

Signal Transduction IV

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
- 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/Thr and Tyr
- What differences occur due to the addition of a phosphoryl group?
- 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) pp 442

- 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)
- PKA is a heterotetramer, not linked together by peptide bond
- 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

Protein Kinase C (PKC)

- Ser/Thr protein kinase
- Monomer - pseudosubstrate 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

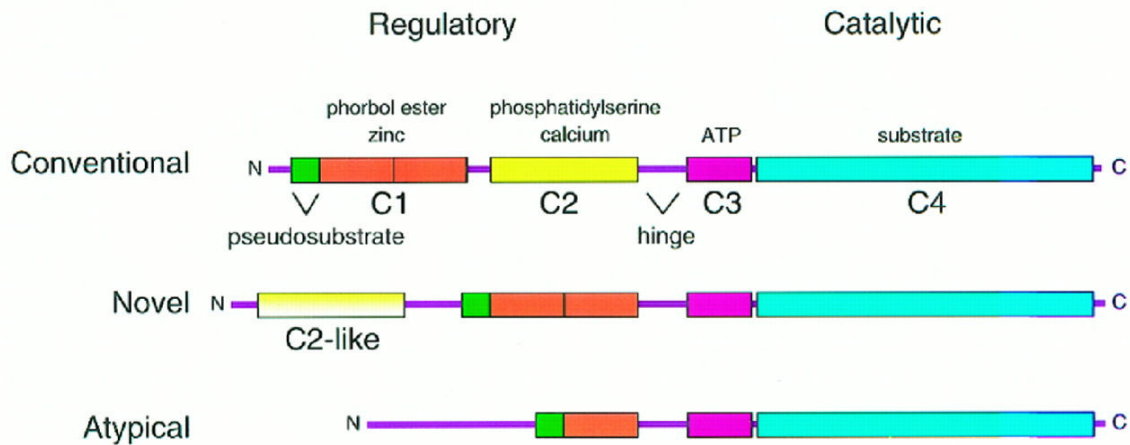
Over 23 isoforms based in three categories

- 1) **conventional PKC** - Ca^{+2} and Lipid regulated

- 2) **novel PKC** - only Ca^{+2} activated
- 3) **Atypical PKC** - not regulated by either Ca^{+2} or DAG possibly activated by sphingosine

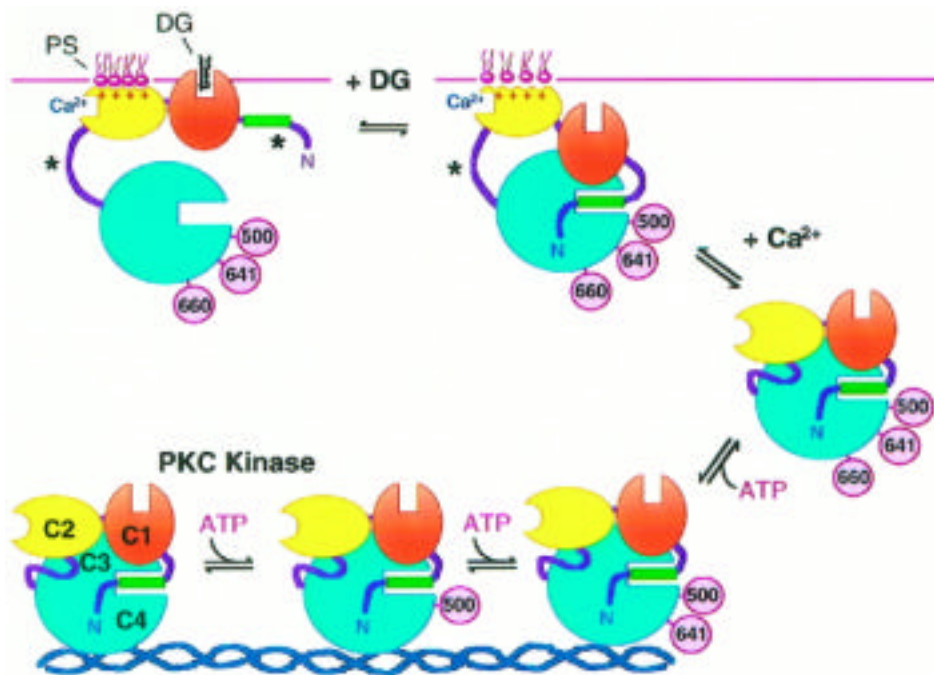
Each of the different forms are generally splice variants (alterations at the gene level)

- 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



Schematic representation of the primary structure of conventional, novel, and atypical protein kinase Cs. Indicated are the pseudosubstrate domain (green), C1 domain comprising one or two Cys-rich motifs (orange), C2 domain (yellow) in the regulatory half, and the ATP-binding lobe (C3, pink) and substrate-binding lobe (C4, teal blue) of the catalytic region. The C2 domain of novel protein kinase Cs lacks amino acids involved in binding calcium but has key conserved residues involved in maintaining the C2 fold (hence its description as "C2-like"). Atypical protein kinase Cs have only one Cys-rich motif, and phorbol ester

binding has not been detected.



from the pseudosubstrate, can interact and phosphorylate the substrate

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
- PKC is activated after ATP, Ca^{+2} and DAG bind. Note the role of Ca^{+2} and PS (- charged membrane p-lipids). Now the catalytic subunit is separated

- Then translocation to membrane occurs when the subunits are free to interact with the membrane.

Model for the regulation of protein kinase C by 1) phosphorylation and 2) membrane binding and 3) pseudosubstrate release.

- Newly synthesized protein kinase C (PKC) associates with the detergent-insoluble fraction of cells (bottom left). It is processed to the mature, cytosolic form by three functionally distinct phosphorylations: transphosphorylation at the activation loop to render the kinase catalytically competent (Thr-500 in II); an autophosphorylation at the C terminus (Thr-641 in II) that stabilizes the catalytically competent conformation; and a second autophosphorylation at the C terminus (Ser-660 in II) that releases protein kinase C into the cytosol. This triple phosphorylated mature form is inactive because the pseudosubstrate occupies the substrate-binding cavity (middle).
- Generation of diacylglycerol (DG or DAG) causes the affinity of protein kinase C for membranes to increase dramatically. Membrane translocation is mediated by diacylglycerol binding to the C1 domain and phosphatidylserine (PS) binding to the C2 domain (top right).
- The affinity for acidic lipids is increased by Ca for conventional protein kinase Cs, likely by structuring the lipid-binding surface, but not for novel protein kinase Cs, whose lipid binding surface may already be structured. Protein kinase C can bind to membranes with low affinity with either C1 domain ligands (not shown) or with C2 domain ligands (top middle). However, it is the high affinity binding (top left) mediated by both domains that results in pseudosubstrate release and maximal activation.
- 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).

Taken from Alexandra C. Newton Protein Kinase C: Structure, Function, and Regulation. Volume 270, Number 48, Issue of December 1, 1995 pp. 28495-28498 The Journal of Biological Chemistry

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
 - 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)

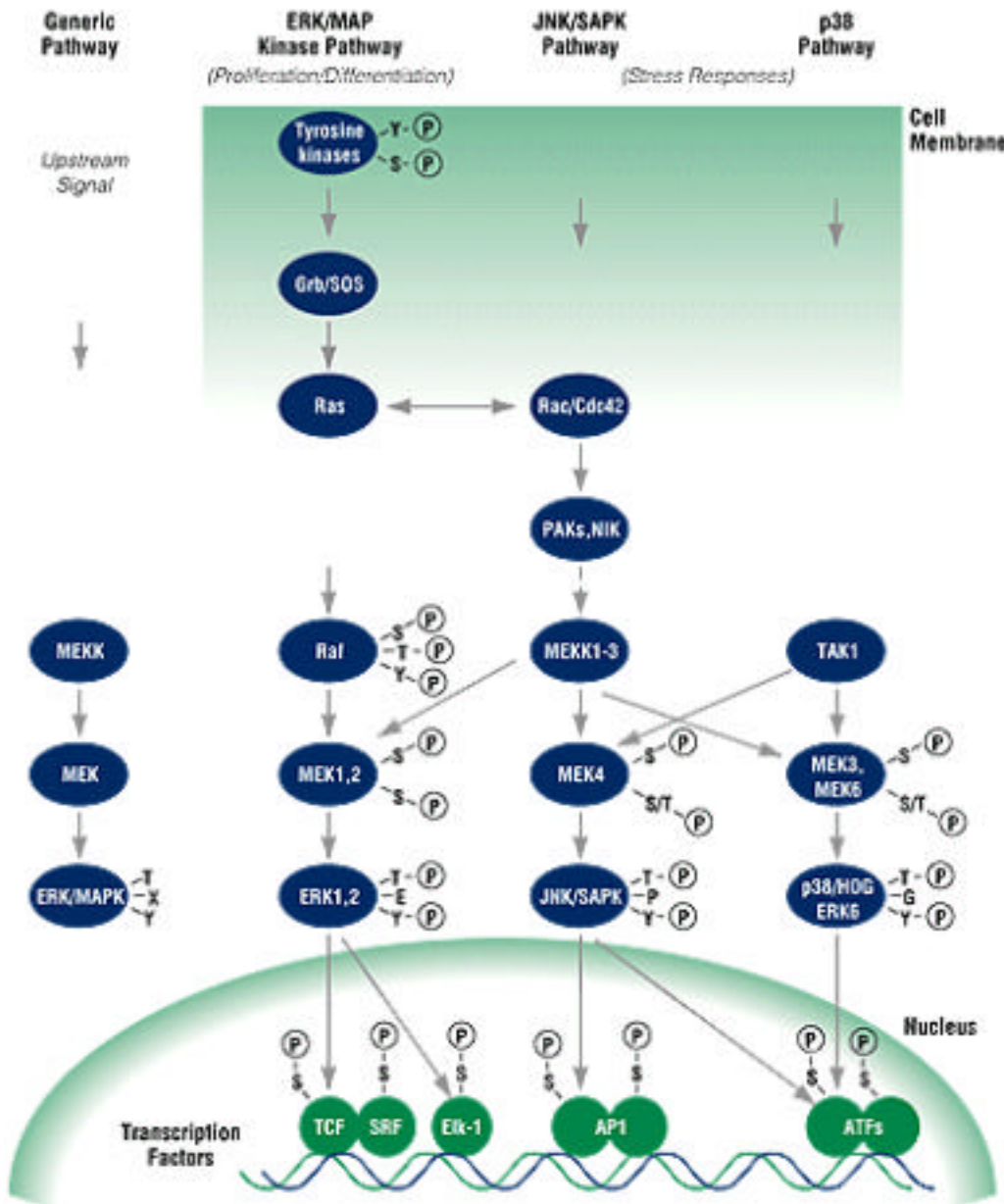
- Newer type of kinase - not much known (1996)
- Protein kinase B is now better known as Akt and is a serine threonine kinase that was first found from a virus that induces T-cell lymphomas in rats.
- Prevents apoptosis, induces glucose uptake by increasing Glut 4 translocation to the membrane, regulates glucose metabolism through phosphorylation of glycogen synthase kinase and can alter protein expression by phosphorylation of ribosomal kinases.
 - 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
 - Exact mechanism of activation remains unclear

MAP Kinases (MAPK)

MAP kinases were identified by virtue of their activation in response to growth factor stimulation of cells in culture, hence the name mitogen activated protein kinases. MAP

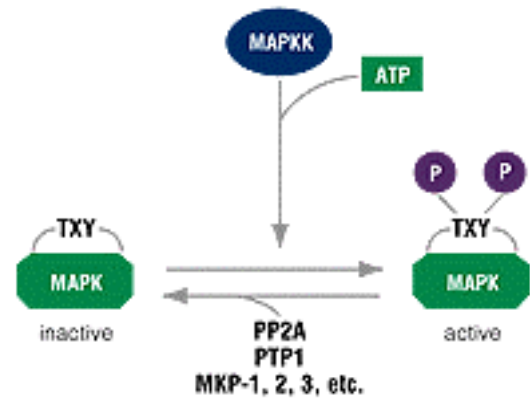
kinases are also called ERKs for extracellular-signal regulated kinases. On the basis of in vitro substrates the MAP kinases have been variously called microtubule associated protein-2 kinase (MAP-2 kinase), myelin basic protein kinase (MBP kinase), ribosomal S6 protein kinase (RSK-kinase: i.e. a kinase that phosphorylates a kinase). Although the latter is now not considered a MAP Kinase and is known as a separate class of kinases whose activity is regulated by MAPK.

There are three other isoforms of MAP kinases one protein of a molecular mass of 38 kDa (p38) and a protein whose function was initially identified as a protein kinase that phosphorylates a protein called Jun on its N-termini. (thus the name Jun N terminal kinase or JNK).



All of these proteins have similar biochemical properties, immuno-crossreactivities, amino acid sequence and ability to in vitro phosphorylate similar substrates. However 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. While 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).

Maximal MAP kinase activity requires that both tyrosine and threonine residues are phosphorylated. This indicates that MAP kinases act as switch kinases that transmits information of increased intracellular tyrosine phosphorylation to that of serine/threonine phosphorylation. Although 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 such as T cell activation (which signals through the Lck [lick] tyrosine kinase), 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.



Dual Phosphorylation of Erk – Notice the TXY (also known as a TEY) consensus sequence.

MAP kinases are, however, 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.

Ultimate targets of the MAP kinases are several transcriptional regulators e.g. serum response factor (SRF), and the proto-oncogenes Fos, Myc and Jun as well as members of the steroid/thyroid hormone receptor super family of proteins.

Ca²⁺/CaM dependent protein kinase II (CaM-KII) Calmodulin-dependent protein kinase II

This is another class of protein kinases that are activated by increases in calcium. CaM-KII 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 Ca²⁺ with high affinity. (see review from text in the glycogen phosphorylase kinase pp 444). The interaction of Ca²⁺ with calmodulin is required for a significant alteration in shape. 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 Ca²⁺ 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 its pseudosubstrate in a fashion similar to that of PKA. In the absence of bound calcium/calmodulin, the kinase is maintained in an inactive conformation by interaction with the autoinhibitory domain. Upon increases in Ca²⁺ binding, the kinase undergoes autophosphorylation. This phosphorylation results in a decreased dissociation rate of the Ca²⁺/CaM and there is a prolonged response to the short Ca²⁺ increase.