## Phylogeny

PRKAA1 has conserved orthologs across eukaryotes, including the SNF1 kinase in *Saccharomyces cerevisiae* (steinberg2023newinsightsinto pages 6-9, carling2012ampactivatedproteinkinase pages 1-2, witczak2008ampactivatedproteinkinase pages 13-14). The mammalian α1 and α2 isoforms share approximately 90% sequence identity within the kinase domain (kurumbail2016structureandregulation pages 4-6). Based on the Manning et al. classification, PRKAA1 belongs to the CAMK-like kinase family (steinberg2023newinsightsinto pages 6-9, steinberg2023newinsightsinto pages 9-13, yan2018structureandphysiological pages 14-15). It is also described as a member of the RD (Arg-Asp) kinase family (yan2018structureandphysiological pages 1-4).

## Reaction Catalyzed

The enzyme catalyzes the transfer of the γ-phosphate from ATP to serine or threonine residues on target proteins (steinberg2023newinsightsinto pages 6-9, carling2012ampactivatedproteinkinase pages 2-3, yan2018structureandphysiological pages 1-4). ATP + protein -> ADP + phosphoprotein (yan2018structureandphysiological pages 1-4, gu2017deconvolutingampactivatedprotein pages 11-13).

## Cofactor Requirements

Catalytic activity requires divalent cations, such as Mg²⁺ or Mn²⁺ (steinberg2023newinsightsinto pages 6-9, steinberg2023newinsightsinto pages 9-13, yan2018structureandphysiological pages 1-4). Mg²⁺ is typically used to coordinate ATP in the catalytic site (yan2018structureandphysiological pages 1-4, woods2003identificationofphosphorylation pages 3-4).

## Substrate Specificity

PRKAA1 phosphorylates serine/threonine residues within a consensus motif characterized by basic residues N-terminal to the phosphorylation site (johnson2023anatlasof pages 2-3). The optimal substrate motif features a hydrophobic residue (methionine preferred) at the P-5 position, a basic residue (arginine preferred) at P-3, and another hydrophobic residue at P+4 (dale1995similarsubstraterecognition pages 3-4, steinberg2023newinsightsinto pages 9-13). The consensus sequence is generally recognized as [Hydrophobic]-X-Arg/Lys-X-X-Ser/Thr-X-[Hydrophobic] (steinberg2023newinsightsinto pages 6-9). A comprehensive kinome-wide analysis by Johnson et al. placed PRKAA1 into a major motif class that prefers basic residues N-terminal to the phospho-acceptor site (johnson2023anatlasof pages 2-3, johnson2023anatlasof pages 1-2). An example of this motif is L-X-R-X-X-S/T (ducommun2015motifaffinityand pages 4-5).

## Structure

PRKAA1 is the catalytic α subunit of the heterotrimeric AMPK complex, which also contains regulatory β and γ subunits (steinberg2023newinsightsinto pages 6-9, carling2012ampactivatedproteinkinase pages 1-2). The α1 subunit consists of an N-terminal kinase domain (KD), an autoinhibitory domain (AID), and a C-terminal regulatory domain that interacts with the other subunits (kurumbail2016structureandregulation pages 4-6, russell2020ampactivatedproteinkinase pages 4-6, carling2012ampactivatedproteinkinase pages 1-2). The KD contains the ATP-binding cleft and key regulatory features, including the activation loop and the αC helix (yan2018structureandphysiological pages 1-4, russell2020ampactivatedproteinkinase pages 4-6). The αC helix is a dynamic structural element whose conformation is critical for activity; it undergoes significant displacement between an autoinhibitory position in the inactive state and a stabilized, active conformation that is required for catalysis (yan2018structureandphysiological pages 14-15, xiao2013structuralbasisof pages 3-4, yan2018structureandphysiological pages 10-12). The β subunit contains a carbohydrate-binding module (CBM) that docks onto the N-lobe of the KD, and the γ subunit contains nucleotide-binding sites that sense the cellular energy status (kurumbail2016structureandregulation pages 4-6, carling2012ampactivatedproteinkinase pages 1-2).

## Regulation

PRKAA1 activity is tightly controlled by allosteric regulation and post-translational modifications (steinberg2023newinsightsinto pages 6-9, steinberg2023newinsightsinto pages 9-13).

**Allosteric and Conformational Regulation:** Adenine nucleotides binding to the γ subunit allosterically regulate the complex (steinberg2023newinsightsinto pages 6-9). AMP binding induces an activating conformational change, promotes phosphorylation at Thr172, and protects this site from dephosphorylation (steinberg2023newinsightsinto pages 9-13, carling2012ampactivatedproteinkinase pages 2-3). ADP also protects Thr172 from dephosphorylation (carling2012ampactivatedproteinkinase pages 2-3). ATP competes with AMP/ADP and antagonizes activation (steinberg2023newinsightsinto pages 9-13). An autoinhibitory domain (AID) in the α subunit also negatively regulates basal activity (kurumbail2016structureandregulation pages 4-6, russell2020ampactivatedproteinkinase pages 4-6).

**Post-Translational Modification:** - **Phosphorylation:** Full activation requires phosphorylation of Thr172 in the activation loop by the upstream kinases LKB1 and CAMKK2 (steinberg2023newinsightsinto pages 6-9, steinberg2023newinsightsinto pages 9-13, russell2020ampactivatedproteinkinase pages 2-4). Phosphorylation at Ser487 by kinases like AKT can inhibit Thr172 phosphorylation (russell2020ampactivatedproteinkinase pages 4-6). - **Dephosphorylation:** Protein phosphatases, including PP2C family members, PP1, and PP2A, can dephosphorylate Thr172 to inactivate the kinase (carling2012ampactivatedproteinkinase pages 2-3, gu2017deconvolutingampactivatedprotein pages 11-13). - **Ubiquitination:** The kinase can be targeted for degradation via the ubiquitin-proteasome system by E3 ligases like WWP1 (yan2018structureandphysiological pages 14-15).

## Function

PRKAA1 is a central component of AMPK, a master sensor of cellular energy homeostasis that is widely expressed in tissues including skeletal muscle, liver, and heart (steinberg2023newinsightsinto pages 6-9, witczak2008ampactivatedproteinkinase pages 3-4).

* **Upstream/Downstream Signaling:** Activated by upstream kinases LKB1 and CAMKK2, PRKAA1 phosphorylates a broad network of downstream substrates to restore cellular energy balance (steinberg2023newinsightsinto pages 6-9). Key substrates include the metabolic enzymes acetyl-CoA carboxylase (ACC), HMG-CoA reductase, and hormone-sensitive lipase (LIPE), as well as proteins involved in autophagy (ULK1, TFEB) and transcription factors (FOXO3) (steinberg2023newinsightsinto pages 6-9, steinberg2023newinsightsinto pages 9-13).
* **Cellular Roles:** AMPK activation inhibits energy-consuming anabolic processes (e.g., synthesis of lipids and carbohydrates) while stimulating energy-producing catabolic pathways (e.g., fatty acid oxidation, glucose uptake) (steinberg2023newinsightsinto pages 9-13, kurumbail2016structureandregulation pages 1-4). It also regulates cell proliferation, autophagy, mitochondrial homeostasis, and lysosomal biogenesis (steinberg2023newinsightsinto pages 6-9).

## Inhibitors

Compound C is a well-characterized experimental inhibitor that targets AMPK activity (steinberg2023newinsightsinto pages 6-9, steinberg2023newinsightsinto pages 9-13, witczak2008ampactivatedproteinkinase pages 13-14).

## Other Comments

Dysfunction or mutations in AMPK subunits are implicated in various human diseases, including metabolic disorders like type 2 diabetes, cancer, and cardiovascular disease (steinberg2023newinsightsinto pages 6-9, kurumbail2016structureandregulation pages 1-4). For example, mutations in *PRKAG2* (γ2 subunit) cause inherited cardiomyopathies with glycogen storage defects (gu2017deconvolutingampactivatedprotein pages 11-13). In cancer, loss-of-function mutations in the upstream kinase LKB1, a tumor suppressor, can impair AMPK activation and contribute to tumorigenesis (russell2020ampactivatedproteinkinase pages 2-4).

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