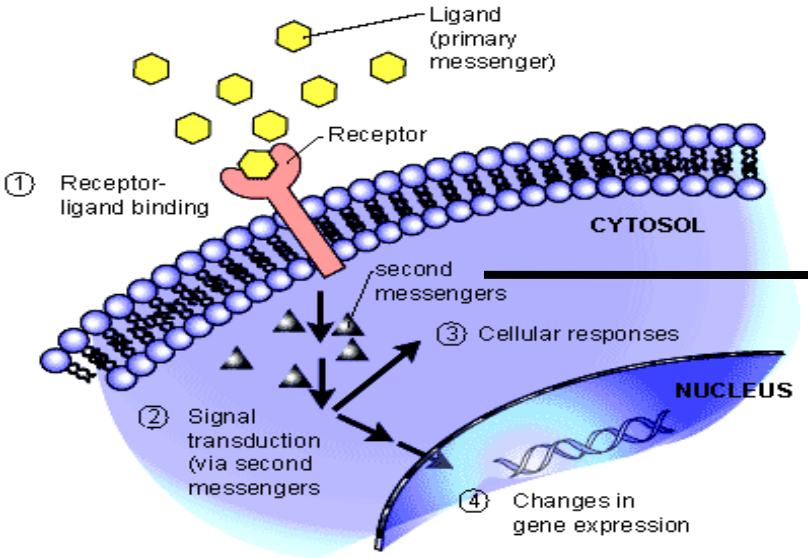
Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

Lecture 18

Regulation of gene expression by Protein Kinase C



GPCRs → Trimeric G proteins

cAMP (PKA)

cGMP (PKG)

IP3 , DAG, Ca^{2+}

PKC

Types of Membrane Receptors

1. Receptors that regulate cyclic AMP formation
2. Receptors that regulate cyclic GMP formation
3. Receptors that regulate diacylglycerol (DAG) and inositol triphosphate (IP3)
4. Receptors that possess protein kinase activity

Trimeric G proteins

$G_s \rightarrow \alpha_s \rightarrow AC \rightarrow cAMP\uparrow$

$G_i \rightarrow \alpha_i \rightarrow AC \rightarrow cAMP\downarrow$

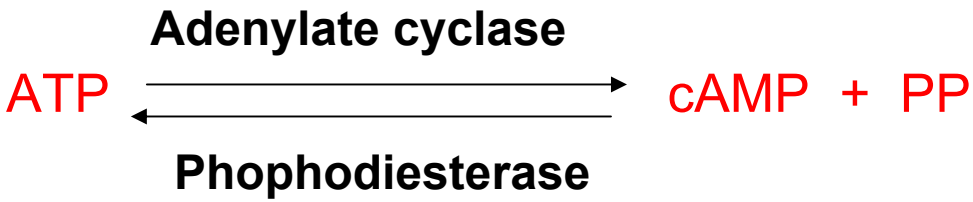
$G_q \rightarrow \alpha_q \rightarrow PI\text{-}PLC \rightarrow IP_3 + DAG$

cAMP dependent-protein kinase A pathway

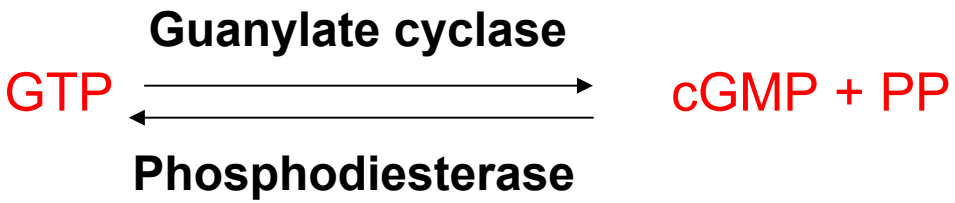
cGMP dependent PKG pathway

Ca^{2+} dependent PKC pathway

1. Adenylate cyclase



2. Guanylate cyclase



3. Phospholipase C

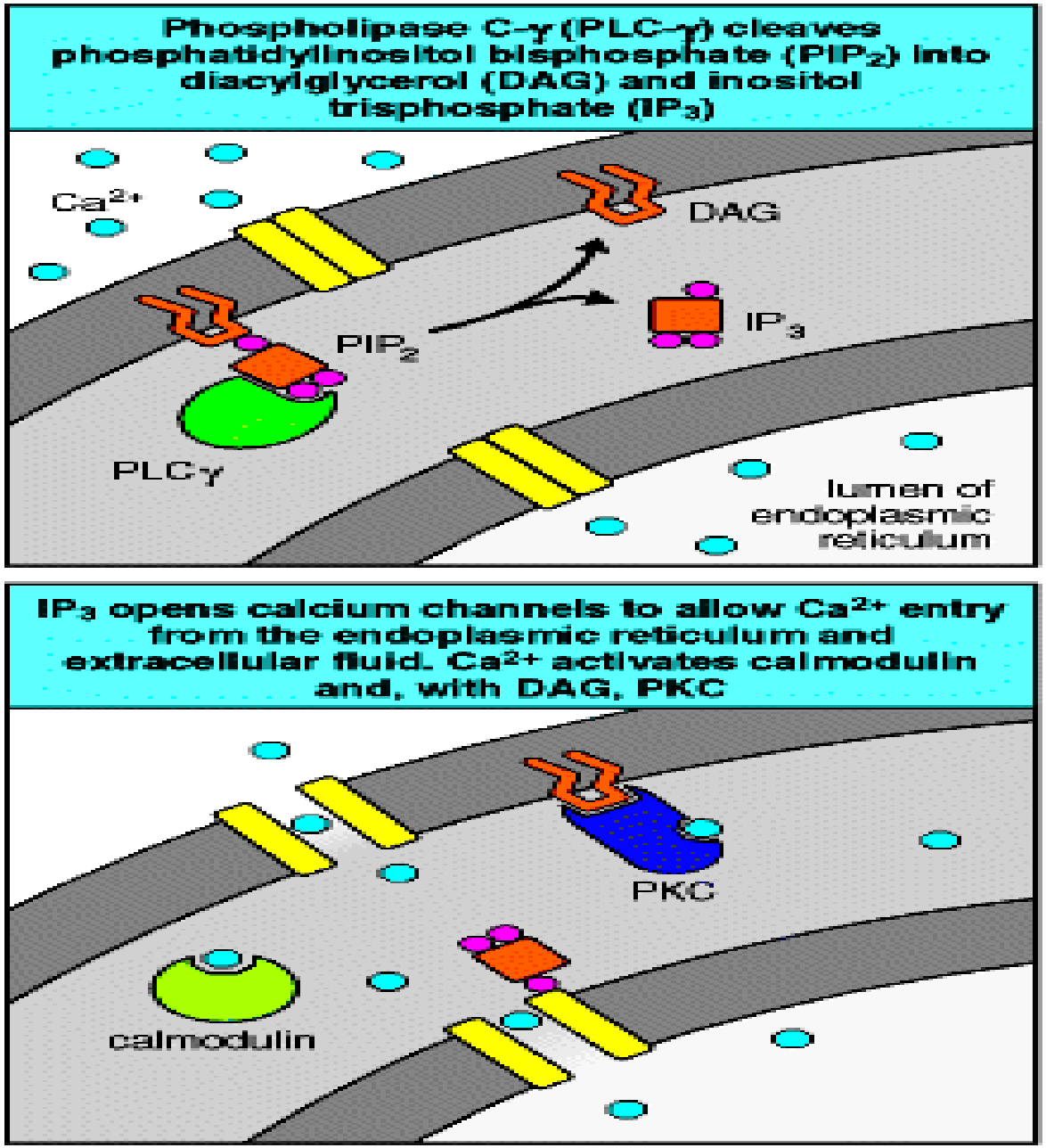
Phosphotidylinositol 4,,5-bisphosphate (PIP_2) —**Phospholipase C (PLC)**→ Inositol triphosphate (IP3) + diacylglycerol (DAG)

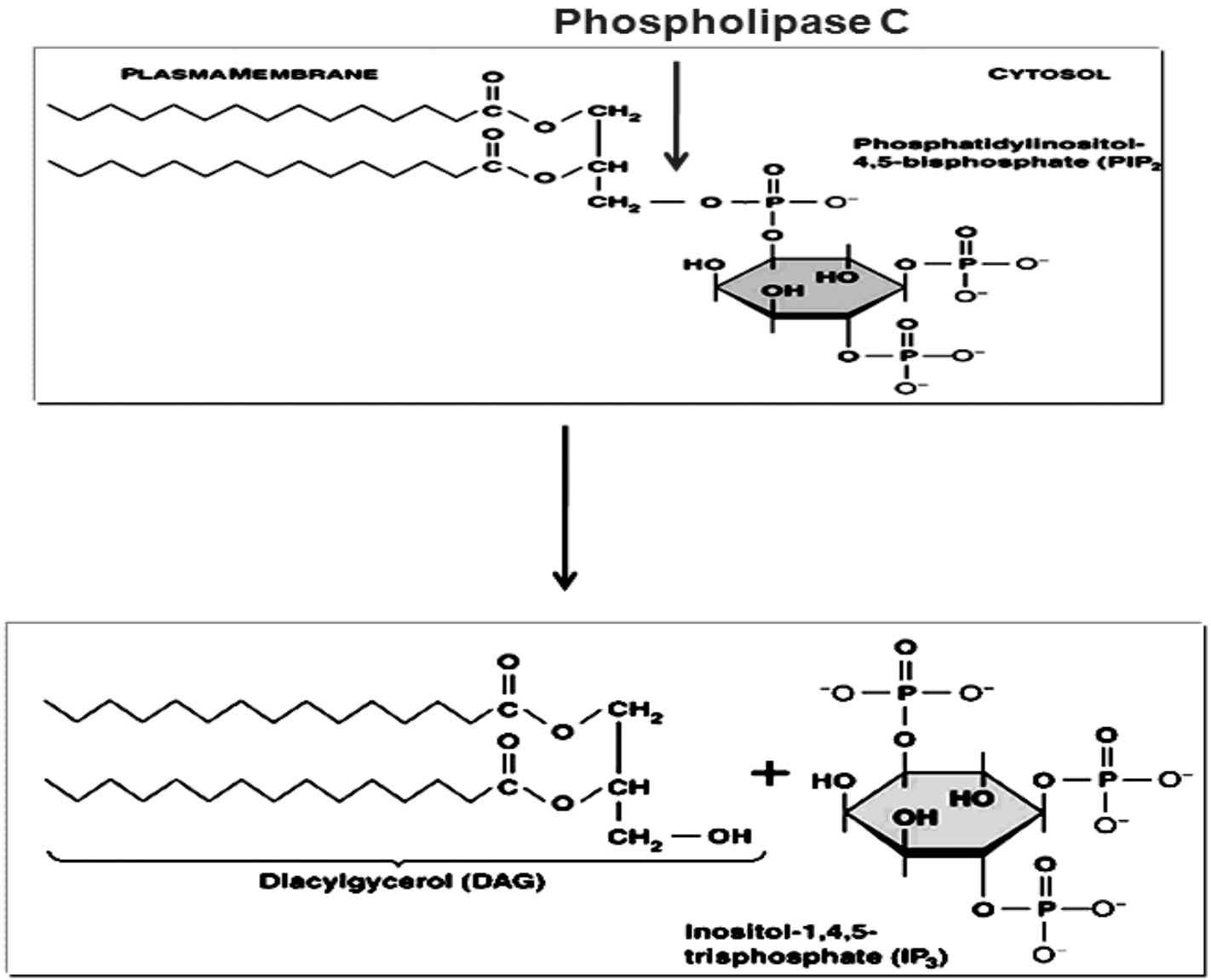
Signal transduction through the receptor-triggered hydrolysis of phosphatidyl inositol 4,5-bisphosphate.

1. An activated receptor stimulates the GTP for GDP exchange on a trimeric G protein (Gq). Activated Gq activates phospholipase C.

2. Membrane bound phospholipase C hydrolyzes the phosphodiester bond linking the phosphorylated inositol unit to the acylated glycerol moiety.

3. The hydrolysis of PIP2 leads to the production of 2 intracellular signaling molecules: IP3 and DAG.


Phospholipase C-γ (PLC-γ) cleaves phosphatidylinositol bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol trisphosphate (IP_3)
Ca^{2+}
DAG
IP_3
PIP_2
PLC-γ
lumen of endoplasmic reticulum
IP_3 opens calcium channels to allow Ca^{2+} entry from the endoplasmic reticulum and extracellular fluid. Ca^{2+} activates calmodulin and, with DAG, PKC
PKC
calmodulin


Phospholipase C
PLASMA MEMBRANE
CYTOSOL
Phosphatidylinositol-
4,5-bisphosphate (PIP2)
Diacylgycerol (DAG)
Inositol-1,4,5-
trisphosphate (IP3)

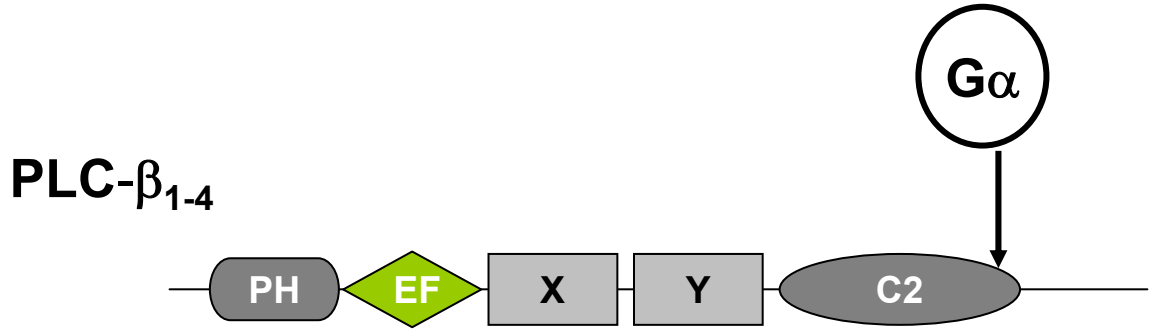
Phospholipase C (PLC)

- cytosolic enzyme
- acts on membrane-inserted phosphoinositide substrates.

Types: beta and gamma

PLC-beta is activated by G-protein-coupled receptors,

PLC-gamma is activated by receptor tyrosine kinases.



PH domain binds to PIP2

Both DAG and IP3 act as second messengers in many types of cells

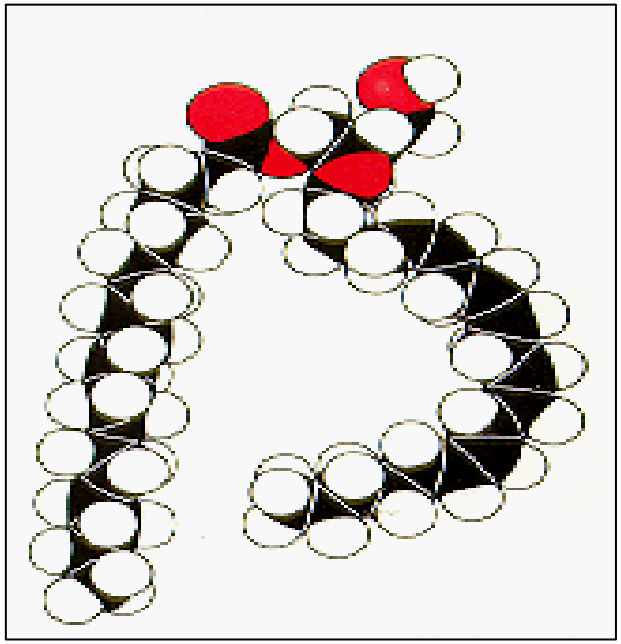
Discovered in insect salivary glands (early 1980s)

Other functions:

- platelet activation
- muscle contraction
- antibody secretion

DAG

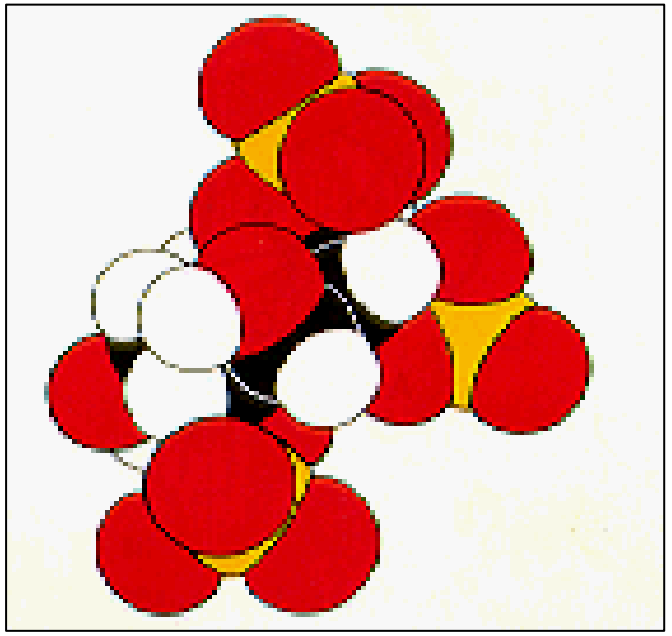
- very nonpolar, in contrast to IP3.
- Remains membrane bound.



IP3

very high density of negatively charged and polar groups.

IP3 is soluble in the cytosol and diffuses through it.



IP3

rapidly diffuses through the cytoplasm

induces the rapid release of calcium from intracellular stores-the endoplasmic reticulum and, in smooth muscle cells, the sarcoplasmic reticulum.

DAG

remains in the membrane

two potential signaling roles

1. **Can be cleaved to release arachidonic acid** ⟵
2. **Activates protein kinase C (major function)**

Arachidonic acid is the precursor for the synthesis of Eicosanoids

The first enzyme involved in their synthesis (cyclooxygenase, COX) is the target of ASPIRIN.

Aspirin actions:

-reduces inflammation and pain (inhibition of prostaglandins)

- reduces platelet aggregation and blood clotting (tromboxanes)

Protein kinase C (PKC) was discovered in the year 1977 in the laboratory of Nishizuka, Department of Biochemistry, University of Kobe, Japan.

It was originally referred to as a cyclic nucleotide-independent, proteolytically modified protein kinase from mammalian brain that was named PKM (M for Mg^{2+} required for activation) .

The full length enzyme was later shown to be activated by calcium and phospholipids and was named protein kinase C (C, for calcium ions, which fully activated the enzyme at low concentrations, and thus differentiate it from cyclic nucleotide-dependent kinases, protein kinase A and G).

Later, unsaturated diacylglycerol (DAG) was shown to be an essential activator of PKC linking the receptor-dependent inositol phospholipid hydrolysis to protein phosphorylation.

This led to the identificaiton of PKC as a key player in intercellular signal transduction research.

The PKC family

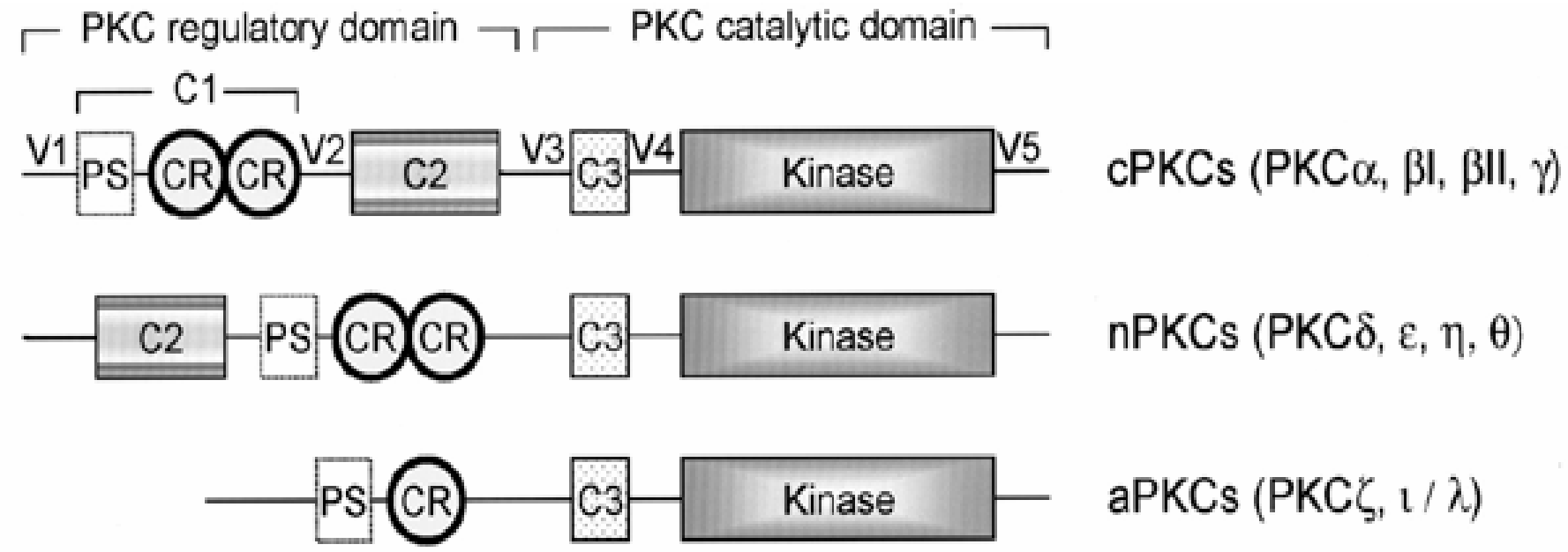
Calcium-dependent PKCs (conventional, cPKCs) :

PKC-I, -II, and -III (cPKCγ, β and α, respectively)

Calcium-independent PKCs (novel, nPKCs)

Atypical PKCs (aPKCs)

Protein kinase C (PKC)

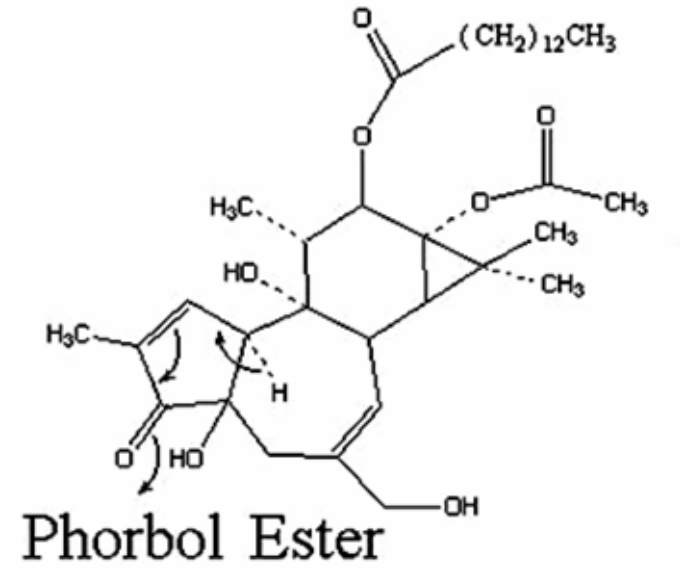


PKC is involved in regulation of diverse cellular processes including growth, differentiation, neural development, synaptic transmission; axonal regeneration, smooth muscle contraction and relaxation, endocrine and exocrine secretion, tumor promotion, and aging.

Only some of these processes involve regulation of gene expression

The importance of protein kinase C in controlling cell division and proliferation was revealed by the action of compounds known as phorbol esters.

In the year 1982, it was demonstrated that phorbol derivatives directly activate PKC and activation with phorbol esters leads to translocation of PKC from the cell soluble to the cell particulate fraction.



Phorbol Ester

How do phorbol esters act as tumour promoters?

Tumor-promoting phorbol esters such as phorbol 12-myristate 13-acetate (PMA), possess a molecular structure that is similar to that of DAG. These agents can therefore substitute for DAG and activate PKC directly in vitro and in vivo.

Like DAG, phorbol esters dramatically increase the affinity of PKC for Ca2+, resulting in its complete activation at physiological Ca2+ concentrations.

Since the phorbol esters are slowly metabolised, they persist in tissues for much longer times than DAG and cause a prolonged activation of PKC leading to uncontrolled cell proliferation

PKC is anchored to membranes by specific proteins and the individual isozymes are often localized to specific subcellular sites following activation.

In the year 1991, Mochly-Rosen and co-workers identified PKC anchoring proteins and named them **‘receptors for activated C kinases (RACKs)’**

Mochly-Rosen, H. Khaner and J. Lopez, Identification of intracellular receptor proteins for activated protein kinase C, *Proc Natl Acad Sci USA* **88** (1991), pp. 3997–4000

Regulation

of

cell proliferation

By

PKC

Gene expression

PKC

↓

Phosphorylation of Transcription factors

↓

Activation of genes

↓

Cell proliferation

Early response:

PKC → Trans-acting factor-P

↓

Immediate early genes → Third messenger

Late response:

PKC → Third messenger-P

↓

Activate genes

↓

Cell proliferation

Mammalian cells are usually cultured in presence of serum since it contains growth factors that promote cell proliferation

Activation of specific kinases such as PKC

Phosphorylation of transcription factors (Early response phase) Ex. Serum Response Factor (SRF)

genes controlling cell proliferation such as *Fos*, *Junb*, *Fosb*, and *Egr1* were regulated by SRF

Phosphorylation of transcription factors (Late response phase) Ex. c-fos, NFκβ

PKC IS INVOVLED IN THE PHOSPHORYLATION OF TRANSCRIPTION FACTORS OF BOTH EARLY AND LATE RESPONSE PHASES.

Immediate early genes (IEGs) are genes which are activated transiently and rapidly in response to a wide variety of cellular stimuli.

They represent a standing response mechanism that is activated at the transcription level in the first round of response to stimuli, before any new proteins are synthesized.

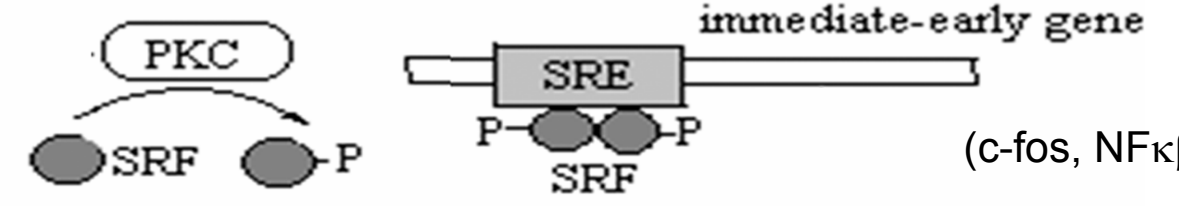
Thus IEGs are distinct from "late response" genes, which can only be activated later following the synthesis of early response gene products.

Thus IEGs have been called the "gateway to the genomic response".

Many IEG products are naturally transcription factors or other DNA-binding proteins.

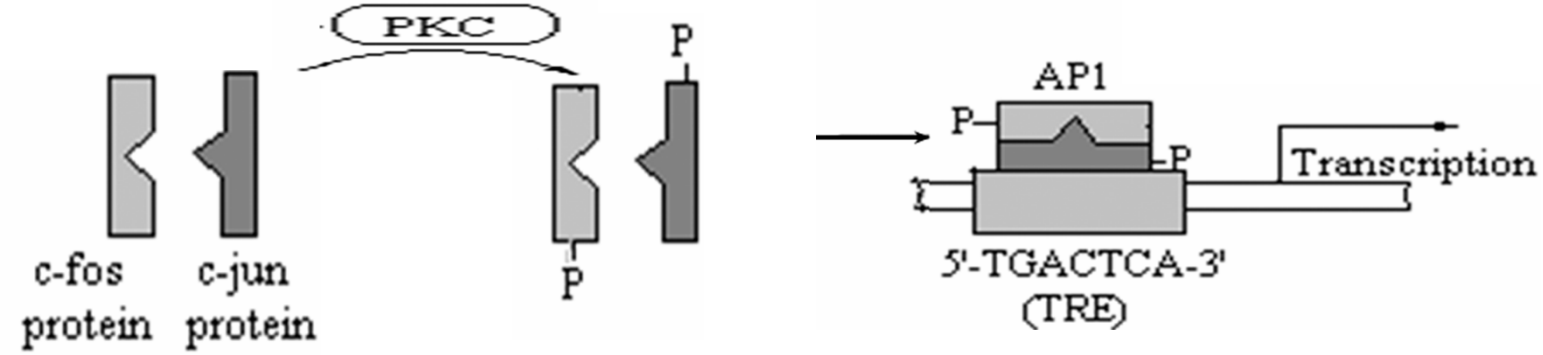
The earliest known and best characterized include *c-fos*, *c-myc* and *c-jun*, genes

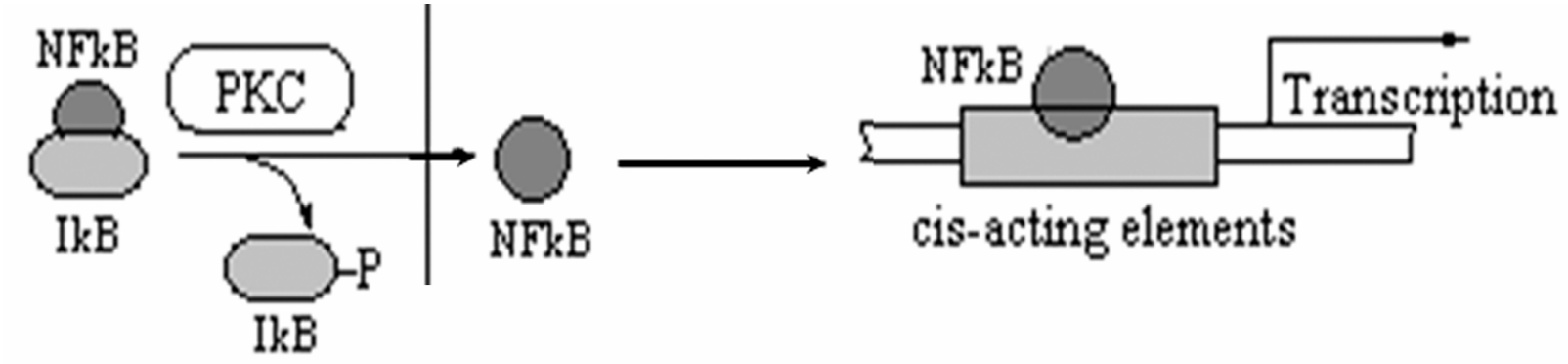
PKC plays a key role in the activation of transcription of IEGs.



(c-fos, NFκβ , Egr-1, etc.)

Late response phase



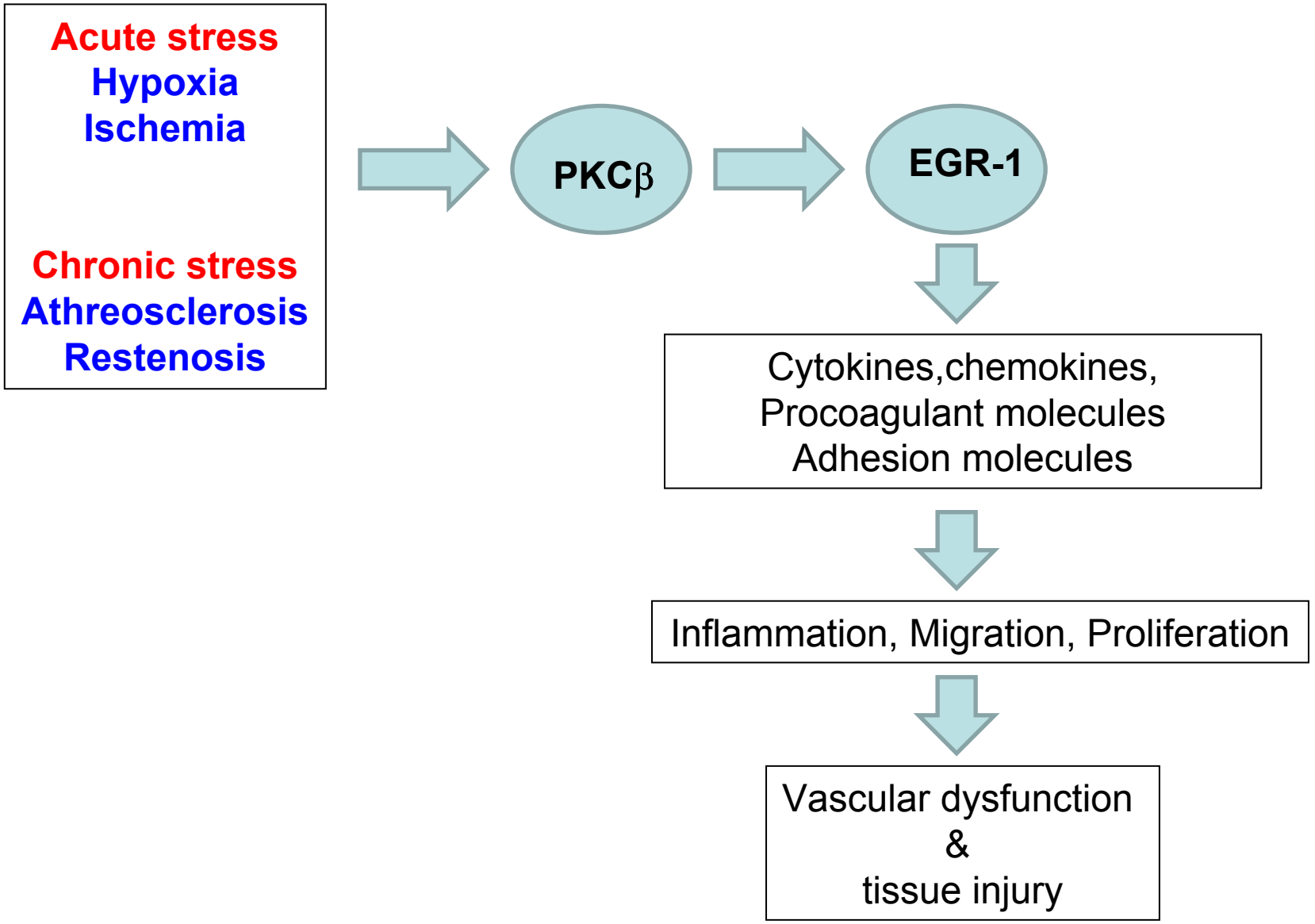


Egr-1

The early growth response gene product (Egr-1), also known as Zif268, NGF1-A, Krox24, or TIS8, is a zinc finger transcription factor first identified due to its characteristic pattern of expression after exposure of cells to mediators associated with growth and differentiation.

Egr1 is induced rapidly, within minutes of a stimulus, and is rapidly decayed, often within hours.

(PKCβ-Egr-1): implications for vascular stress



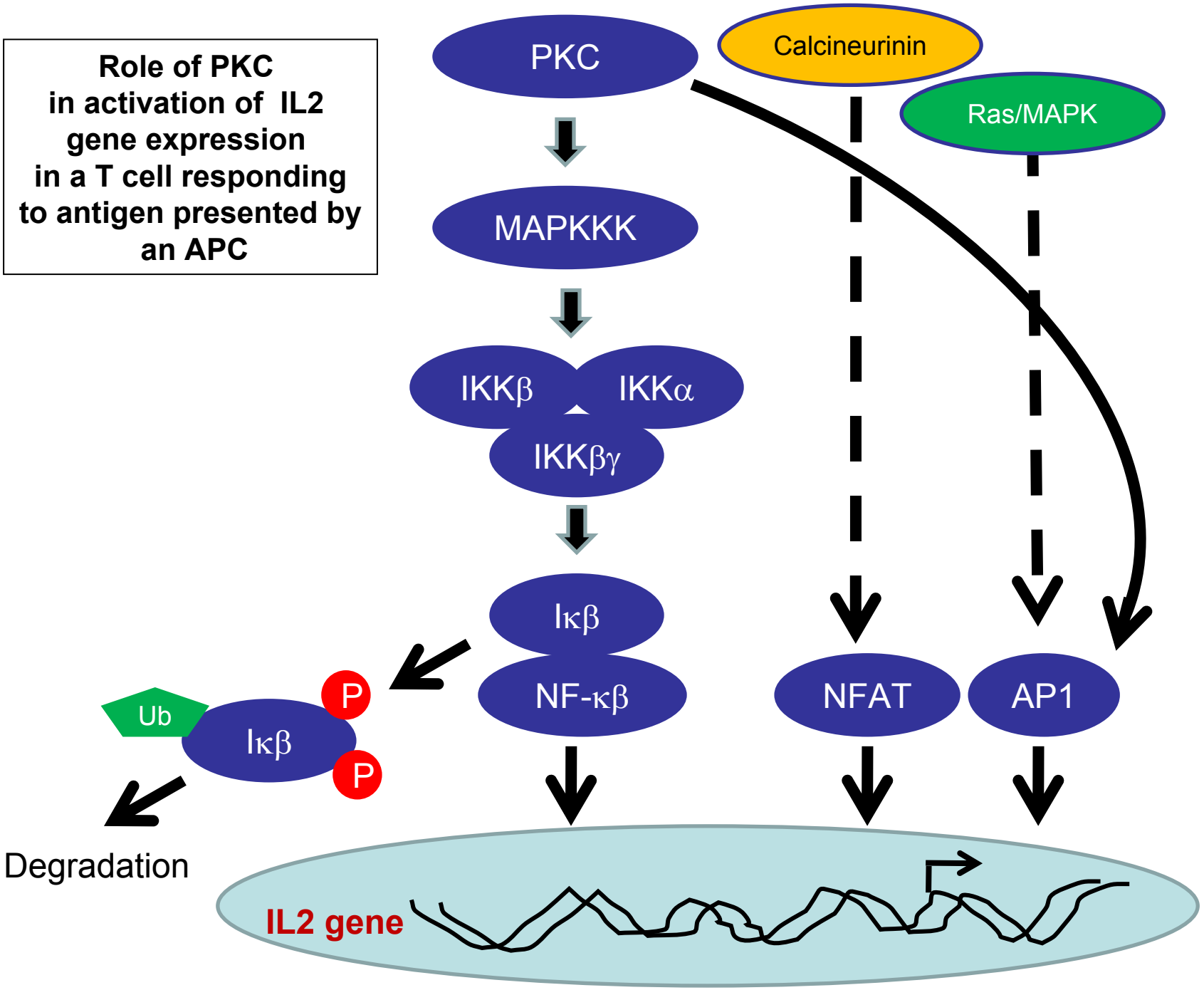
Adapted from: Yan, S.-F. et al. J Am Coll Cardiol 2006;48:A47-A55

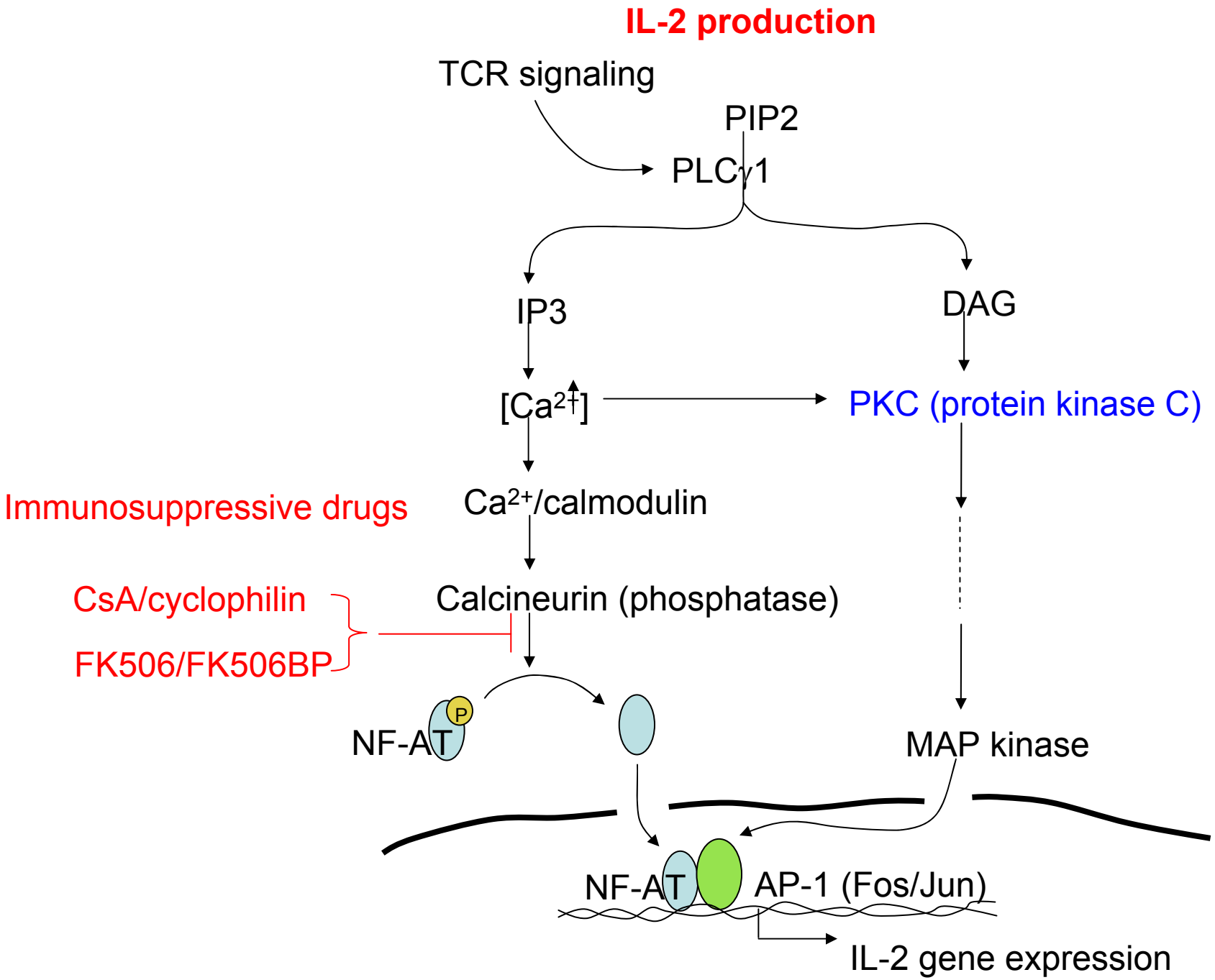
Role of PKC

in the regulation of

T cell functions

Role of PKC in activation of IL2 gene expression in a T cell responding to antigen presented by an APC




IL-2 production
TCR signaling
PIP2
PLCγ1
IP3
DAG
[Ca2+]
PKC (protein kinase C)
Immunosuppressive drugs
Ca2+/calmodulin
CsA/cyclophilin
FK506/FK506BP
Calcineurin (phosphatase)
P
NF-AT
MAP kinase
NF-AT
AP-1 (Fos/Jun)
IL-2 gene expression

Cyclosporine A (CsA)

Immunosuppresive drug used in transplant surgery patients.

Inhibits calcineurin by blocking the active site of calcineurin and preventing it from acting on any molecules within the cell.

-major consequence is the inhibition of activation of the transcription factor, NFAT, thus preventing the rejection of foreign organs or bone marrow by disrupting the signaling pathway that activates T cells.

Evidence for a prominent role for PKC in T cell function

PKC-θ, a member of novel PKC family is expressed primarily in T lymphocytes and muscle.

In PKC-θ knockout mice, *PKC-θ -/-* naïve T cells display defects in T cell activation due to lack of NFAT, NF-κB and AP1 activation.

The transcription factors NF-κB and AP-1 are the primary physiological targets of PKCθ, and efficient activation of these transcription factors by PKCθ requires integration of TCR and CD28 costimulatory signals.

PKCθ cooperates with the protein Ser/Thr phosphatase, calcineurin, in transducing signals leading to activation of JNK, NFAT, and the *IL-2* gene.

PKC-θ plays important roles in T cell activation, survival, apoptosis and IL-2 production.

PKC-θ

T cell activation: NFκβ, AP1, NFAT

T cell survival: Bcl-xL, Bcl2

Apoptosis: Fas/FasL

T cell proliferation: IL2

Protein kinase C-mediated signalling pathways

Myocyte Adrenergic Pathway

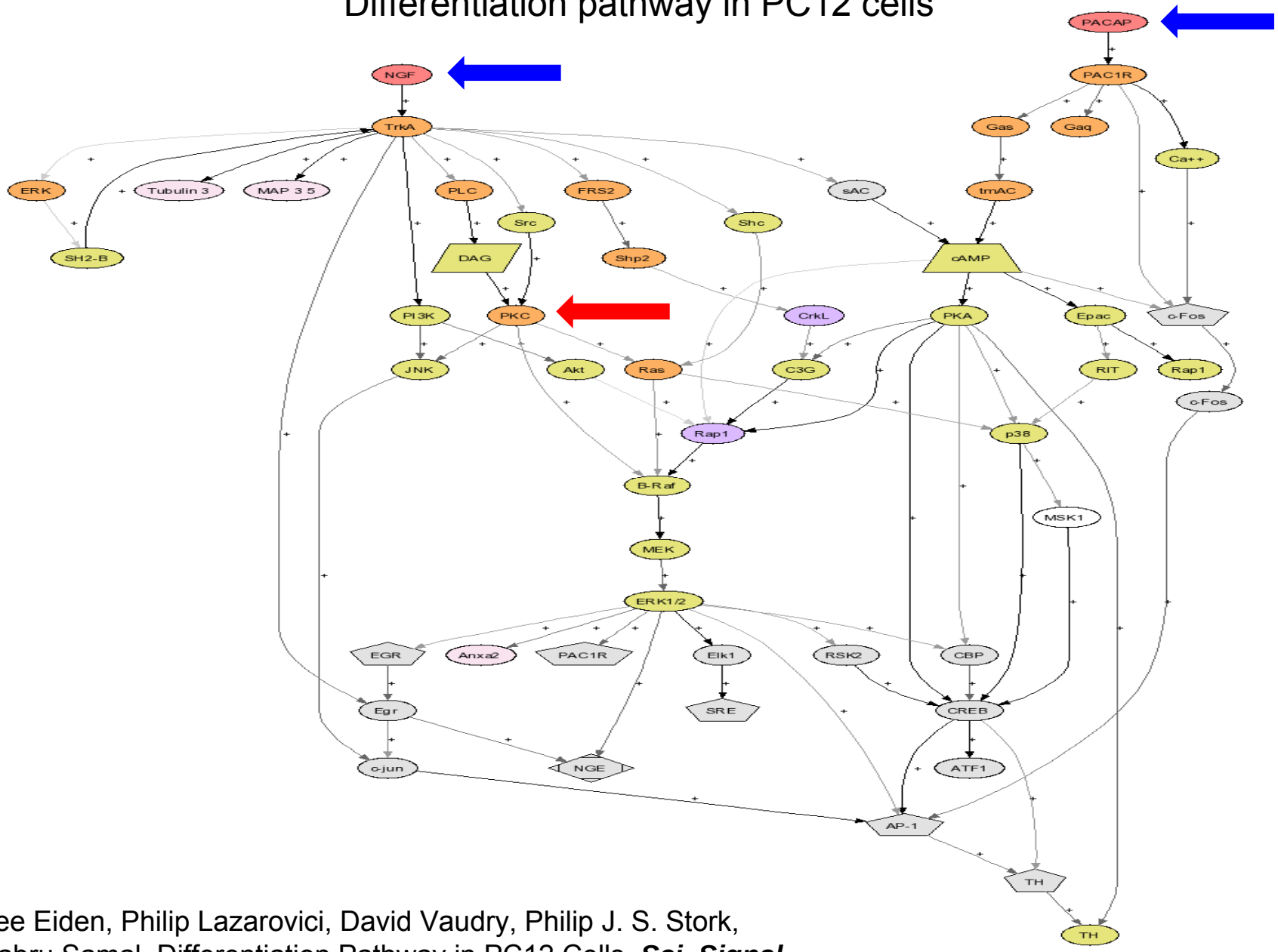
Granule Cell Survival Pathway

Differentiation Pathway in PC12 Cells

PC12 is a clonal cell line derived from a transplantable rat adrenal pheochromocytoma, which responds reversibly to nerve growth factor (NGF).

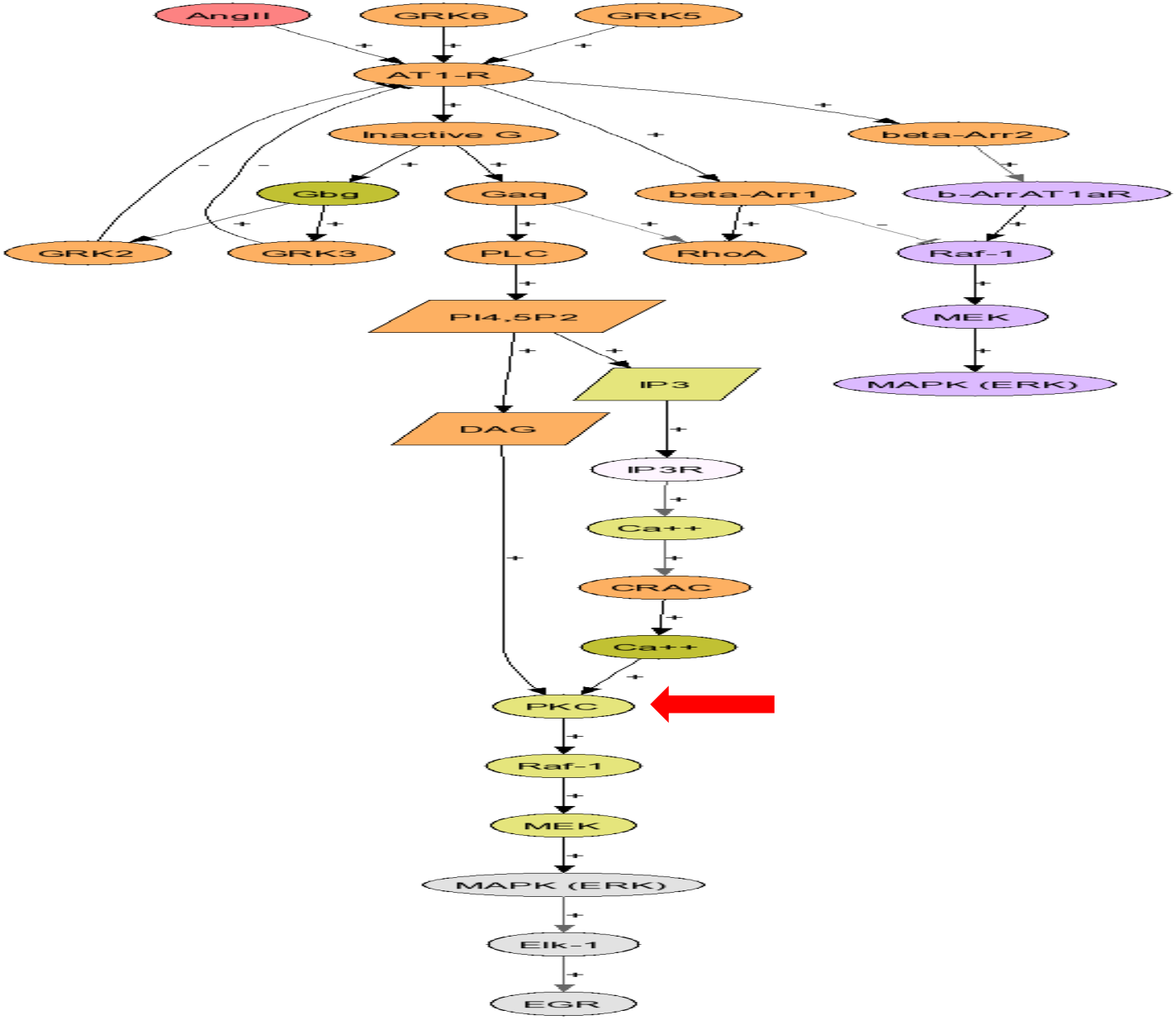
Pituitary adenylate cyclase-activating polypeptide (PACAP) is an adrenomedullary neurotransmitter that has also been shown to cause PC12 cell differentiation.

Differentiation pathway in PC12 cells



Lee Eiden, Philip Lazarovici, David Vaudry, Philip J. S. Stork, Babru Samal, Differentiation Pathway in PC12 Cells. ***Sci. Signal.***

Angiotensin II-Stimulated Signaling through G Proteins and beta-Arrestin

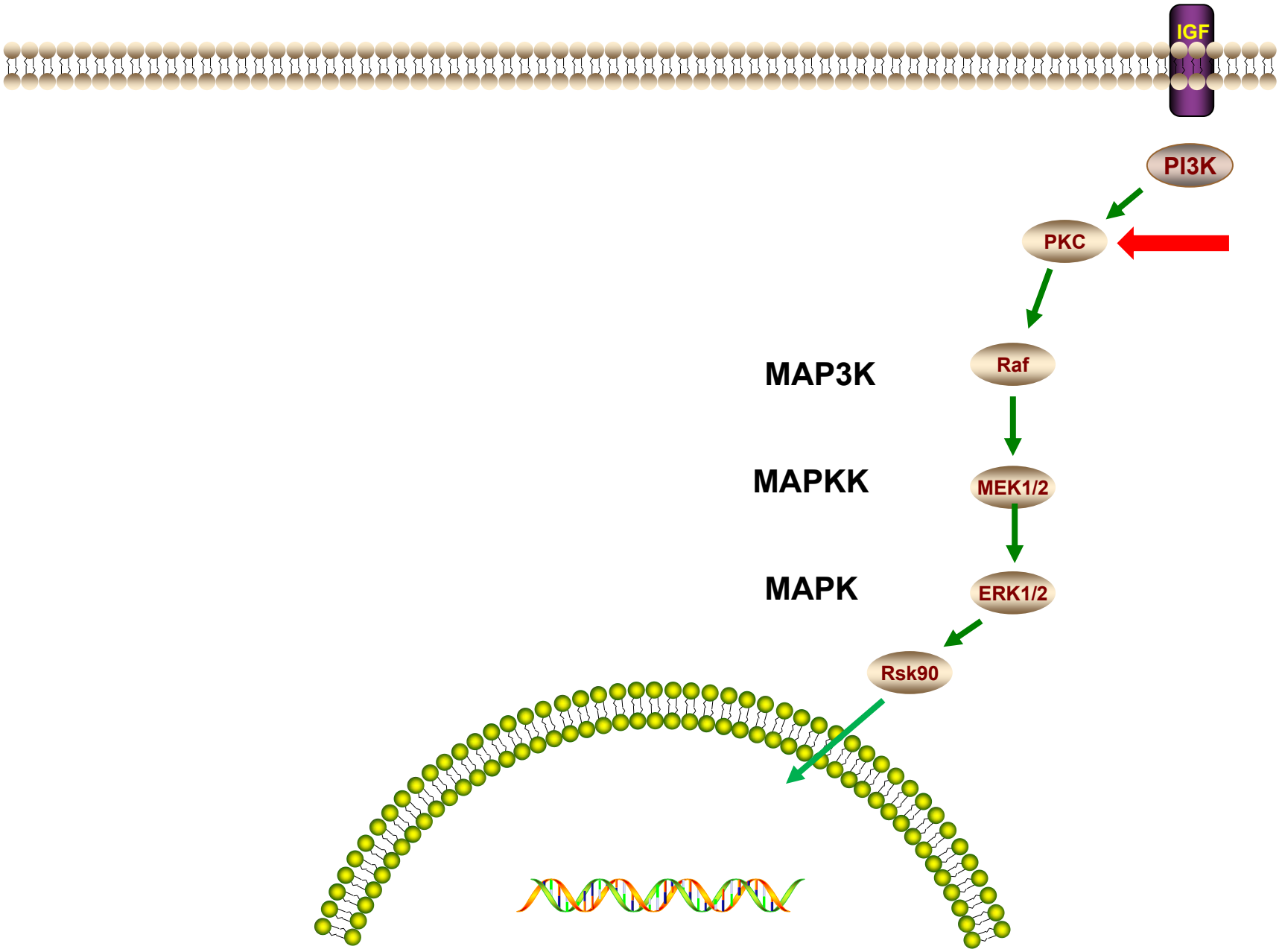


Robert J. Lefkowitz, Sudha K. Shenoy, Angiotensin II-Stimulated Signaling through G Proteins and beta-Arrestin. ***Sci. Signal.***

The angiotensin 1a receptor mediates various angiotensin II (AngII)-dependent physiological responses such as vasoconstriction, smooth muscle cell motility and growth, and aldosterone secretion.

Stimulation of the receptor with its peptide ligand AngII results in the activation of Gaq/11 and the downstream protein kinase C (PKC). This leads to the activation of the ERK cascade.

The active ERK translocates to the nucleus to stimulate transcriptional pathways governed by Elk-1 activity and early growth response I (EGR-1) induction.


IGF
PI3K
PKC
MAP3K
Raf
MAPKK
MEK1/2
MAPK
ERK1/2
Rsk90

PKC and insulin resistance

Obese Individuals → FAs↑+ acyl-coAs, ceramides,
diacyglycerol → activated protein kinase (PKC, JNK,etc)→
increasing the inhibitory serine phosphorylation of IRS →
impair insulin signaling

Hyperglycemic control in diabetes is key to preventing the development and progression of vascular complications such as retinopathy, nephropathy and neuropathy.

Increased activation of the diacylglycerol (DAG)-protein kinase C (PKC) signal transduction pathway has been identified in vascular tissues from diabetic animals, and in vascular cells exposed to elevated glucose.

Vascular abnormalities associated with glucose-induced PKC activation leading to increased synthesis of DAG include altered vascular blood flow, extracellular matrix deposition, basement membrane thickening, increased permeability and neovascularization.

Preferential activation of the PKCbeta isoform by elevated glucose is reported to occur in a variety of vascular tissues.

This has lead to the development of LY333531, a PKCbeta isoform specific inhibitor, which has shown potential in animal models to be an orally effective and nontoxic therapy able to produce significant improvements in diabetic retinopathy, nephropathy, neuropathy and cardiac dysfunction.

PKC and diabetes

Gonadotropins

GnRH acts via G_q-coupled seven-transmembrane (7TM) receptors to stimulate the synthesis and secretion of LH and FSH and thereby mediates central control of reproduction.

Like many other 7TM receptors, GnRH receptors (GnRHRs) activate the prototypic MAPK, ERK.

In quiescent cells, ERKs are typically anchored in the cytosol.

Upon dual phosphorylation and activation by MAPK/ERK kinase (MEK), ERKs can translocate to the nucleus where they can in turn phosphorylate transcription factors and immediate-early gene products.

Typically, G_q-dependent PKC activation plays a major role in GnRH-stimulated ERK1/2 activation.

- The most common PKC isoforms deregulated in cancer are α, β, and δ, but abnormal expression of other isoforms may also take place.

- The two main effects of deregulation of PKC in cancer are on *Mitogenic Signals* and *Apoptotic Signals.*

Mitogenic signals

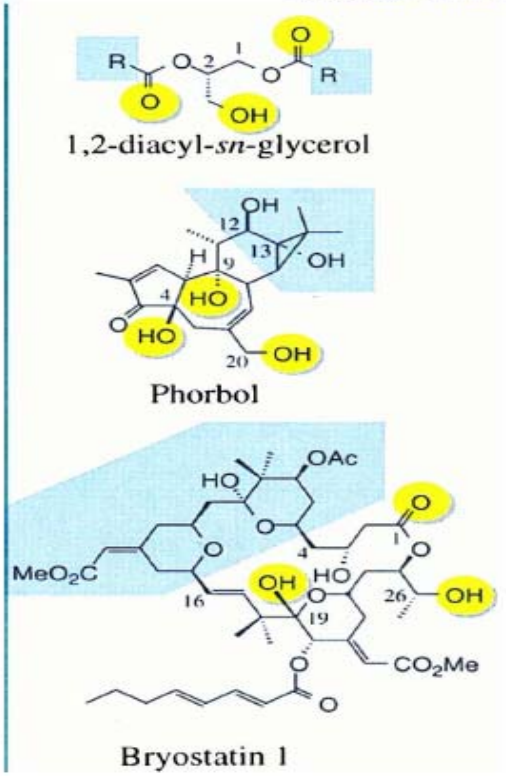
- Aggressive breast cancer cell lines have elevated PKCε levels that make them more proliferative, invasive, and motile
- PKCα has been linked to decreased proliferation in gastric cancer and increased proliferation in gliomas
- PKCδ is usually associated to growth arrest and tumor suppression . However, it can also promote survival, and in some cases metastatic potential, such as in melanoma, breast and lung cancer cells

Apoptotic signals

- PKCε suppresses apoptosis in prostate cancer cells, and promotes survival through inhibition of mitochondria-induced caspase activation in lung cancer
- Inhibition of PKCα induces apoptosis of glioma cells
- PKCδ plays a critical role in the apoptotic mechanisms induced by many anticancer drugs

The bryostatins : DAG analogs with promising antitumor activity

- **Bryostatin 1 is a potent activator of PKC**
- **Bryostatin 1 only mimics some of the phorbol ester responses and antagonizes those actions of phorbol esters that it cannot produce**



From Wender *et. al.* Proc. Natl. Acad. Sci. USA 95:6624, 1998

- Bryostatin 1 shows antitumor activity *in vitro* on P388 leukemia cells
- It has shown very modest activity *per se* in clinical trials
- It appears to contribute with the activity of chemotherapeutic agents, by reducing chemoresistance

- Some Bryostatin derivatives have been synthesized and are currently licensed and undergoing preclinical tests as anticancer drugs

- There are several clinical trials evaluating Bryostatin 1 as a coadjuvant agent in cancer treatment

Discovery of Protein kinase C - original research papers

[1] Y. Takai, A. Kishimoto, M. Inoue and Y. Nishizuka, Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. I. Purification and characterization of an active enzyme from bovine cerebellum, *J Biol Chem* **252** (1977), pp. 7603–7609.

[2] M. Inoue, A. Kishimoto, Y. Takai and Y. Nishizuka, Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. II. Proenzyme and its activation by calcium-dependent protease from rat brain, *J Biol Chem* **252** (1977), pp. 7610–7616.

[3] Y. Takai, A. Kishimoto, Y. Iwasa, Y. Kawahara, T. Mori and Y. Nishizuka, Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids, *J Biol Chem* **254** (1979), pp. 3692–3695.

[4] Y. Takai, A. Kishimoto, U. Kikkawa, T. Mori and Y. Nishizuka, Unsaturated diacylglycerol as a possible messenger for the activation of calcium-activated, phospholipid-dependent protein kinase system, *Biochem Biophys Res Commun* **91** (1979), pp. 1218–1224.

[5] M.R. Hokin and L.E. Hokin, Enzyme secretion and the incorporation of P32 into phospholipids of pancreas slices, *J Biol Chem* **203** (1953), pp. 967–977.

Activation of PKC by phorbol esters

M. Castagna, Y. Takai, K. Kaibuchi, K. Sano, U. Kikkawa and Y. Nishizuka, Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters, *J Biol Chem* **257** (1982), pp. 7847–7851.

A.S. Kraft, W.B. Anderson, H.L. Cooper and J.J. Sando, Decrease in cytosolic calcium/phospholipid-dependent protein kinase activity following phorbol ester treatment of EL4 thymoma cells, *J Biol Chem* **257** (1982), pp. 13193–13196.

PKC-θ is required for TCR-induced NF-κB activation in mature but not immature T lymphocytes

Zuoming Sun*, Christopher W. Arendt*, Wilfried Ellmeier*†, Edward M. Schaeffer‡§, Mary Jean Sunshine*†, Leena Gandhi*, Justin Annes*, Daniela Petrzilka*†, Abraham Kupfer‖, Pamela L. Schwartzberg‡ & Dan R. Littman*†

Nature **404**, 402-407 (23 March 2000)

Nature **404**, 402-407 (23 March 2000)

Productive interaction of a T lymphocyte with an antigen-presenting cell results in the clustering of the T-cell antigen receptor (TCR) and the recruitment of a large signalling complex to the site of cell–cell contact[1,2]. Subsequent signal transduction resulting in cytokine gene expression requires the activation of one or more of the multiple isoenzymes of serine/threonine-specific protein kinase C (PKC)[3]. Among the several PKC isoenzymes expressed in T cells, PKC-θ is unique in being rapidly recruited to the site of TCR clustering[4]. Here we show that PKC-θ is essential for TCR-mediated T-cell activation, but is dispensable during TCR-dependent thymocyte development. TCR-initiated NF-κB activation was absent from *PKC-θ*$^{-/-}$ mature T lymphocytes, but was intact in thymocytes. Activation of NF-κB by tumour-necrosis factor α and interleukin-1 was unaffected in the mutant mice. Although studies in T-cell lines had suggested that PKC-θ regulates activation of the JNK signalling pathway[5,6], induction of JNK was normal in T cells from mutant mice. These results indicate that PKC-θ functions in a unique pathway that links the TCR signalling complex to the activation of NF-κB in mature T lymphocytes.

Up-Regulation of Intracellular Signalling Pathways May Play a Central Pathogenic Role in Hypertension, Atherogenesis, Insulin Resistance, and Cancer Promotion – the 'PKC Syndrome'

M. F. McCARTY

Medical Hypotheses (1996) **46**, 191–221

It can be anticipated that various aspects of the modem diet and lifestyle - high-fat diet, low omega-3 intake, abdominal obesity, enhanced adipocyte lipolysis secondary to insulin resistance, diabetic hyperglycaemia - will tend to increase DAG production and thus enhance the activity of PKC.

Comprehensive nutritional / lifestyle measures designed to down-regulate calcium- and PKC-dependent signalling pathways, constitute a novel strategy for the prevention and control of vascular disease and cancer.

Happy birthday protein kinase C: past, present and future of a superfamily
Battaini F, Mochly-Rosen D.
Pharmacol Res. 2007 Jun;55(6):461-6. Epub 2007 May 18.

Protein kinase C: poised to signal
Alexandra C. Newton
Am J Physiol Endocrinol Metab 298: E395-E402, 2010.

Protein Kinase C ß/Early Growth Response-1 Pathway

A Key Player in Ischemia, Atherosclerosis, and Restenosis

J Am Coll Cardiol, 2006; 48:47-55

VIEWPOINT

G Protein Pathways

Susana R. Neves, Prahlad T. Ram, Ravi Iyengar*

SCIENCE (2002) 296:1636-1639