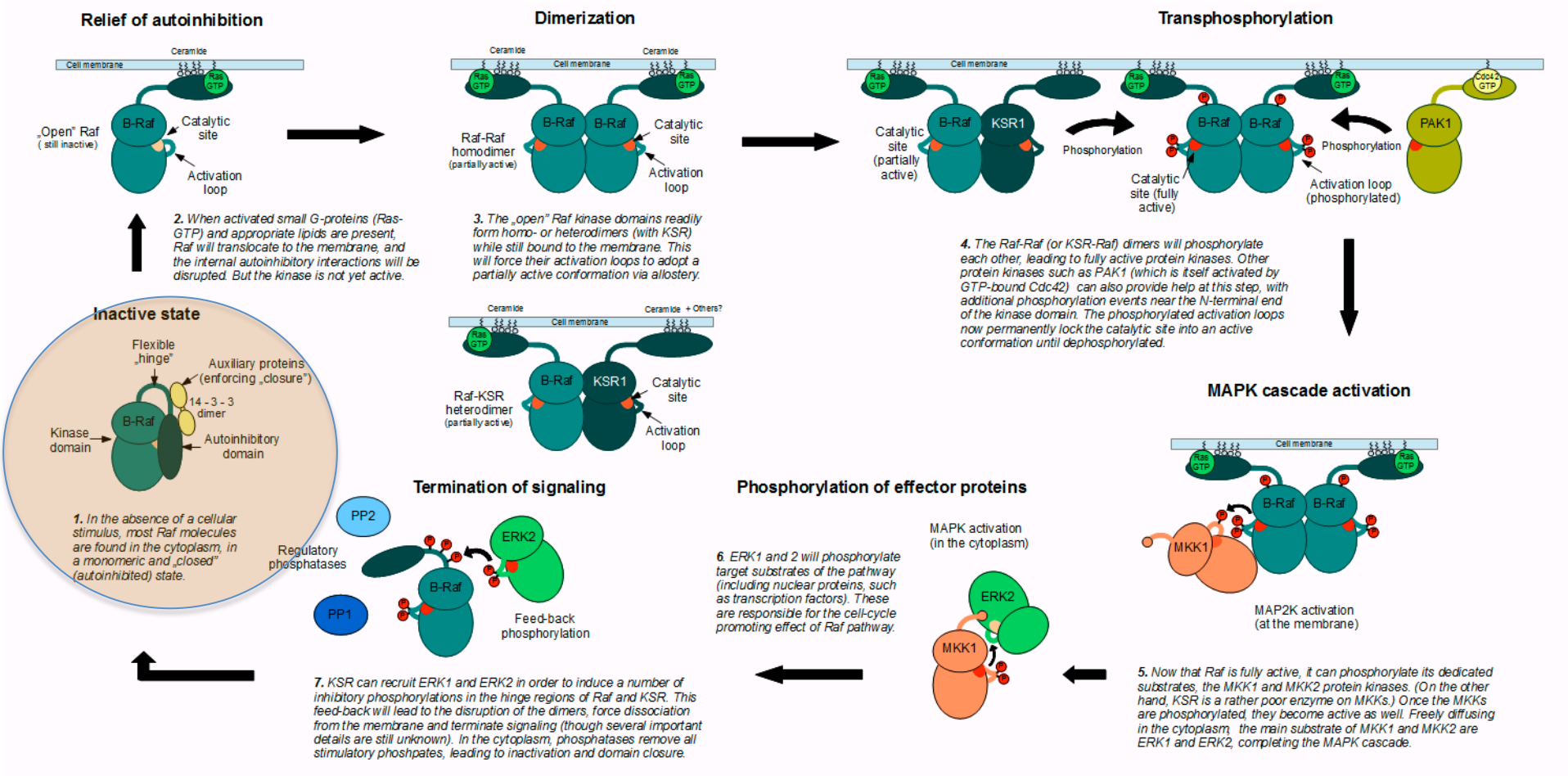
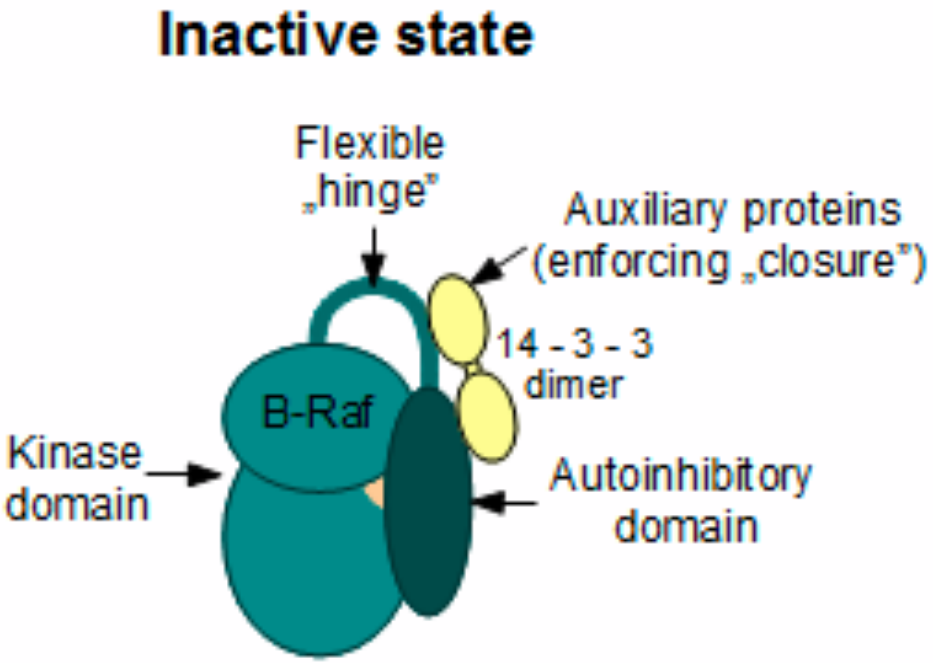
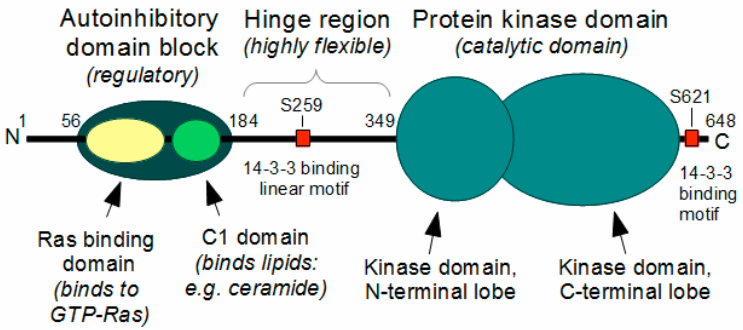
The activation cycle of mammalian c-Raf



The activation cycle of mammalian c-Raf



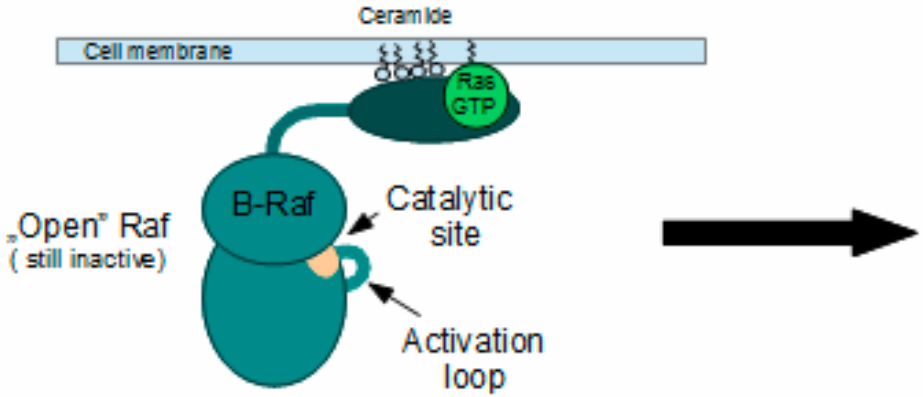


***1.** In the absence of a cellular stimulus, most Raf molecules are found in the cytoplasm, in a monomeric and „closed" (autoinhibited) state.*

In quiescent cells, Raf-1 is phosphorylated on both 14-3-3 binding sites (by PKA?) and 14-3-3 maintains the Raf closed inactive conformation

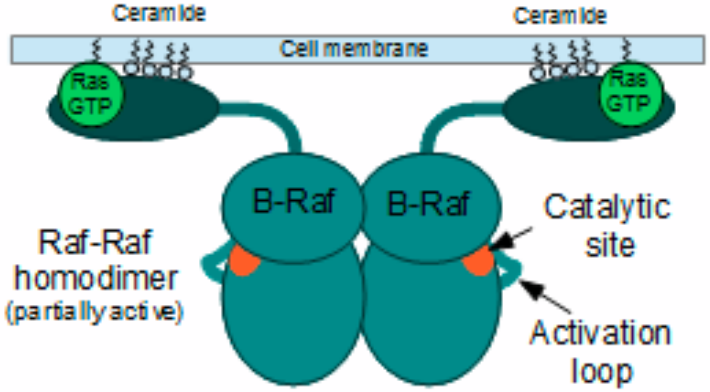
Regulation and function of Raf kinases / *Matallanas et al.* *Genes & Cancer* / vol 2 no 3 (2011)

Relief of autoinhibition

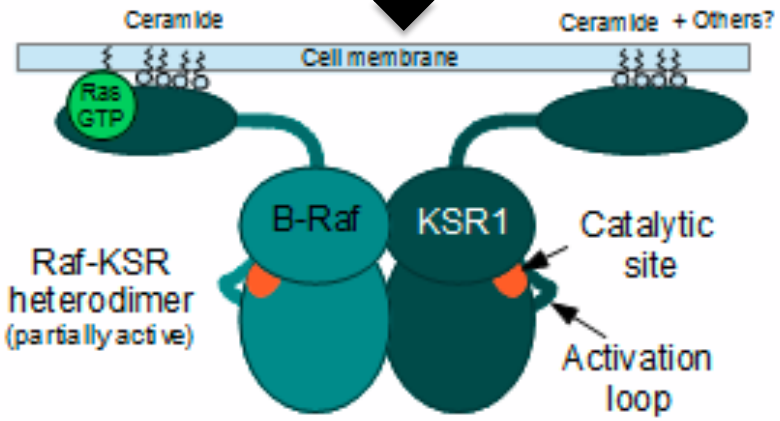


2. *When activated small G-proteins (Ras-GTP) and appropriate lipids are present, Raf will translocate to the membrane, and the internal autoinhibitory interactions will be disrupted. But the kinase is not yet active. Phosphatases PP1 or PP2A are co-recruited to the plasma membrane and de-phosphorylate the inhibitory residues; 14-3-3 proteins are released in the cytoplasm.*

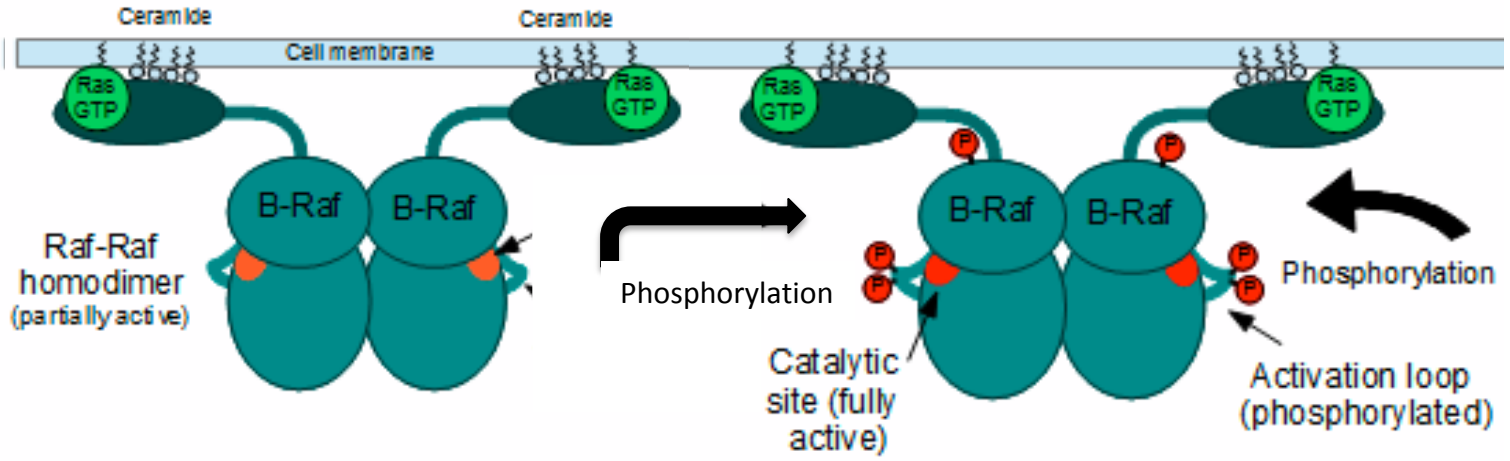
Dimerization



3. *The „open" Raf kinase domains readily form homo- or heterodimers (with KSR) while still bound to the membrane. This will force their activation loops to adopt a partially active conformation via allostery.*

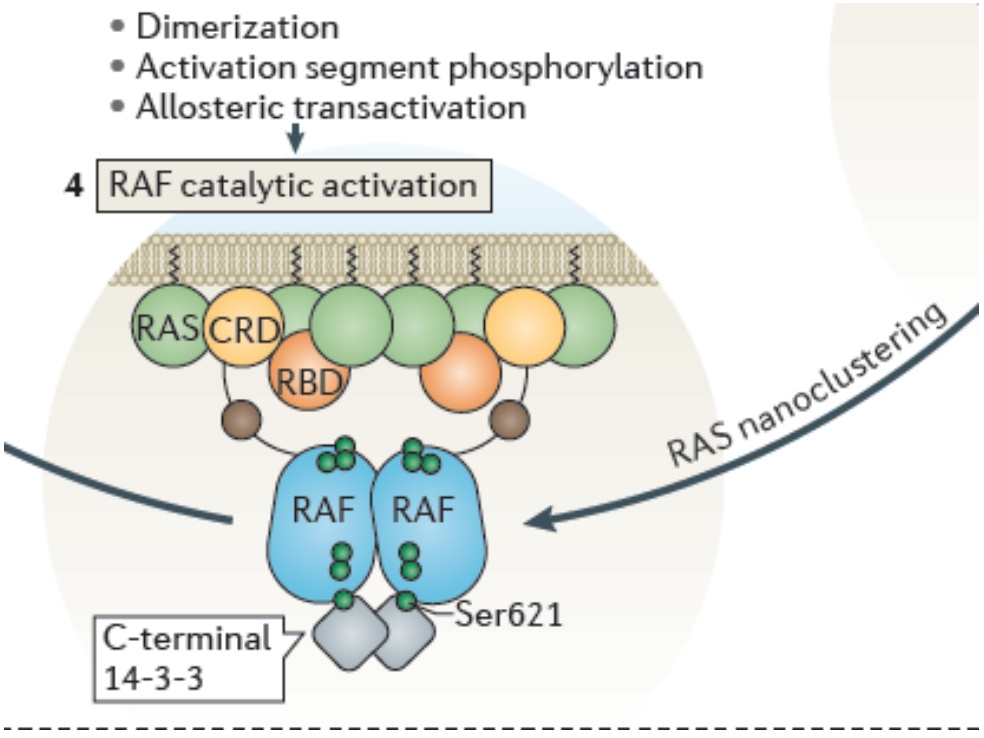


Transphosphorylation



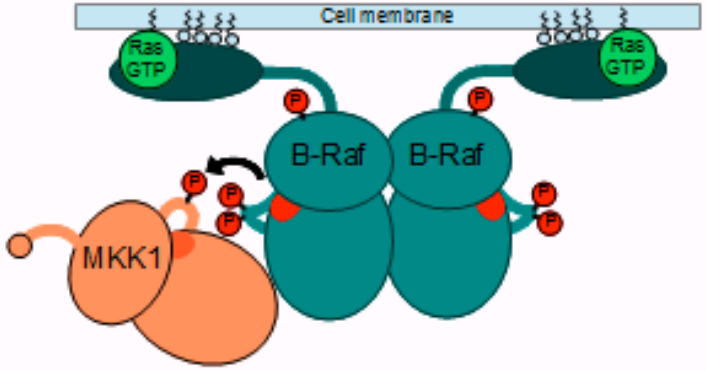
4. *The Raf-Raf (or KSR-Raf) dimers will phosphorylate each other, leading to fully active protein kinases.*

The phosphorylated activation loops now permanently lock the catalytic site into an active conformation until dephosphorylated.



Membrane binding and RAS nanoclustering augment the effective concentration of RAF and thereby contribute to RAF dimerization.

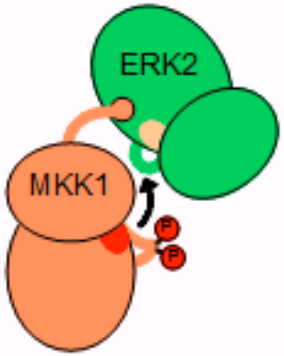
MAPK cascade activation



MAP2K activation
(at the membrane)

5. *Now that Raf is fully active, it can phosphorylate its dedicated substrates, the MKK1 and MKK2 protein kinases. (On the other hand, KSR is a rather poor enzyme on MKKs.) Once the MKKs are phosphorylated, they become active as well. Freely diffusing in the cytoplasm, the main substrate of MKK1 and MKK2 are ERK1 and ERK2, completing the MAPK cascade.*

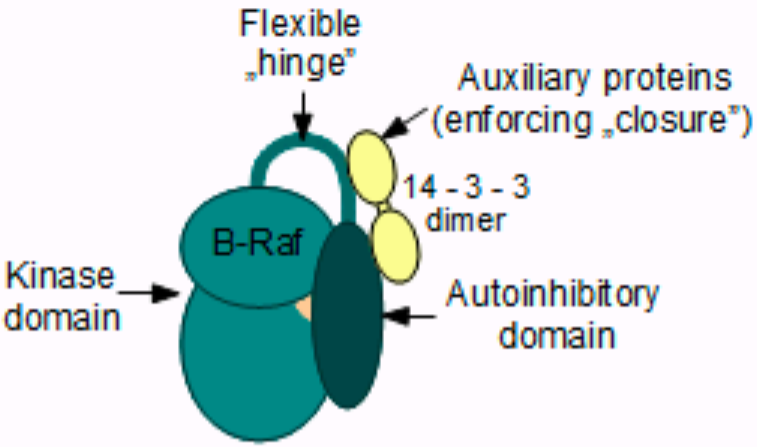
MAPK activation
(in the cytoplasm)



Phosphorylation of effector proteins

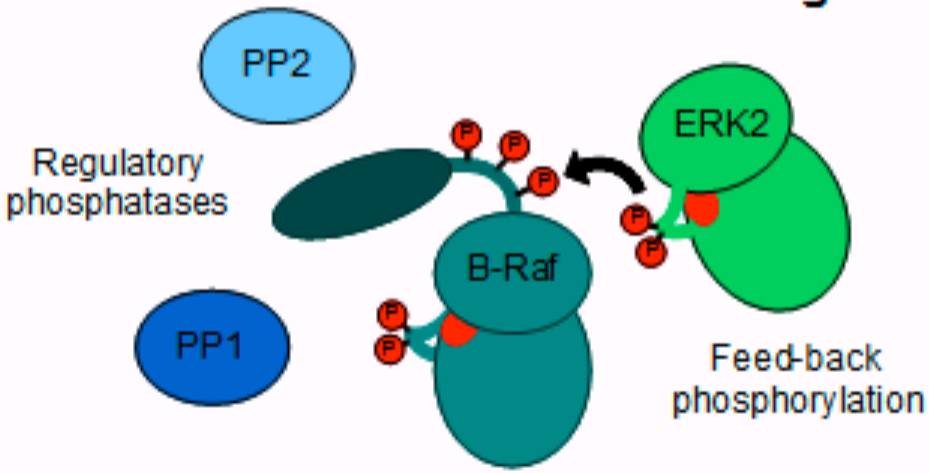
6*. ERK1 and 2 will phosphorylate target substrates of the pathway (including nuclear proteins, such as transcription factors). These are responsible for the cell-cycle promoting effect of Raf pathway.*

Inactive state



1. *In the absence of a cellular stimulus, most Raf molecules are found in the cytoplasm, in a monomeric and „closed" (autoinhibited) state.*

Termination of signaling



ERK signalling implements a **negative feedback loop** in which ERK phosphorylates several inhibitory sites in distinct regions of activated RAF, causing a release from activated RAS and the disruption of RAF dimers. In the cytoplasm, phosphatases remove stimulatory phosphates leading to inactivation and domain closure

Come e' possibile che le MAPKinasi, solubili nel citoplasma, riescano a trovarsi esattamente nei paraggi di Raf attivato in membrana e subirne la fosforilazione?

Non puo' essere un evento regolato dal caso!!

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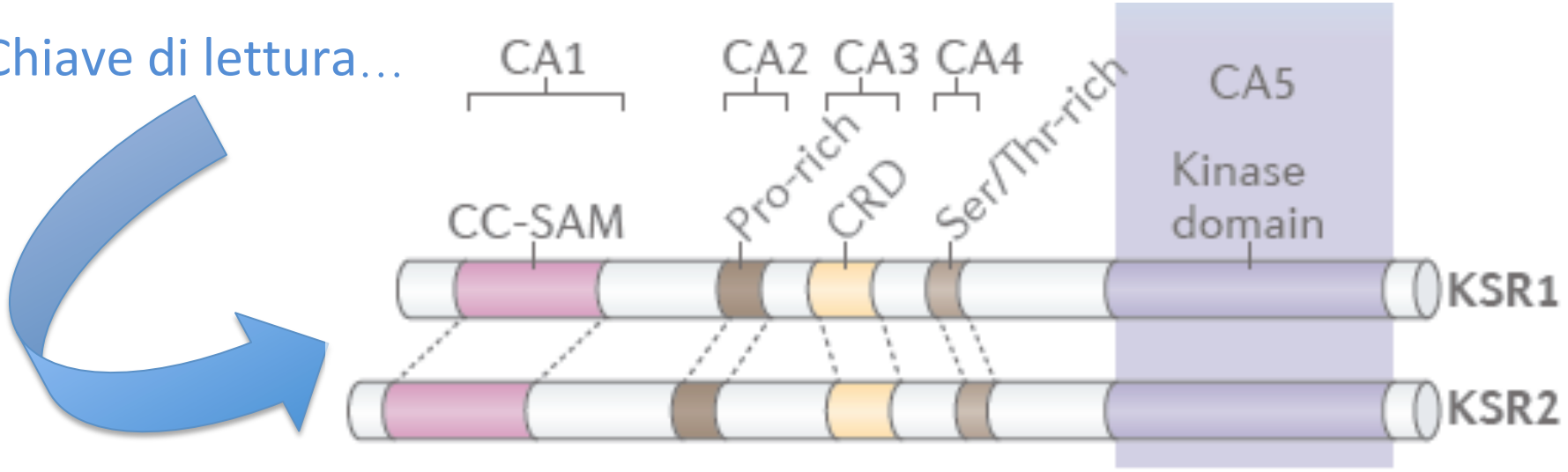
Non puo' essere un evento regolato dal caso!!

Chiave di lettura…



Come e' possibile che le MAPKinasi, solubili nel citoplasma, riescano a trovarsi esattamente nei paraggi di Raf attivato in membrana e subirne la fosforilazione?

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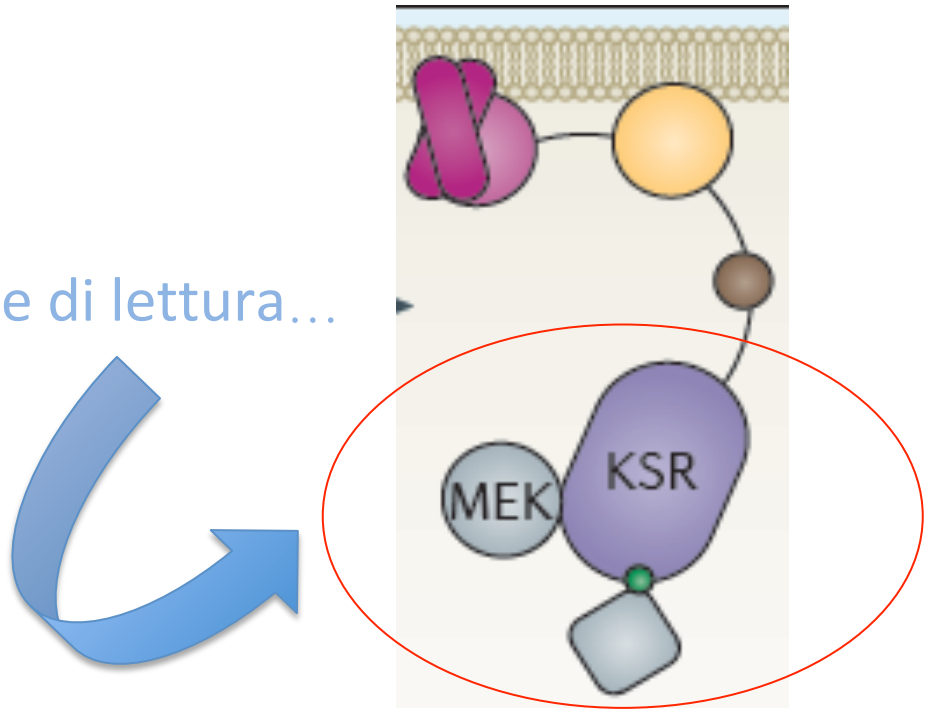


(KSR-1)

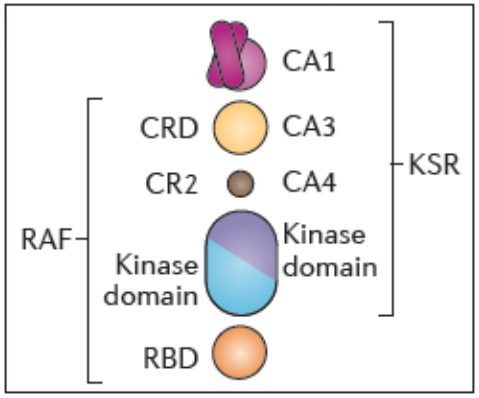
The Kinase Suppressor of RAS (KSR-1) was originally identified in genetic screens in Drosophila and Caenorhabditis elegans designed to isolate mutations in genes that modify the phenotypes associated with oncogenic RAS alleles.

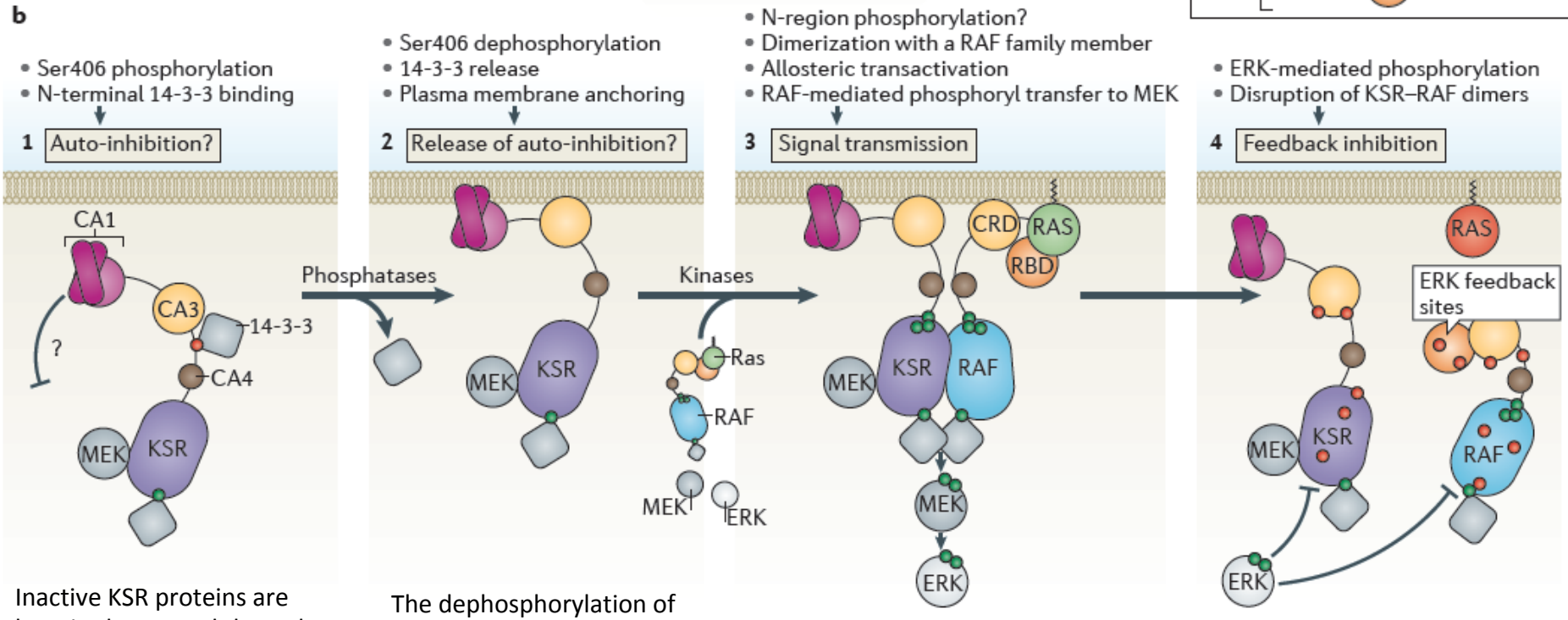
In mammalian cells, KSR-1 acts as a molecular scaffold to assemble a macromolecular complex of MAPK pathway components to facilitate efficient signal transmission and is required for mutant RAS-mediated cellular transformation.

In quiescent cells, KSR-1 is phosphorylated on S297 and S392 by C-TAK1 and held in an inactive state in the cytosol by 14-3- 3 proteins.

RAS activation stimulates the dephosphorylation of KSR-1 on S392, resulting in its translocation to the plasma membrane where it potentiates MAPK signaling (Ory et al., 2003).

Steps involved in KSR regulation often parallel those defined for RAF proteins





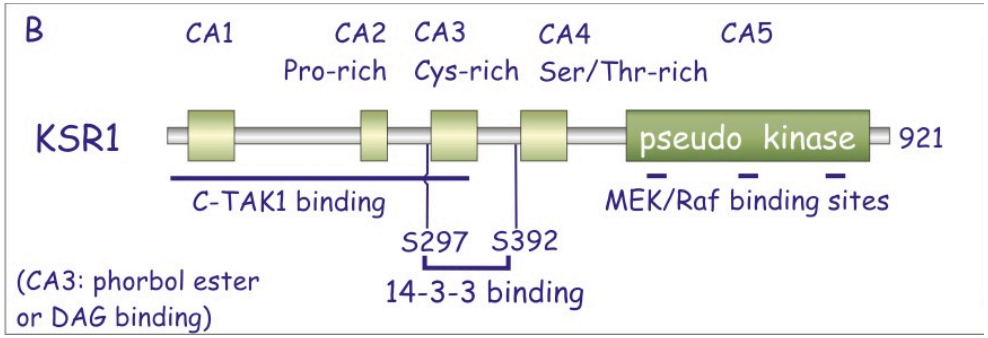
Inactive KSR proteins are kept in the cytosol through interaction with inhibitory 14-3-3 proteins in their N-terminal region. KSR and MEK proteins form constitutive complexes

The dephosphorylation of Ser406 allows 14-3-3 release and plasma membrane anchoring of KSR proteins via conserved area 1 (CA1) and CA3.

KSR proteins heterodimerize with other RAF proteins, leading to RAF transactivation and MEK–ERK signalling.

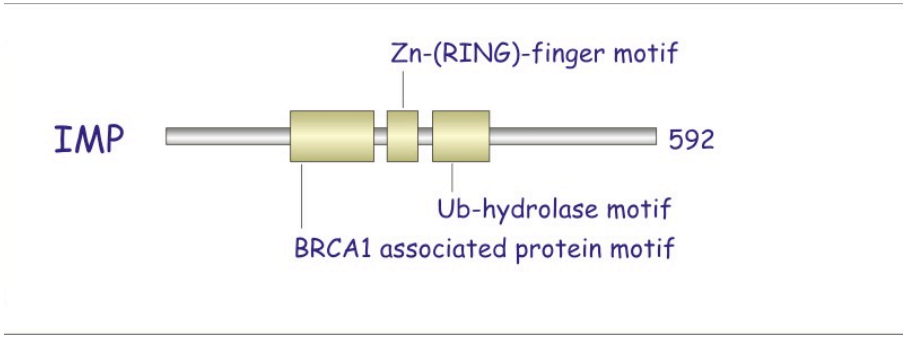
ERK-mediated negative feedback phosphorylation of several sites in RAF and KSR disrupts RAF–KSR dimers, leading to signal attenuation.

REGULATION OF THE MAPK SIGNALING CASCADE

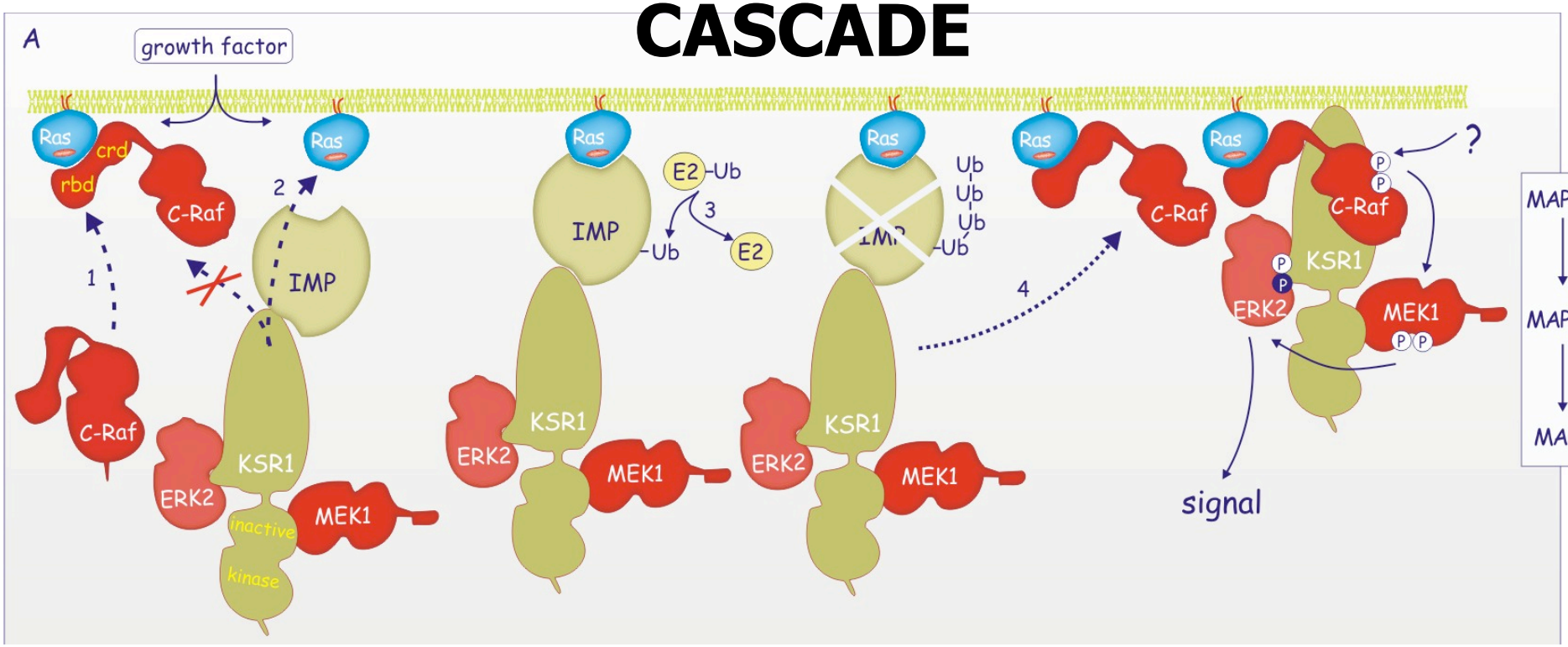


KSR1 has a number of conserved domains, and a kinase domain that resembles that of Raf, but lacks an essential lysine and is therefore inactive.

IMP (impedes mitogenic signal propagation) is an E3-ligase and binds E2-ubiquitin. The E3-ligase activity is activated by binding to RasGTP and this results in the auto-ubiquitylation of IMP, followed by its destruction.



REGULATION OF THE MAPK SIGNALING CASCADE



In order to activate the ERK pathway, Ras has not only to recruit C-Raf, but also to remove **IMP**, the inhibitor which prevents formation of the Raf-MEK-ERK signalling cassette.

RasGTP binds IMP and this initiates a series of autoubiquitylations that mark the protein for destruction by the proteasome.

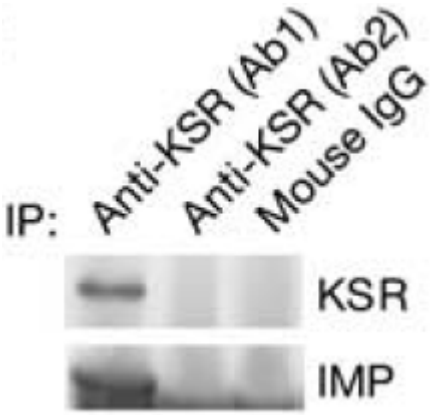
MEK1 and ERK2, linked to the scaffold protein KSR1, are now able to join C-Raf, enabling the signal to pass from one kinase to another

NATURE | VOL 427 | 15 JANUARY 2004 | www.nature.com/nature

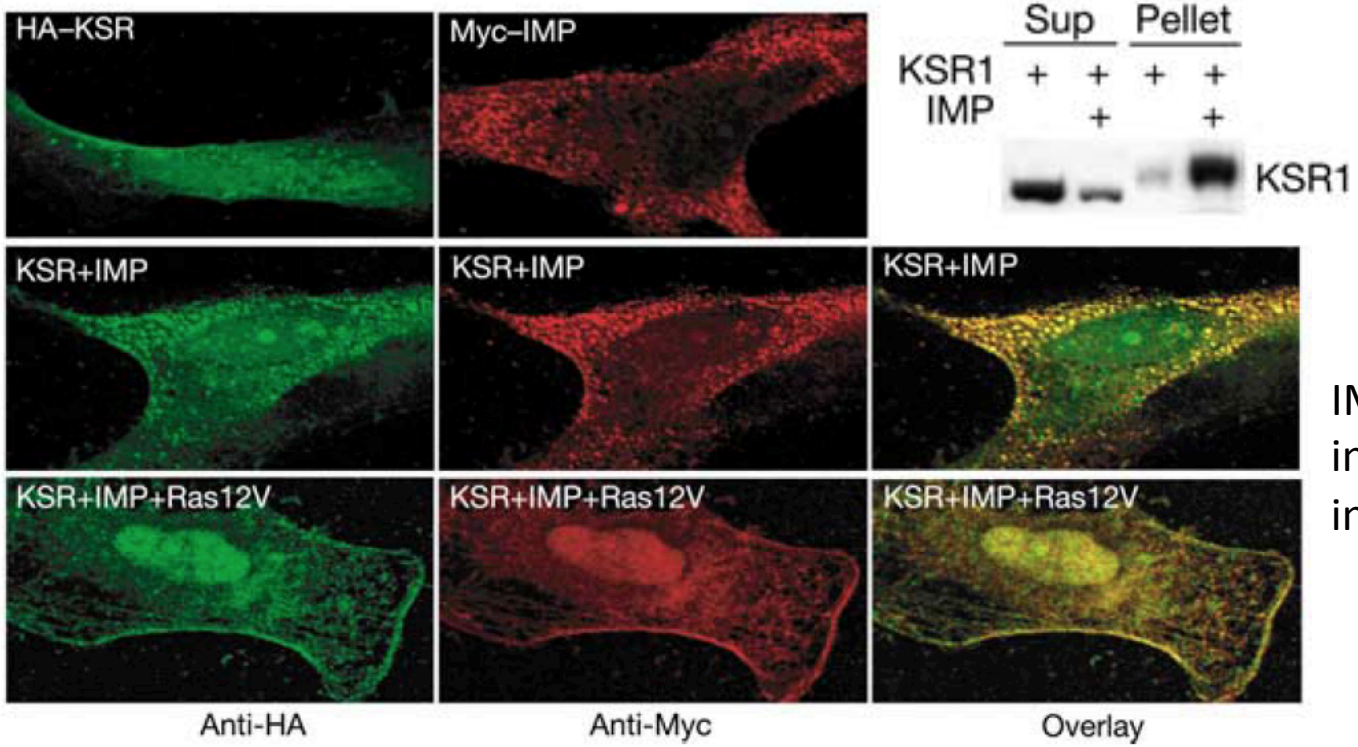
Ras regulates assembly of mitogenic signalling complexes through the effector protein IMP

Sharon A. Matheny[1], Chiyuan Chen[1], Robert L. Kortum[2], Gina L. Razidlo[2], Robert E. Lewis[2] & Michael A. White[1]

[1]*Department of Cell Biology, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390-9039, USA*
[2]*Eppley Institute for Research in Cancer and Allied Diseases, Department of Pathology, University of Nebraska Medical Center, Omaha, Nebraska 68198-6805, USA*



IMP co-immunoprecipitates with KSR

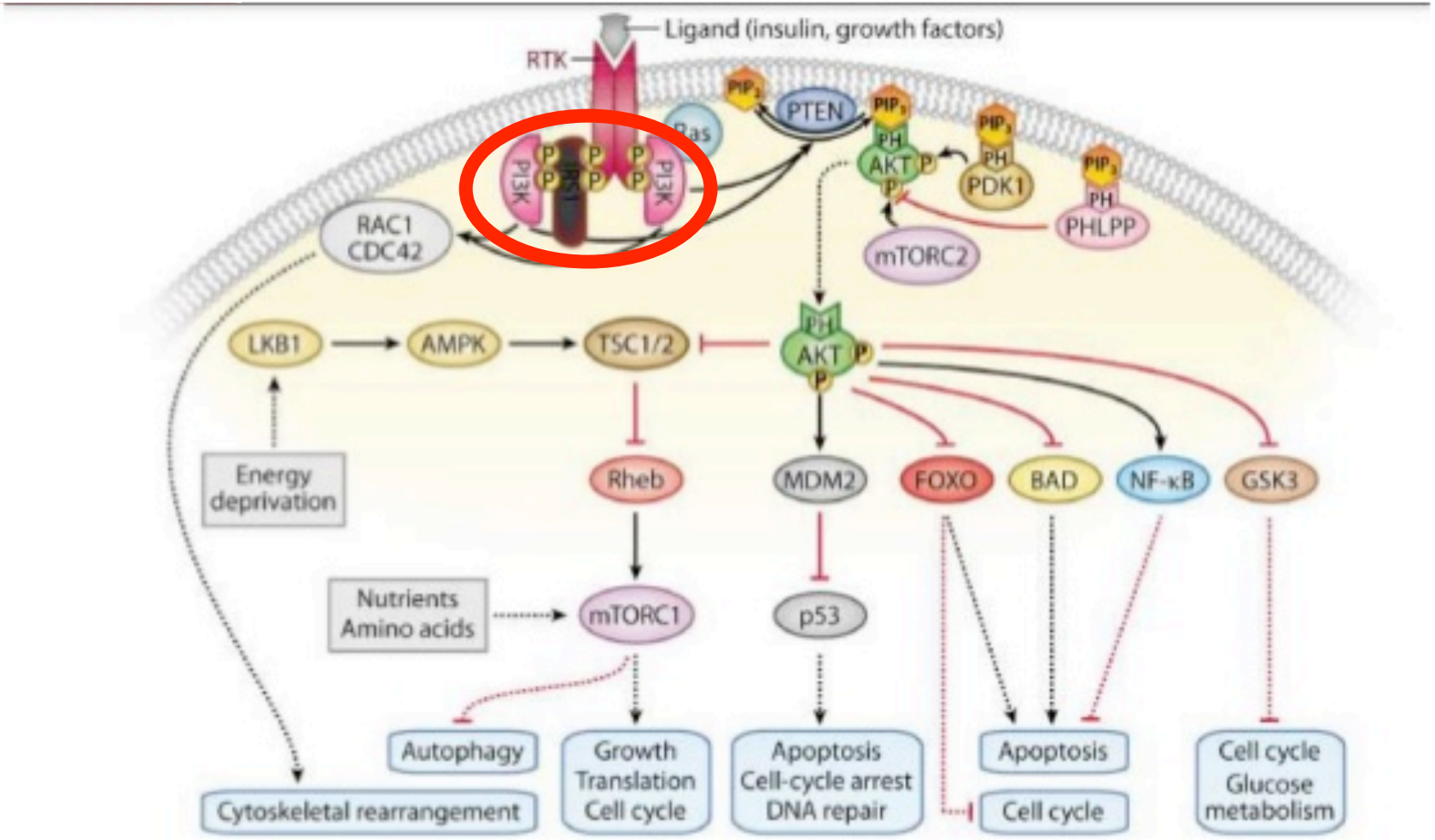


IMP and KSR1 colocalize in Ras-sensitive Triton-insoluble structures

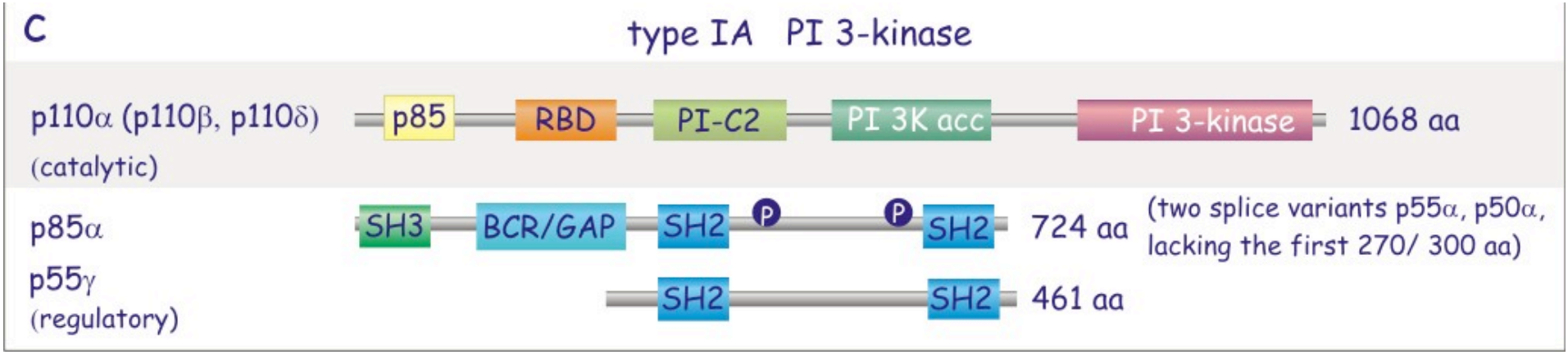
Paradigms for activation of RTKs signaling cascade

As many protein targets of RTKs are located at the cell membrane, translocation to the plasmalemma is essential for activation of many effector proteins

Signaling Through PI3-Kinase Pathway



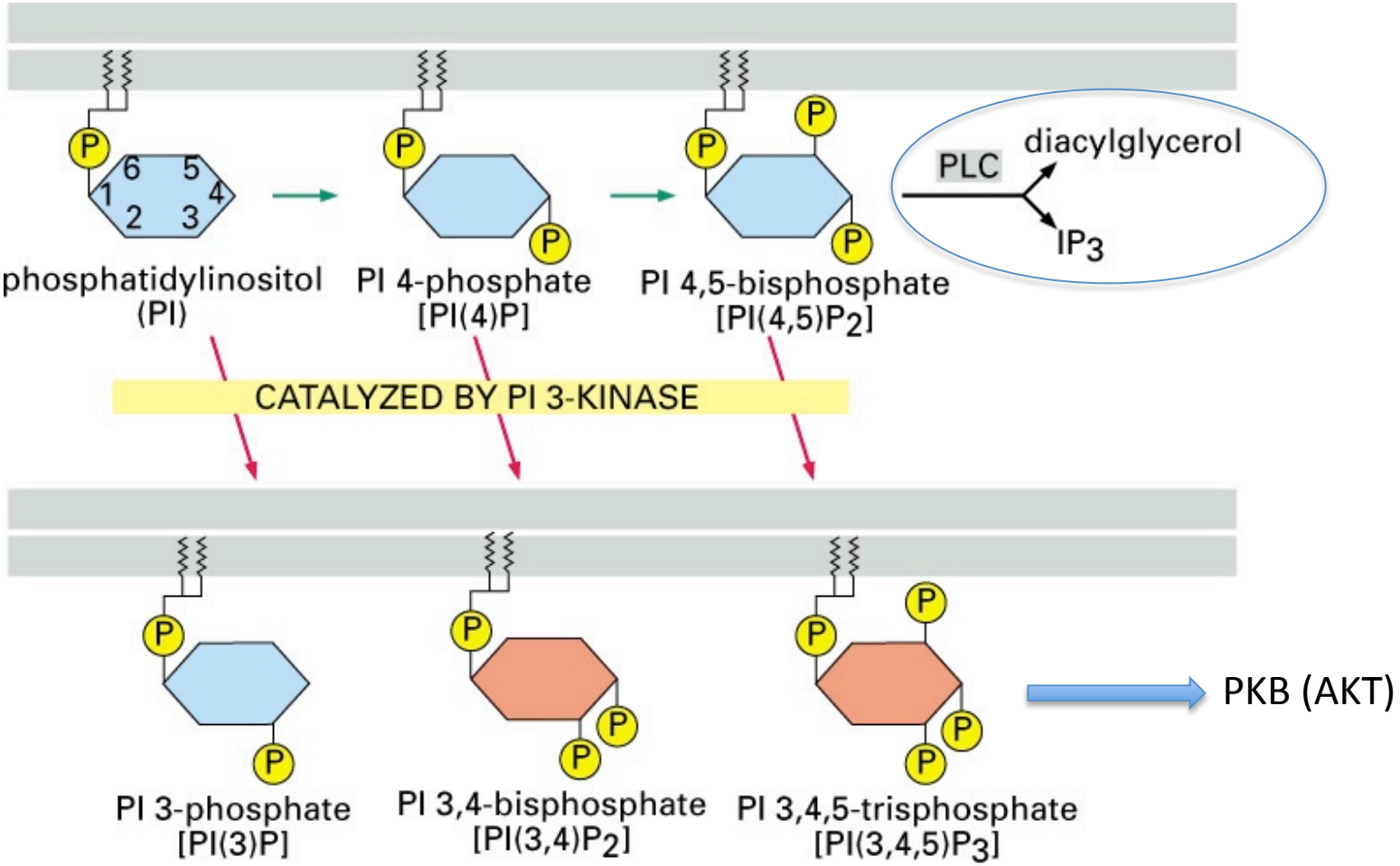
PI3-KINASES



- The catalytic subunits all possess a p85- and Ras-binding site.
- They also have a PI-C2 domain to interact with phospholipids.
- The PI 3K accessory domain serves as a spine on which the other domains are fastened.

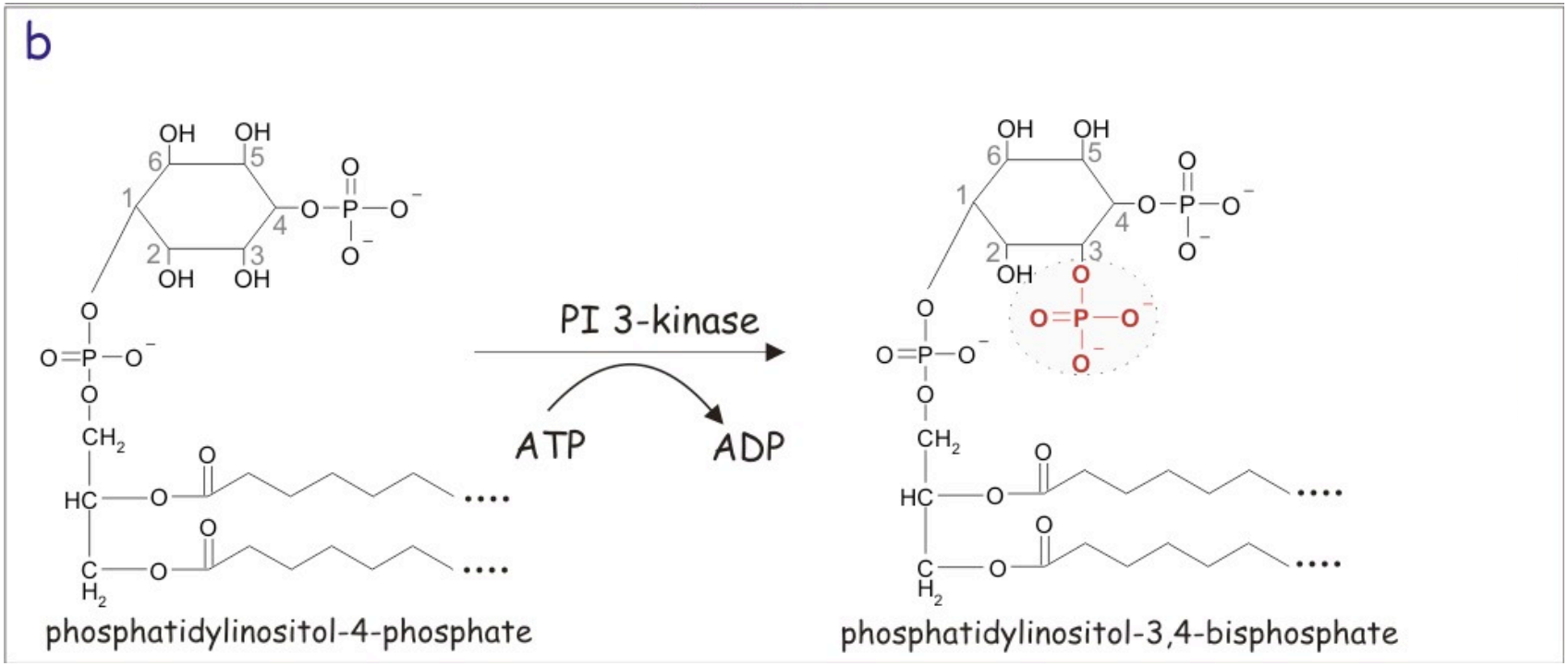
- The regulatory subunits, p85α is particularly versatile: its SH3 domain interacts with proline rich sequences, its BCR/GAP domain interacts with monomeric GTPases of the Rho family (Cdc42 and Rac), whereas its SH2 domain interacts with phosphotyrosines.

Lipids formed by PI3Kinase

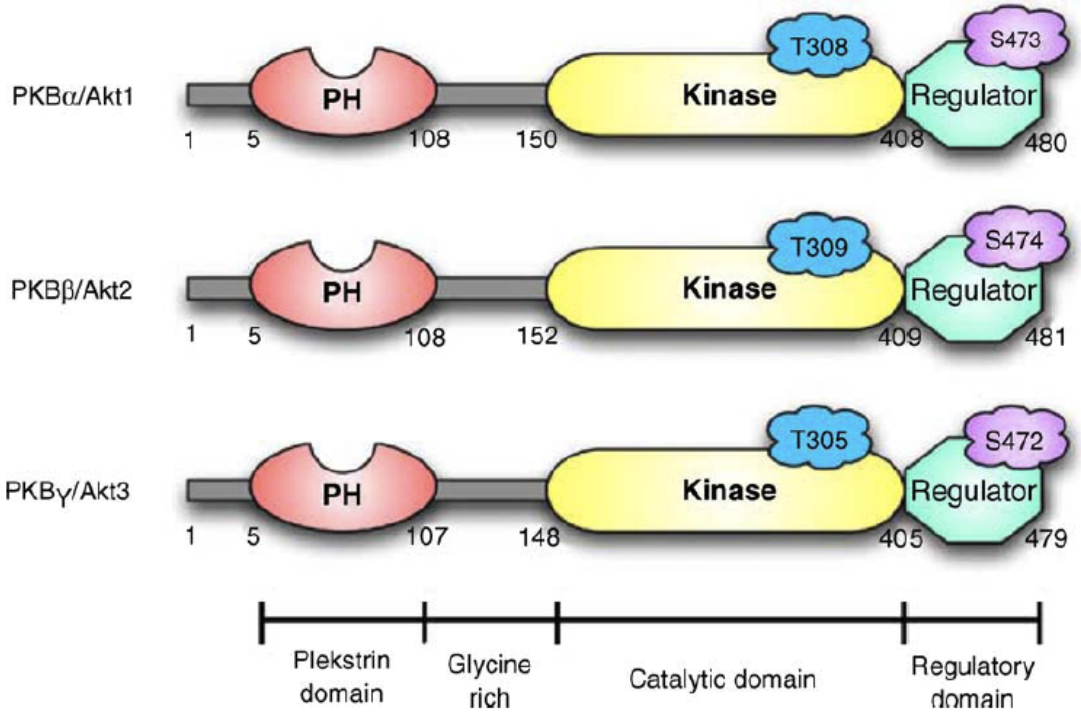


- The PI 3-kinases phosphorylate the 3-OH-position in the inositol ring of the phosphatidylinositol lipids.
- The PI 3-phosphate compounds synthesized by PI-3 kinase activate protein kinase B (PKB).

Composition of inositol lipids before and after phosphorylation by PI 3-kinase.

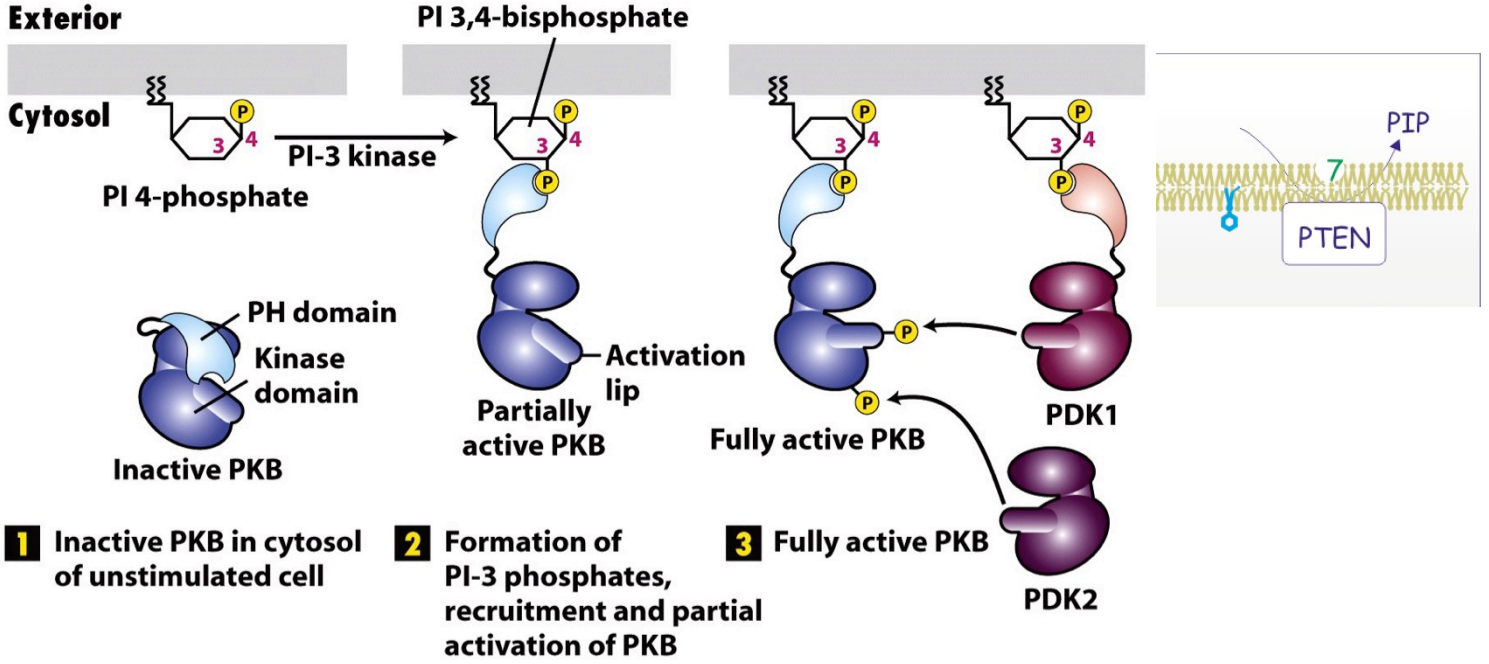


PKB (AKT)



- Akt/PKB, the cellular homologue of the viral oncogene v-Akt/PKB.
- Akt1 and 2 are ubiquitously expressed; Akt3 is mainly expressed in the brain and testis
- Akt/PKB is an ~57-kDa serine/threonine kinase containing an N-terminal pleckstrin homology
- (PH) domain that mediates binding to phosphatidylinositol (3,4,5) P3 phosphate (PIP3) and a catalytic domain containing a threonine residue (T308 for Akt1/PKB) whose phosphorylation is necessary for activation of Akt/PKB. Next to the kinase domain there is a hydrophobic C-terminal tail containing a second regulatory phosphorylation site (S473 in Akt1/PKB).
- Both phosphorylation events are required for the full activation of Akt/PKB.

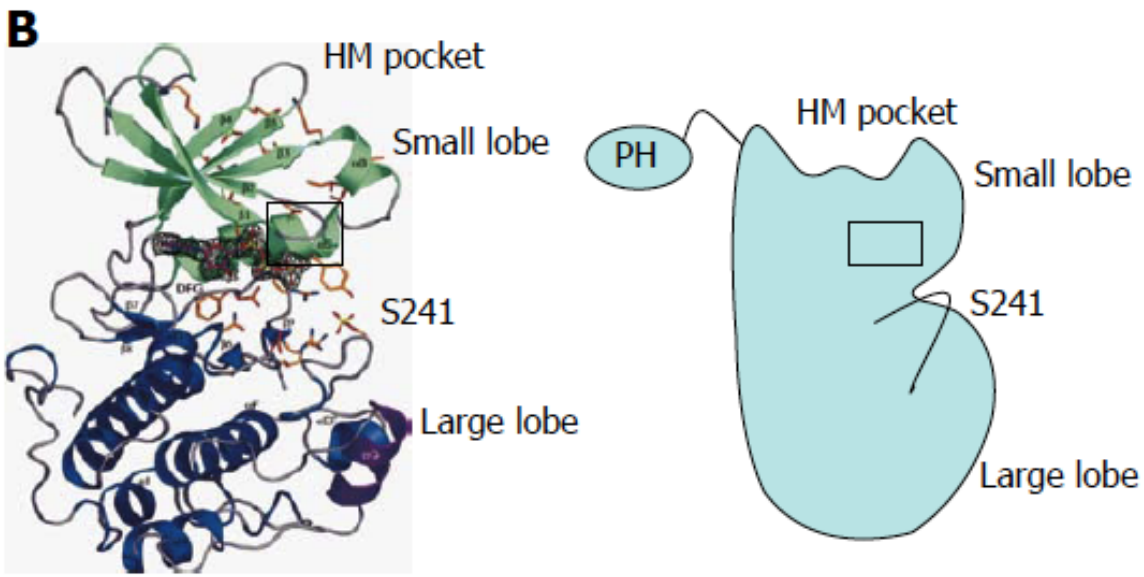
Activation of Protein S/T Kinase B (Akt)



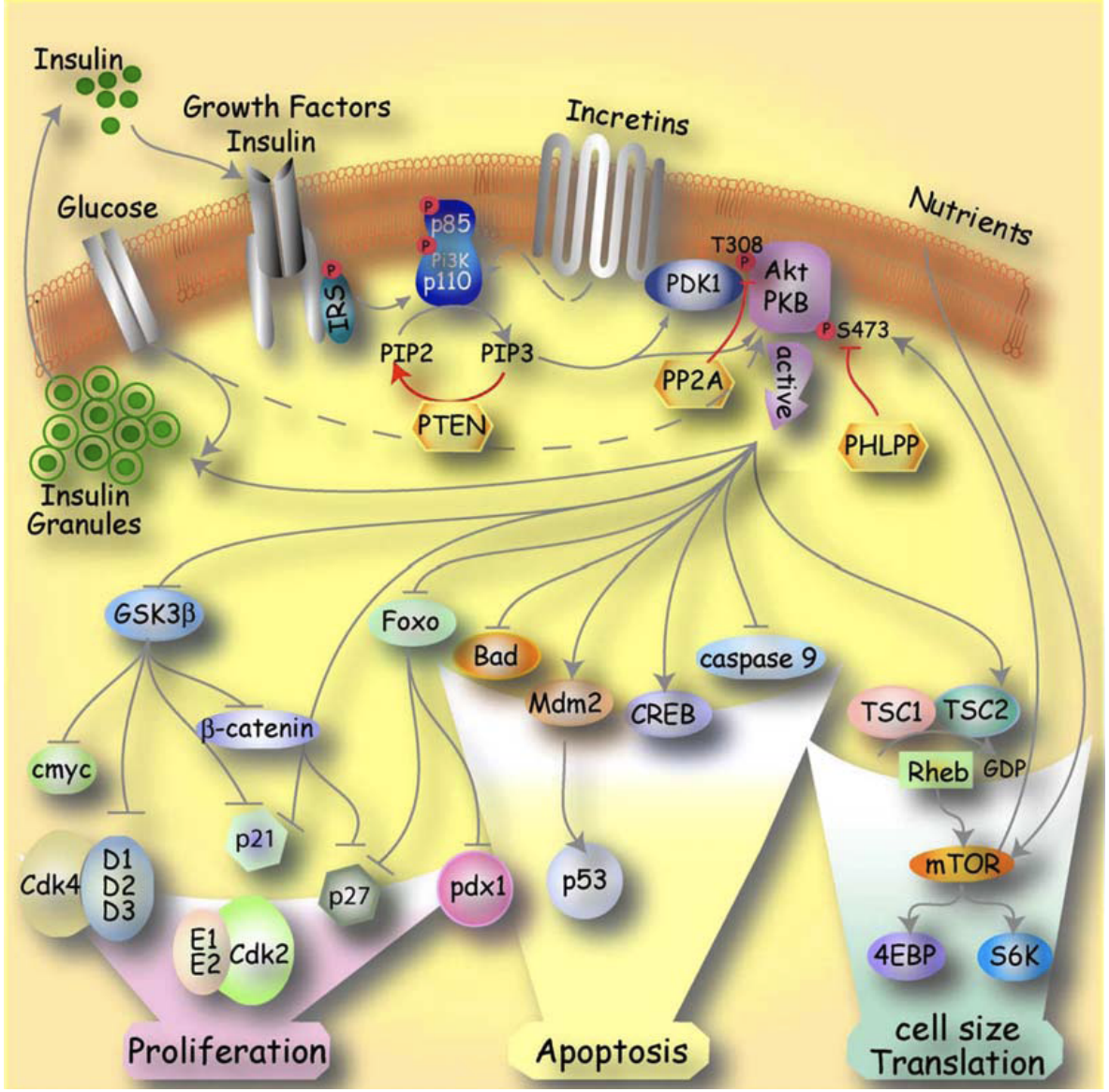
Signaling downstream of PI 3-phosphates is driven by **PKB**.

- Generation of 3-phosphoinositides (PIP3) by PI3K recruits Akt/PKB and PDK1 to the membrane. Akt/PKB is subsequently phosphorylated at Thr 308 and at Ser 473 by PDK1 and PDK2, respectively.
- Akt/PKB translocation to the nucleus results in phosphorylation of many substrates that control various biological signaling cascades.

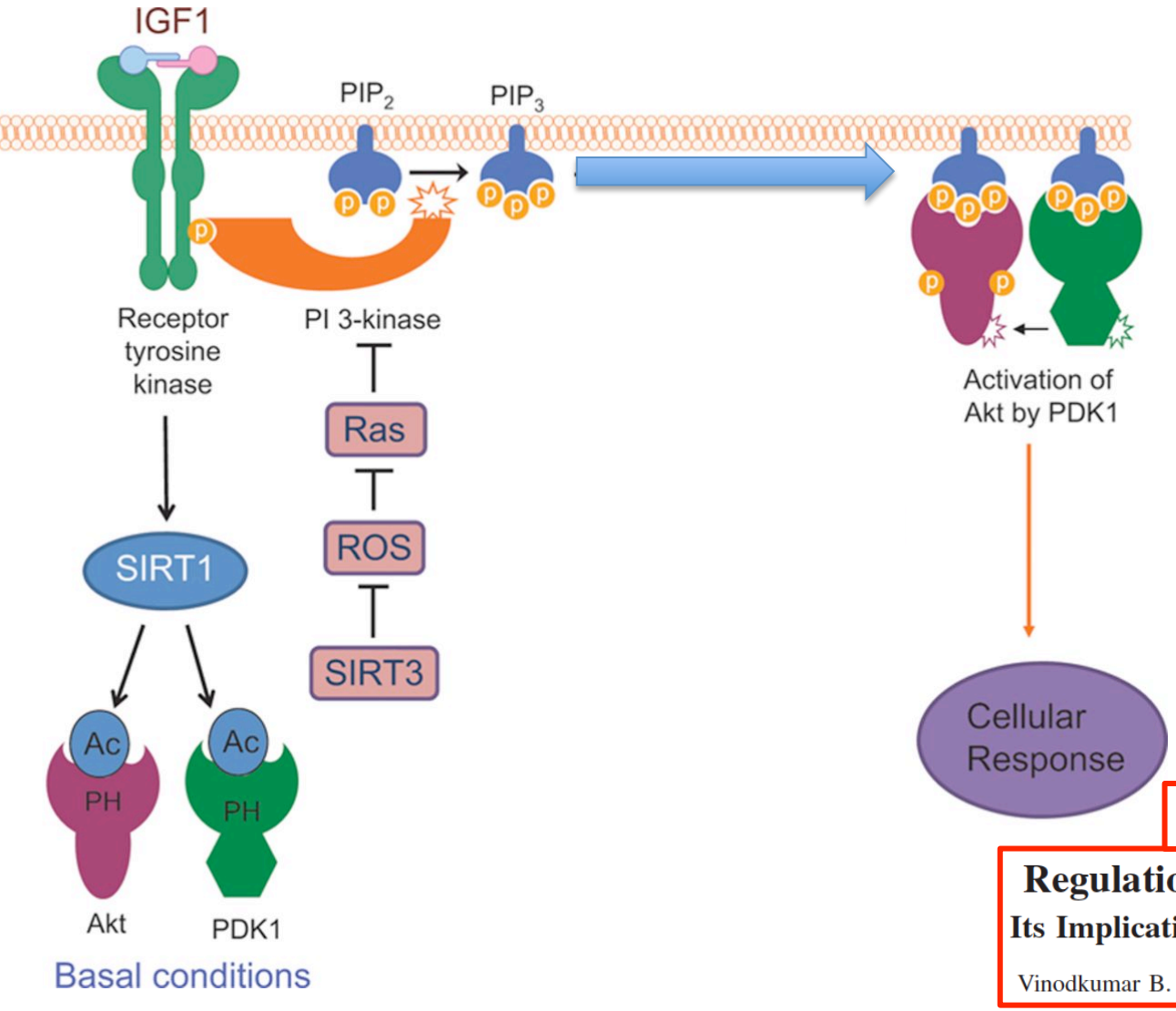
Phosphoinositide-dependent kinase-1 (PDK1)



- PDK1 is a **master kinase**, crucial for the activation of AKT/PKB and many other kinases including **PKC**, S6K, SGK.
- Mice lacking PDK1 die during early embryonic development, indicating that this enzyme is critical for transmitting the growth-promoting signals necessary for normal mammalian development.
- The structure of PDK1 can be divided into two domains; the kinase or catalytic domain and the PH domain.
- The PH domain functions mainly in the interaction of PDK1 with phosphatidylinositol (3,4)-bisphosphate and phosphatidylinositol (3,4,5)-trisphosphate.
- The kinase domain has crucial binding sites: the substrate binding site, the ATP binding site.
- **PDK1 is constitutively active and at present, there is no known inhibitor for PDK1**.



- Akt/PKB is negatively regulated (shown in red arrows) by the phospholipid phosphatase PTEN. PTEN down-regulates Akt/PKB by dephosphorylation of the PI3K product PIP3.
- The PP2A and PHLPP phosphatases inactivate and negatively regulate Akt/PKB by dephosphorylating T308 and S473, respectively.



Circulation Research **January 17, 2014**

Regulation of Akt Signaling by Sirtuins

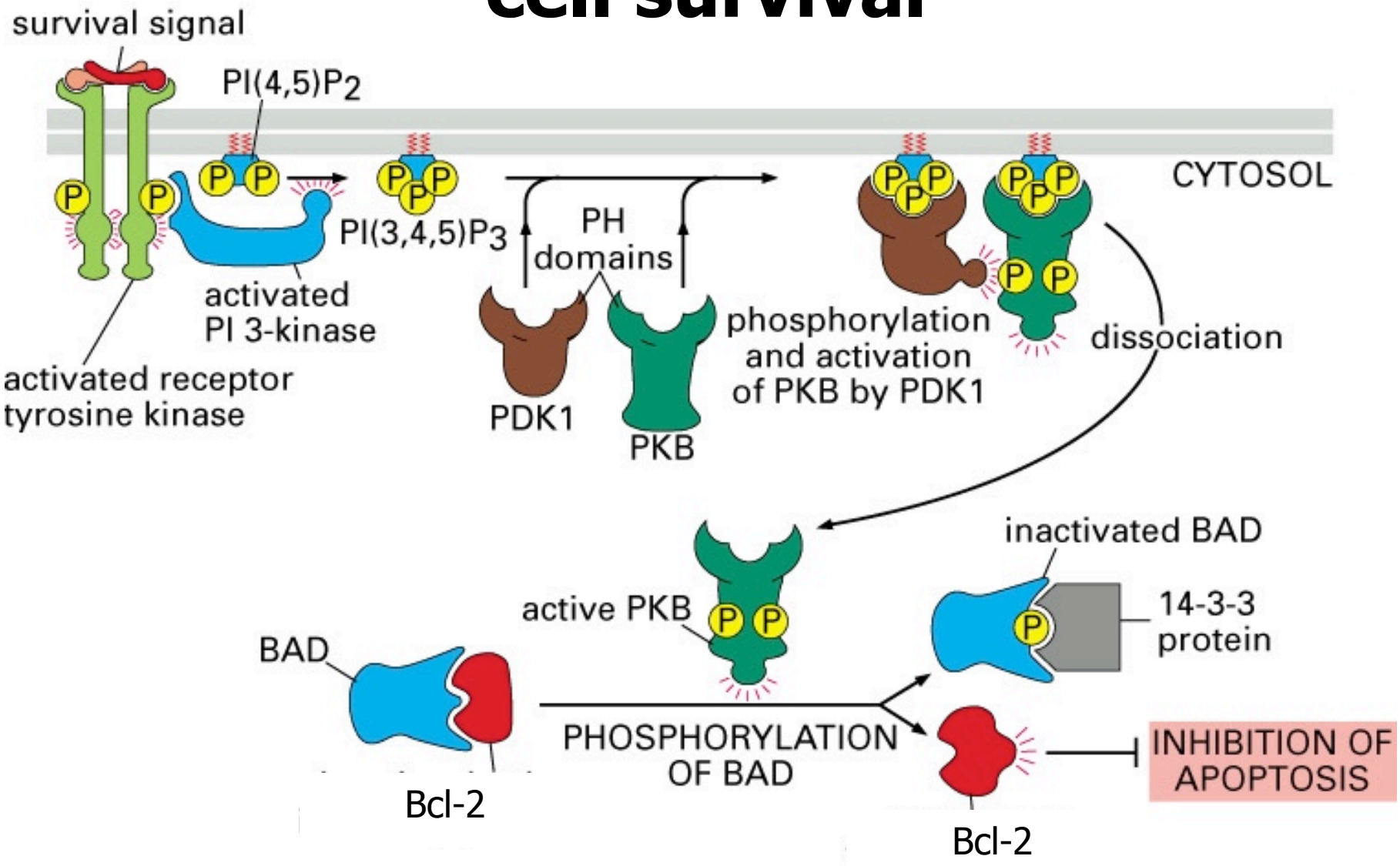
Its Implication in Cardiac Hypertrophy and Aging

Vinodkumar B. Pillai, Nagalingam R. Sundaresan, Mahesh P. Gupta

Under basal conditions, the pleckstrin homology (PH) domains of Akt and PDK1 are acetylated, leading to inhibition of their binding to PIP3 and, hence, inactivation.

During growth factor stimulation of cells, SIRT1 binds to and deacetylates Akt and PDK1 PH domains. This change enables them to bind to PIP3, generated by the activation PI3K.

The PI3K pathway to regulate cell survival

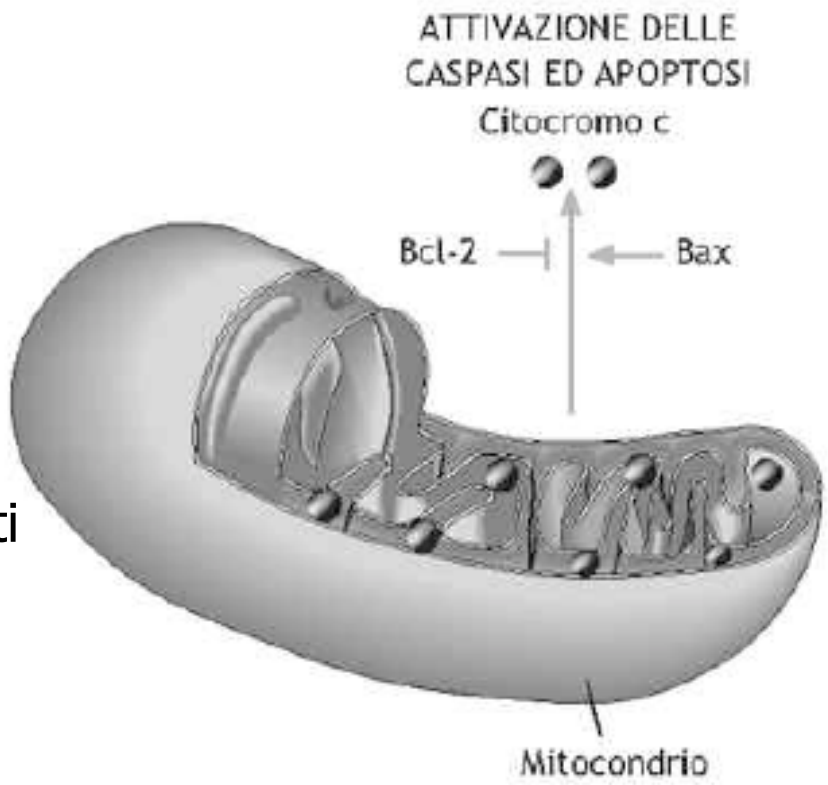


Le proteine Bcl-2 regolano l'equilibrio tra morte e sopravvivenza

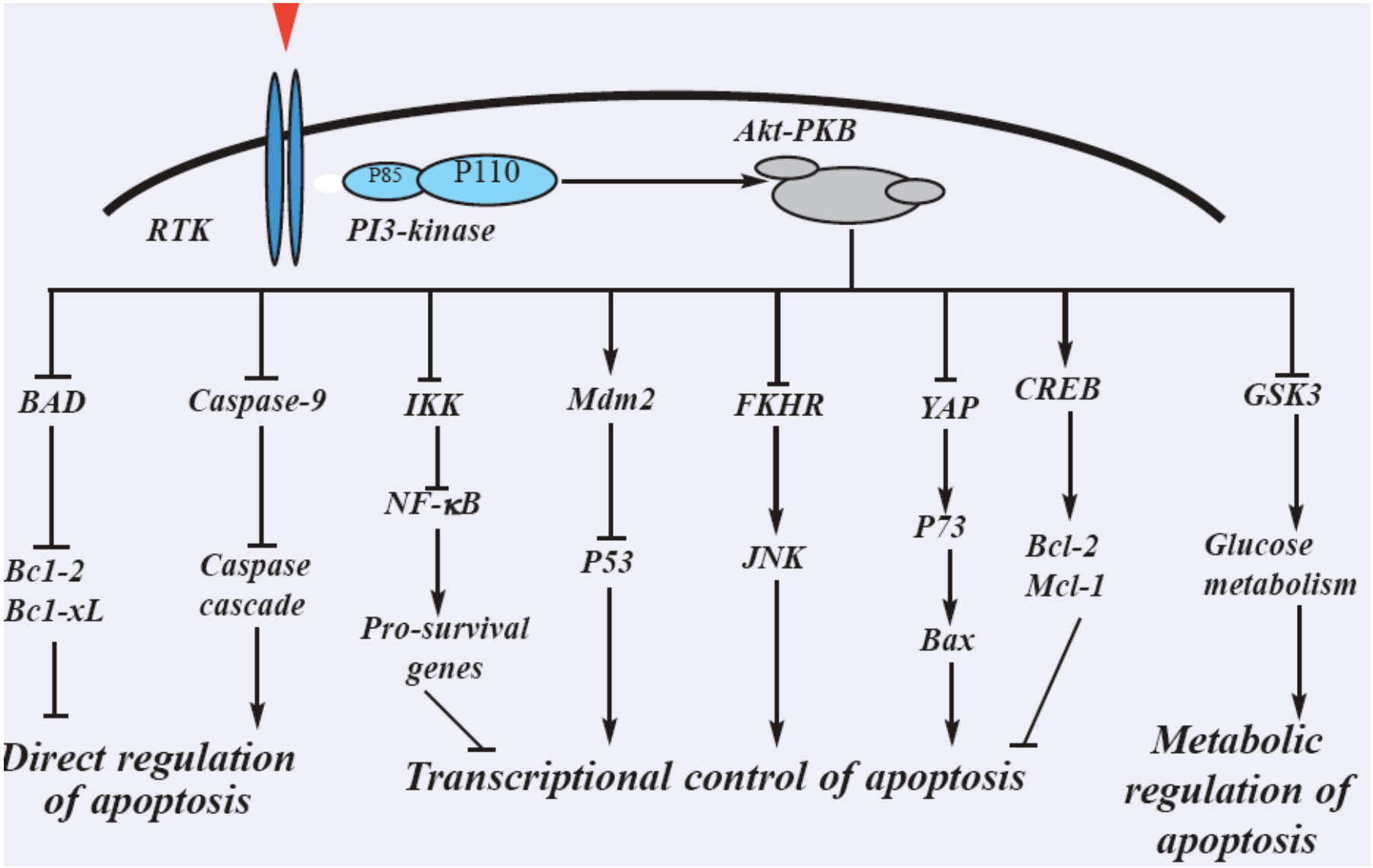
♦ FIGURA 17.3
I membri della famiglia Bcl2 regolano l'equilibrio tra sopravvivenza e morte cellulare. Variazioni dei livelli tra le proteine che inibiscono o inducono la morte cellulare condizionano la risposta cellulare ai diversi stimoli (vedi testo per i dettagli).

Controllo della permebilita' mitocondriale da parte di Bcl-2

- Le proteine Bcl-2 possono associarsi in oligomeri e modulare la permeabilita' di membrana dei mitocondri
- L'effetto anti o pro-apoptotico dipende dalla combinazione di particolari domini proteici (BH1-2-3-4)
- Bcl-2, antiapoptotica, contiene tutti e 4 I domini BH e blocca la fuoriuscita del citocromo c dal mitocondrio
- Bax, pro-apoptotica, possiede I domini BH1, 2 e 3 e facilita la fuoriuscita del citocromo.



Multiple mechanisms of cell survival regulation by Akt/PKB



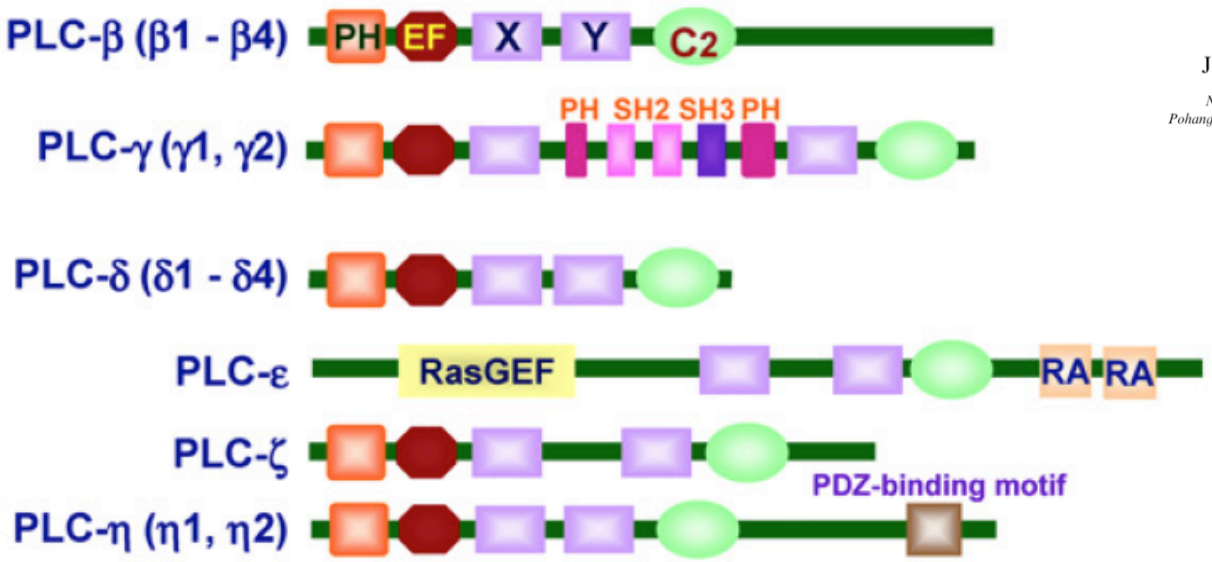
Phospholipase C (PLC) isozymes

ELSEVIER
Advan. Enzyme Regul. 47 (2007) 104–116
ADVANCES IN ENZYME REGULATION
www.elsevier.com/locate/advenzreg

On/Off-regulation of phospholipase C-γ1-mediated signal transduction

Jang Hyun Choi, Sung Ho Ryu, Pann-Ghill Suh*

National Research Laboratory of Signaling Network, Department of Life Science, Pohang University of Science and Technology, Pohang, Kyungbuk 790-784, Republic of Korea

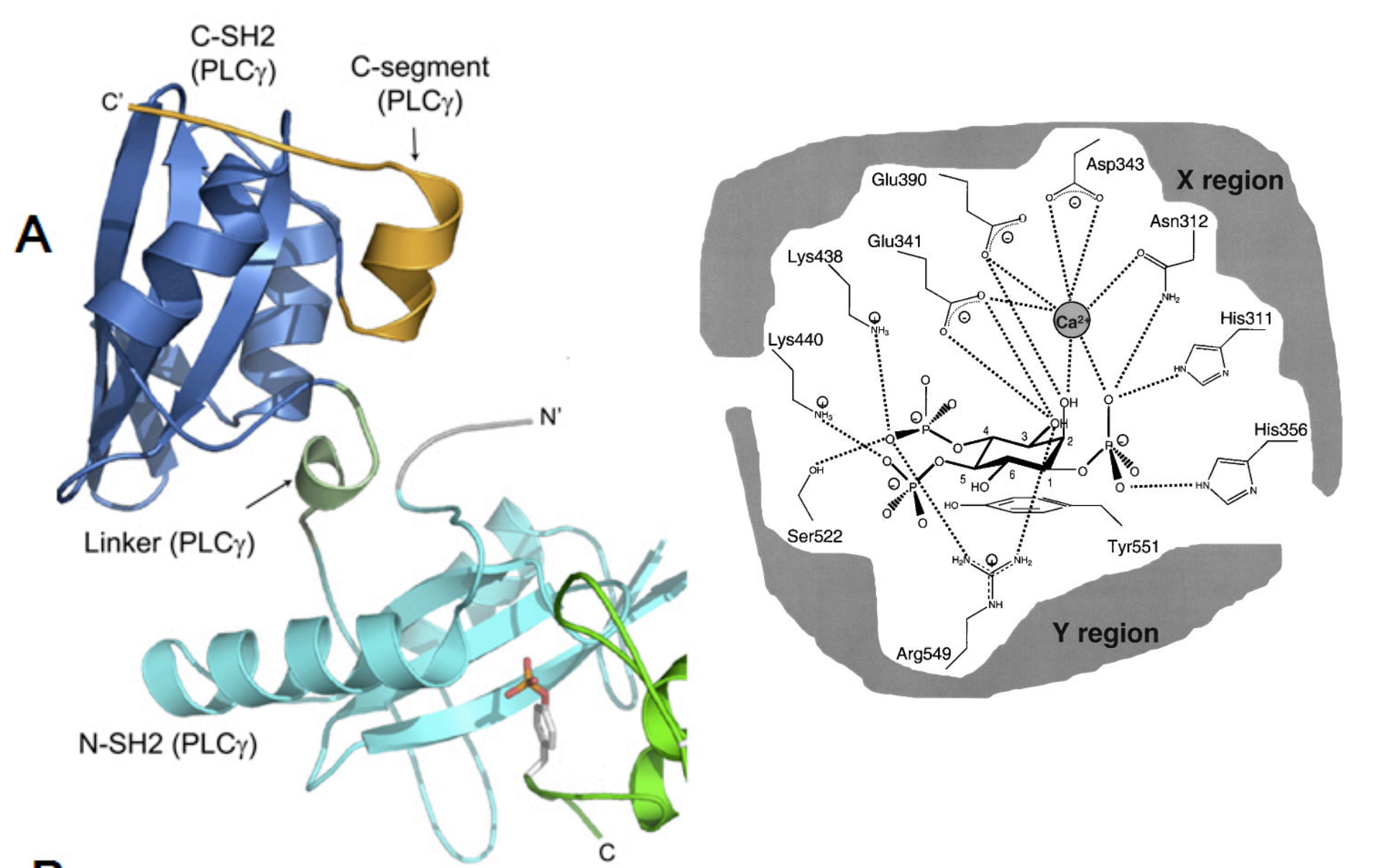


Dominio PH (*Pleckstrin Homology*): in grado di legarsi a diversi polifosfoinositidi.

Dominio "a mano EF": in grado di legare il Ca2+, generalmente in misura di uno ione per dominio (Tutte le fosfolipasi infatti necessitano del Ca2+ come cofattore per la loro attività catalitica).

Dominio catalitico: è costituito dall'associazione dei domini X ed Y, ognuno dei quali va a costituire una metà di una struttura simile ad un "*TIM-barrel*" distorto e chiuso.

Dominio C2: rappresenta un modulo proteico, costituito da circa 120 aminoacidi, presente in copia singola o multipla in numerose proteine, molte delle quali sono coinvolte nella trasduzione del segnale o nell'interazione con le membrane lipidiche.


A
C-SH2
(PLCγ)
C-segment
(PLCγ)
C'
N'
Linker (PLCγ)
N-SH2 (PLCγ)
C
X region
Y region
Asp343
Glu390
Asn312
Glu341
Lys438
Lys440
His311
His356
Ser522
Tyr551
Arg549
Ca2+
B
PLCγ
EF
PH
PLC
P
SH2
SH2
SH3
H
PLC
C2
Y783

Review TRENDS in Biochemical Sciences Vol.30 No.12 December 2005

Phospholipase C-γ: diverse roles in receptor-mediated calcium signaling

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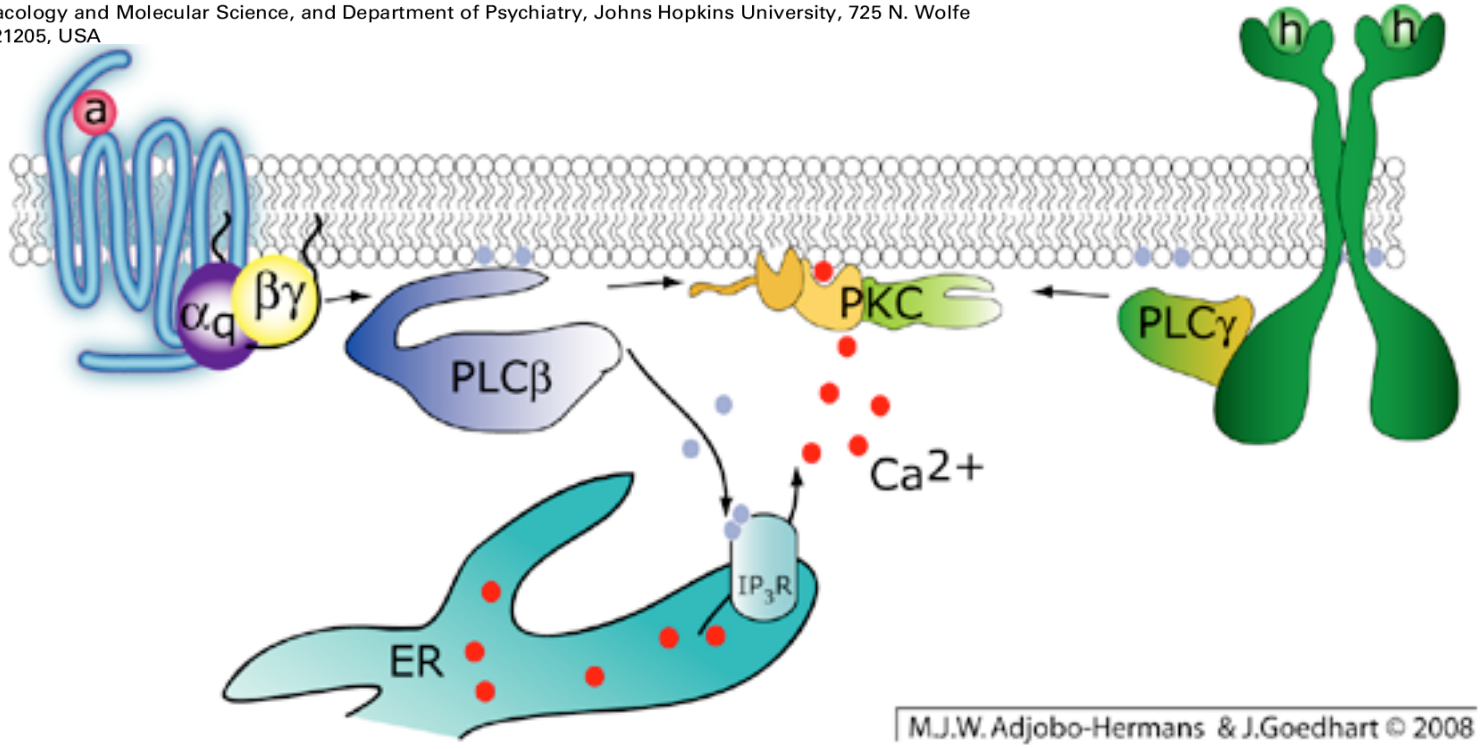
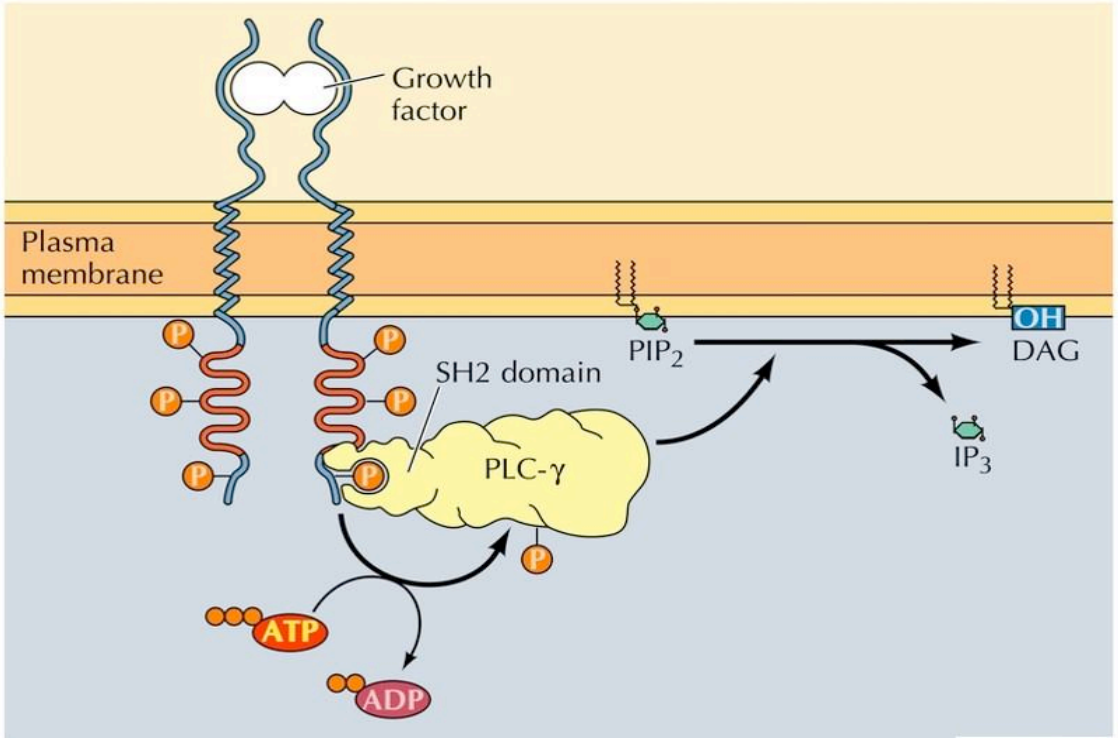


G-protein-coupled receptors (GPCRs) signal to **PLC-β** via activation of G proteins.
PLCs transform PIP2 to DAG and inositol (1,4,5)-triphosphate. IP3 activates the IP3R to cause Ca^{2+} release and Ca^{2+} entry.

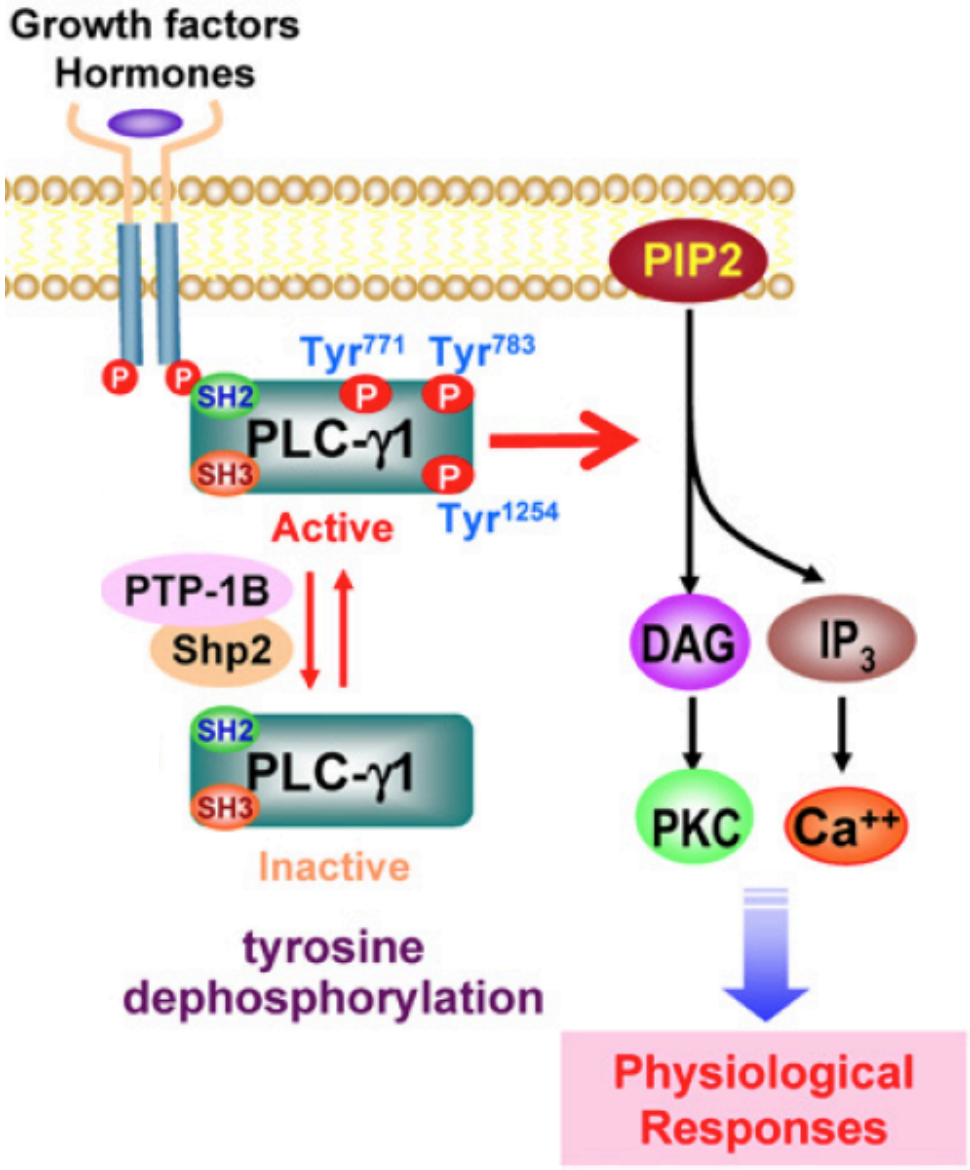
PLC_{γ}

- PLC-gamma is recruited to receptor tyrosine kinases or to adaptor proteins through the interaction between its SH2 domain
- PLC-gamma has two SH2 domains, and the one in the NH2-terminal side is responsible for the recruitment.
- Then, the PLC is phosphorylated on Tyr residues by the RTK, or by a non-receptor tyrosine kinase associated with the adaptor protein and the phosphorylated protein becomes active.



Regulation mechanism of PLC-γ1 by tyrosine phosphorylation.

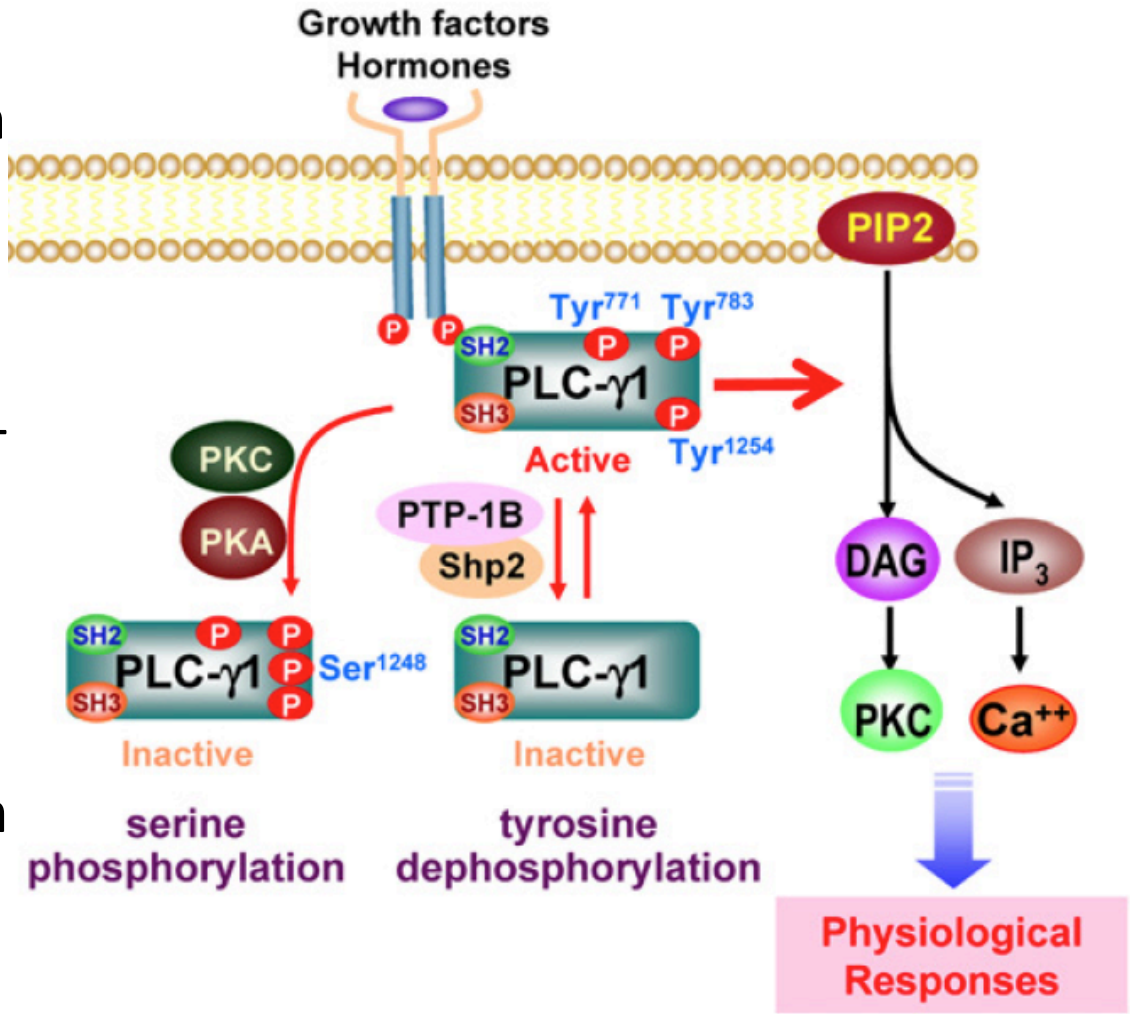
Association of PLC-γ1 via SH2 domain with phosphorylated receptor tyrosine kinases is followed by tyrosine phosphorylation of PLC-γ1 at specific tyrosine residues, necessary for its activation. PLC-γ1 hydrolyzes phosphatidylinositol 4, 5-bisphosphate (PIP2) to inositol 1, 5-trisphosphate (IP3) and 1, 2-diacylglycerol (DAG), implicated i the mobilization of intracellular Ca2+ and protein kinase C activation.



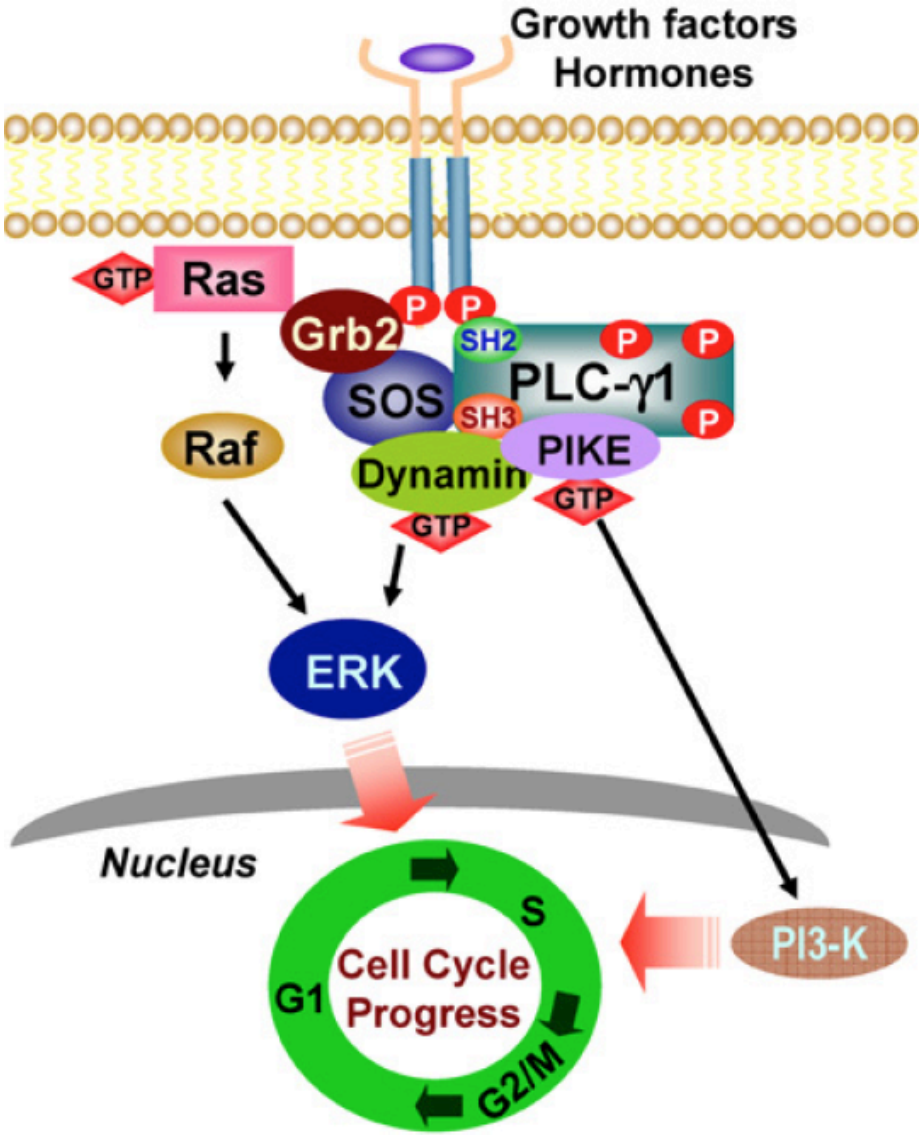
Regulation mechanism of PLC-γ1 by tyrosine phosphorylation.

To inactivate PLC-γ1, some tyrosine phosphatases such as PTP-1B and Shp2 can interact with PLC-γ1.

In addition, Ser1248 of PLC-γ1 is phosphorylated by activation of PKC or PKA upon growth factor activation.
This serine phosphorylation inhibits the enzymatic activity of PLC-γ1.



Activation mechanism of PLC-γ1-mediated signaling pathway

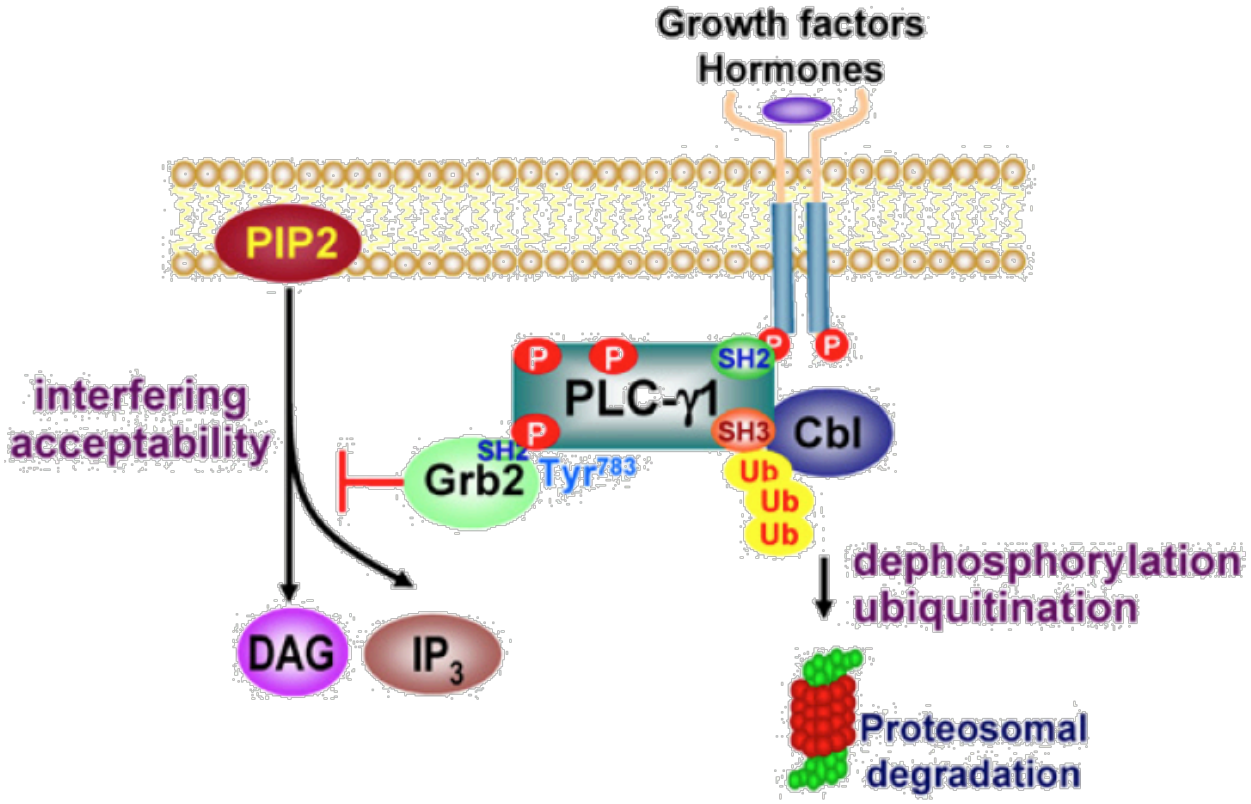


After recruiting of PLC-γ1 to receptor tyrosine kinases, PLC-γ1 can interact with various effector proteins via its SH2 or SH3 domains.

The SH3 domain mediates interactions with proteins containing proline-rich sequences such as SOS, which can activate the Ras-mediated signaling pathway and cell cycle progression.

In addition, the SH3 domain of PLC-γ1 directly interacts with dynamin and PIKE, potentiating growth factor-induced mitogenesis.

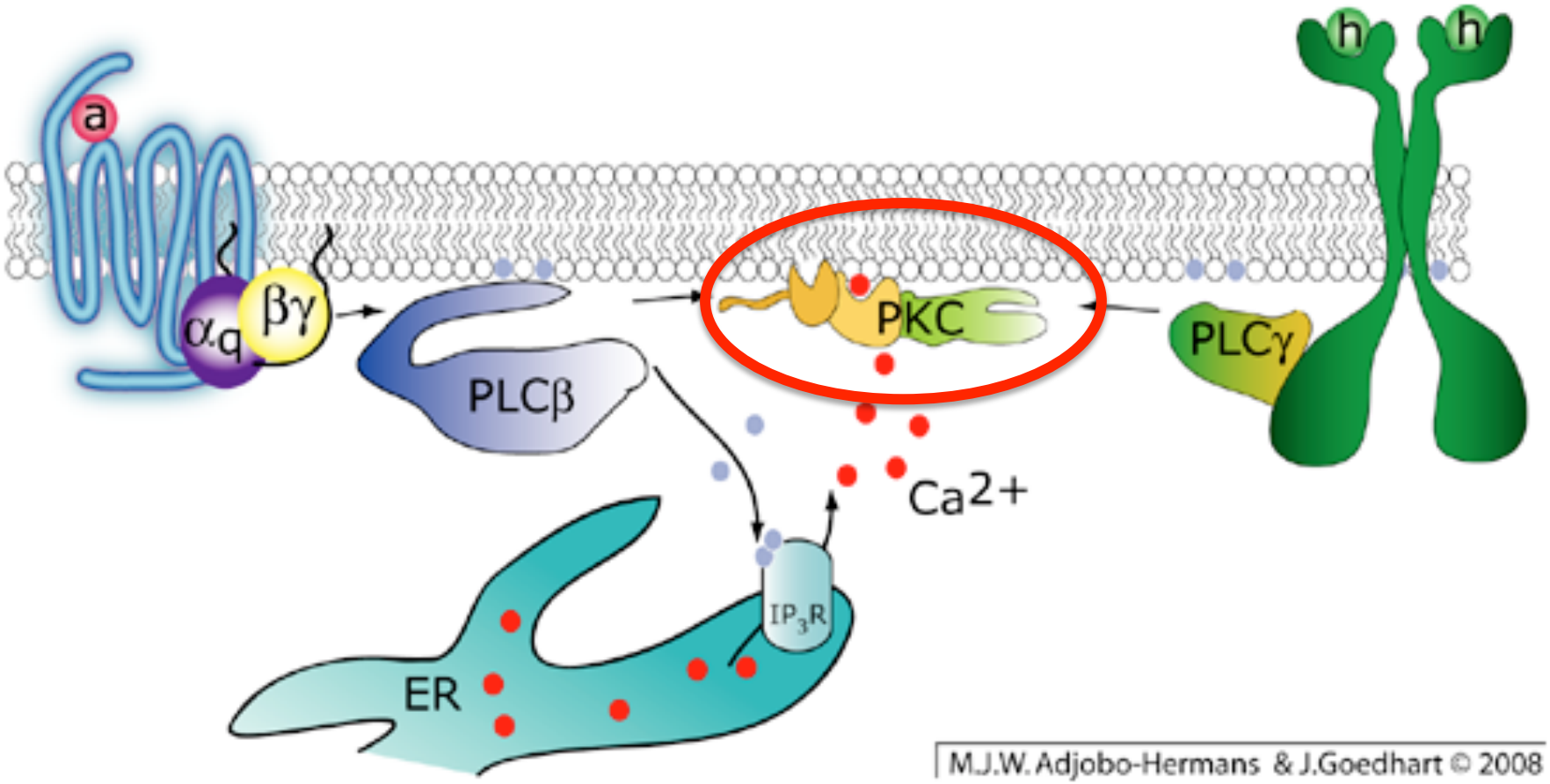
Inactivation mechanism of PLC-γ1-mediated signaling pathway.



PLC-γ1 can interact with several protein such as Grb2 and Cbl.

Cbl directly associates with SH3 domain of PLC-γ1 and potentiates the ubiquitination and proteosomal degradation of PLC-γ1.

Grb2 directly interacts with tyrosine-phosphorylated PLC-γ1 at Tyr783, and thereby inhibits the EGF-induced PLC-γ1 activity by interfering with the acceptability of PLC-γ1 to its substrates.


a
αq
βγ
PLCβ
PKC
PLCγ
h
h
Ca2+
IP3R
ER
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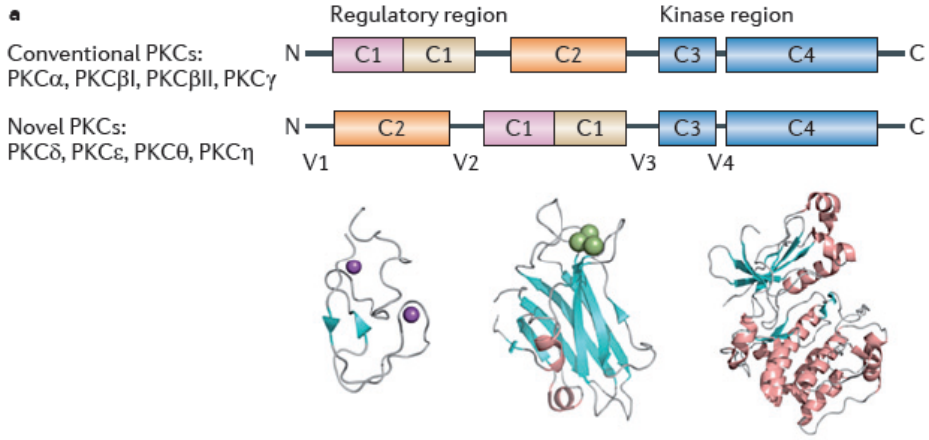
Protein kinase C and other diacylglycerol effectors in cancer

Erin M. Griner and Marcelo G. Kazanietz

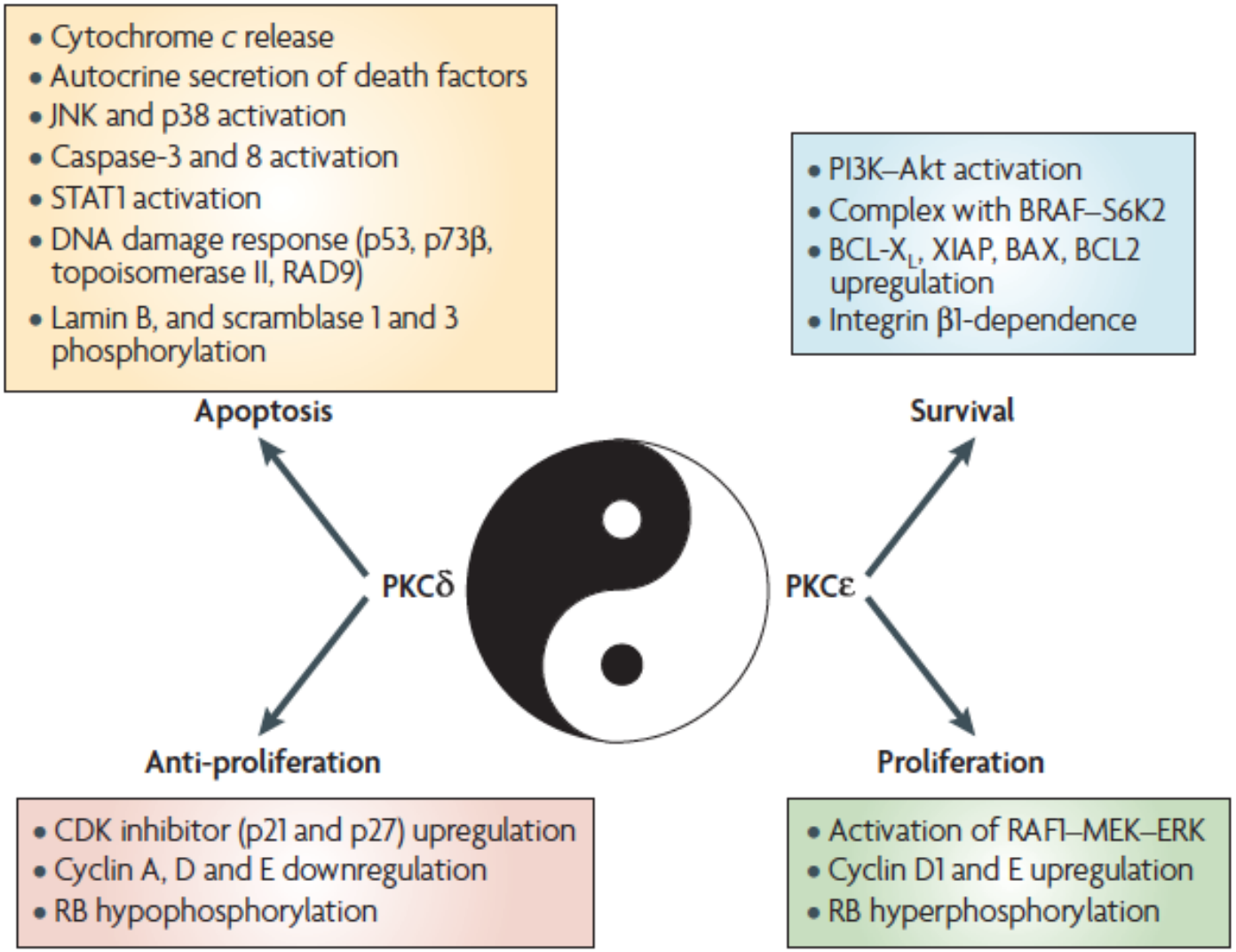


Isozyme	Overall homology (%)	C1 domain (%)	C2 domain (%)	Kinase domain (%)
PKCβ	38	51	8	65
PKCε	41	44	13	62
PKCθ	64	85	52	67

At a glance

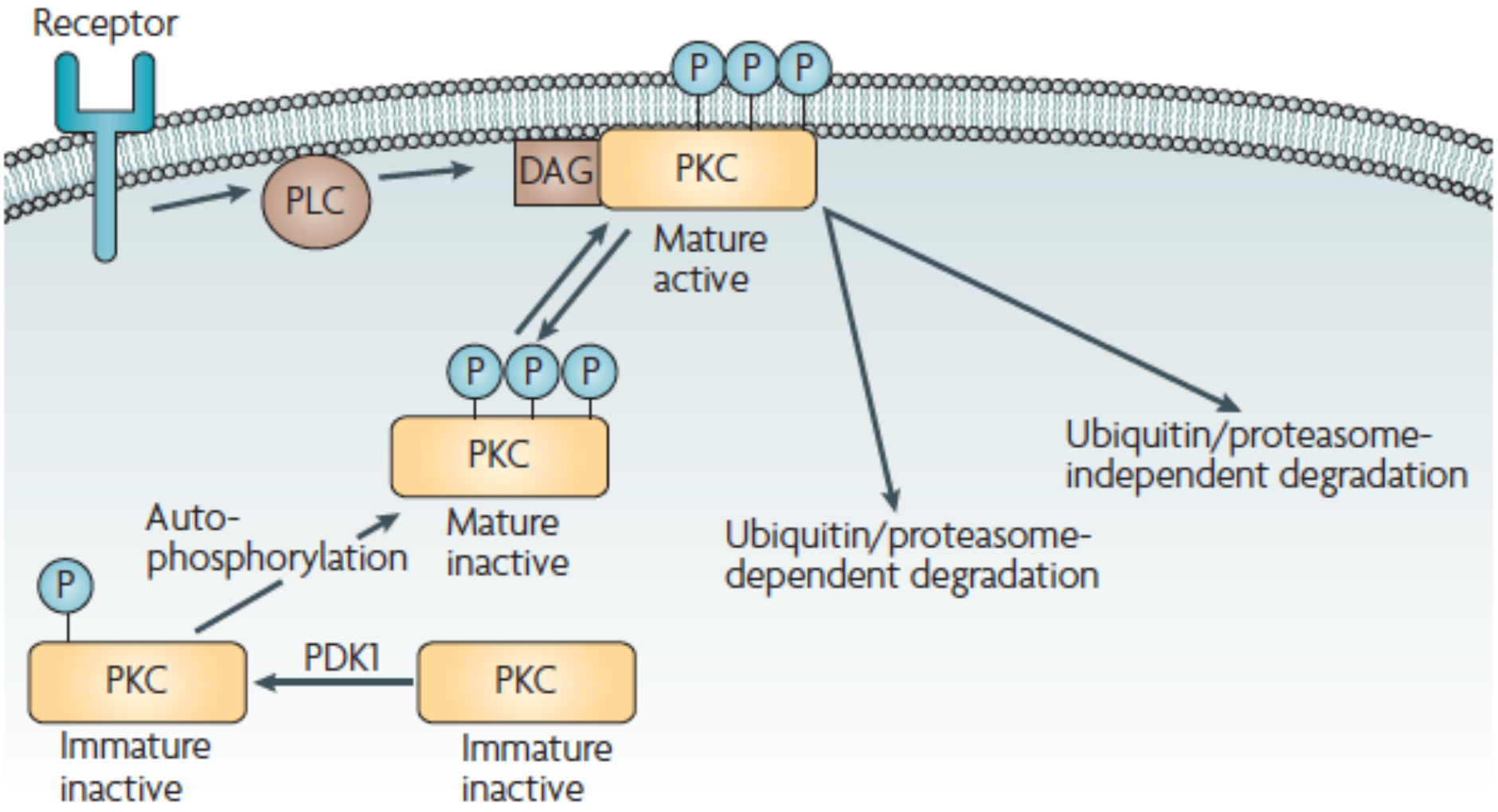
- Protein kinase C (PKC) is a family of serine/threonine kinases that regulates a diverse set of cellular processes including proliferation, apoptosis, cell survival and migration, and there is a substantial amount of evidence linking PKC to tumorigenesis. Studying PKC regulation of these processes and how misregulation might contribute to tumorigenesis is complicated by the fact that each individual PKC isozyme has a distinct role in these processes in a cell-type-dependent manner.
- There is a limited number of instances in which mutation of PKCs in humans is linked to a cancer phenotype; however, altered levels of PKC isoforms can be found in many types of human cancers. In many cases, altered expression of PKC can also be linked to disease progression.
- PKCs were originally thought to be pro-mitogenic kinases, but this effect seems to be PKC-isozyme-dependent and cell-type-dependent, as many PKCs can also inhibit cell-cycle progression. Several PKCs have been shown to be anti-proliferative in various cell types, generally through upregulation of cell-cycle inhibitors.
- PKCε promotes cell survival in many cell types through increased activation of the Akt pathway and upregulation of pro-survival factors. Furthermore, PKCε overexpression has been linked to chemotherapeutic resistance in various cell types.
- PKCδ is generally considered a growth inhibitory or pro-apoptotic PKC, and many types of apoptotic stimuli can induce PKCδ translocation to mitochondria, leading to cytochrome c release, caspase-3 cleavage and generation of a constitutively active PKCδ catalytic fragment that is important for phosphorylation of nuclear PKC substrates. Activation of PKCδ can also trigger the autocrine secretion of death factors and kill cells through the activation of the extrinsic apoptotic pathway.
- Several PKCs have been implicated in invasion and metastasis of cancer cells; however, knowledge of the molecular mechanisms through which PKC might contribute to these processes is still vague.
- Emerging evidence indicates that PKC, specifically PKCβII, might be an important mediator of vascular endothelial growth factor (VEGF)-induced angiogenesis and have a role in VEGF-induced endothelial-cell proliferation.
- Several other classes of proteins can be activated by phorbol esters or DAG, including protein kinase D, Ras guanyl nucleotide-releasing proteins, chimaerins, diacylglycerol kinases and Munc13s. Several of these proteins have also been implicated in cancer progression.

Disease or indication	Main PKC isozyme implicated in the pathology	Pathology associated with PKC	Refs
Cancer	PKCα	Proliferation, intravasation and metastasis	228
	PKCβ	Vasculogenesis and cancer cell invasion	54
	PKCδ	Angiogenesis	30
	PKCε	Proliferation, tumour survival, metastasis and resistance to chemotherapy	229
	PKCθ	Gastrointestinal stromal cell proliferation	58
	PKCη	Glioblastomal cancer; increased proliferation and resistance to radiation	89
Diabetic complications	PKCβII	Vascular complications	2
	PKCβ	Knockout attenuates obesity and increased glucose transport (role of PKCβI?)	230,231
	PKCδ	Stimulation of islet cell function	60
Ischaemic heart disease	PKCδ (mediates injury)	Increased ROS production, decreased ATP generation and increased apoptosis and necrosis	52,160, 225,232
	PKCε (protective effect; useful for predictive ischaemia such as in surgery or organ transplantation)	Protection of mitochondrial functions and proteasomal activity, activation of ALDH2 and reduction of aldehydic load	4,118,160, 195,233
Heart failure	PKCα	Decreased cardiac contractility, force of myofilaments, uncoupling of β-adrenergic receptors	201,234
	PKCβII	In rats: decreased proteasomal activity, removal of misfolded proteins in several models and disregulation of calcium handling	5,108,209
	PKCβII	Conflicting data in mice‡: overexpression either results in hypertrophy or it is not required for hypertrophy; PKCβII has also been shown to decrease or increase contractility	204–208
	PKCε	Increased fibrosis, fibroblast proliferation and inflammation	4,5,35
Psoriasis	PKCδ	Increased inflammation, increased proliferation and disregulation of angiogenesis	12,66
Pain	PKCγ	Key mediator of pain in dorsal root ganglia	44
	PKCε	Key mediator of pain in spinal cord	44
Autoimmunity and inflammation	PKCδ	B cell development and inflammation	235,236
	PKCθ	Involved in many T cell responses	6,237
Stroke	PKCδ	Increased mitochondrial fission, ROS production and dysfunction of blood–brain barrier	41, 238–240
	PKCε	Cytoprotective effect; increased cerebral blood flow	41,241
Bipolar disorders	PKCα	Altered gene expression	10,11, 65,112
	PKCε	Altered neuronal transmission	63
Asthma and other lung diseases	PKCθ	Inflammation and airway hyper-responsiveness	75
	PKCδ (loss of other isozymes may also contribute to disease pathology)	Eosinophil activation	242
Parkinson's disease	PKCδ	Inflammation and neuronal cell death	8,243,244

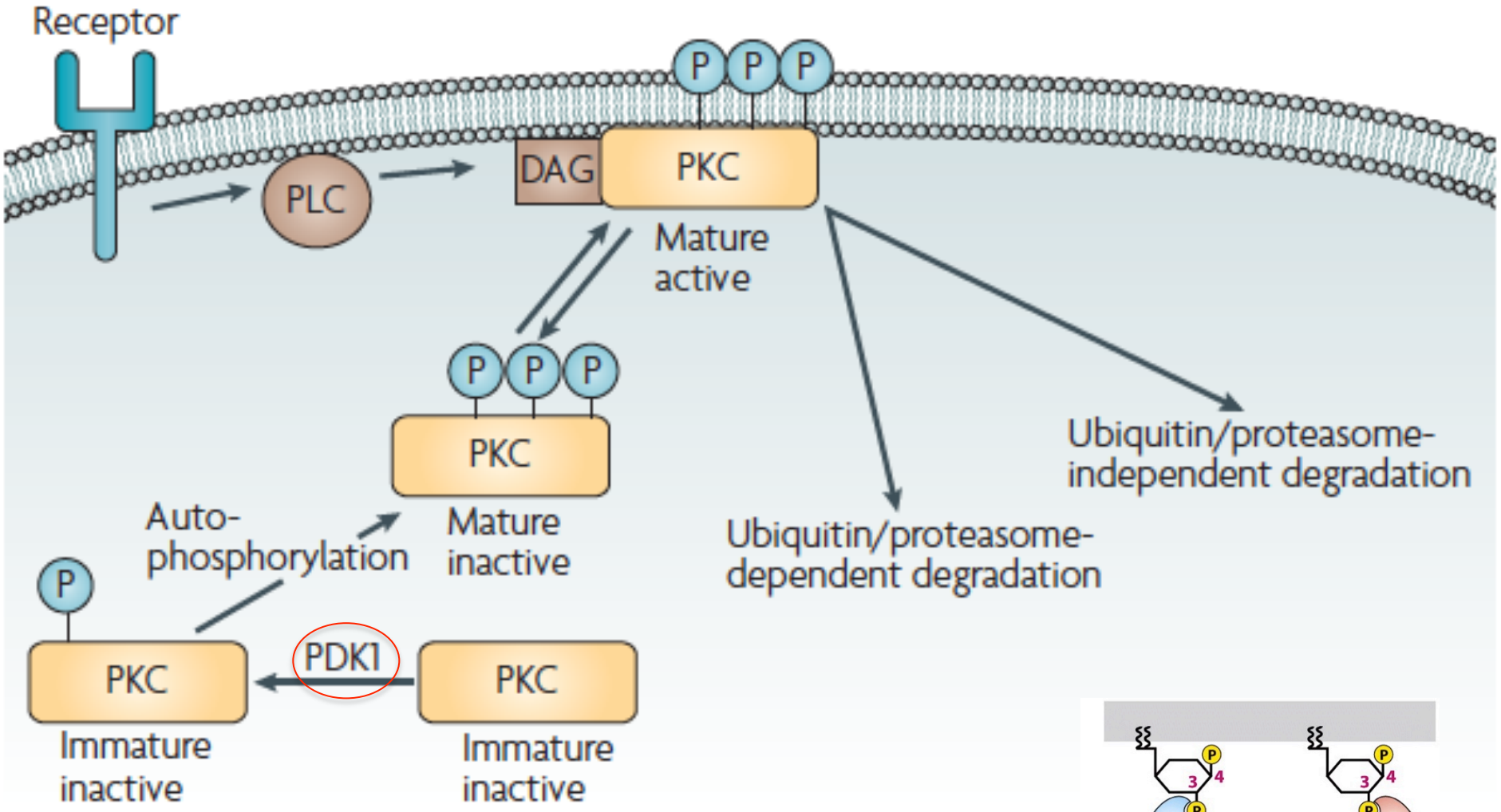


Protein kinase Cδ (PKCδ) and PKCε have opposing roles in regulating apoptosis, survival and proliferation.

- **PKCδ** is pro-apoptotic and negatively regulates proliferation
- **PKCε** is a pro-mitogenic and pro-survival kinase.



Inactive conformation by intramolecular interactions between the N-terminal region and the kinase domain



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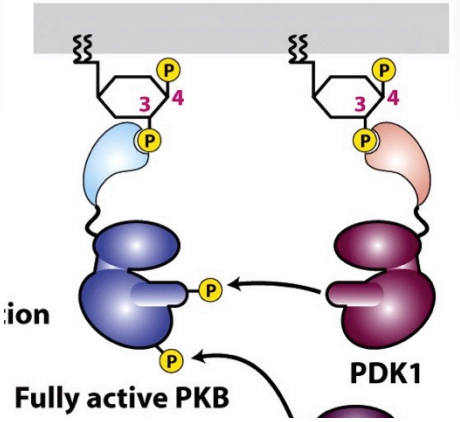
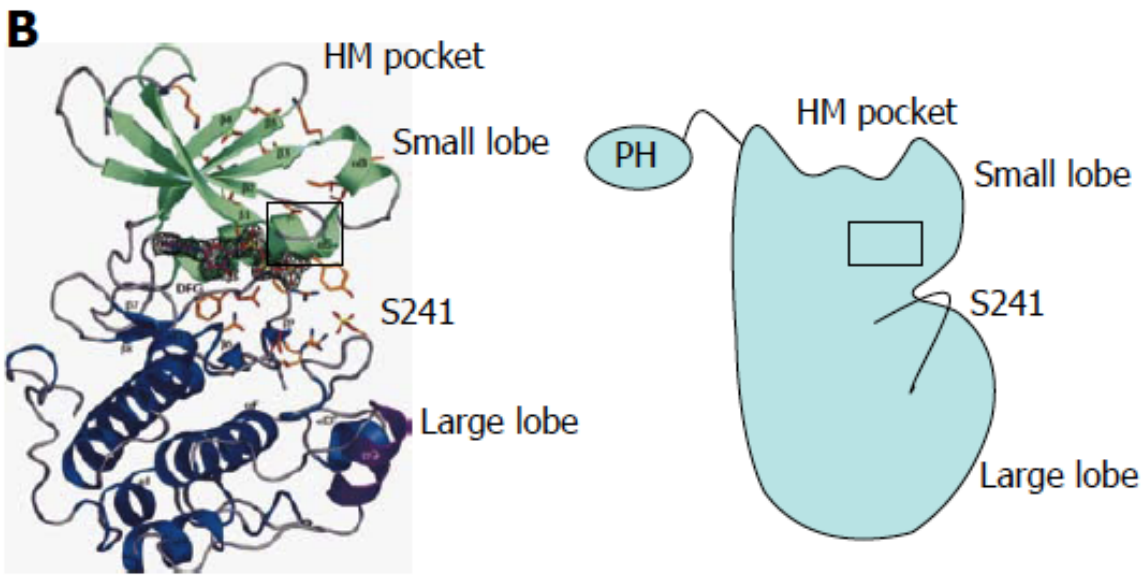


Phosphoinositide-dependent kinase-1 (PDK1)



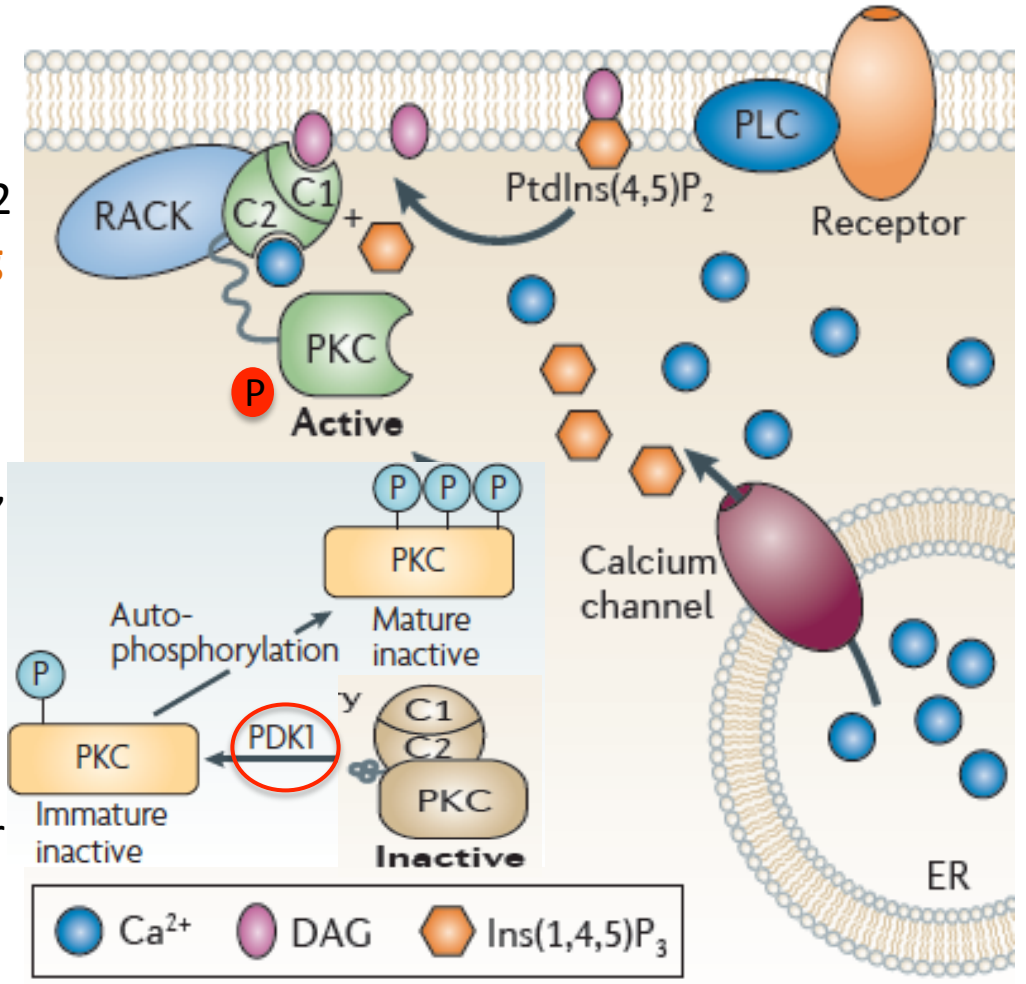
- PDK1 is a **master kinase**, crucial for the activation of AKT/PKB and many other kinases including **PKC**, S6K, SGK.
- Mice lacking PDK1 die during early embryonic development, indicating that this enzyme is critical for transmitting the growth-promoting signals necessary for normal mammalian development.
- The structure of PDK1 can be divided into two domains; the kinase or catalytic domain and the PH domain.
- The PH domain functions mainly in the interaction of PDK1 with phosphatidylinositol (3,4)-bisphosphate and phosphatidylinositol (3,4,5)-trisphosphate.
- The kinase domain has crucial binding sites: the substrate binding site, the ATP binding site.
- **PDK1 is constitutively active and at present, there is no known inhibitor for PDK1**.

- PDK1 phosphorylates the activation-loop
- Autophosphorylation leads to stabilization of the enzyme.
- PKC, 'primed' for activation by DAG and calcium, is released into the cytosol and kept in an inactive conformation by intramolecular interactions between the N-terminal region and the kinase domain.
-On RTK activation, PKC is tethered to the membrane through calcium binding to the C2 domain, where it interacts with its **anchoring protein**, receptor of activated C-kinase (**RACK**).
-DAG binding confers a high-affinity interaction between PKC and the membrane, leading to a massive conformational change, allowing for substrate binding, phosphorylation and the activation of downstream signalling effectors.

-The short half-life of DAG is probably key for reversing the activation of PKC, down-regulated through internalization (caveolae or ubiquitin–proteasome-dependent)

Protein kinase C, an elusive therapeutic target?

Daria Mochly-Rosen[1,2], Kanad Das[2] and Kevin V. Grimes[1,2]



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Cell Signaling by Receptor Tyrosine Kinases

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