

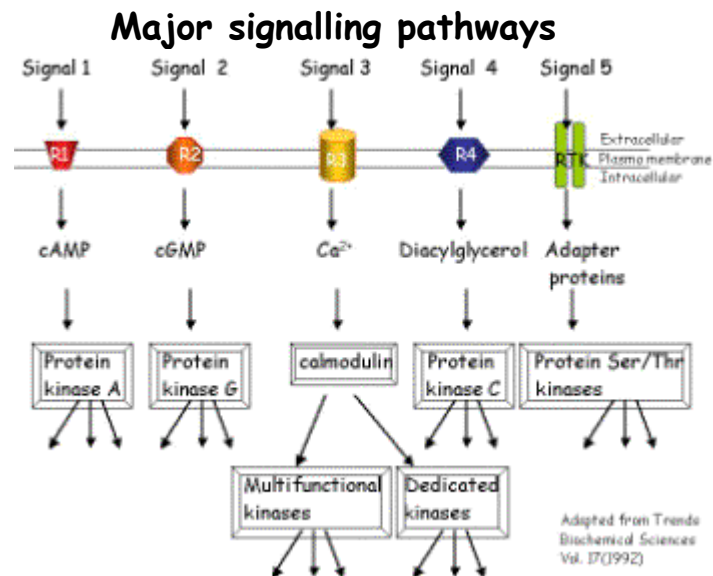
THE PROTEIN KINASE FAMILY

A protein kinase is an enzyme that modifies other proteins by chemically adding phosphate groups to them (phosphorylation). This usually results in a functional change of the target protein (substrate), by changing enzyme activity, cellular location or association with other proteins. Up to 30% of all proteins may be modified by kinase activity, and kinases are known to regulate the majority of cellular pathways, especially those involved in signal transduction, the transmission of signals within the cell. The human genome contains about 500 protein kinase genes; they constitute about 2% of all eukaryotic genes.

The chemical activity of a kinase involves removing a phosphate group from ATP and covalently attaching it to one of three amino acids that have a free hydroxyl group. Most kinases act on both Serine and Threonine, others act on Tyrosine, and a number (dual specificity kinases) act on all three.

Because protein kinases have profound effects on a cell, their activity is highly regulated. Kinases are turned on or off by phosphorylation (sometimes by the kinase itself - *cis*-phosphorylation/autophosphorylation), by binding of activator proteins or inhibitor proteins, or small molecules, or by controlling their location in the cell relative to their substrates.

Disregulated kinase activity is a frequent cause of disease, particularly cancer, where kinases regulate many aspects that control cell growth, movement and death. Drugs which inhibit specific kinases are being developed to treat several diseases, and some are currently in clinical use, including Gleevec (imatinib) and Iressa (gefitinib).



Serine/threonine-specific protein kinases

Serine/threonine protein kinases (EC 2.7.1.37) phosphorylate the OH group of serine or threonine (which have similar sidechains). These protein kinases can be regulated by:

- cAMP/cGMP
- Diacylglycerol
- Ca^{2+} /calmodulin

These kinases are not specific to a similar *consensus sequence* (a consensus sequence is a group of flanking amino acids that determines whether the protein kinase can act on it). Since the substrate to be phosphorylated aligns with the kinase by several key amino acids (usually through hydrophobic forces and ionic bonds), a kinase

is usually specific, not to a single substrate, but to a whole "substrate family" having common properties. Most kinases are inhibited by a pseudosubstrate that binds to the kinase like a real substrate but lacks the amino acid to be phosphorylated. When the pseudosubstrate is removed, the kinase can perform its normal function.

The catalytic domain of these kinases is highly conserved.

Many serine/threonine protein kinases do not have their own individual EC numbers and use "2.7.1.37", which is a general EF number for any enzyme that phosphorylates proteins while converting ATP to ADP (i.e. ATP:protein phosphotransferases.) This category is currently being reviewed by the Nomenclature Committee of IUBMB (NC-IUBMB), and it is believed that the various serine/threonine-kinases will get their own EC numbers eventually.

Phosphorylase kinase

Phosphorylase kinase (EC 2.7.1.38) was in fact, the first Ser/Thr protein kinase to be discovered (in 1959 by Krebs *et al.*).

Protein kinase A

Protein kinase A (EC 2.7.1.37) consists of two domains, a small domain with several β sheet structures and a larger domain containing several α helices. The binding sites for substrate and ATP are located in the catalytic cleft between the domains (or lobes). When ATP and substrate bind, the two lobes rotate so that the terminal phosphate group of the ATP and the target amino acid of the substrate move into the correct positions for the catalytic reaction to take place.

Regulation

Protein kinase A has several functions in the cell, including regulation of glycogen, sugar, and lipid metabolism. It is controlled by cAMP: in the absence of cAMP, the kinase is a tetramer of two regulatory and two catalytic subunits (R_2C_2), with the regulatory subunits blocking the catalytic center of the catalytic subunits. Binding of cAMP to the regulatory subunit leads to dissociation of active RC dimers. Also, the catalytic subunit itself can be regulated by phosphorylation.

Downregulation of protein kinase A occurs by a feedback mechanism: one of the substrates that is activated by the kinase is a phosphodiesterase, which converts cAMP to AMP, thus reducing the amount of cAMP that can activate protein kinase A.

Protein kinase C

Protein kinase C ('PKC', EC 2.7.1.37) is actually a family of protein kinases consisting of ~15 isozymes. They are divided into three subfamilies: conventional, novel, and atypical based on their second messenger requirements. Conventional PKCs require Ca^{2+} , diacylglycerol (DAG), and a phospholipid such as phosphatidylcholine for activation. Novel PKCs require DAG, but do not require Ca^{2+} for activation. Thus, conventional and novel PKCs are activated through the same signal transduction pathway as phospholipase C. Atypical PKCs, on the other hand, require neither Ca^{2+} nor diacylglycerol for activation. The term "protein kinase C" usually means the protein kinase C α enzyme, a conventional PKC.

Structure and regulation

The structure of all PKCs consists of a regulatory domain and a catalytic domain tethered together by a hinge region. The catalytic region is highly homologous among the different isoforms, as well as to a lesser degree the

catalytic region of other serine/threonine kinases. The second messenger requirement differences in the isoforms are a result of the regulatory region, which are similar within the classes, but differ among them. The crystal structure of the catalytic region of PKC has not been determined. Due to its similarity to other kinases whose crystal structure have been determined, the structure can be strongly predicted.

The regulatory domain or the amino-terminus of the PKCs contains several shared subregions. The C1 domain, present in all of the isoforms of PKC has a binding site for DAG as well as non-hydrolysable analogues called phorbol esters. This domain is functional and capable of binding DAG in both conventional and novel isoforms, however, the C1 domain in atypical PKCs is incapable of binding to DAG or phorbol esters. The C2 domain acts as a Ca^{2+} sensor and is present in both conventional and novel isoforms, but functional as a Ca^{2+} sensor only in the conventional. The pseudosubstrate region, which is present in all three classes of PKC, is a small sequence of amino acids that mimic a substrate and bind the substrate-binding cavity in the catalytic domain keeping the enzyme inactive. When Ca^{2+} and DAG are present in sufficient concentrations, they bind to the C2 and C1 domain, respectively, and recruit PKC to the membrane. This interaction with the membrane results in release of the pseudosubstrate from the catalytic site and activation of the enzyme. In order for these allosteric interactions to occur, however, PKC must first be properly folded and in the correct conformation permissive for catalytic action. This is contingent upon phosphorylation of the catalytic region, discussed below.

The catalytic region or kinase core of the ABC kinases contains approximately 40% amino acid sequence similarity. This similarity increases to ~ 70% across PKCs and even higher when comparing within classes. For example, the two atypical PKC isoforms, ζ and ι/λ , are 84% identical (Selbie et al., 1993). Of the over 30 protein kinase structures whose crystal structure has been revealed, all of them have the same basic organization. They are a bilobal structure with a β sheet comprising the N-terminal lobe and an α helix constituting the C-terminal lobe. Both the ATP- and substrate-binding sites are located in the cleft formed by these two lobes. This is also where the pseudosubstrate domain of the regulatory region binds. Another feature of the PKC catalytic region that is essential to the viability of the kinase is its phosphorylation. The catalytic and novel PKCs have three phosphorylation sites, termed: the activation loop, the turn motif, and the hydrophobic motif. The atypical PKCs are phosphorylated only on the activation loop and the turn motif. Phosphorylation of the hydrophobic motif is rendered unnecessary by the presence of a glutamic acid in place of a serine, which, as a negative charge, acts similarly to a phosphorylated residue. These phosphorylation events are essential for the activity of the enzyme, and 3-phosphoinositide-dependent protein kinase-1 (PDK1) is the upstream kinase responsible for initiating the process by transphosphorylation of the activation loop.

Upon activation, protein kinase C enzymes are translocated to the plasma membrane by RACK proteins (membrane-bound receptor for activated protein kinase C proteins). The protein kinase C enzymes are known for their long-term activation: they remain activated after the original activation signal or the Ca^{2+} -wave is gone. This is presumably achieved by the production of diacylglycerol from phosphatidylcholine by a phospholipase; fatty acids may also play a role in long-term activation.

Function

The consensus sequence of protein kinase C enzymes is similar to that of protein kinase A, since it contains basic amino acids close to the Ser/Thr to be phosphorylated. Their substrates are MARCKS proteins, MAP kinase, transcription factor inhibitor I κ B, the vitamin D₃ receptor VDR, Raf kinase, calpain, and the epidermal growth factor receptor.

Ca^{2+} /calmodulin-dependent protein kinases

Also called *CaM kinases* (EC 2.7.1.123), these kinases are primarily regulated by the Ca^{2+} /calmodulin complex. These kinases show a memory effect on activation. Two types of CaM kinases are:

- *Specialized CaM kinases*. An example is the myosin light chain kinase (MLCK) that phosphorylates myosin, causing muscles to contract.

- *Multifunctional CaM kinases*. Also collectively called *CaM kinase II*, which play a role in many processes, such as neurotransmitter secretion, transcription factor regulation, and glycogen metabolism. Between 1% and 2% of the proteins in the brain are CaM kinase II.

Structure and autoregulation

The CaM kinases consist of an N-terminal catalytic domain, a regulatory domain, and an associative domain. In the absence of Ca^{2+} /calmodulin, the catalytic domain is autoinhibited by the regulatory domain, which contains a pseudosubstrate sequence. Several CaM kinases aggregate into a homooligomer or heterooligomer. Upon activation by Ca^{2+} /calmodulin, the activated CaM kinases autophosphorylate each other in an intermolecular reaction. This has two effects:

1. An increase in affinity for the calmodulin complex, prolonging the time the kinase is active.
2. Continued activation of the phosphorylated kinase complex even after the calmodulin complex has dissociated from the kinase complex, which prolongs the active state even more.

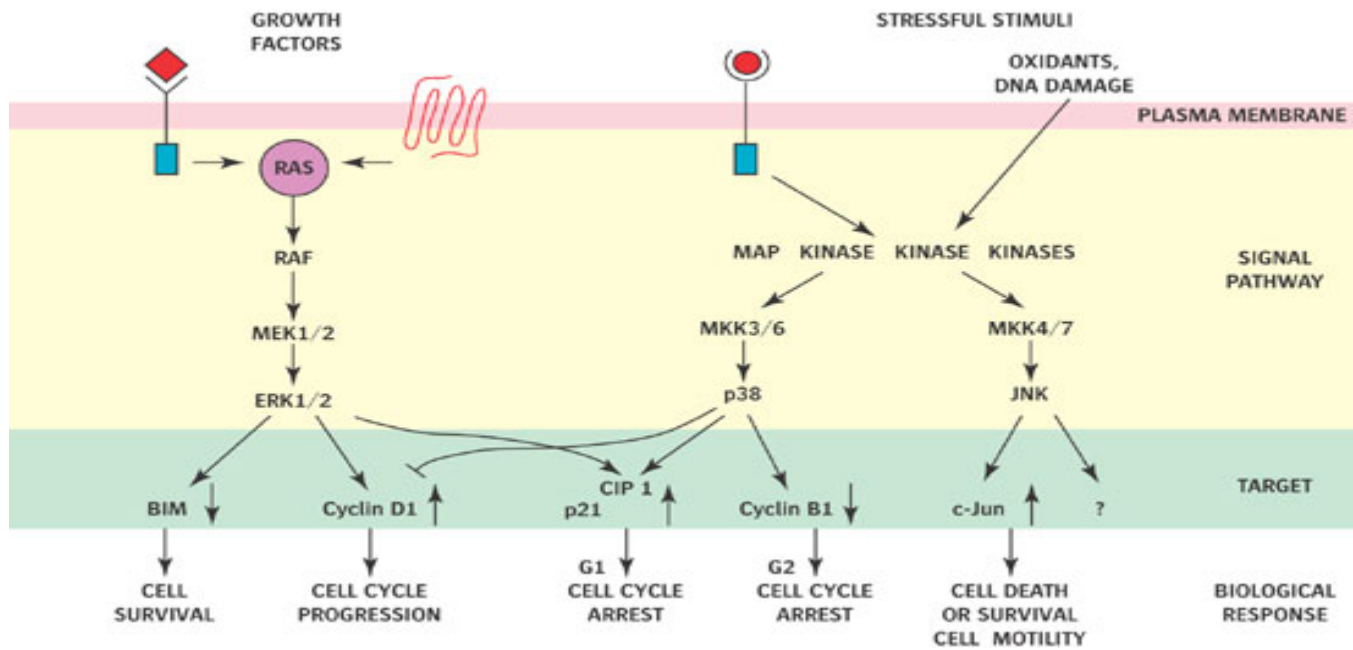
MAP kinases

Mitogen-activated protein kinases (MAPKs) (EC 2.7.1.37) respond to extracellular stimuli (mitogens) and regulate various cellular activities, such as gene expression, mitosis, differentiation, and cell survival/apoptosis. Extracellular stimuli lead to activation of a MAPK via a signaling cascade composed of MAPK, MAPK kinase (MAPKK), and MAPKK kinase (MAPKKK). A MAPKKK that is activated by extracellular stimuli phosphorylates a MAPKK on its serine and threonine residues, and then this MAPKK activates a MAPK through phosphorylation on its serine and tyrosine residues. This MAPK signaling cascade has been evolutionarily well-conserved from yeast to mammals.

To date, four distinct groups of MAPKs have been characterized in mammals: (1) extracellular signal-regulated kinases (ERKs), (2) c-Jun N-terminal kinases (JNKs), (3) p38 isoforms, and (4) ERK5. The ERKs (also known as classical MAPKs) signaling pathway is preferentially activated in response to growth factors and phorbol ester (a tumor promoter), and regulates cell proliferation and cell differentiation. The JNKs (also known as stress-activated protein kinases; SAPKs) and p38 signaling pathways are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and are involved in cell differentiation and apoptosis. And ERK5, which has been found recently, is activated both by growth factors and by stress stimuli, and it participates in cell proliferation.

Mos/Raf kinases

Mos/Raf kinases form part of the MAPKK Kinase family and are activated by growth factors. The enzyme functions to stimulate growth of cells. Raf inhibition has become the target for new anti-metastatic cancer drugs as they inhibit the MAPK cascade and reduce cell proliferation.



Tyrosine-specific protein kinases

Tyrosine-specific protein kinases (EC 2.7.1.112) phosphorylate tyrosine amino acid residues, and are, like serine/threonine-specific kinases, used in signal transduction. They act primarily as growth factor receptors and in downstream signaling from growth factors; some examples:

- Platelet-derived growth factor (PDGF) receptor;
- Epidermal growth factor (EGF) receptor;
- Insulin receptor and insulin-like growth factor (IGF1) receptor;
- Stem cell factor (*scf*) receptor (also called *c-kit*, see the article on gastrointestinal stromal tumor).

Receptor tyrosine kinases

These kinases consist of a transmembrane receptor with a tyrosine kinase domain protruding into the cytoplasm. They play an important role in regulating cell division, cellular differentiation, and morphogenesis. More than 50 receptor tyrosine kinases are known in mammals.

Structure

The extracellular domain serves as the ligand receptor. It can be a separate unit that is attached to the rest of the receptor by a disulfide bond. The same mechanism can be used to bind two receptors together to form a homo- or heterodimer. The transmembrane element is a single α helix. The intracellular or cytoplasmic domain is responsible for the (highly conserved) kinase activity, as well as several regulatory functions.

Regulation

Ligand binding causes two reactions:

1. Dimerization of two monomeric receptor kinases or stabilization of a loose dimer. Many ligands of receptor tyrosine kinases are multivalent. Some tyrosine receptor kinases (e.g., the platelet-derived growth factor receptor) can form heterodimers with other similar but not identical kinases of the same subfamily, allowing a highly varied response to the extracellular signal.

2. *Trans*-autophosphorylation (phosphorylation by the other kinase in the dimer) of the kinase.

The autophosphorylation causes the two subdomains of the intrinsic kinase to shift, opening the kinase domain for ATP binding. In the inactive form, the kinase subdomains are aligned so that ATP cannot reach the catalytic center of the kinase. When several amino acids suitable for phosphorylation are present in the kinase domain (e.g., the insulin-like growth factor receptor), the activity of the kinase can increase with the number of phosphorylated amino acids; in this case, the first phosphorylation is said to be a *cis*-autophosphorylation, switching the kinase from "off" to "standby".

Signal transduction

The active tyrosine kinase phosphorylates specific target proteins, which are often enzymes themselves. An important target is the ras protein signal-transduction chain.

Histidine-specific protein kinases

Histidine kinases are structurally distinct from most other protein kinases and are found mostly in prokaryotes as part of two-component signal transduction mechanisms. A phosphate group from ATP is first added to a histidine residue within the kinase, and later transferred to an aspartate residue on a 'receiver domain' on a different protein, or sometimes on the kinase itself. The aspartyl phosphate residue is then active in signaling.

Histidine kinases are found widely in prokaryotes, as well as in plants and fungi. The pyruvate dehydrogenase family of kinases in animals is structurally related to histidine kinases, but instead phosphorylate serine residues, and probably do not use a phospho-histidine intermediate.

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