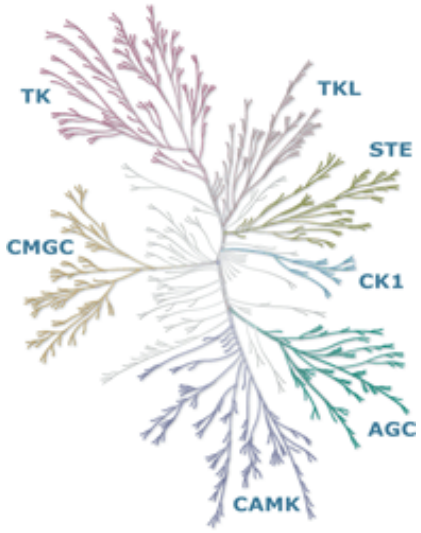
Protein Kinases

Phosphorylation/dephosphorylation Protein phosphorylation is one of the most important mechanisms of cellular responses to growth, stress metabolic and hormonal environmental changes. Most mammalian protein kinases have highly a homologous 30 to 32 kDa catalytic domain.



- Most common method of reversible modification
  - activation and localization
- Up to 1/3 of cellular proteins can be phosphorylated
- Leads to a very fast response to cellular stress, hormonal changes, learning processes, transcription regulation ....
- Different than allosteric or Michealis Menten regulation

Protein Kinome

To date – 518 human kinases known

- 50 kinase families between yeast, invertebrate and mammaliane kinomes
- 518 human PKs, most (478) belong to single super family whose catalytic domain are homologous.

- Kinase dendrogram displays relative similarities based on catalytic domains.
- AGC (PKA, PKG, PKC)
- CAMK (Casein kinase 1)
- CMGC (CDC, MAPK, GSK3, CLK)
- STE (Sterile 7, 11 & 20 kinases)
- TK (Tryosine kinases memb and cyto)
- TKL (Tyrosine kinase-like)

- Phosphorylation stabilized thermodynamically

- only half available energy used in adding phosphoryl to protein
- change in free energy forces phosphorylation reaction in one direction

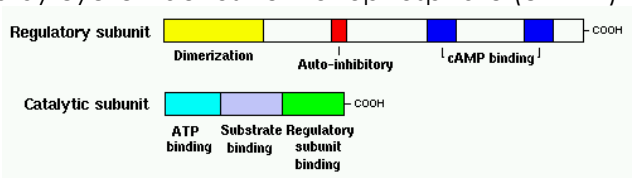
- Phosphatases reverse direction
- The rate of reaction of most phosphatases are 1000 times faster
- Phosphorylation occurs on Ser/The *or* Tyr

Consider…

- Regulation of protein phosphorylation varies depending on protein
  - some turned on or off
  - most kinases are regulated
  - phosphatases generally not regulated
  - can lead to large amplification of original signal
- General classes of protein kinases, based on substrate (both sequence and target amino acid phosphorylated), homology and regulation mechanisms (thousands of kinases)

Protein Kinase A (PKA)

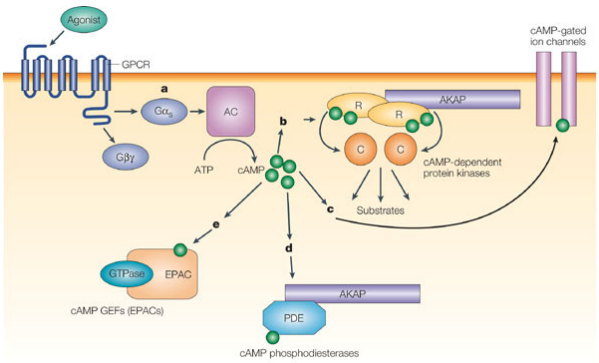
Activated by cyclic Adenosine Monophosphate (c-AMP)



- Recognizes specific sequences in substrate

  Arg-Arg- X - Ser/Thr - Z

  X = small aa, Z = hydrophobic aa (not Tyr)
- Called consensus sequence
- Important in regulation by hormones and neurotransmitters
  - Epinephrine (adrenaline)
- c-AMP produced from ATP by adenylyl cyclase (AKA adenylate cyclase)



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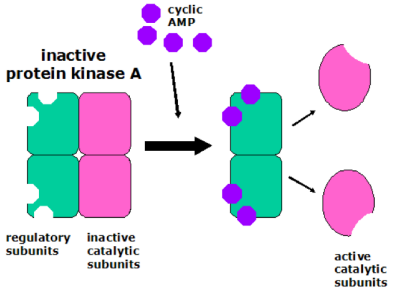
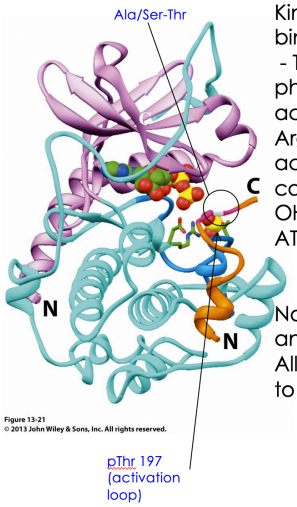
Protein Kinase A (PKA)

- Regulatory subunits - Arg-Arg- Gly - Ala - Ile
- Pseudosubstrate - binds deep in cleft between catalytic subunits
- Competitive inhibitor at active site
- Binding of c-AMP to R subunits shifts Pseudosubstrate away from active site
- Catalytic subunits now active
- Degradation of c-AMP to AMP by another enzyme leads to removal of c-AMP from R subunits and reformation of inactive heterotetramer



Catalytic Subunit of Mouse Protein Kinase A

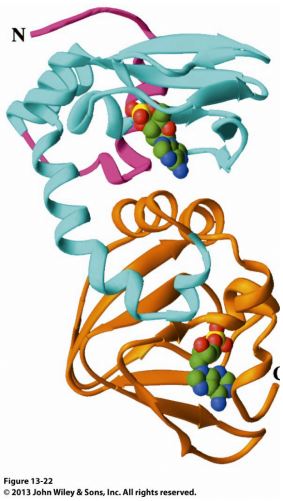


Kinase cleft for substrate binding.
- Thr197 needs to be phosphorylated for max activation.

Arg165 and pThr 197 are activated by Asp acid catalysis – Asp acidifies OH of target to attack ATP

Note locn of ATP, T197 and pseudo target. Allows transfer from ATP to target in active site

cAMP-Bound Bovine Protein Kinase A Regulatory Subunit



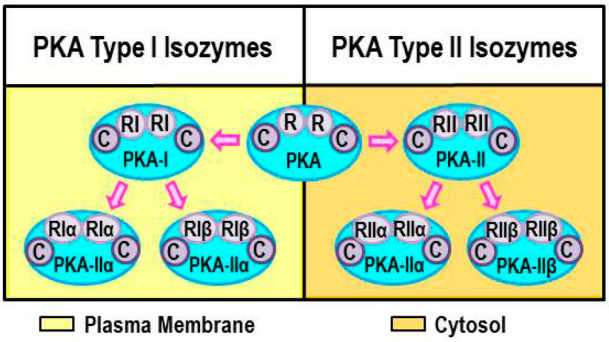
R subunit binds
- cAMP
- Catalytic SU
- AKAP

Two cAMP binding domains in each R subunit
- Domain A Cyan
- Domain B Orange

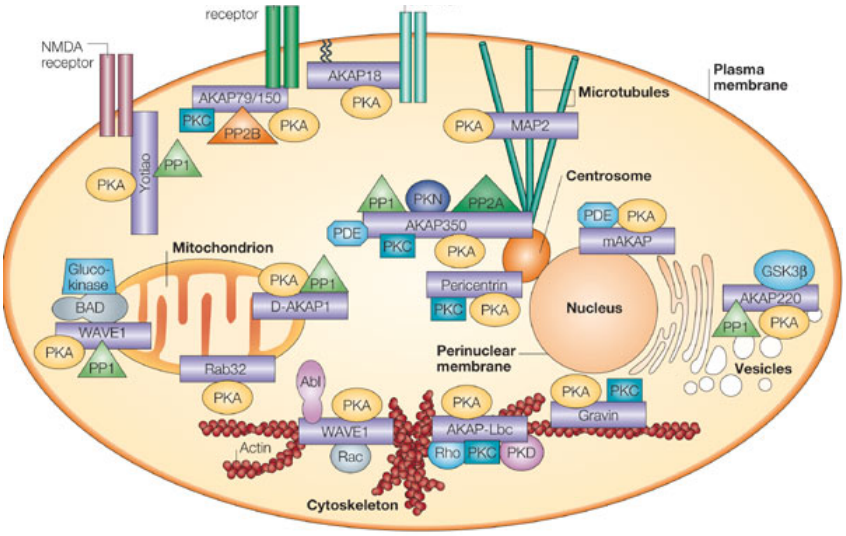
cAMP binds to A domain

Protein Kinase A (PKA)

- PKA is a heterotetramer, not liked together by peptide bond
- Two classes of R subunits
  - Type (RI□ & RI□) and Type II (RII□ & RII□□
  - R subunit is critical for localizing PKA to a specific cellular location – RI localizes to the plasma and organelle membranes, RII localizes to cytosol
  - R subunits bind to anchor proteins allowing specific PKA substrates to be phosphorylated.
  - Anchor proteins called A-kinase anchor proteins (AKAPs).



Nomenclature for PKA isoforms



Protein Kinase C (PKC)

- Ser/Thr protein kinase
- Monomer - psuedosubstrate part of whole protein
- Activated by increases in cellular Ca^{+2} and Lipid activator (DAG)
- Diacylglycerol (DAG) - tumor promoter made by other enzymes in response to hormonal changes.

Very transient molecule, often use phorbol esters (PMA) to study

- No real stringent consensus sequence - usually Arg rich targets

Over 23 isoforms are based in three categories

1) **conventional PKC** - Ca^{+2} and Lipid regulated **α, β_1, β_2 and γ isoforms**

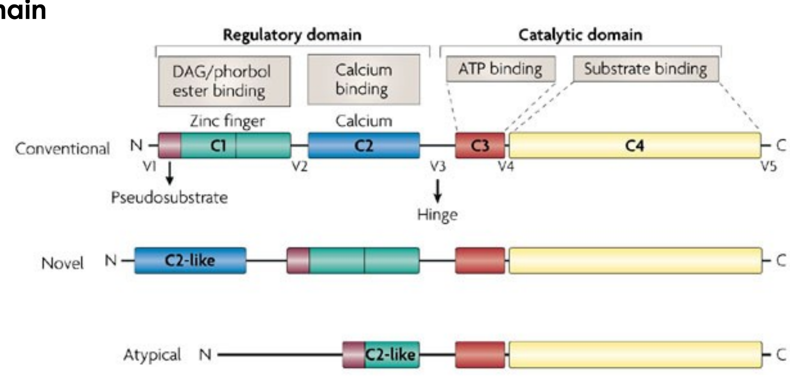
2) **novel PKC** - only Ca^{+2} activated

- □□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□

3) **Atypical PKC** - not regulated by either Ca^{+2} or DAG possibly activated by sphingosine **ζ and τ.**

Each of the different forms are generally splice variants (alterations at the gene level) - several shared domains

- Pseudosubstrate
- C1 - DAG binding domain
- C2 – Ca^{+2} and lipid binding domain which interacts with the PS in the membrane (phosphatidyl serine)
- C3 - ATP binding domain (glycine rich)
- C4 - Catalytic domain



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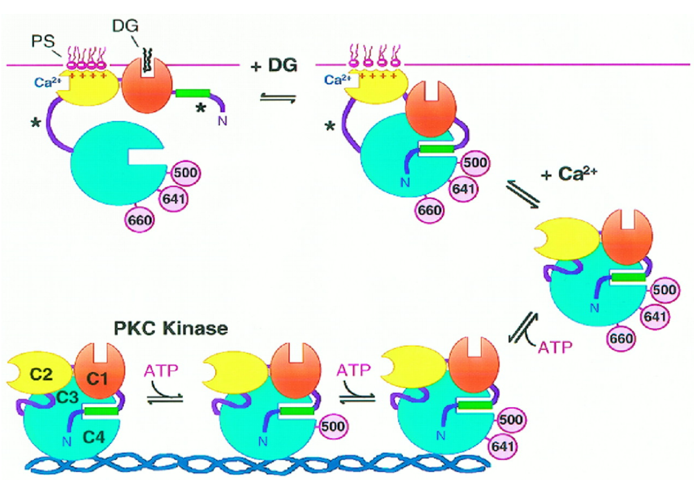
Activation of PKC

•PKC is inactive in the resting state when it is bound to its pseudosubstrate. The conventional isoforms are typically found in cytosol. Note the interactions of the various subunits with each other. At this time the enzyme is cytosolic



- three functionally distinct phosphorylations: transphosphorylations render the kinase catalytically competent - autophosphorylation at the C terminus stabilizes the catalytically competent conformation ; and at the C terminus that releases protein kinase C into the cytosol.
- This triple phosphorylated mature form is inactive because the pseudosubstrate occupies the substrate-binding cavity (middle).

•Now the catalytic subunit is separated from the pseudosubstrate, can interact and phosphorylate the substrate

•Generation of diacylglycerol (DG or DAG) causes the affinity of protein kinase C for membranes to increase dramatically. Membrane translocation

Asterisks indicate the exposed hinge, which becomes proteolytically labile upon membrane binding (independently of pseudosubstrate release), and the exposed pseudosubstrate, which becomes proteolytically labile upon activation (independently of membrane binding).

Protein Tyrosine Kinases (PTK)

Phosphorylates at a tyrosine residue only
Several kinds of cancer are mutated versions of tyrosine kinases
2 classes; receptor or cytosolic
Receptor tyrosine kinases
- receptor of hormones/growth factors
- found on both sides of the cell membrane
- extracellular portion binds hormone and alters conformation through the membrane and the cytosolic portion
- now the kinase part of the receptor is active

Protein Tyrosine Kinases (PTK)

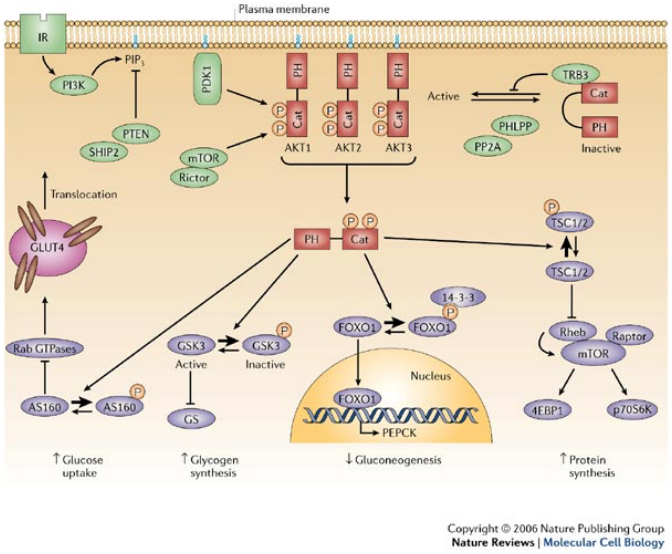
Cytosolic or non-receptor
- Part of the Src family -mutated form originally found in rous sarcoma virus
Usually regulated by other tyrosine kinases (receptor kinases)
- many different soluble tyrosine kinases
- most have SH2 or and SH3 domains

– Murine lymphoma (leukemia) formed when tyrosine kinase of Abl is uncontrolled

Protein Kinase B (PKB/AKT)

Ser/Thr kinasae – of three subfamilies AKT1/2/3
Protein kinase B is now better known as Akt and is a serine threonine kinase that was first found from a virus that induces T-cell lymphomas in rats.

- Prevents apoptosis, induces glucose uptake by increasing Glut 4 translocation to the membrane, regulates glucose metabolism through phosphorylation of glycogen synthase kinase and can alter protein expression by phosphorylation of ribosomal kinases.



Protein Kinase B (PKB/AKT)

- Activated by growth factor receptors such as insulin and epidermal growth factor.
- Binds and is activated by the phospholipid Phosphoinositol 3,4,5 trisphosphate. Binds at the PH domain - pleckstrin homology
- Catalytic domain similar to PKC and PKA
- PDK phosphorylates AKT at two sites on the catalytic domain for activation.
- Targets several downstream proteins including glycogen synthase kinase

Mitogen Activated Protein Kinases

MAP kinases (MAPK) were identified by virtue of their activation in response to growth factor stimulation
- ERKs for extracellular-signal regulated kinases.
  - Also microtubule associated protein-2 kinase (MAP-2 kinase), myelin basic protein kinase (MBP kinase),
- P38
- JNK
- Big MAP Kinase
- Ribosomal S6 protein kinase (RSK-kinase: i.e. a kinase that phosphorylates a kinase)

Each of the three forms of MAP kinases ERK, JNK and p38 are activated by different mechanisms.
- ERK (also commonly referred to as MAPK) is activated by G proteins and most growth factors.
- JNK and p38 are activated by cellular stress such as UV, heat and osmotic changes.

The targets for each of the MAP kinases vary greatly and can be found in the cytosol (where metabolism,

cytoskeletal and other responses are initiated) or in the nucleus (where transcription factors are activated, ultimately leading to altered gene production).

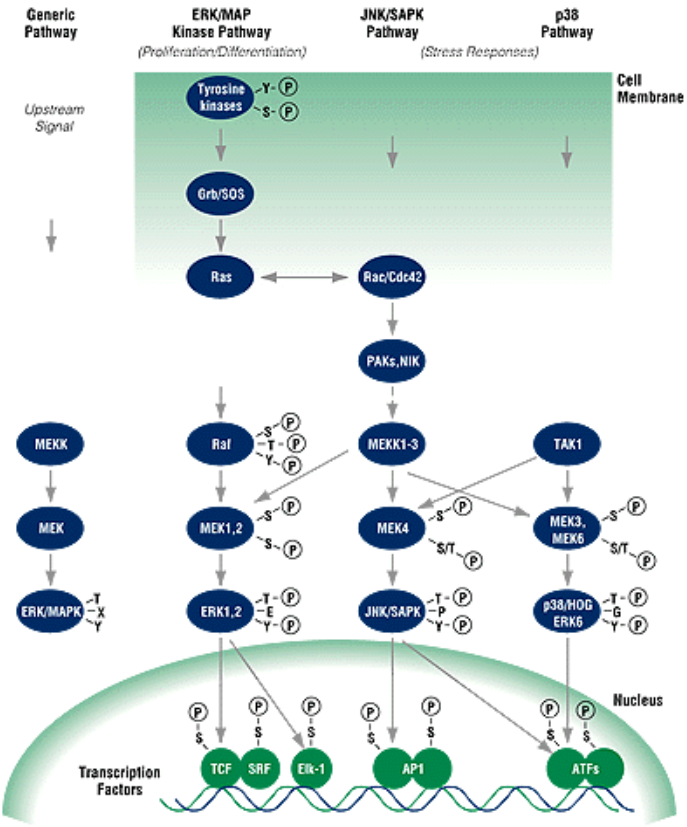
- p90Rsk (ribosomal kinase)
- C-Jun (transcription factor)
- Heat shock proteins
- proto-oncogenes Fos, Myc and Jun
- members of the steroid/thyroid hormone receptor super family of proteins.

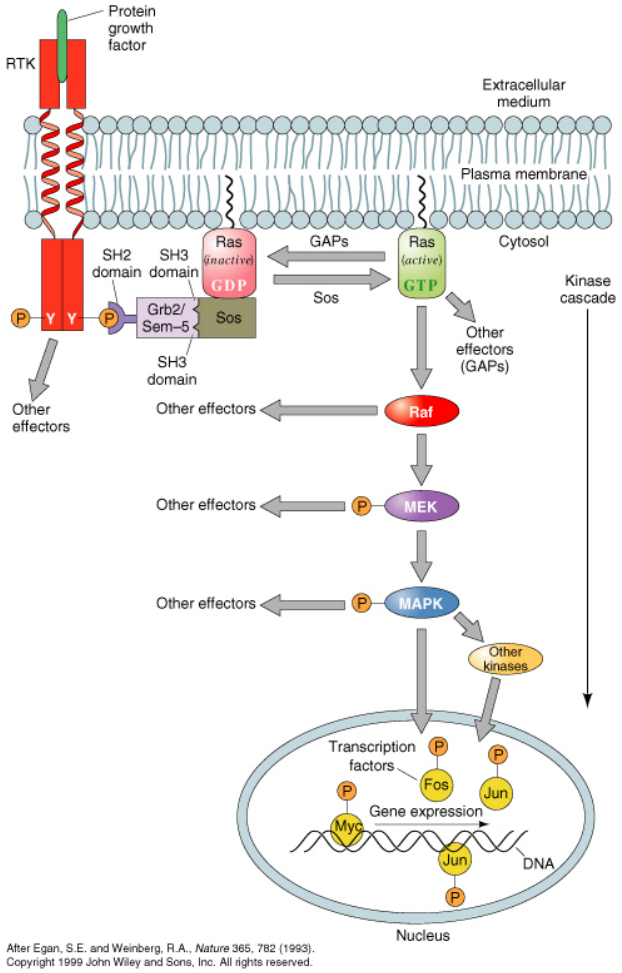
Maximal MAP kinase activity requires that both tyrosine and threonine residues are phosphorylated.

MAP kinase activation was first observed in response to activation of the EGF, PDGF, NGF (epidermal, platelet and nerve growth factors) and insulin receptors,

Other cellular stimuli include: as T cell activation (which signals through the Lck [lick] tyrosine kinase)

And - phorbol esters (that function through activation of PKC), thrombin, epinephrine and lysophosphatidic acid (LPA)(all hormones that function through G-proteins) also rapidly induce tyrosine phosphorylation of MAP kinases.





After Egan, S.E. and Weinberg, R.A., *Nature* 365, 782 (1993).

MAP kinases, however are not the direct substrates for G-proteins, receptor or receptor associated tyrosine kinases but are in fact activated by an additional class of kinases termed MAP kinase kinases (MAPK kinases) and MAPK kinase kinases (MAPKK kinases). One of the MAPK kinases has been identified as the proto-oncogenic serine/threonine kinase, Raf.

Ca2+/CaM dependent protein kinaseII (CaM-KII)

Another class of protein kinases that are activated by increases in calcium.
CaM-K binds tightly to calmodulin and thus is responsive to transient changes in intracellular calcium.
Calmodulin is a small (17 kDa) protein that binds 4 calcium ions by the four EF hand which bind Ca^{+2} with high affinity

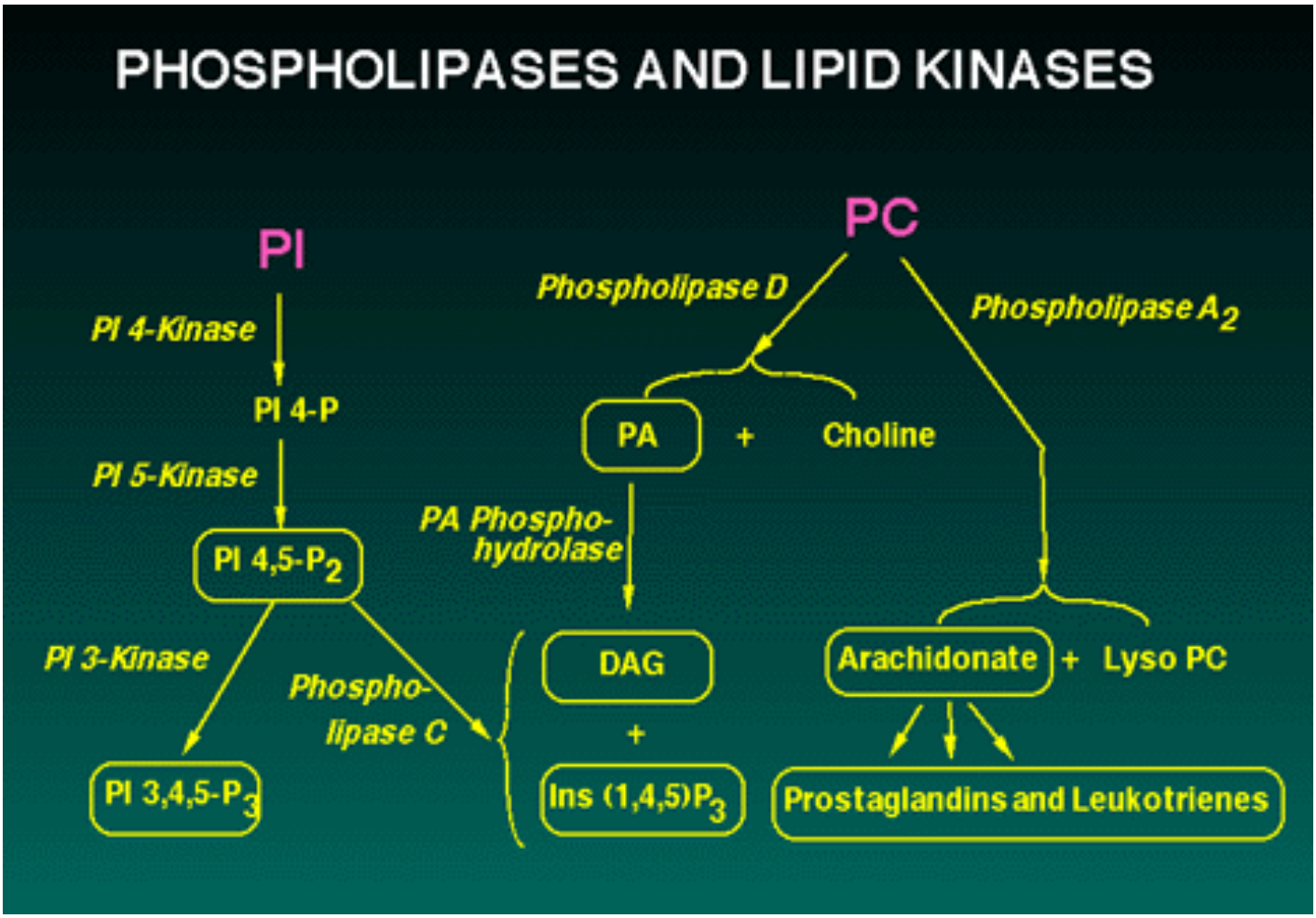
CaM-KII is a Ser/Thr protein kinase that binds and phosphorylates a wide variety of proteins. The protein is found in nearly all tissues and is a key component of Ca+2 signaling but is particularly enriched in neural tissue (up to 2% of the total protein in the hippocampus).

CaMKII is a complex of about 12 subunits

CaM-KII is a Ser/Thr protein kinase that binds and phosphorylates a wide variety of proteins. The protein is found in nearly all tissues and is a key component of Ca+2 signaling but is particularly enriched in neural tissue (up to 2% of the total protein in the hippocampus).

Ca binding to calmodulin activates the kinase by relieving it's pseudosubstrate in a fashion similar to that of PKA.

- In the inactive state there is a strong interaction between the inhibitory and kinase domains
- Ca/Cam allows the catalytic domain to phosphorylate the inhibitory domain
- Enzyme stays active even after calcium is removed, prolonging the duration of kinase activity

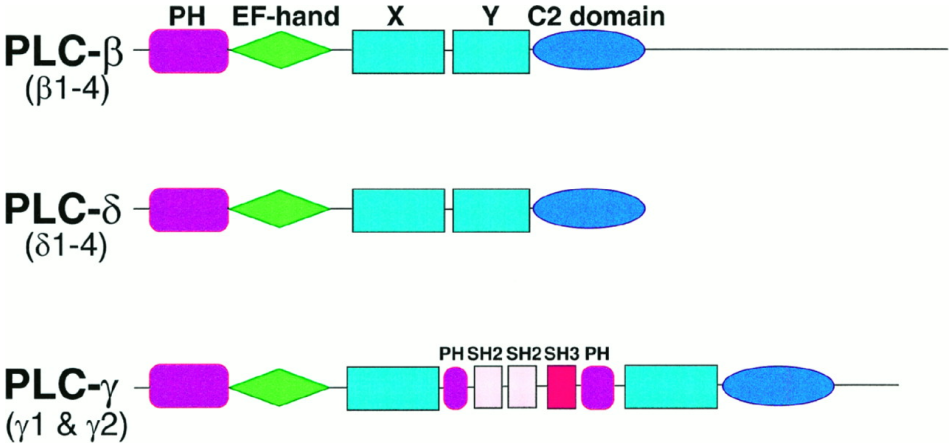


Phospholipase C

The lipase activity regulates PIP_2 signaling and is used by many different hormones.-So how is PLC regulated?

Which signaling systems depends on PLC isozyme
- 8 different in 4 families PLC proteins / genes

- PLC γ - activated by growth factors & insulin -> SH2 SH3 domains
- PLC β enzymes activated by Gq proteins some by βγ subunits
- PLC δ - is not well defined



Differences in structural organization of forms of phospholipase C

- X and Y domains conserved throughout the PLC family - make up the catalytic domains
- Each isozyme contains a PH (plekstrin homology domain) for binding inositol lipids
- Calcium binding occurs through EF hand domain for each form and a C2 domain also interacts with calcium
- PLC γ - has additional SH domains (SH2 and SH3) for interactions with phosphotyrosine signaling
- Calcium is required for activity, binding both at C2 domain and at the active site via histadine residues. Inositol lipids bind to protein, Ca+2 lead to X and Y domain coming together to form active catalytic protein

Activation of PLC beta form

- Activated by hormones which signal through Gq
- Hormones include angiotensin II, alpha adrenergic agonists, acetylcholine
- Lipase acts as GAP for G protein alpha subunit
- G proteins interact at the C terminal

beta gamma subunits of G proteins also can activate PLC beta

Activation of PLC gamma

- receptors which bind growth factors lead to PLC gamma activation
- Interactions between intracellular domain of Receptor and PLC occurs by the SH2 domains
- Once interacting PLC gamma becomes phosphorylated by the receptor on tyrosine residues - increases PLC interactions with other proteins
- SH3 domain targets activated PLC to cytoskeletal proteins (actin myosin)

In immune cells soluble tyrosine kinase can activate by phosphorylation of PLC

PLC inactivation: Additional phosphorylation of PLC leads to inhibition and down regulation of PLC signal - by either PKA or PKC

The phospholipase A2 (PLA2) enzymes hydrolyze fatty acid from the sn-2 position of phospholipid with the concomitant production of lysophospholipid.

Mammalian cells contain structurally diverse forms of PLA2 including secretory PLA2 (sPLA2), calcium-independent PLA2, and the 85-kDa cytosolic PLA2 (cPLA2) (1-4). PLA2 functions in the digestion of dietary lipid, microbial degradation, and regulation of phospholipid acyl turnover either in a housekeeping role for membrane repair or for the production of inflammatory lipid mediators.

Phospholipase activity was first described in pancreatic juice and cobra venom at about the turn of the century.

Another Phospholipase - PLD

- Mammalian isoform- specific for phosphatidylcholine (PC)
- Plant phosphatidylethanolamine (PE)
- Animal form is activated by RhoA, PKC and ARF.

- Involved in PC derived Diacylglycerol (DAG) - thus activating atypical and novel PKC isoforms
- Many hormones activate this enzyme
- PLD is believed to be responsible for chronic activation of PKC AND lipid rearrangement for signaling to exocytosis and Raf activation

Second Messengers

cAMP - adenylate cyclase and phosphodiesterase (caffine inhibited) - ‘

Ca^{2+} - intracellular (mito and ER) and extracellular
Phosphoinositols
Bioactive lipids

- **DAG**
- **PIP_2/PIP_x**
- **PA / LPA**
- **AA (arachadonic acid)**