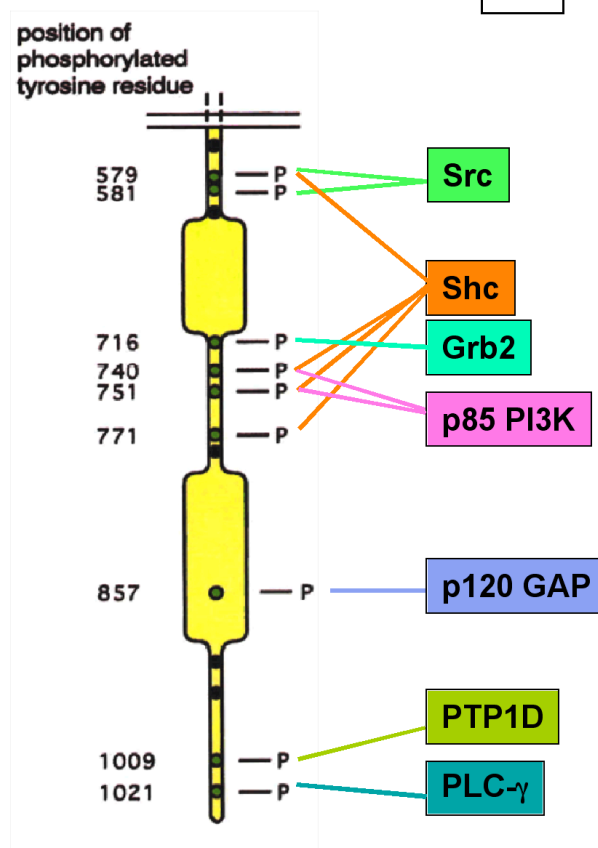
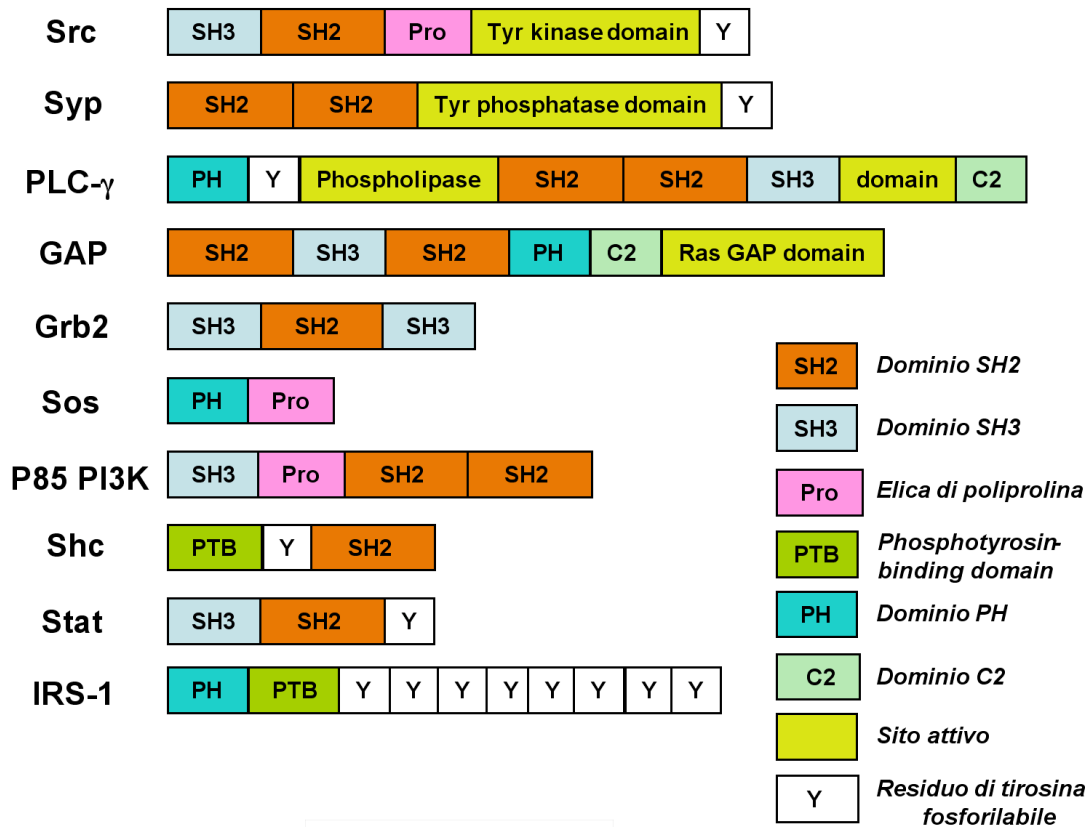
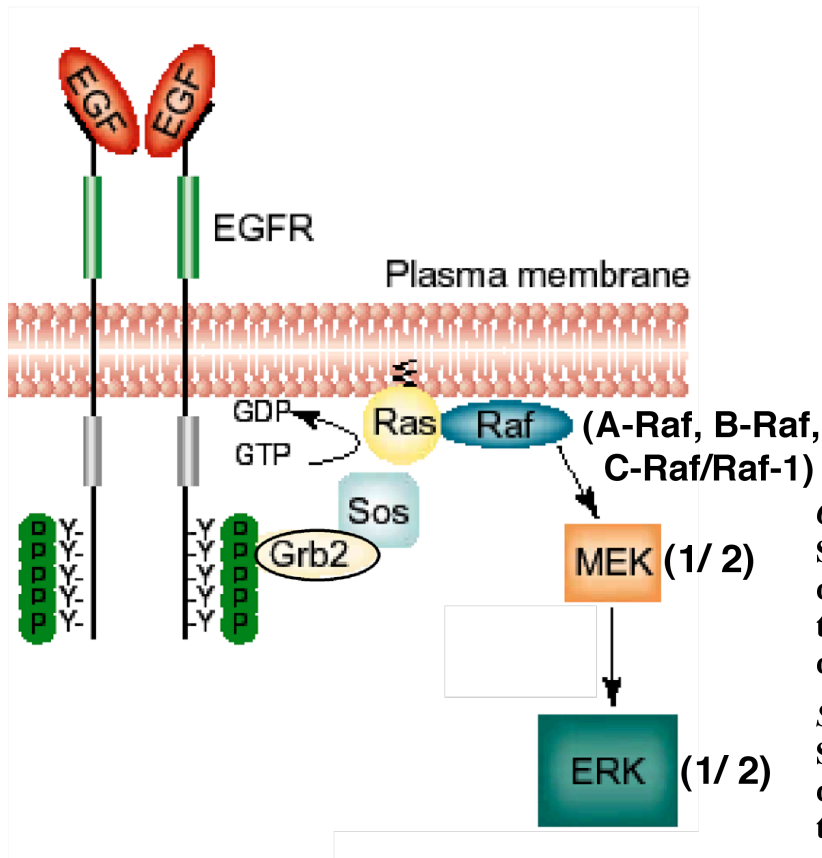


Domini e residui tirosinici di rilievo in proteine reclutate dai recettori per i fattori di crescita



Attivazione di ERK1/2 in seguito a stimolazione dei recettori per i fattori di crescita



Chinasi a funzione mista.

Sono attivate in seguito a fosforilazione di due residui conservati di serina e treonina situati nella *loop* di attivazione del dominio catalitico.

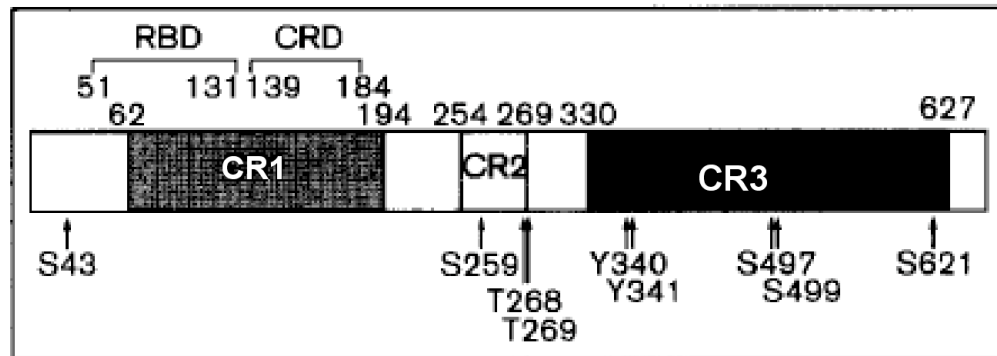
Serina/treonina chinasi.

Sono attivate in seguito a fosforilazione di due residui conservati di treonina e tirosina situati nella *loop* di attivazione del dominio catalitico (T-X-Y)

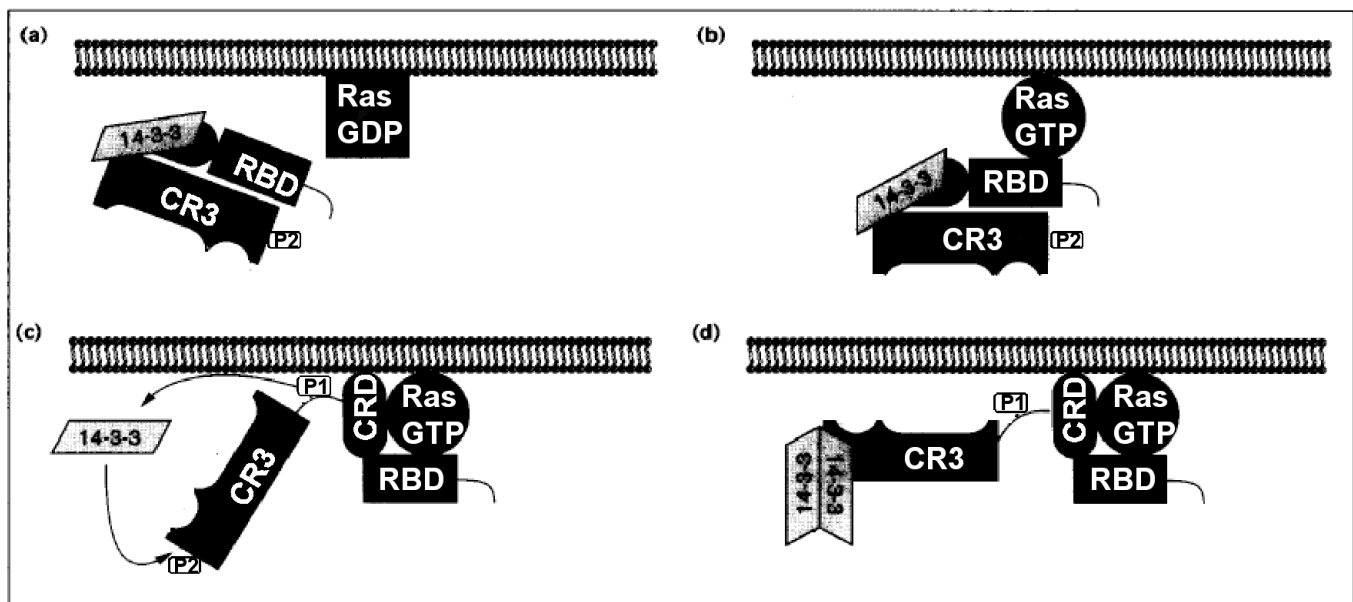
Sequenza degli eventi che portano all' attivazione di ERK:

- 1) Il recettore tirosin-chinasico attivato recluta il complesso Grb2/Sos tramite il dominio SH2 presente su Grb2.
- 2) Sos funge da GEF per la small G protein Ras e la attiva.
- 3) Ras recluta a sua volta in membrana la serina/treonina chinasi Raf (MAPKKK) e la attiva.
- 4) Raf fosforila ed attiva MEK (MAPKK).
- 5) MEK fosforila ed attiva ERK (MAPK), consentendone la traslocazione nel nucleo, ove fosforila ed attiva una serie di fattori di trascrizione.

Struttura ed attivazione di Raf

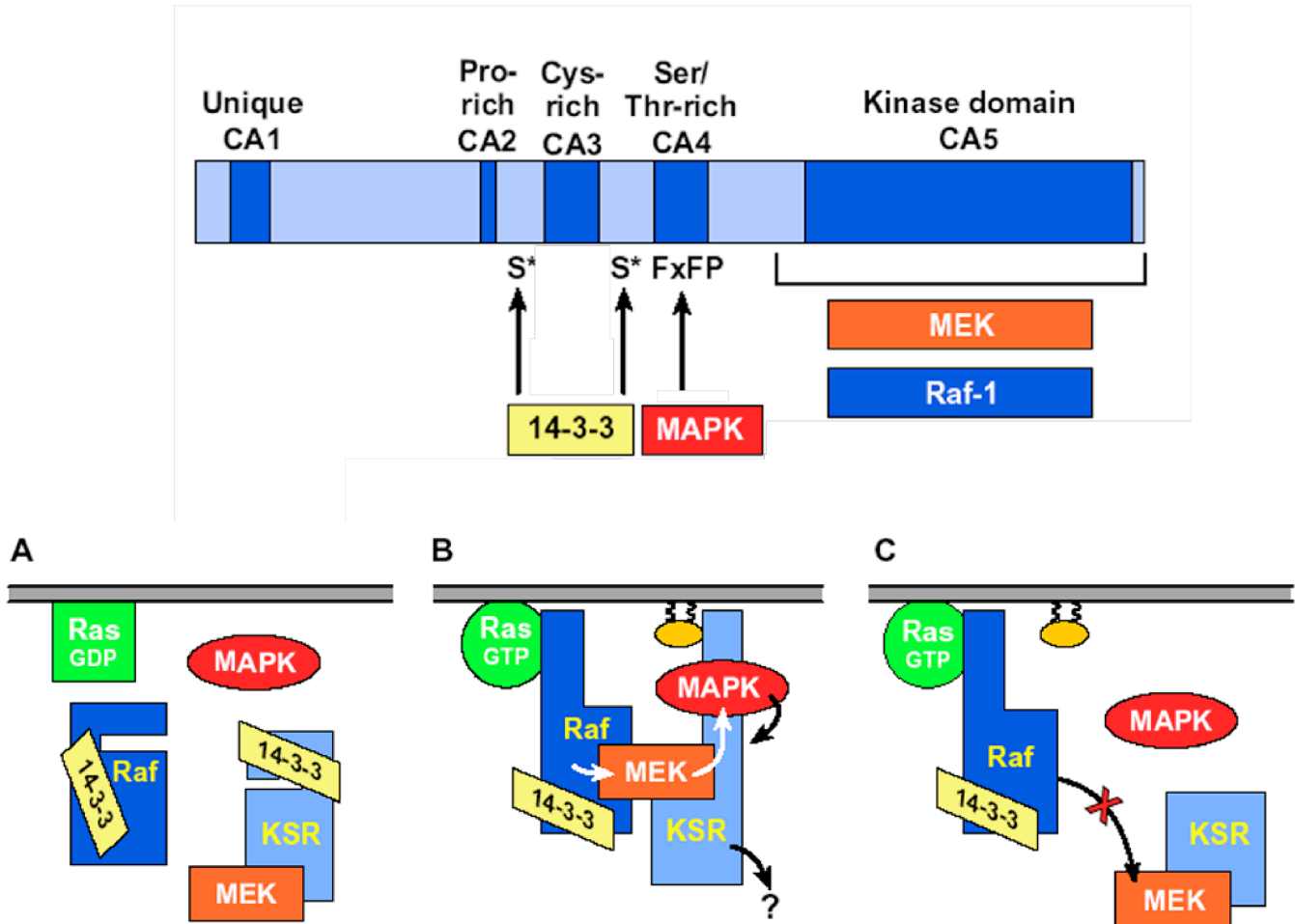


Schematic depiction of the kinase Raf-1. Numbers indicate amino acid position. Three regions are highly conserved among Raf proteins, namely conserved region (CR)1, CR2, and CR3. CR1 is rich in cysteine residues; CR2 is rich in serine/threonine residues; CR3 is the kinase domain. The two Ras-binding domains, RBD and CRD, are indicated. Sites of Raf-1 phosphorylation are also shown. S259 and S621 each constitute part of the consensus motif for 14-3-3 binding. T268 and T269 are the Raf-1 autophosphorylation sites. Y340 and Y341 are *in vivo* sites of tyrosine phosphorylation by Src family of kinases, that enhance the catalytic activity of Raf-1. S497 and S499 have been implicated to be sites phosphorylated by PKC.



Putative model for Raf-1 activation. (a) When Raf-1 is in an inactive state, 14-3-3 is bound to it through the Ser259 phosphorylation site (P1) and perhaps the CRD. This interaction obscures the CRD and stabilizes the inactive Raf-1 conformation. Ras is GDP-bound at this stage. (b) When Ras becomes GTP-loaded, Raf-1's RBD binds to Ras. This will displace 14-3-3 from the Ser259 site and unmask the CRD. (c) The CRD of Raf-1 then becomes accessible for Ras binding. The binding of the RBD and CRD to Ras results in a change in the Raf-1 conformation that serves to expose the kinase domain (CR3). 14-3-3 displaced from the Ser259 site is then free to bind to the higher-affinity Ser 621 site (P2). (d) The interaction between the Raf-1 kinase domain and 14-3-3 may serve to stabilize an active 'open' Raf-1 conformation.

KSR



Model for KSR function.

(A) In a quiescent cell, inactive GDP-bound Ras is located at the plasma membrane, and inactive Raf and KSR proteins are found in the cytoplasm. Inactive MEK stably interacts with the KSR CA5 domain; 14-3-3 proteins bind to both KSR and Raf and perhaps stabilize their inactive conformations.

(B) In response to membrane signaling events, Ras becomes GTP-loaded, thereby inducing the translocation of Raf to the plasma membrane, where it becomes activated. Through the exposure of the CA3 domain and/or by the generation of binding partners for the CA3 domain, KSR also translocates to the membrane, bringing MEK with it. As a result, MEK is localized with Raf-1, its physiological activator. Release of 14-3-3 proteins and localization of KSR at the membrane may also expose the MAPK docking sites, allowing MAPK to bind and become phosphorylated by activated MEK. KSR also serves as a substrate for activated MAPK, and the phosphorylation of KSR may result in the release of MAPK from the complex. Whether KSR phosphorylates a substrate remains inconclusive.

(C) The dominant-inhibitory effect of the isolated KSR kinase domain. MEK is bound to the CA5 domain of KSR and is sequestered in the cytosol since truncation of the N-terminus has removed the CA3 domain required for membrane localization. As a result, activation of MEK and MAPK is blocked.

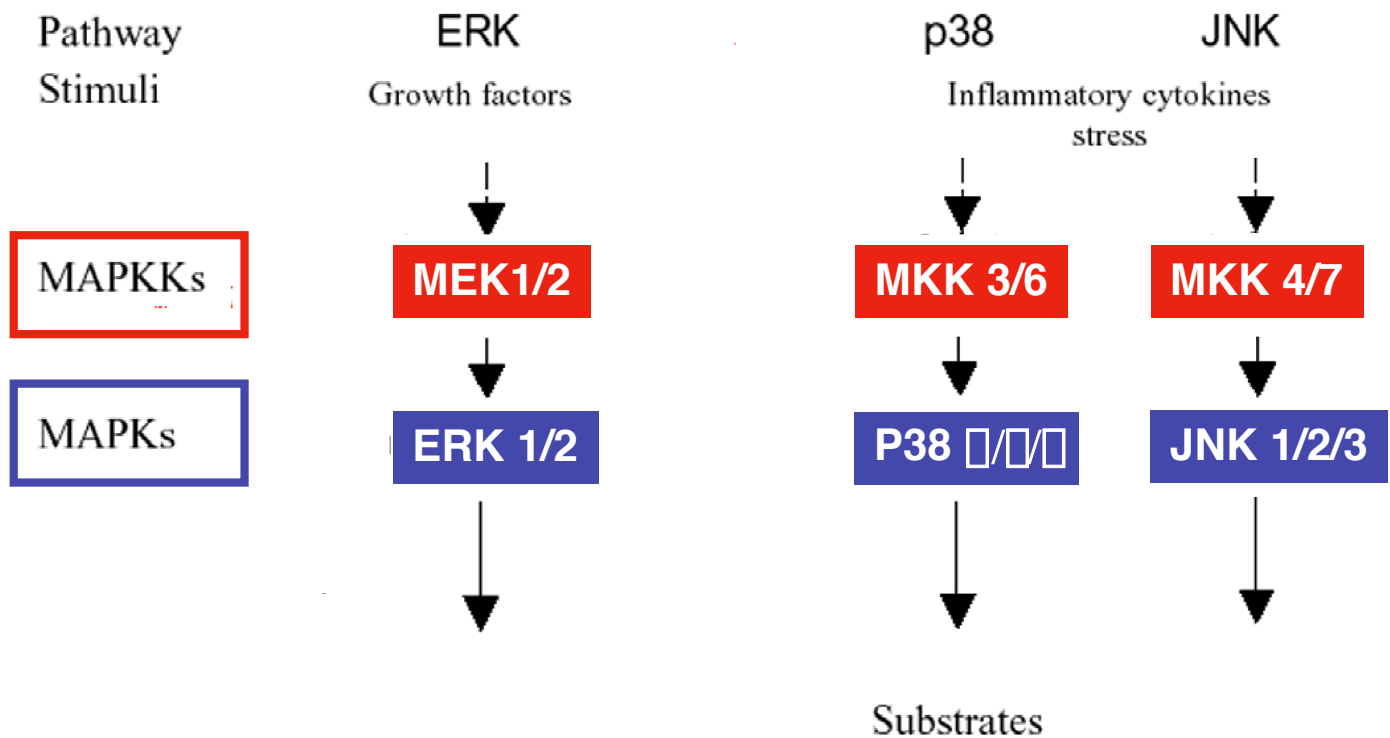
MAP KINASE FAMILIES

Five families of MAPKs have been defined in mammalian cells:

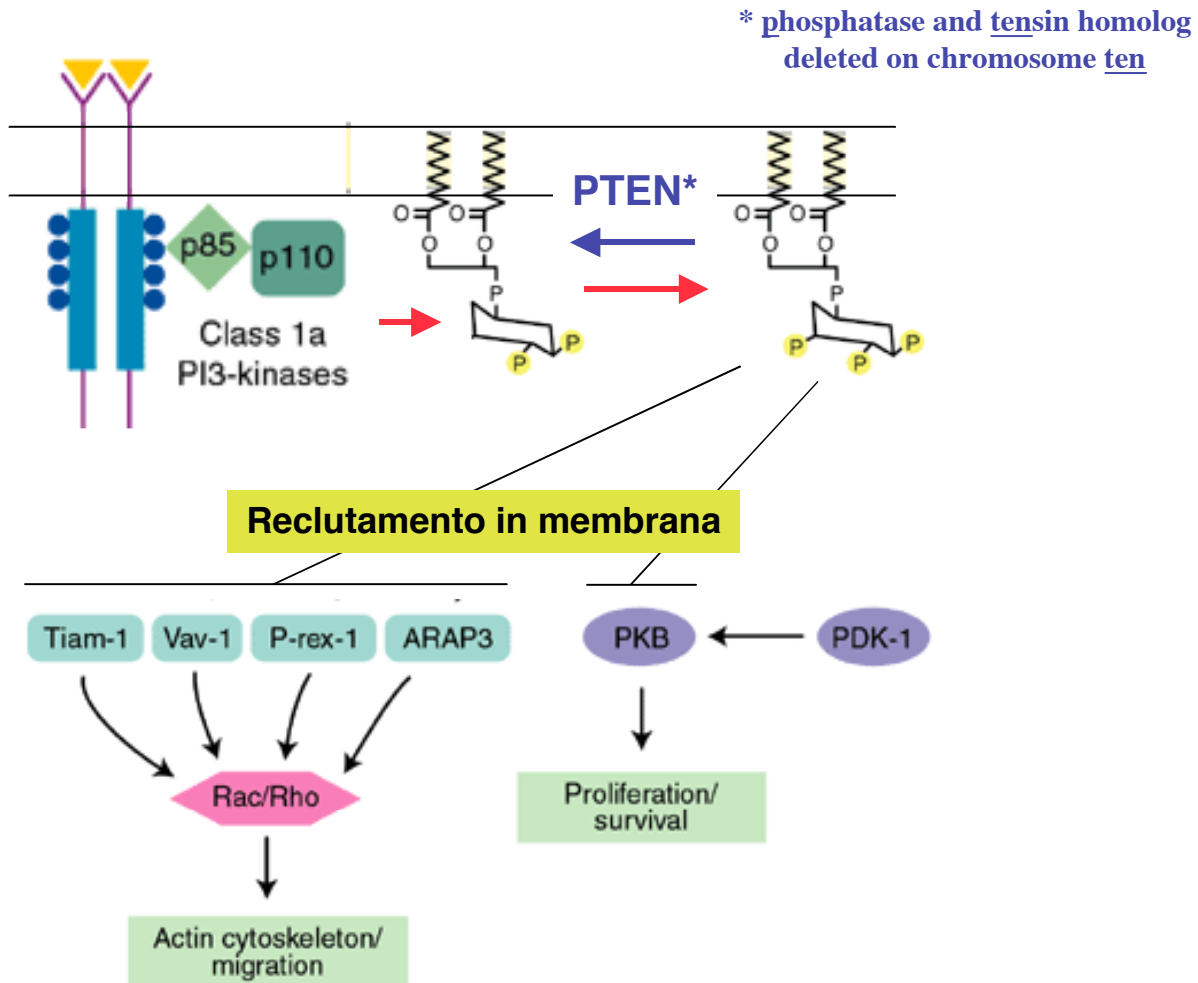
- a) extracellular signal-regulated kinases (ERK1 and ERK2);
- b) p38kinase isozymes (p38 α , p38 β , p38 γ and p38 δ);
- c) Jun N-terminal kinases (JNK1, JNK2 and JNK3);
- d) ERK3/ERK4;
- e) ERK5.

The first three families are best characterized.

Mammalian MAPK pathways



Phosphatidylinositol 3'-kinase

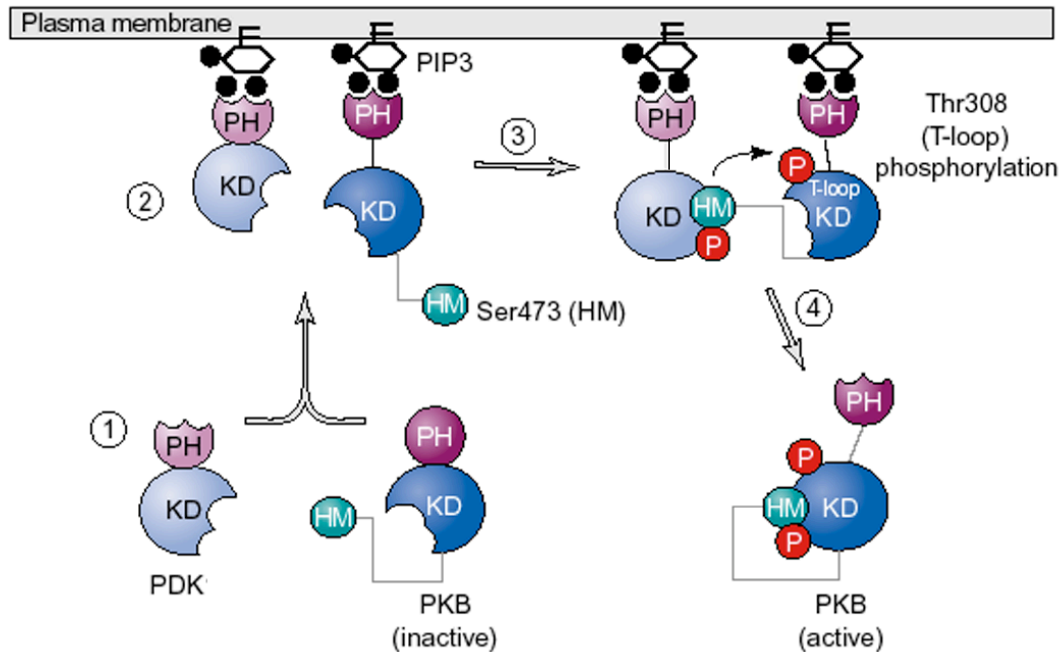


There are multiple isoforms of PI3Ks in mammalian cells, subdivided into three classes. Class I is the best studied and is further subdivided into Class IA and Class IB.

Class IA

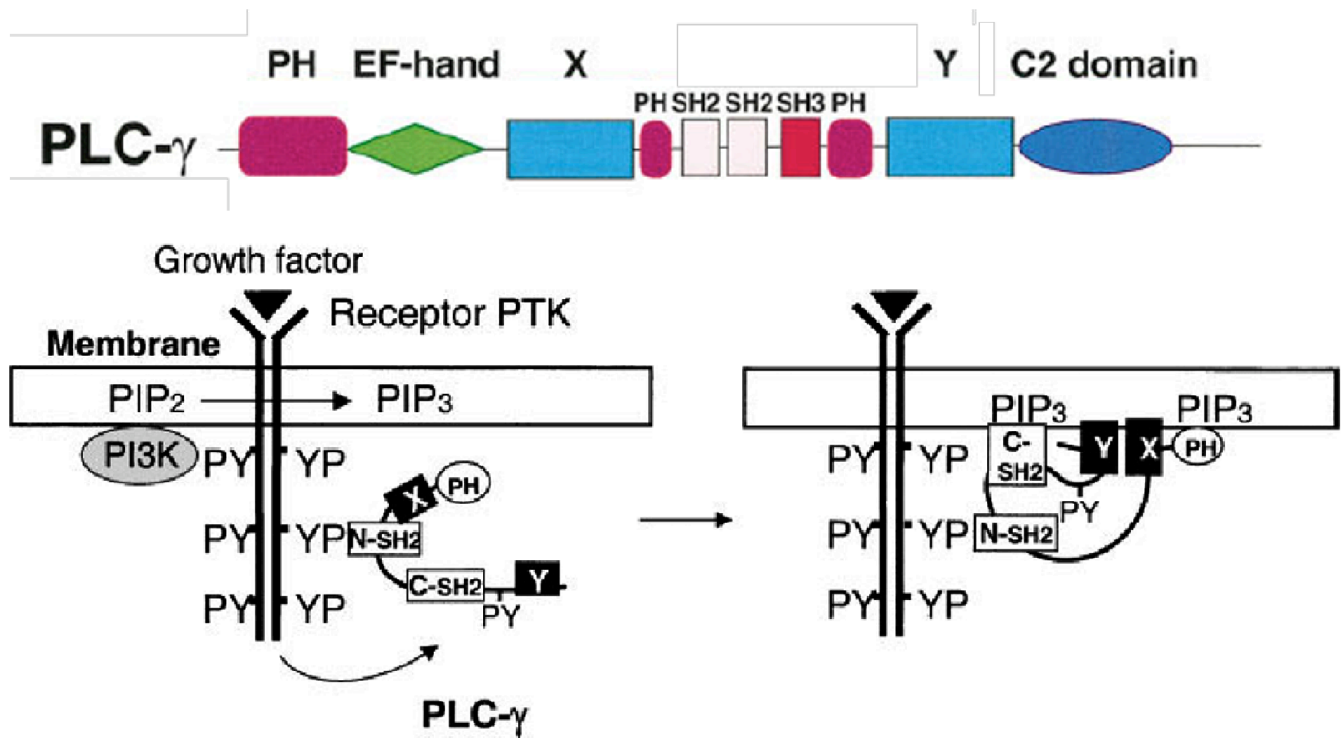
There are three class IA catalytic subunits: p110 α , p110 β e p110 δ . All of these PI3Ks physically interact with a family of SH2 domain-containing regulatory adaptor proteins (p85 α , p85 β and p55 γ). All members of this 'p85' family of adaptors bind to the N-terminal 100 amino acids of the class IA PI3Ks and mediate their activation by binding to posphotyrosine residues of activated growth factor receptors.

Meccanismo di attivazione di PKB/Akt



- 1) La PDK (phosphoinositide-dependent kinase), nella forma attiva, e la PKB/Akt, in quella inattiva, sono entrambe presenti nel citosol ma non interagiscono.
- 2) La produzione di PIP3, operata dall'attivazione di una PI3K, induce il reclutamento in membrana di entrambe le chinasi, mediante i loro domini PH.
- 3) La PDK fosforila inizialmente PKB/Akt sulla serina 473 del dominio HM. La PDK interagisce quindi con il dominio HM fosforilato e viene posizionata in modo da poter fosforilare PKB/Akt nella *loop di attivazione* del dominio catalitico (KD, treonina 308).
- 4) PKB/Akt è ora nella forma attiva.

Fosfolipasi C- γ



Experimental data

- 1) PLC- γ is phosphorylated by activated TKRs at tyrosine residues 771, 783 and 1254. Replacement of Tyr783 with Phe prevents the activation of PLC- γ , but not its association with TKRs.
- 2) Mutation of specific autophosphorylation sites of TKRs prevents receptor association with PLC- γ and growth factor-induced production of IP₃, but not tyrosine phosphorylation of PLC- γ on Tyr783.
- 3) Mutation of PLC- γ at the N-terminal SH2 domain prevents TRK association with PLC- γ and growth factor-induced PIP₂ hydrolysis. By contrast, PLC- γ mutated at the C-terminal SH2 domain is able to hydrolyze PIP₂ to an extent only slightly less than that observed with the wild-type enzyme.
- 4) In growth factor-stimulated cells, prevention of PIP₃ generation, by pharmacological inhibition of PI 3-kinase, results in about 40% decrease in both intracellular generation of IP₃ and the release of intracellular Ca²⁺.

Model for TRK-mediated activation of PLC- γ

(Left) Growth factor stimulation triggers autophosphorylation of TRK of tyrosine residues that function as docking sites for PI 3-kinase and PLC- γ . The TRK phosphorylates and activates the PLC- γ and also activates the PI 3-kinase, which then catalyzes the conversion of PIP₂ to PIP₃. (Right) Phosphorylated PLC- γ likely undergoes substantial conformational changes and is maintained in proximity to the membrane through association of its N-terminal SH2 domain with the receptor and of its PH domain (and possibly its C-terminal SH2 domain) with PIP₃.

Legend: XY, catalytic domain of PLC- γ ; PY/YP, phosphotyrosine. The EF-hand, SH3 and C2 domains of PLC- γ are not shown.