTP state: Thr35 and Gly60 make hydrogen bonds with the γ-phosphate, holding the switch I and switch II in the active conformation**.

- Removal of the phosphate group allows these regions to relax and adopt an inactive conformation.

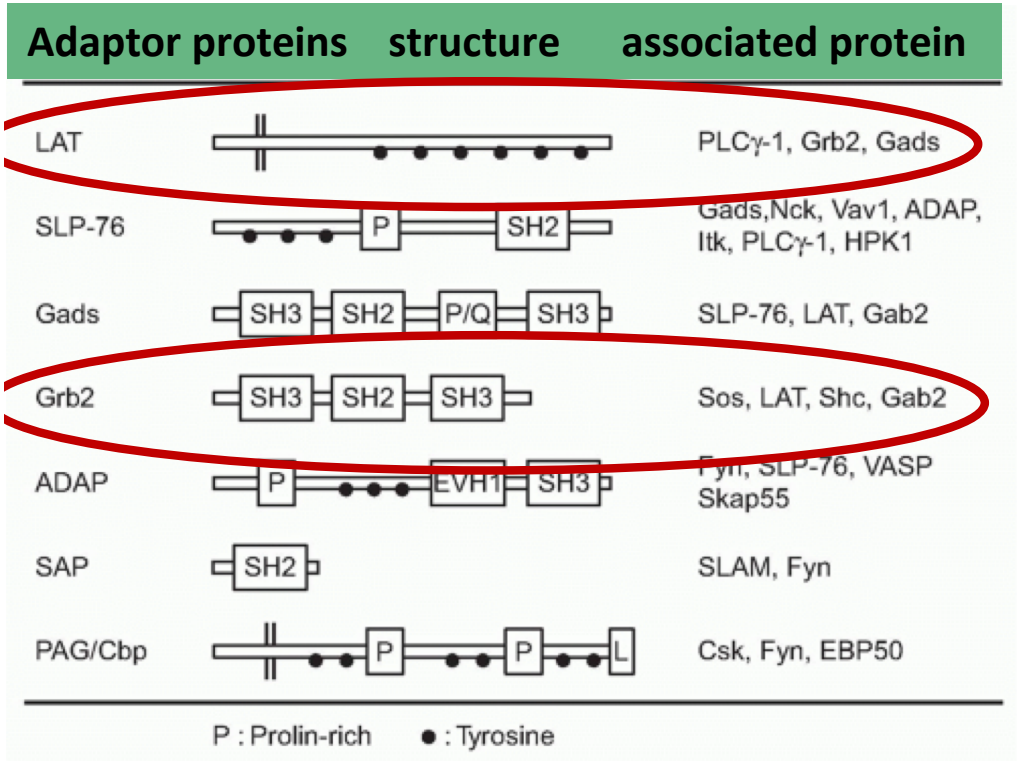
Pivotal role of RAS in receptor signal transduction



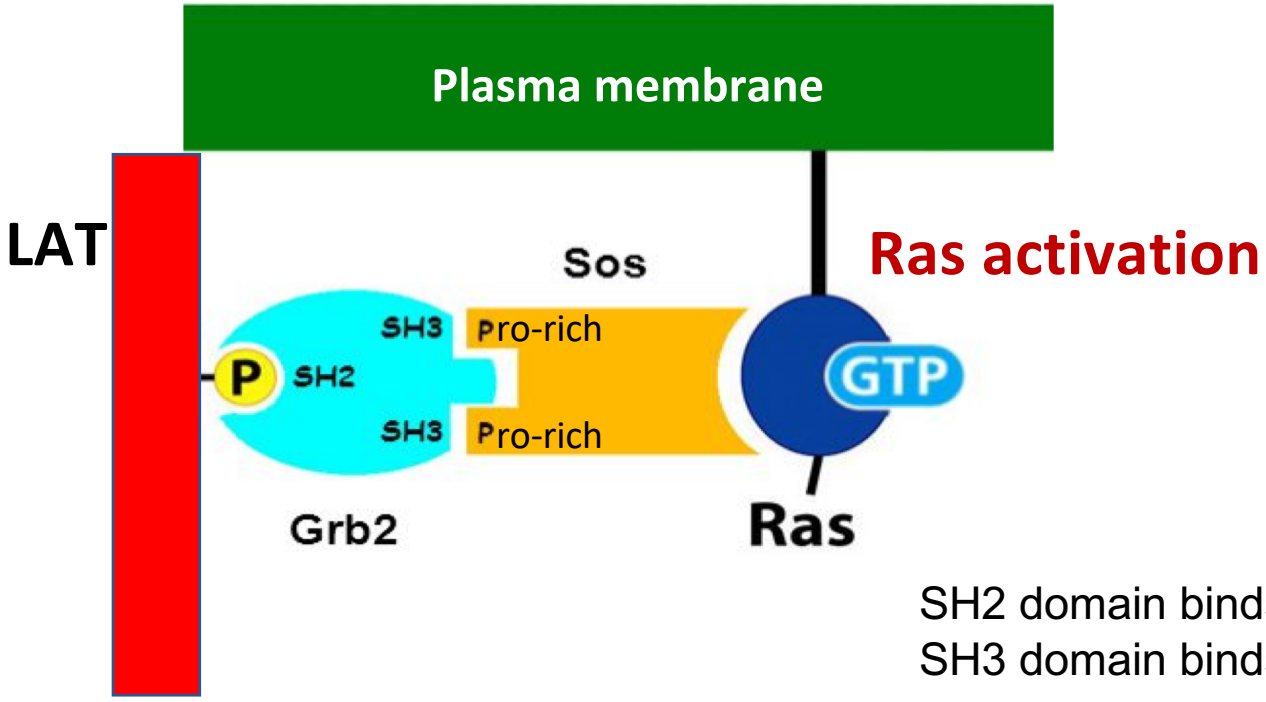
Attivazione dei linfociti T: la via di Ras e delle MAP chinasi

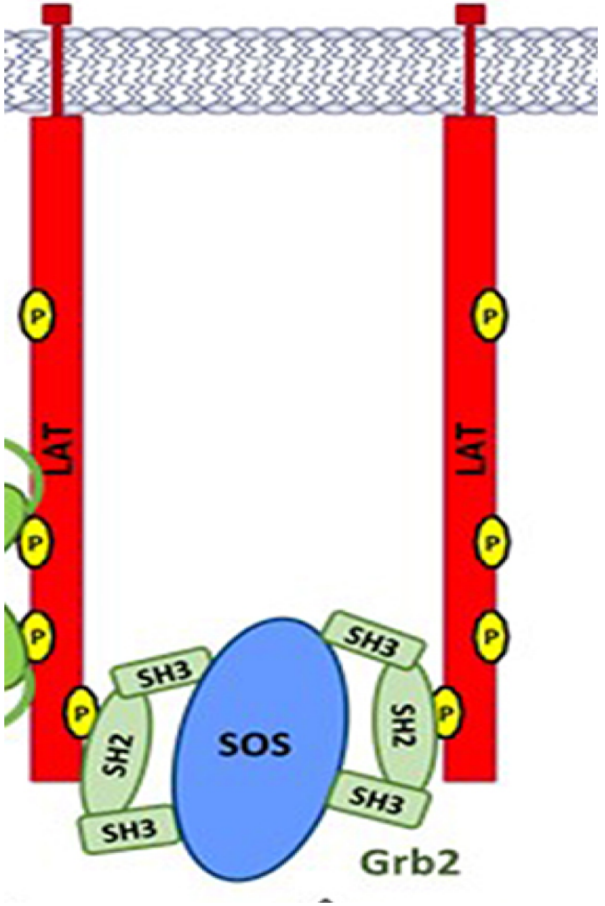


SOS = son of sevenless



SOS: (GDP-GTP exchange factors) GEF capable of activating Ras





SH2 domain binds phosphotyrosine containing motif;
SH3 domain binds proline-rich motif;

Classical Ras/Raf/MEK/ERK mitogen kinase cascade

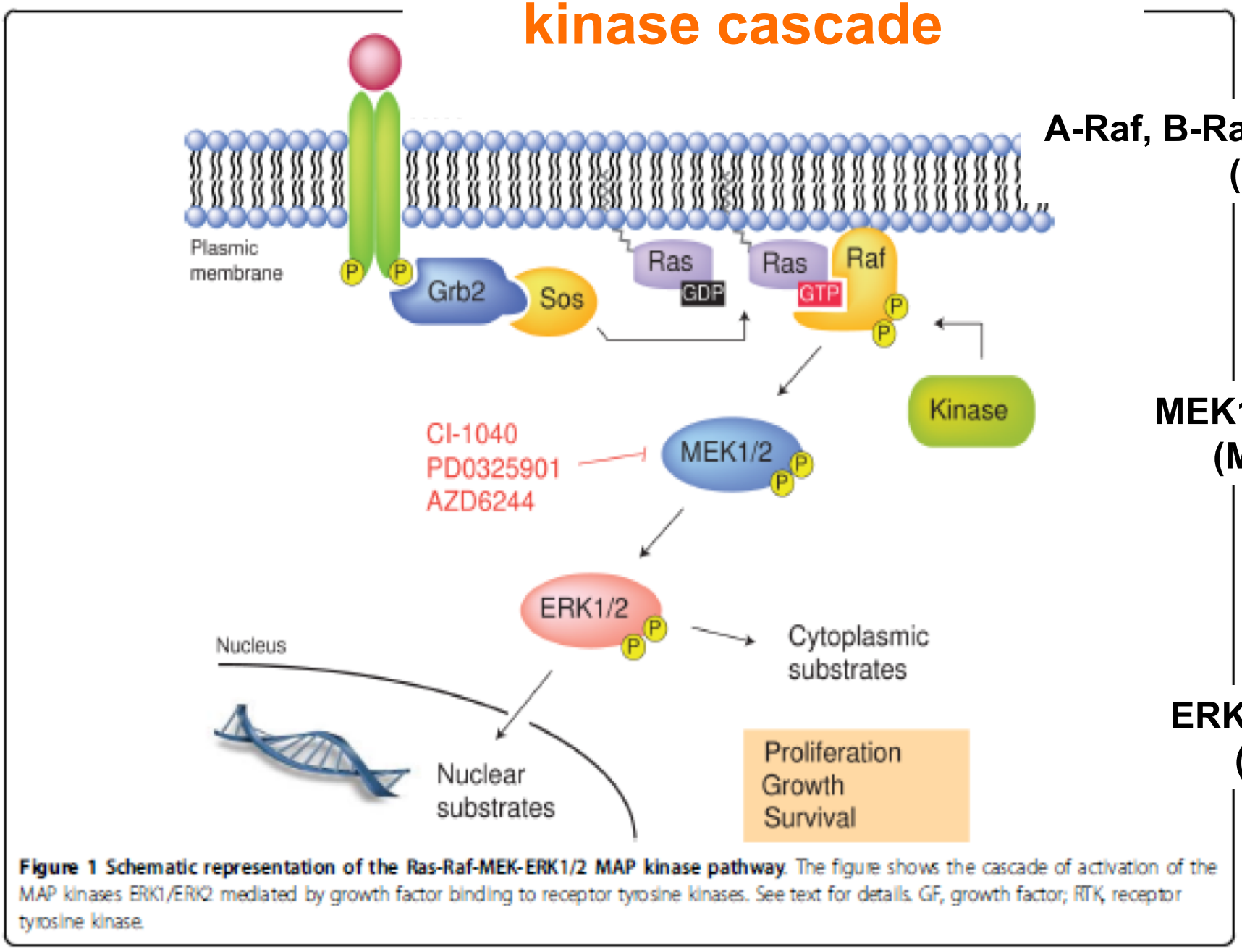
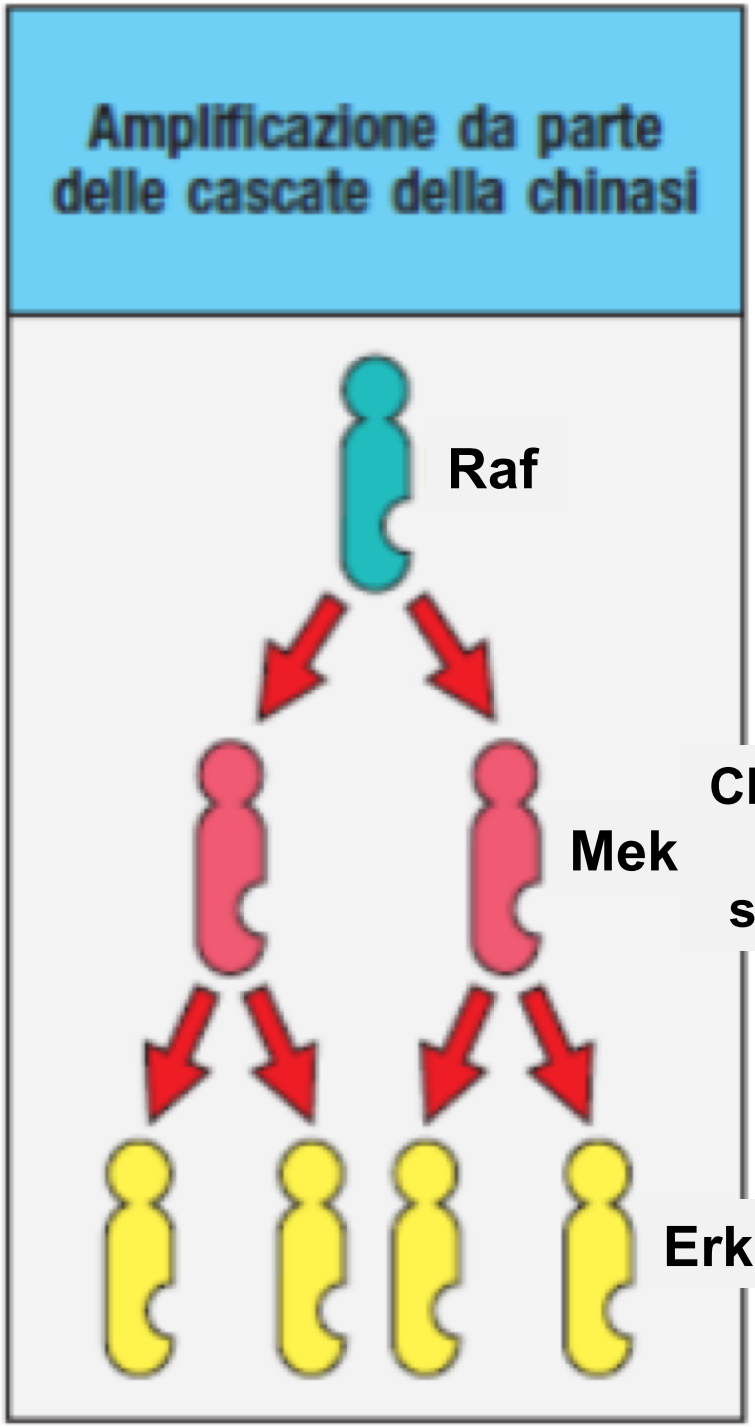


Figure 1 Schematic representation of the Ras-Raf-MEK-ERK1/2 MAP kinase pathway. The figure shows the cascade of activation of the MAP kinases ERK1/ERK2 mediated by growth factor binding to receptor tyrosine kinases. See text for details. GF, growth factor; RTK, receptor tyrosine kinase.

A-Raf, B-Raf, and C-Raf (MAP3Ks)

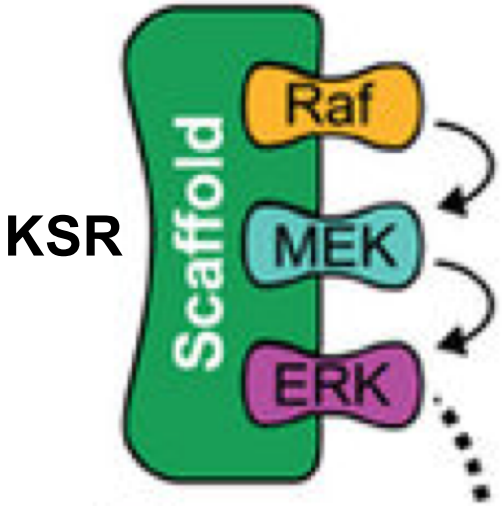
MEK1 and MEK2 (MAP2Ks)

ERK1 and ERK2 (MAPKs)



I processi di trasmissione del segnale amplificano il segnale iniziale

Chinasi con doppia specificità



ERK = extracellular signal-related kinase

RAF

(serine-threonine Kinases)

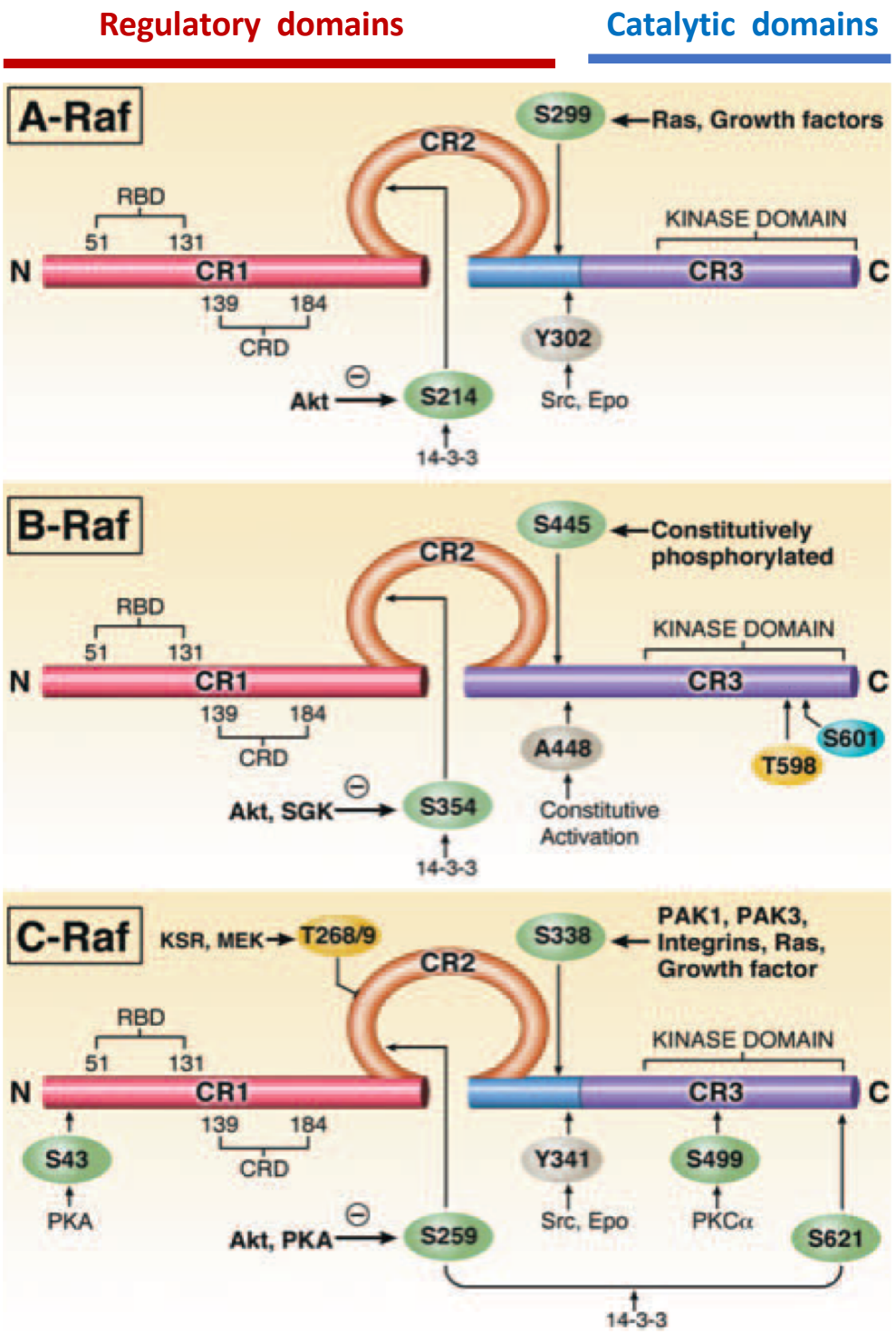
- ***A-raf, B-raf, C-raf***, chromosome Xp11, 7q32 and 3p25.
- Three proteins (68- to 74-kDa) **A-Raf**, **B-Raf** and **C-Raf** (or RAF1), with high conserved motives at the N- and C-termini.
- Serine-threonine kinases that phosphorylate and activate MEK, thus inducing MAPK/ERK cascade.

RBD: **Ras binding domain** induces the activation of Raf by **releasing CR1 from CR3 binding**

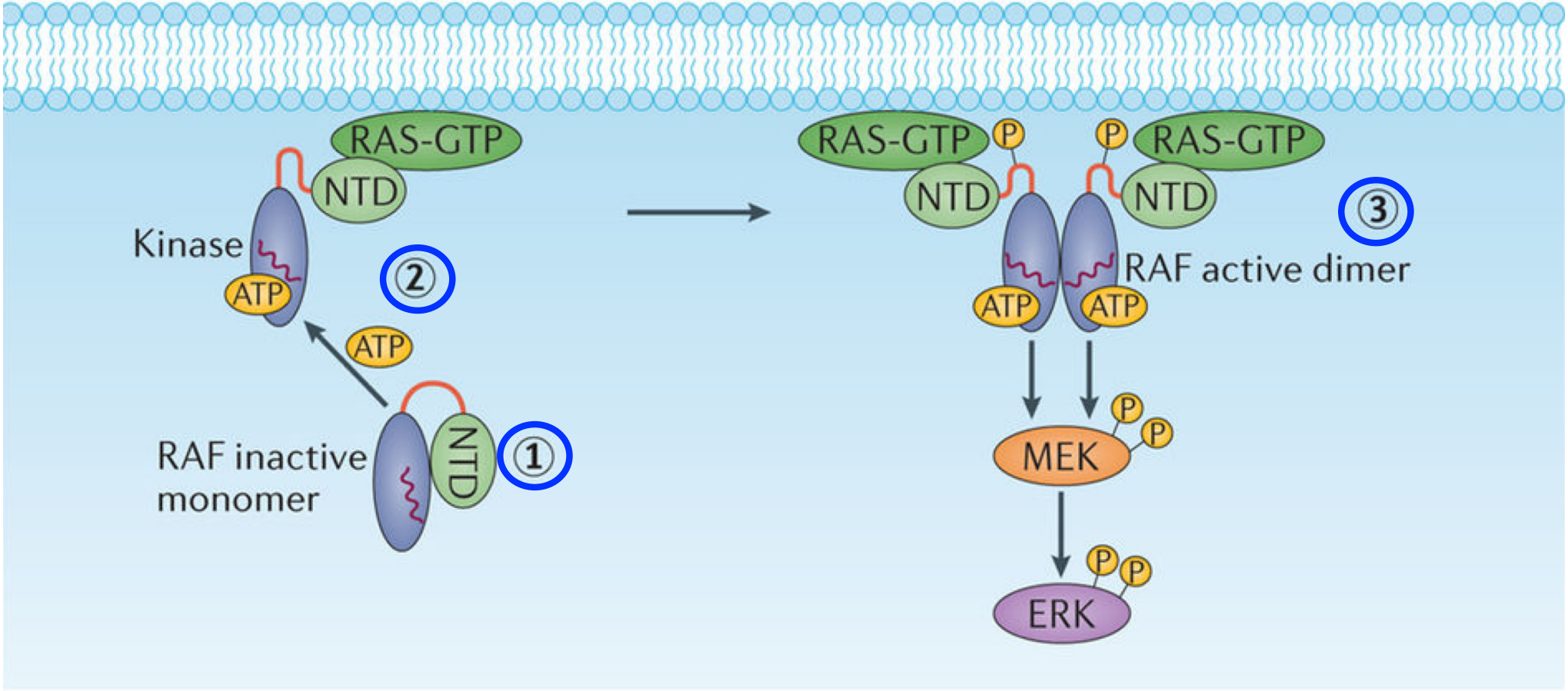
CR1: **inhibitory domain** that binds the **CR3** domain and blocks Raf activity

CR2: flexible **linker between CR1 and CR3; binds 14-3-3 protein** (regulatory protein)

CR3: **kinase domain with ATP binding sites** and **Ser residues** pivotal for Raf activity



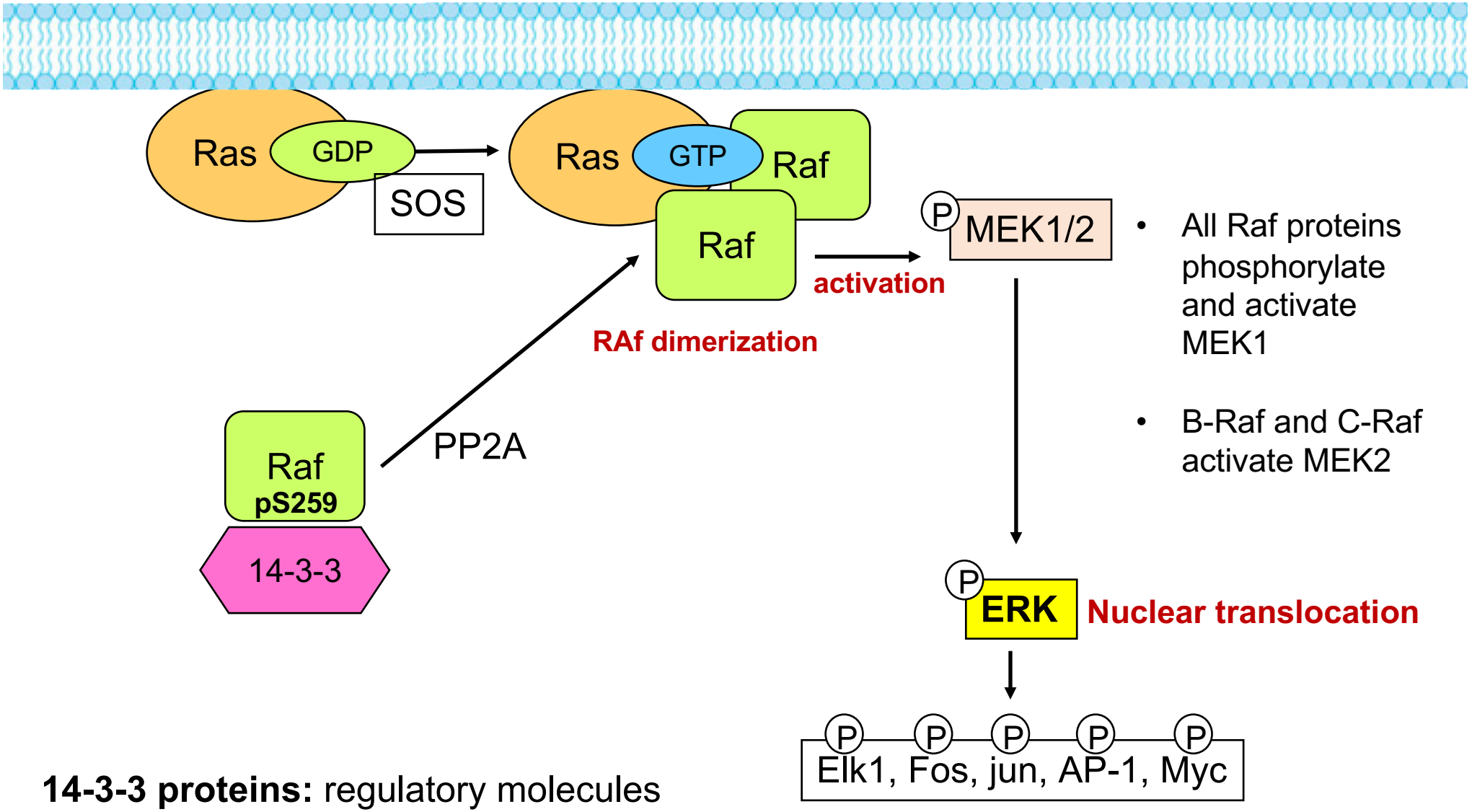
RAF activation



- With low RAS-GTP, RAF is monomeric and inactive in the cytosol due to intramolecular interaction between the CR1 and the CR3 domains.
- Upregulation of RAS-GTP promotes the formation of the RAF–RAS-GTP complex in the membrane due to the high affinity of RAS-GTP for the RAS-binding domain (RBD) present in the CR1
- Dimerization and full RAF activation

NTD= N-terminal domain

Regulation of Raf activity



14-3-3 proteins: regulatory molecules

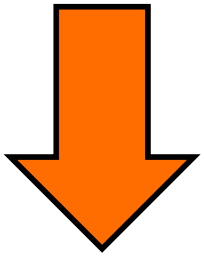
PP2A: serine/threonine phosphatase

ERK (MAPK) function

Activated ERK regulates cell function by acting on more than **50 substrates** in the cytosol and the nucleus.

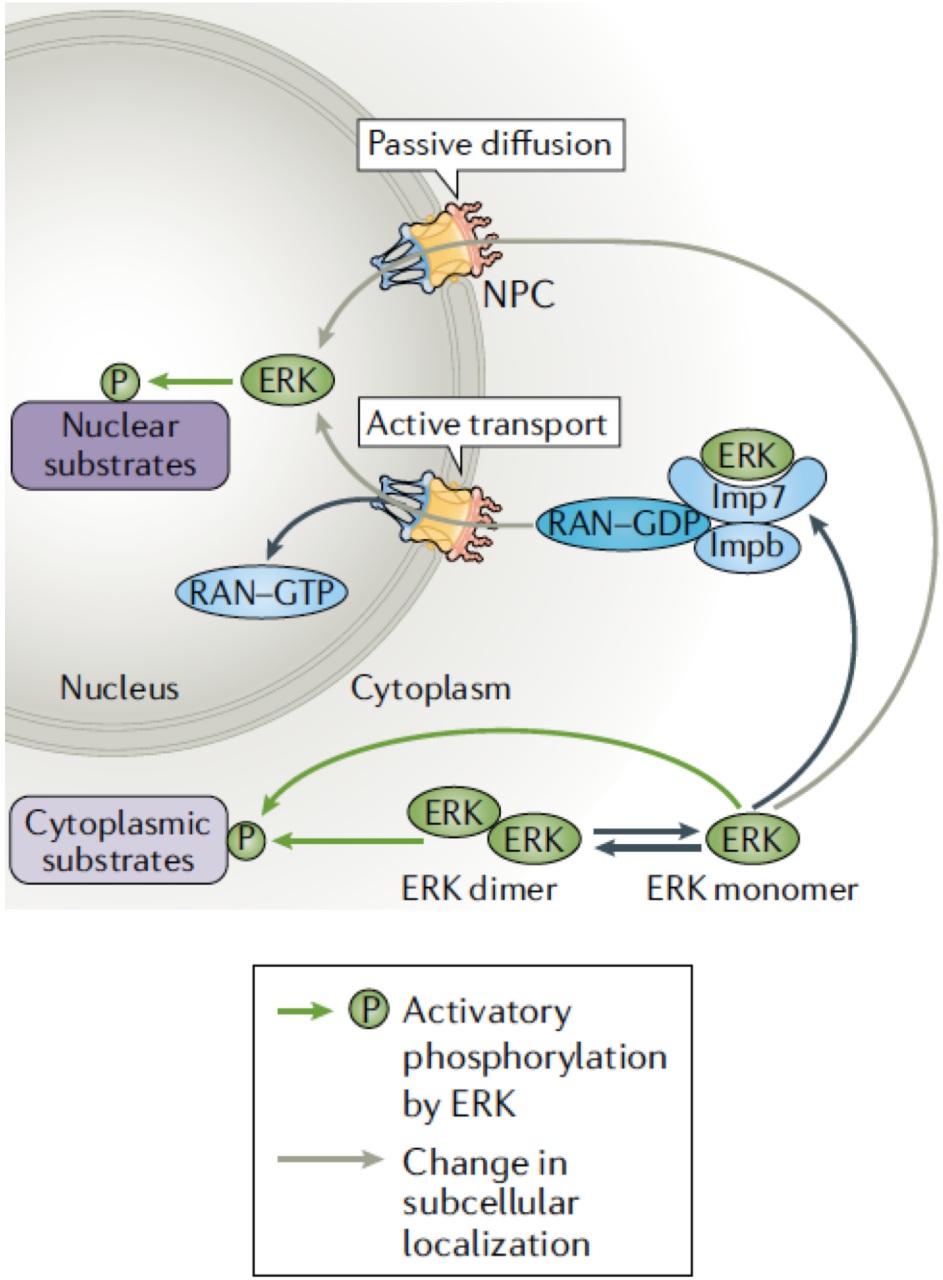
Substrates phosphorilated by ERK include:

- **Transcription factors: Elk-1** (Ets-like transcription factor-1) →**Fos** *(Schulze A. et al. Genes Dev 2001)*
- **Cell-cycle related proteins: Mdm2; p27kip, ARF**
  (Halaschek-Wiener J. et al. Cell Signal 2004; Xaus J. et al. Immunobiology 2001)
- **Apoptotic regulatory proteins: Bim, Bad, Caspase-9** (inactivation)



The biological outcomes of signaling through Raf/MEK/ERK are the increase of cellular proliferation and prolonged cell survival

Subcellular localization of activated ERK

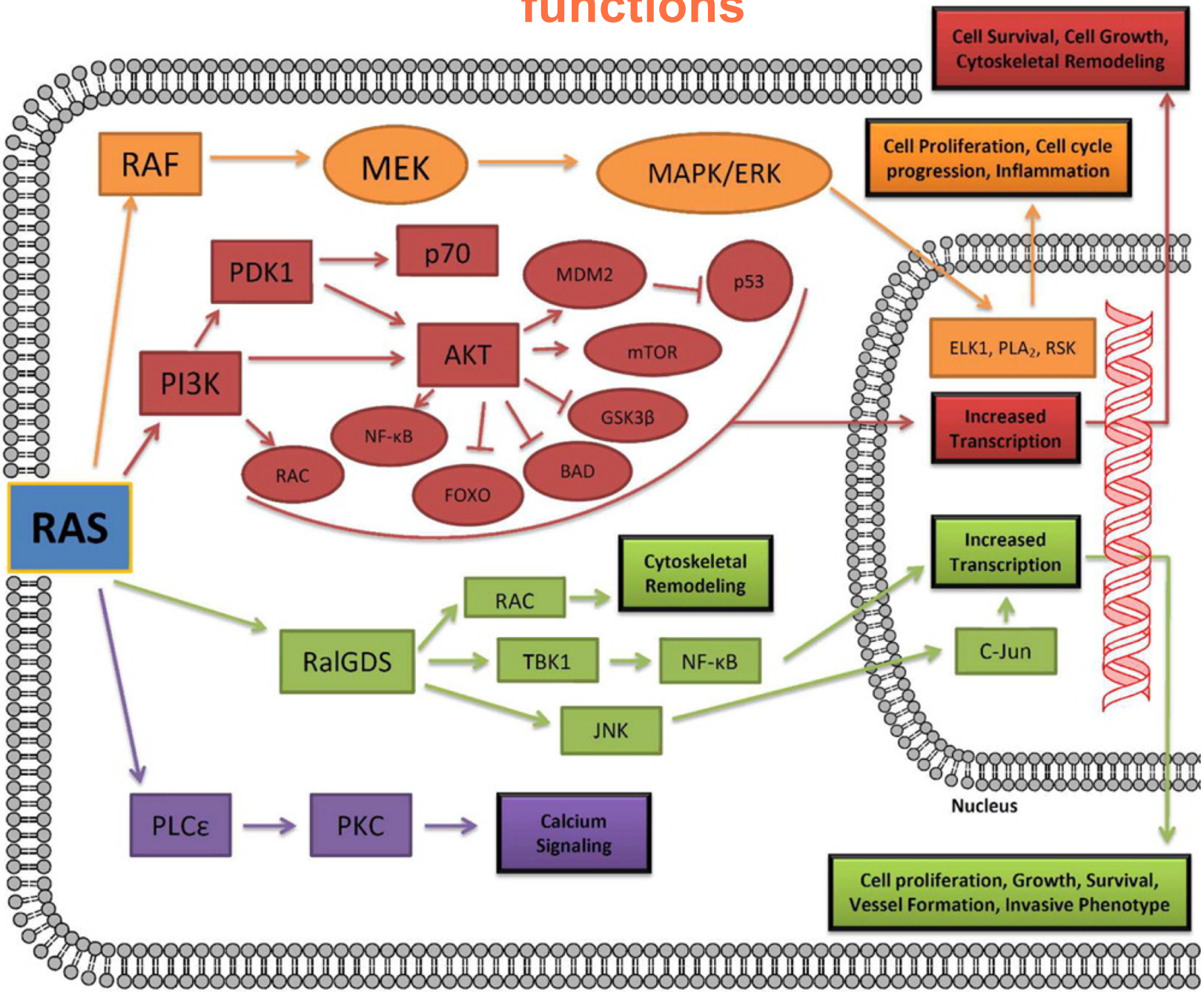


ERK localization in the cell membrane and endomembranes is dictated by interactions with various scaffolding proteins, but the mechanisms are poorly characterized.
Inactive ERK is thought to be excluded from the nucleus and retained in the cytoplasm through interactions with several factors.
This process is dependent on RAN GTPase activity.

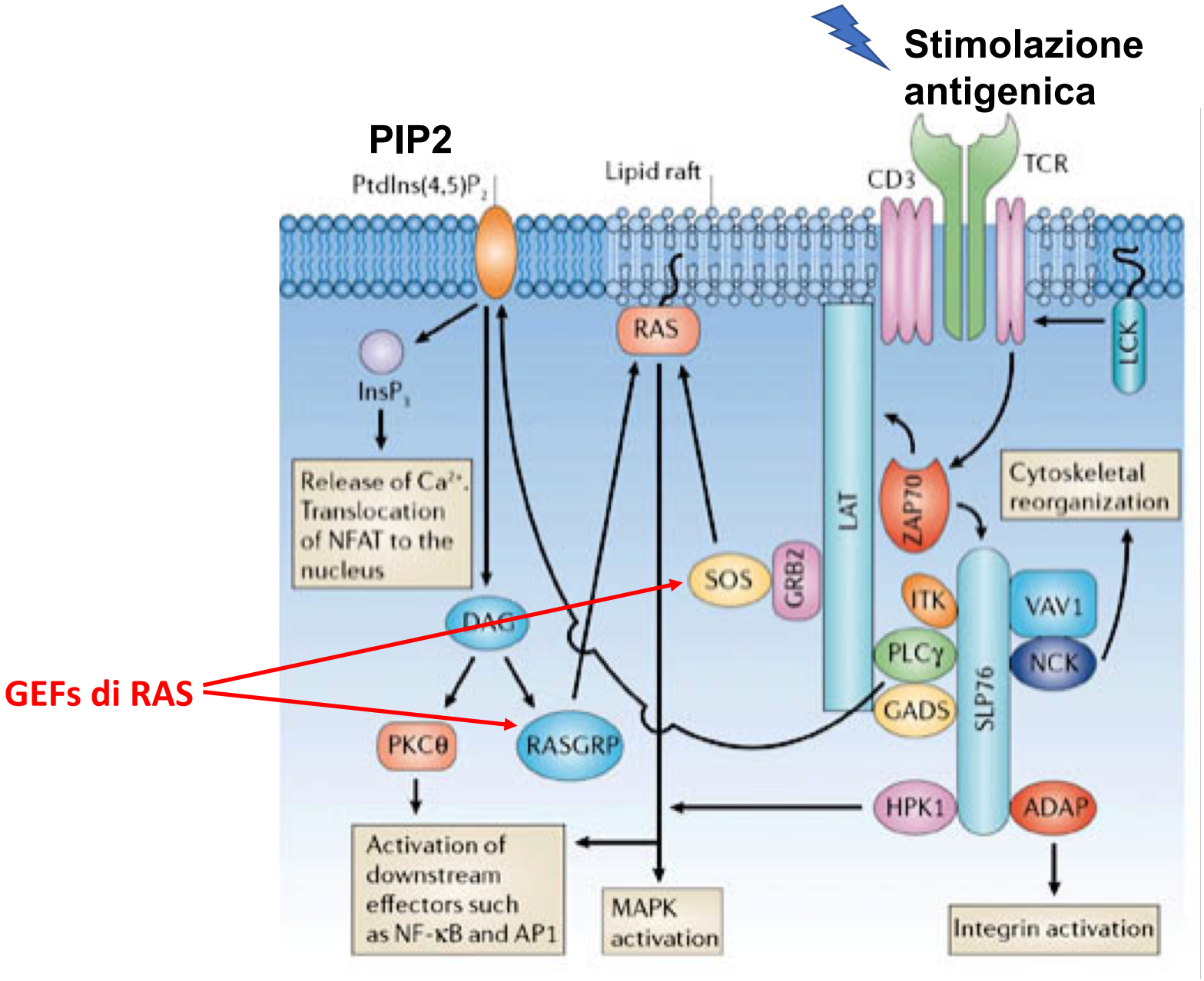
ERK activation triggers rapid nuclear entry.
ERK can passively diffuse through its interaction with nuclear pore complex (NPC) subunits.
ERK can also be actively transported by a RAN–importin-7 (Imp7)- dependent mechanism.

ERK autophosphorylation of the activation segment residue Thr188 (human Thr190) in the cytoplasm was also proposed to promote its nuclear localization. Nuclear ERK phosphorylates a variety of nuclear targets. Activated ERK is also thought to form homodimers in the cytoplasm, which are primarily involved in the phosphorylation ofcytoplasmic substrates.

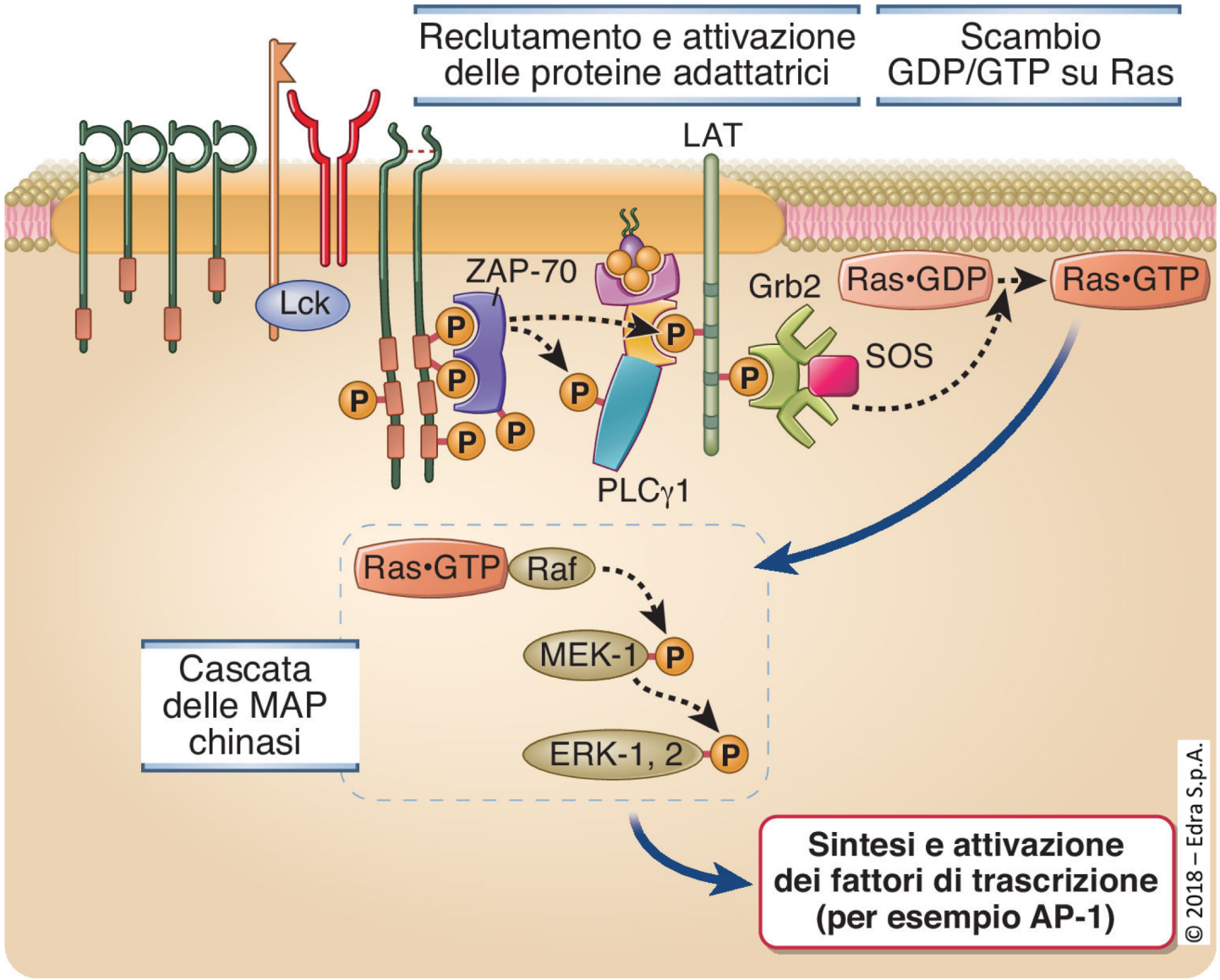
RAS-regulated signaling pathways and biological functions



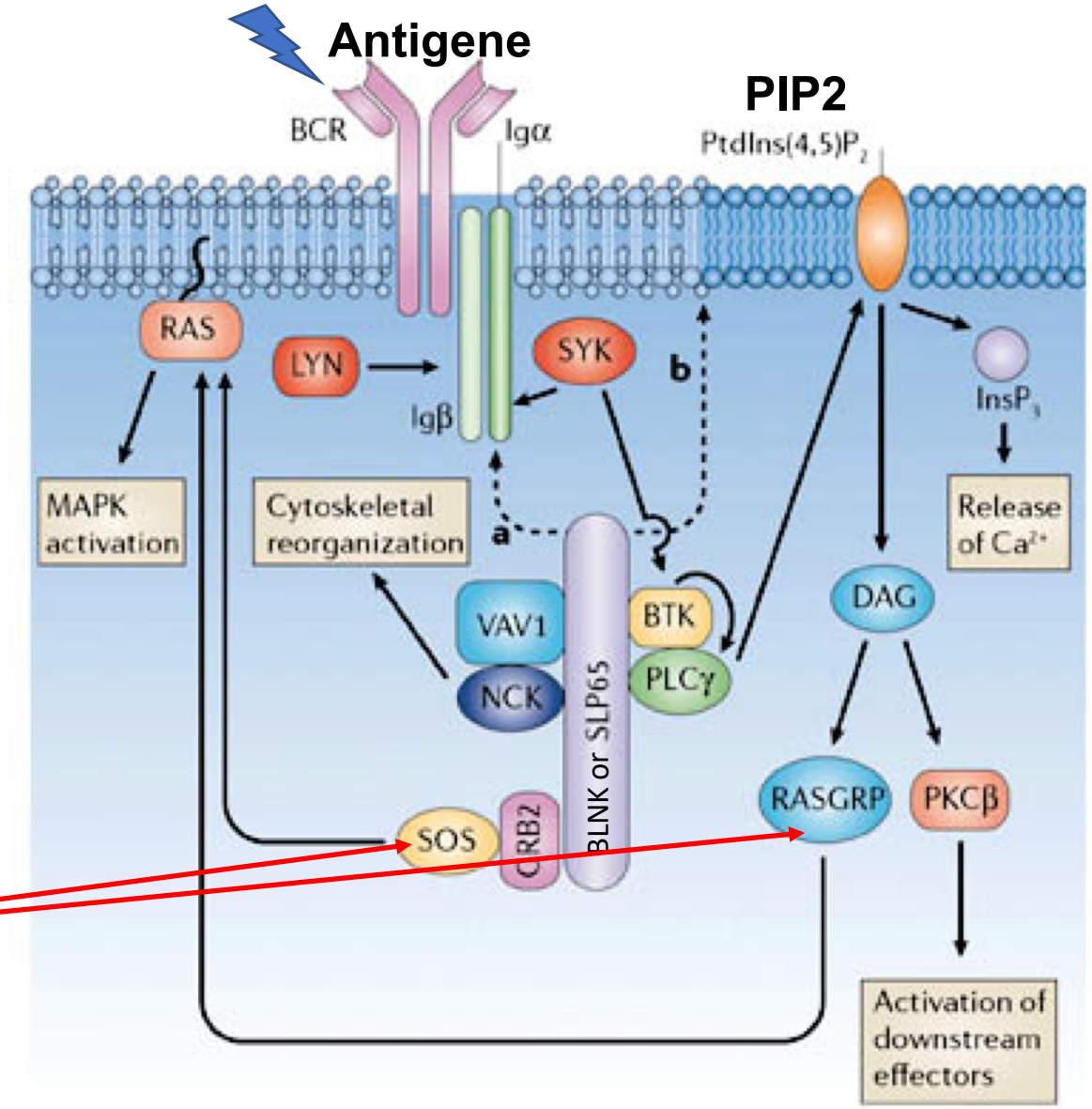
TCR e la cascata RAS/RAF/MEK/ERK



Attivazione dei linfociti T : la via di Ras e delle MAP chinasi



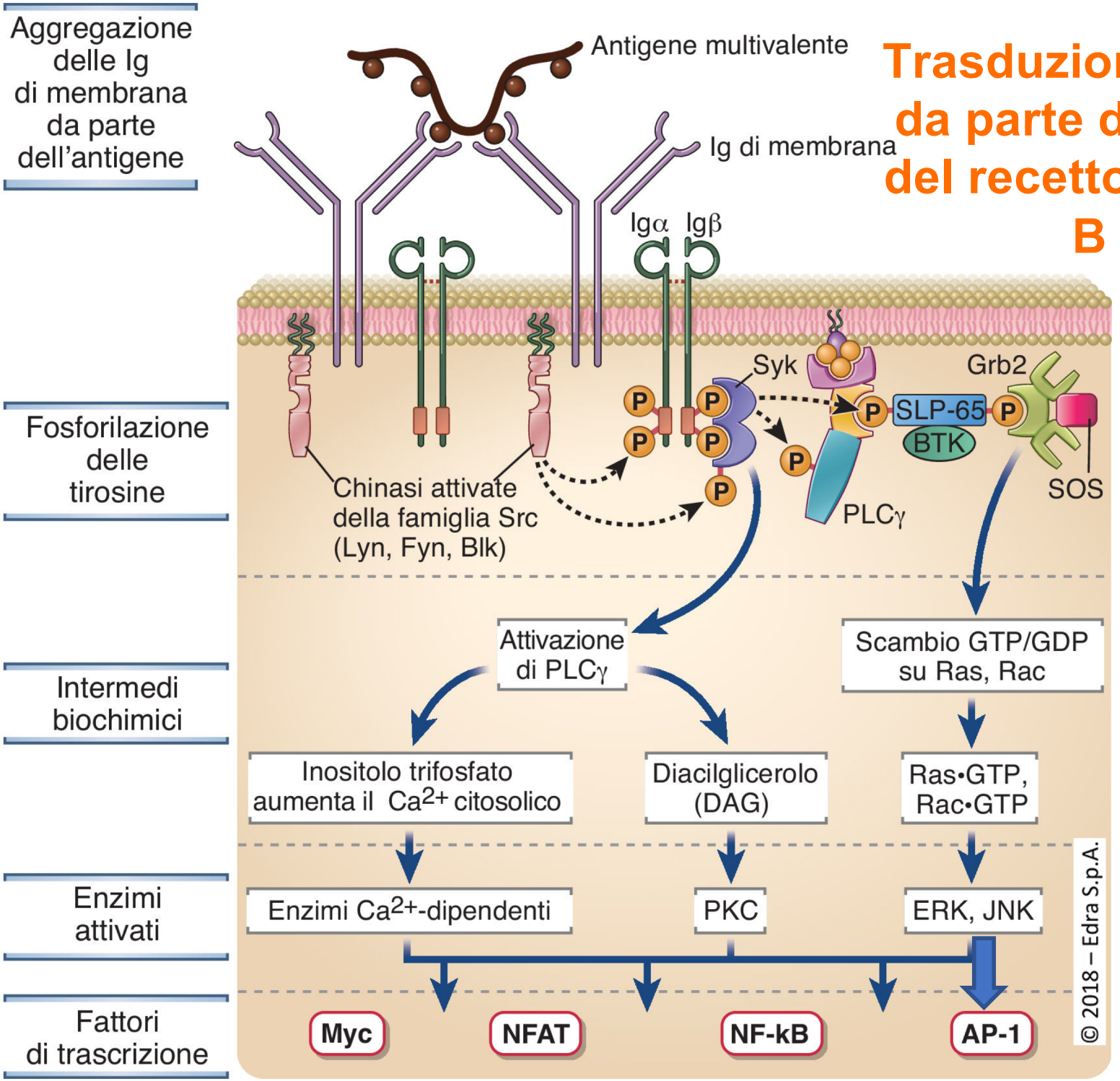
BCR and RAS/RAF/MEK/ERK

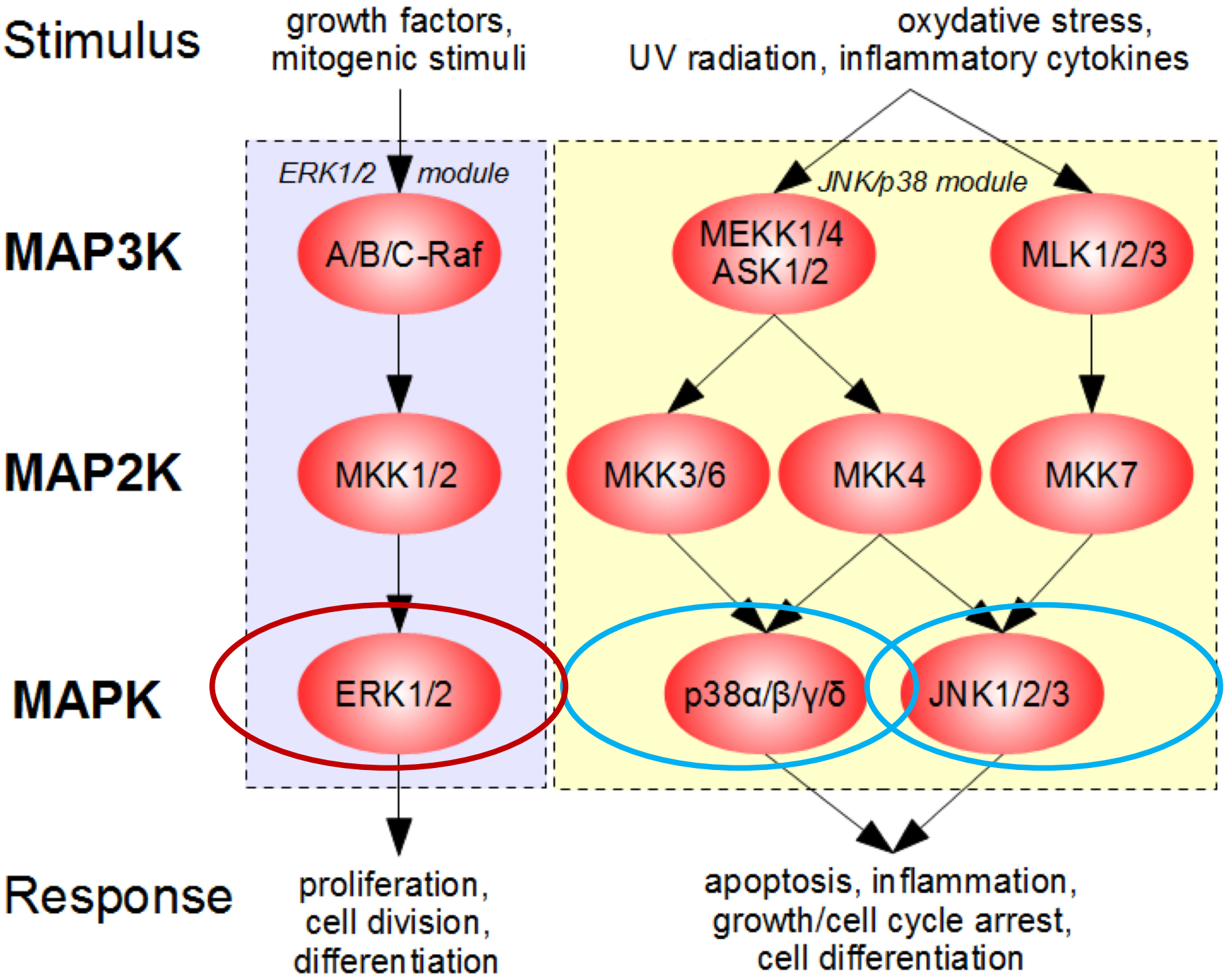


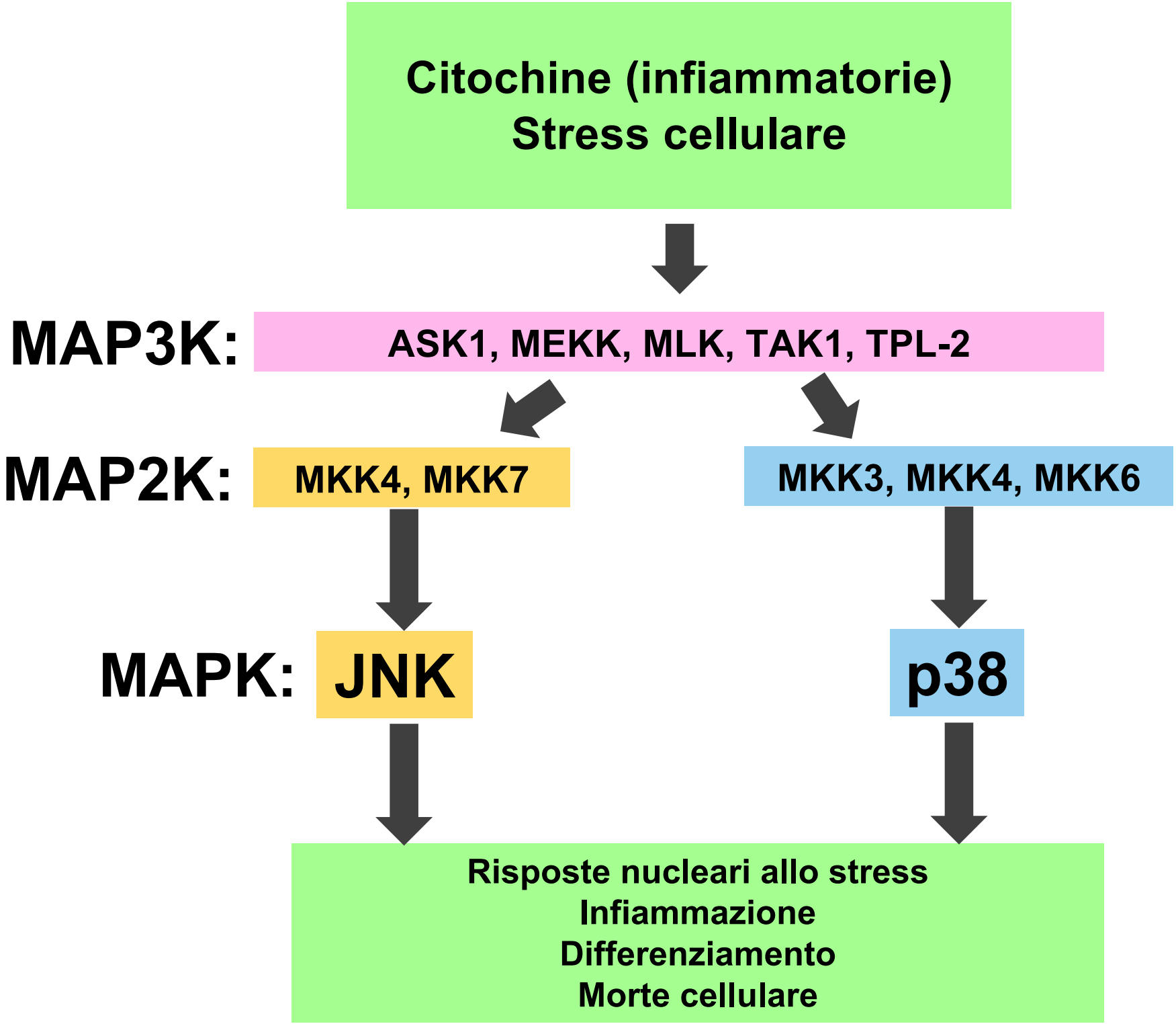
GEFs di RAS

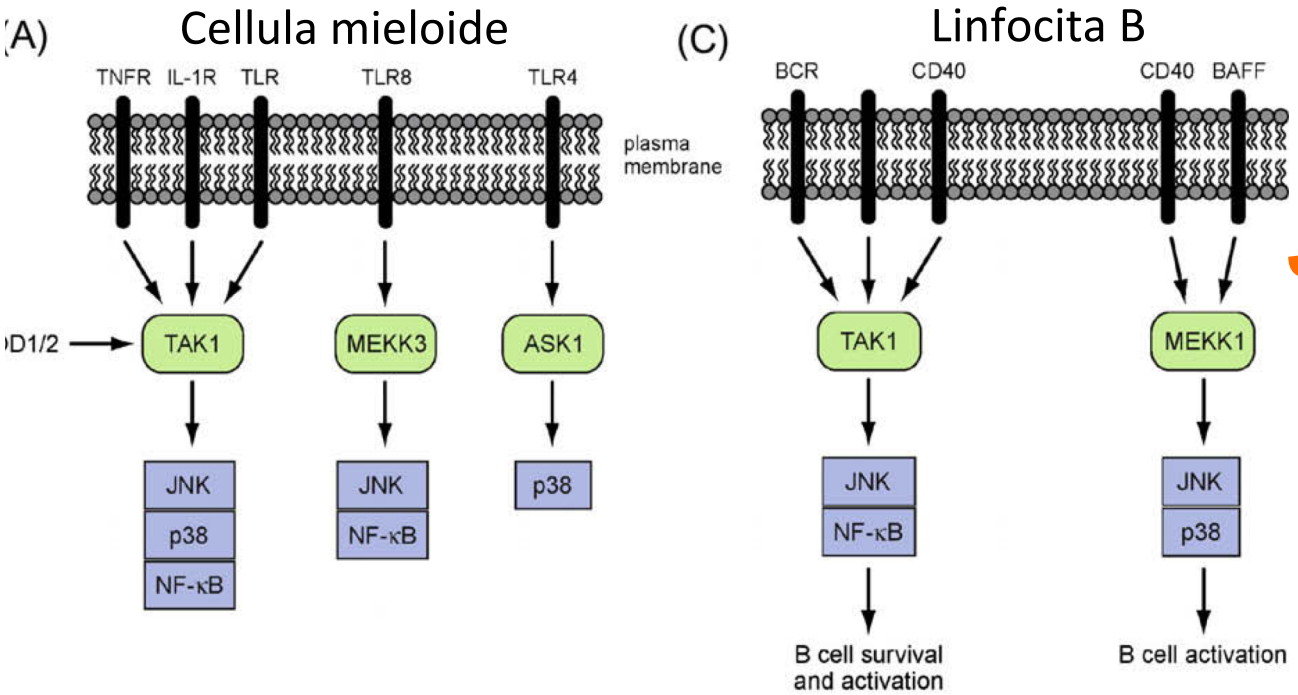
SLP65 o BLNK o B cell linker

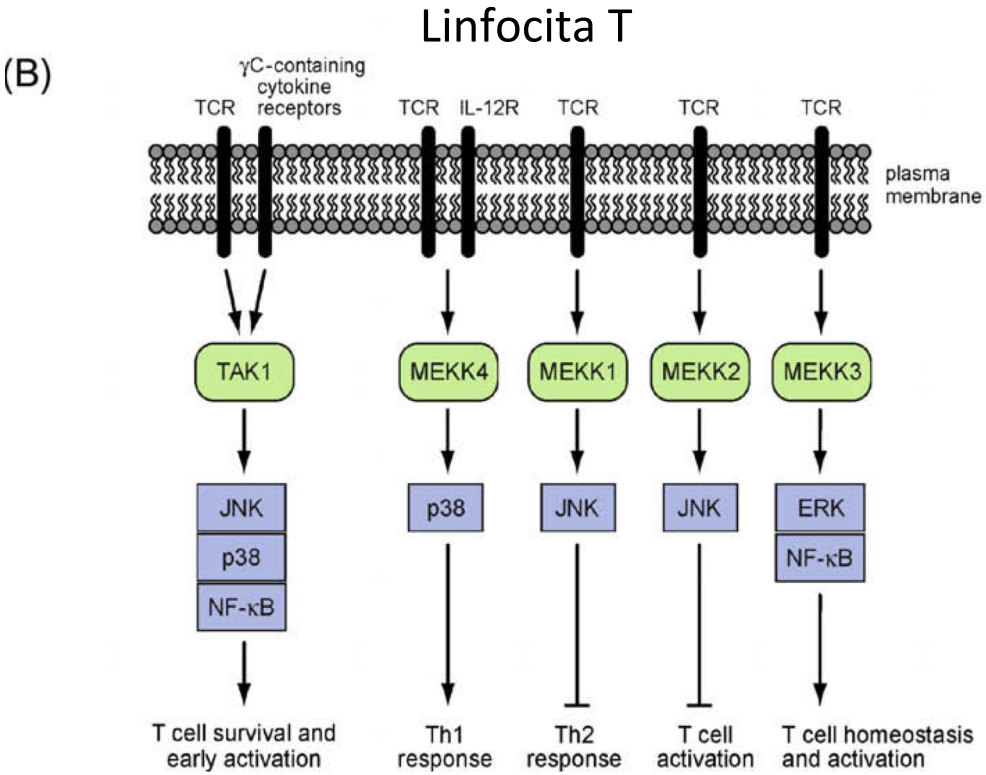
Trasduzione del segnale da parte del complesso del recettore dei linfociti B (BCR)




Stimulus
growth factors,
mitogenic stimuli
oxydative stress,
UV radiation, inflammatory cytokines
ERK1/2 module
JNK/p38 module
MAP3K
A/B/C-Raf
MEKK1/4
ASK1/2
MLK1/2/3
MAP2K
MKK1/2
MKK3/6
MKK4
MKK7
MAPK
ERK1/2
p38α/β/γ/δ
JNK1/2/3
Response
proliferation,
cell division,
differentiation
apoptosis, inflammation,
growth/cell cycle arrest,
cell differentiation


Citochine (infiammatorie)
Stress cellulare
MAP3K:
ASK1, MEKK, MLK, TAK1, TPL-2
MAP2K:
MKK4, MKK7
MKK3, MKK4, MKK6
MAPK:
JNK
p38
Risposte nucleari allo stress
Infiammazione
Differenziamento
Morte cellulare





JNK e p38 MAPK

TLRs, TNFR, IL-1R, BCR, TCR, CD40, BAFF: Attivazione di **MAP3Ks,** Ser/Thr chinasi che fosforilano **MAP2K** attivandole che a loro volta fosforilano e attivano **JNK** e/o **p38 MAPK**.

MAP3K comuni: **TAK1, MEKK1, MEKK2, e ASK1**

The p38 MAPK family

- **p38 MAPK is a family of four isoforms: p38α, p38β, p38γ and p38δ**
- **p38γ is not expressed by immune or inflammatory cells**

p38 isoform	Distribution*	Inhibited by SB203580	Activated by TAB1	Activated by Tyr323 phosphorylation
p38α	All cells	+	+	+
p38β	T cells	+	–	+
p38δ	T cells, macrophages/ monocytes and neutrophils	–	–	–

*Tissue distribution as determined in REF. 15. p38γ is not expressed by immune or inflammatory cells. There is little detailed information about expression of p38 isoforms by lymphocyte subsets. TAB1, TGFβ-activated-protein-kinase-1-binding protein 1.

p38 MAP chinasi

- Originariamente descritte come SAPK (stress-activated protein kinases) perché capaci di modulare la produzione di TNFα (tumor necrosis factor α; citochina pro-infiammatoria) in monociti stimolati da LPS (Lee et al. 1994) ora considerate mediatori chiave dell'infiammazione.

- Più recentemente le p38 MAP chinasi sono state coinvolte anche nel **ciclo cellulare**, nella **morte cellulare**, nel **differenziamento** e nella **senescenza**.

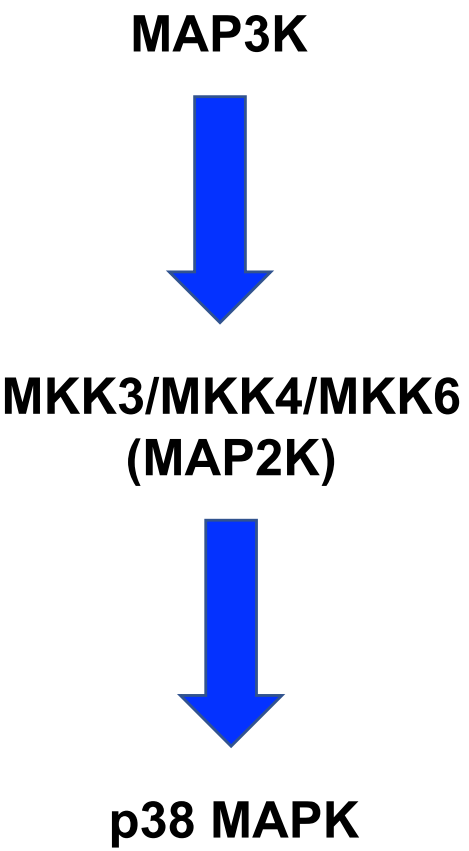
- Quattro isoforme: p38α e p38β sono espresse ovunque; p38γ nel muscolo scheletrico e p38δ nei testicoli, nel pancreas e nell'intestino tenue.

- Attivate da un'ampia gamma di stimoli di stress e citochine.

- Targets terapeutici per lo sviluppo di terapie antinfiammatorie in diverse patologie: **Artrite Reumatoide, morbo di Crohn, psoriasi e asma.**

Attivazione di p38 MAPKs

- Le P38 MAPKs contengono la sequenza caratteristica –**TGY**–, **Treonina**-**Glicina**-**Tirosina**.
- La **fosforilazione** sia di **T** che di **Y** all'interno di questa sequenza caratteristica è necessaria per l'attivazione di p38 MAPK.
- Questa fosforilazione si ottiene tramite una cascata di segnali che coinvolge una MAPK chinasi (MAPKK o **MAP2K**) responsabile della fosforilazione della MAPK appropriata e una MAPK chinasi (MAPKKK o **MAP3K**) che fosforila e attiva MAP2K.
- Le principali MAP2Ks che mediano l'attivazione di p38 sono MKK3, MKK4 e MKK6



p38 signalling pathway

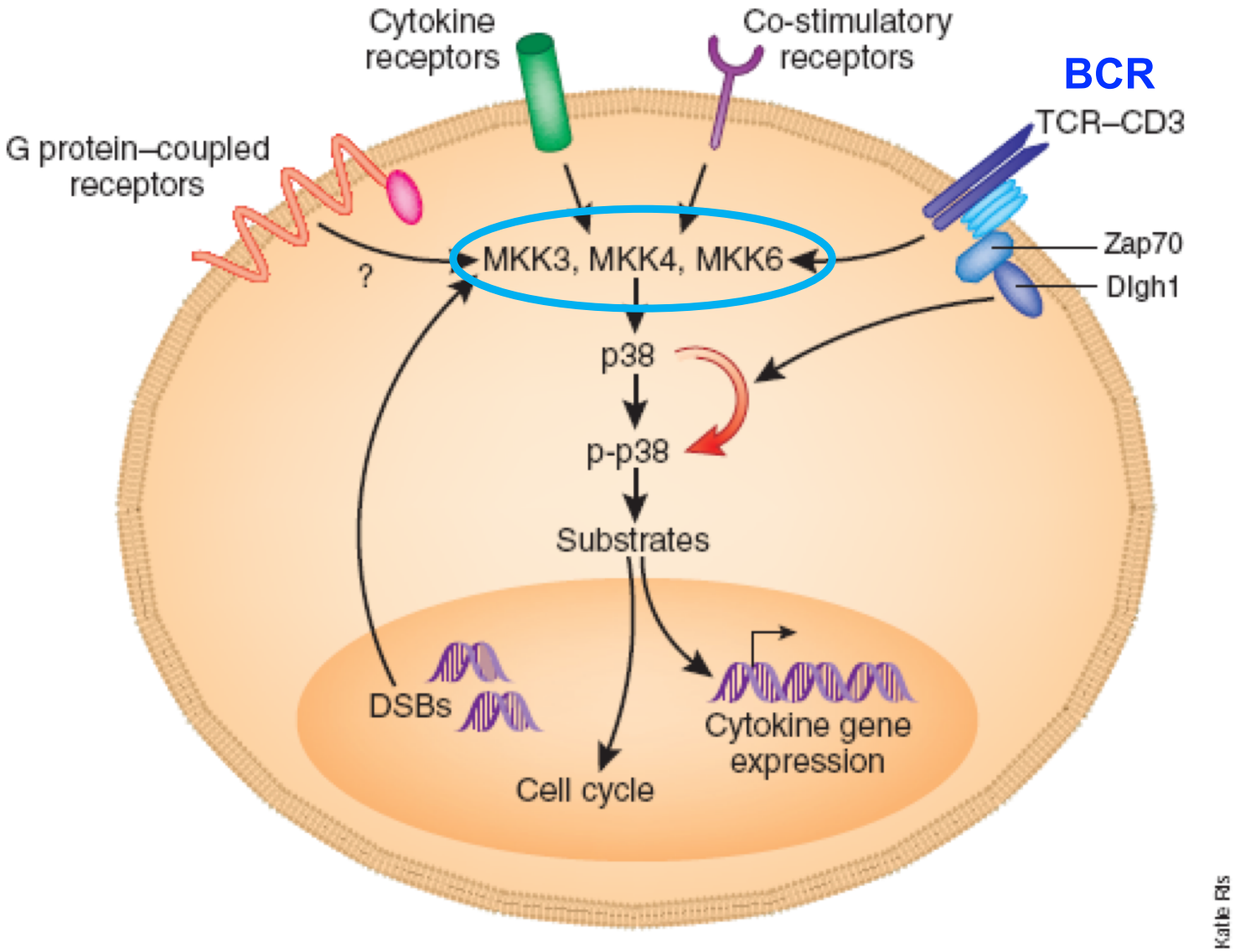
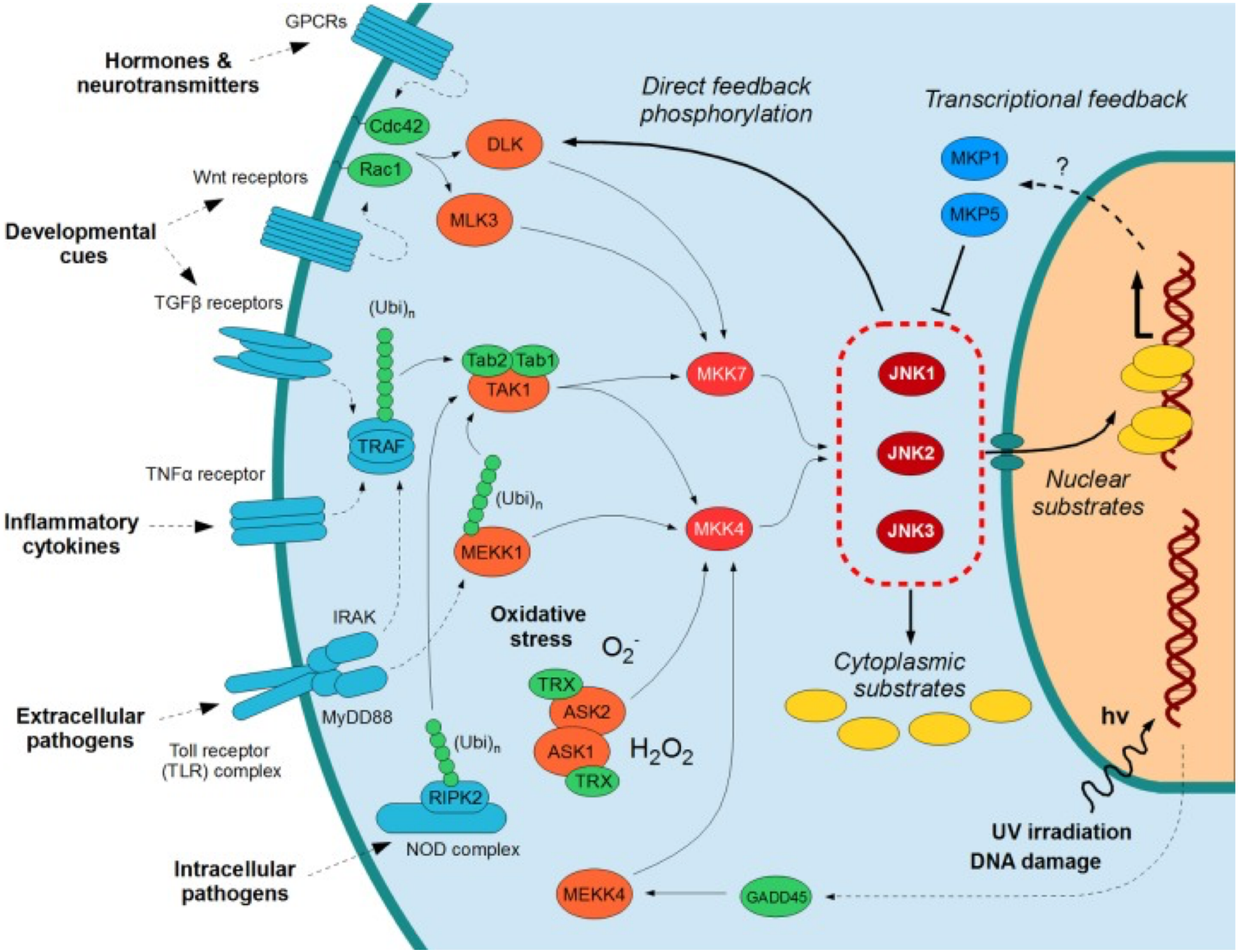


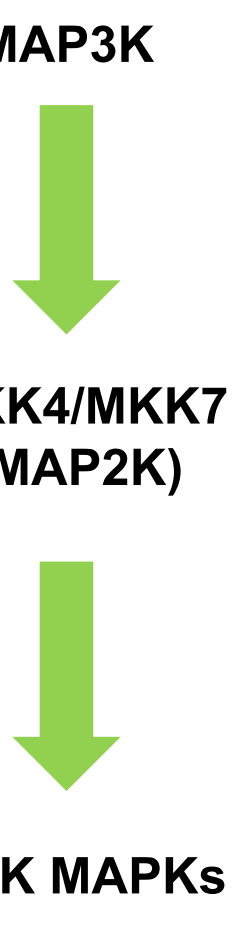
Figure 1 Activation of p38 in T cells. The canonical pathway activates the MAPK kinase isoforms MKK3, MKK4 and MKK6, which then phosphorylate and activate p38. The TCR-mediated pathway requires the scaffold protein Dlgh1, which 'assembles' a signaling module that activates the alternative p38 pathway by phosphorylating (red arrow) p38 (p-p38). DSB, double-stranded break.

JNK signalling pathway



Attivazione di JNK MAPKs

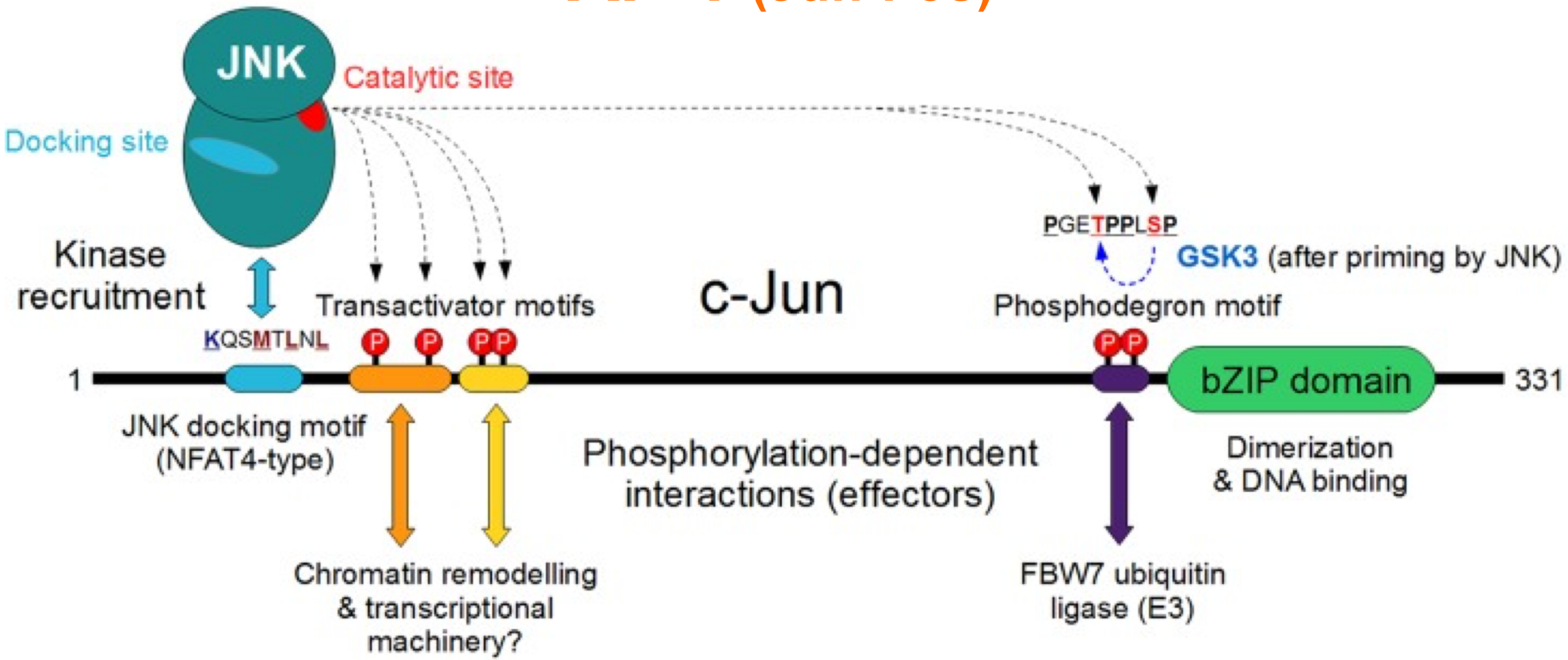
- Le JNK MAPKs contengono la sequenza caratteristica –**TPY**–, **Treonina**-**Prolina**-**Tirosina**.
- La **fosforilazione** di **T** e **Y** all'interno di questa sequenza caratteristica è richiesta per l'attivazione delle JNK MAPKs.
- La fosforilazione delle MAPK si ottiene tramite una cascata di segnali che coinvolge una MAPK chinasi (MAPKK o **MAP2K**) responsabile della fosforilazione della MAPK appropriata e una MAPK chinasi (MAPKKK o **MAP3K**) che fosforila e attiva le MAP2Ks.
- Le principali **MAP2Ks** che mediano l'attivazione di JNK sono MKK4 e MKK7



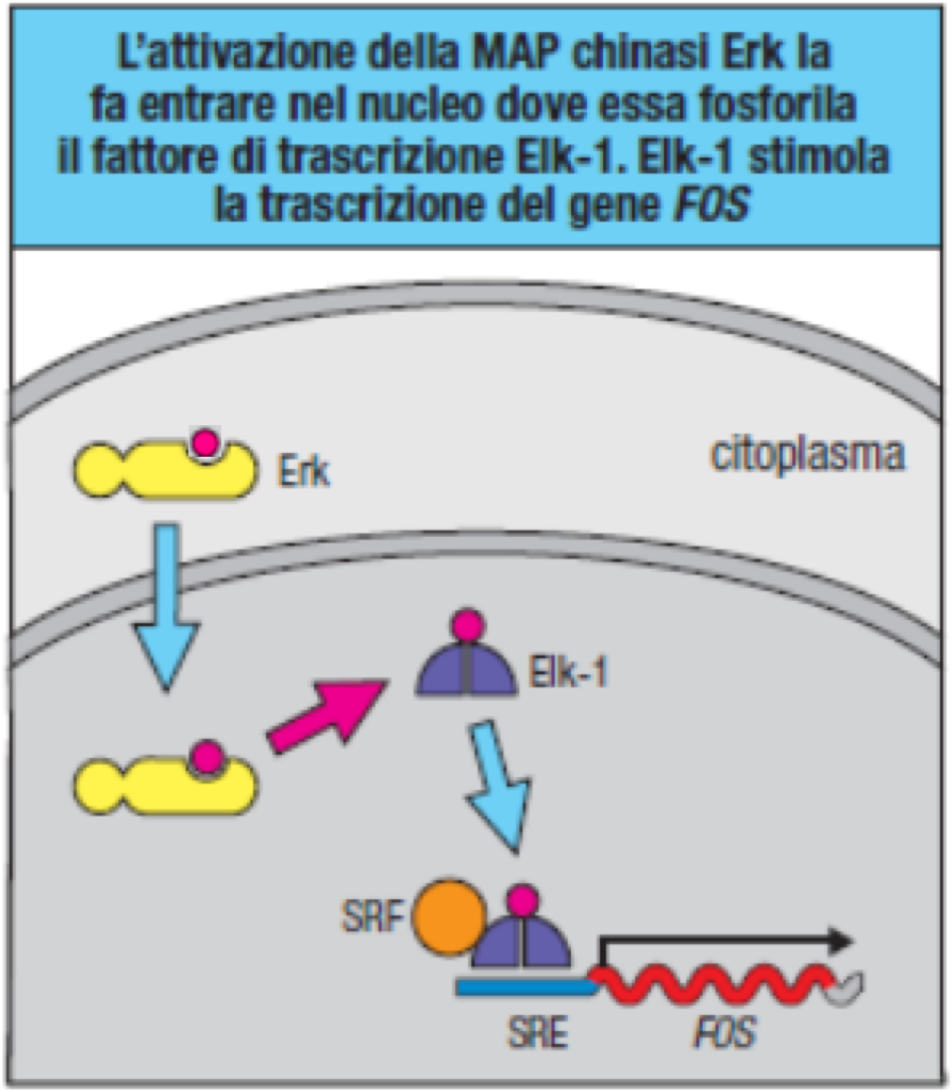
JNK fosforila c-Jun

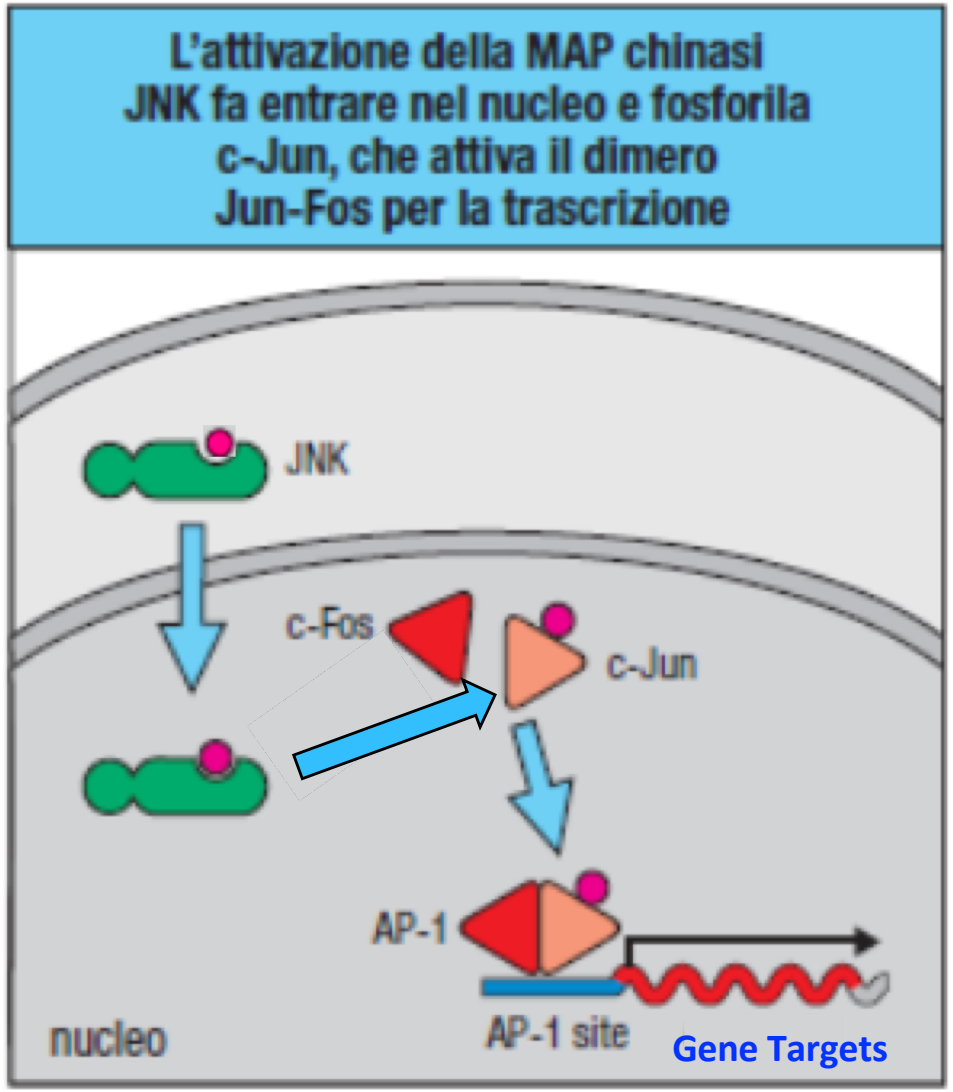
C-JUN è un componente del fattore di trascrizione

AP-1 (Jun-Fos)

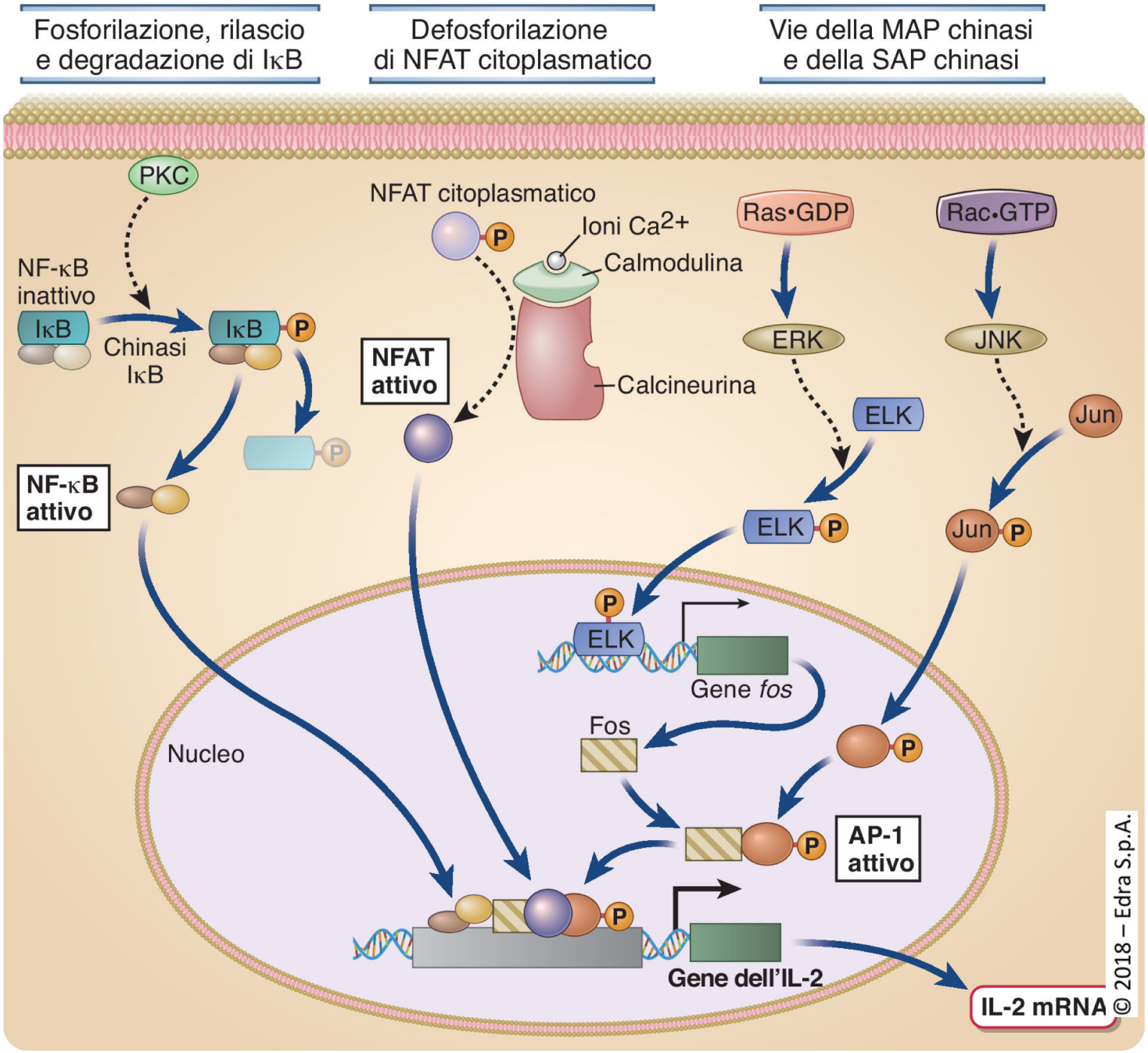


Attivazione del fattore di trascrizione AP1 (Jun/Fos)

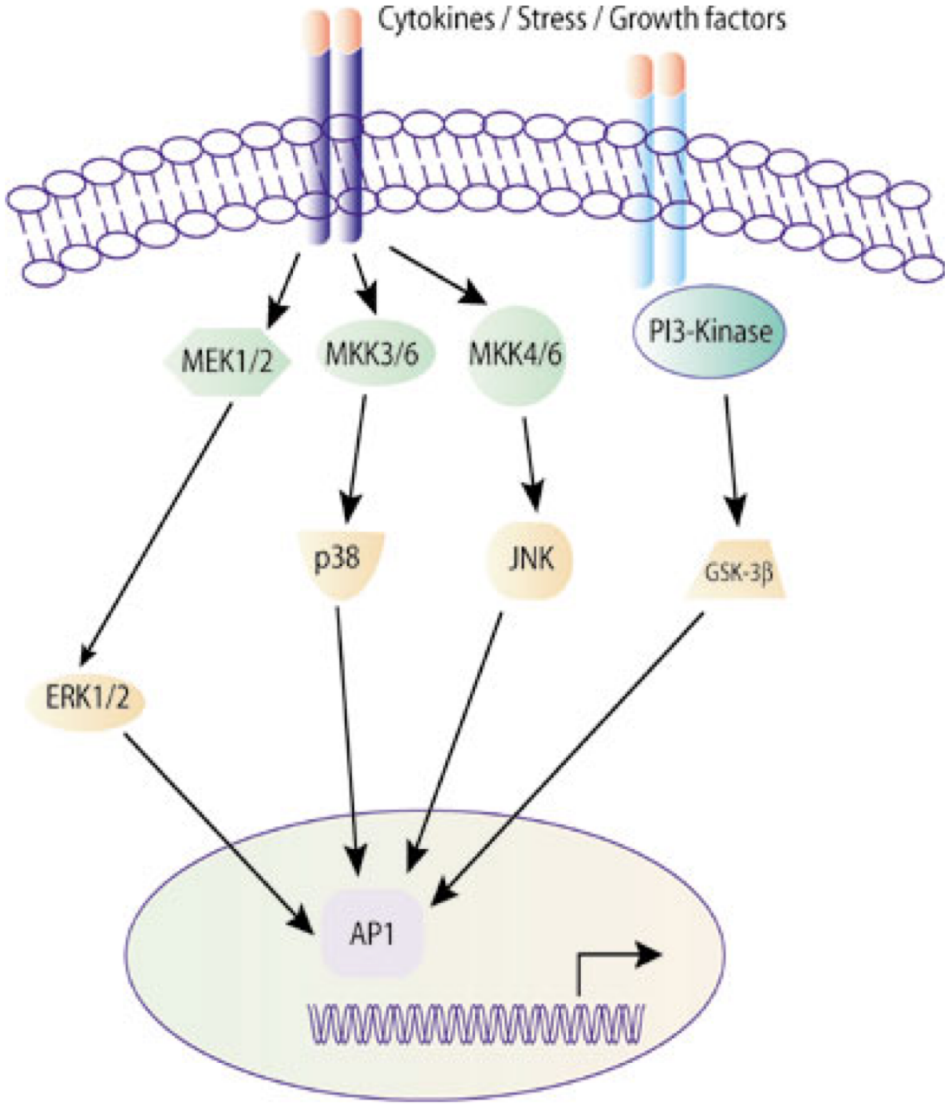




Attivazione di fattori di trascrizione nei linfociti T



Funzioni di AP-1



Attivazione:

Via TCR: AP-1 regola la trascrizione di **IL-2, IL-4, IFNγ**

Via BCR: AP-1 promuove la trascrizione dei geni che codificano **per le catene leggere e pesanti delle Ig**

Via TLR: AP-1 promuove la trascrizione di **TNFα, IL-8 e IL-1**

Inoltre, AP-1 regola la **differenziazione dei monociti in macrofagi**