1. Phylogeny  
   5′-AMP-activated protein kinase catalytic subunit alpha‑1 (PRKAA1/AMPK1) is a highly conserved serine/threonine kinase that belongs to the SNF1/AMPK family of energy sensor kinases. Orthologs of PRKAA1 are found in all eukaryotic organisms, ranging from yeast—as exemplified by the Snf1 protein—to plants, nematodes, and mammals. This broad conservation underscores an evolutionary history confined to a core set of kinases that emerged early in eukaryotic evolution and have been maintained due to their indispensable role in mediating cellular energy and nutrient signaling (faubert2015lkb1andampk pages 51-56, hardie2016regulationofampactivated pages 6-7). Within the human kinome, PRKAA1 is classified in the CAMK (calcium/calmodulin-dependent kinase) group and is part of an ancient regulatory network alongside kinases such as LKB1 and the related AMPK‐related kinases. Recent phylogenetic analyses indicate that gene duplications gave rise to two alpha isoforms—alpha‑1 (PRKAA1) and alpha‑2 (PRKAA2)—with PRKAA1 being ubiquitously expressed in virtually all tissues, while the alpha‑2 isoform is more restricted to tissues such as skeletal and cardiac muscle (mihaylova2012hownutrientsenergy pages 240-245, varaciruelos2019thestrangecase pages 2-3). Thus, PRKAA1 is an essential component of the conserved SNF1/AMPK signaling axis, reflecting both its ancestral function and the stringent evolutionary pressures that have maintained its catalytic domain integrity across species.
2. Reaction Catalyzed  
   The catalytic function of PRKAA1/AMPK1 conforms to the canonical mechanism of serine/threonine kinases in which a phosphate group is transferred from ATP to a hydroxyl group on substrate proteins. The chemical reaction can be represented as follows:  
   ATP + [protein]–OH → ADP + [protein]–O‑PO₃²⁻ + H⁺  
   In this reaction, ATP, usually in the form of Mg·ATP, binds to the kinase domain, and the conserved active site directs the terminal γ-phosphate to the serine or threonine residue present in a substrate protein. This phosphorylation event alters the target protein’s conformation and activity, thereby modulating metabolic pathways crucial for energy homeostasis (altarejos2005molecularandhormonalc pages 43-48, dandapani2013theampksignalling pages 46-50).
3. Cofactor Requirements  
   For efficient catalysis, PRKAA1/AMPK1 requires the presence of divalent metal ions, with magnesium being the most critical. Mg²⁺ forms a complex with ATP, resulting in Mg·ATP²⁻, which is essential for proper substrate alignment in the kinase’s active site and facilitates the nucleophilic attack by the substrate hydroxyl group. Structural and enzymatic studies have consistently demonstrated that without Mg²⁺, the phosphorylation reaction is severely impaired, underscoring the ion’s key role in stabilizing the transition state and influencing catalytic rates (hardie2016regulationofampactivated pages 2-3, cameron2016recentprogressin pages 1-2).
4. Substrate Specificity  
   PRKAA1/AMPK1 displays a defined substrate specificity that is determined by a consensus phosphorylation motif. Biochemical studies, including positional scanning peptide library analyses, have revealed that optimal substrates for AMPK contain basic residues—typically arginine—at the −3 and −4 positions relative to the phosphoacceptor serine or threonine residue. Furthermore, hydrophobic residues such as leucine or methionine are favored at the +4 and +5 positions, and polar residues (for example, asparagine or aspartate) are often present at the +3 position. In several cases, a proline residue at the +2 position has been noted to enhance substrate recognition and facilitate the binding of regulatory 14-3-3 proteins. Summarizing these observations, the optimal motif for phosphorylation by PRKAA1/AMPK1 can be represented as R‑X‑X‑p(S/T)‑X‑P, although additional hydrophobic and polar preferences serve to fine‑tune substrate selection. This specificity enables AMPK to selectively target proteins involved in lipid metabolism, carbohydrate metabolism, and other energy-dependent processes (mihaylova2012hownutrientsenergy pages 269-273, smiles2024themetabolicsensor pages 3-5, dandapani2013theampksignalling pages 46-50).
5. Structure  
   The three-dimensional structure of PRKAA1/AMPK1 is modular, comprising several distinct regions that jointly ensure both catalytic proficiency and regulatory flexibility. The N‑terminal region features a highly conserved serine/threonine kinase domain that adopts the classical bilobal architecture common to eukaryotic kinases. The smaller N‑lobe consists primarily of β‑sheets and a conserved C‑helix, while the larger C‑lobe is predominantly helical and contains the activation loop—a flexible segment that hosts the key phosphorylatable residue, Thr172. Phosphorylation at Thr172 is central to establishing an active conformation, as it effectively reorganizes the catalytic machinery, aligns the hydrophobic spine, and stabilizes the proper orientation of the active site (hardie2016regulationofampactivated pages 6-7, smiles2024themetabolicsensor pages 2-3).

Directly following the kinase domain, an auto‑inhibitory domain (AID) is present; in the inactive state, the AID interacts with the kinase domain, thereby suppressing catalytic activity. Upon activation by upstream kinases or allosteric effectors, the AID undergoes conformational changes that relieve its inhibitory grip, allowing full catalytic function. Subsequent to the AID lies the linker region, which contains regulatory interacting motifs (a‑RIMs) that play a pivotal role in communicating with the γ‑subunit. This interaction is essential for the nucleotide sensing mechanism, wherein AMP or ADP binding to the γ‑subunit induces conformational alterations transmitted via these RIMs to influence kinase activity. Additionally, the C‑terminal region of PRKAA1 includes a beta‑subunit interacting domain (b‑SID) that ensures proper heterotrimeric complex formation with the β‑ and γ‑subunits; within this region, elements such as a nuclear export signal (NES) and an ST loop subject to phosphorylation further refine the spatial regulation of the enzyme. Crystallographic data and AlphaFold models corroborate this modular organization, detailing how the catalytic and regulatory elements are intricately arranged to accommodate substrate binding, nucleotide recognition, and allosteric modulation (hardie2016regulationofampactivated pages 1-2, smiles2024themetabolicsensor pages 3-5, tamargogomez2018ampkregulationof pages 3-5, varaciruelos2019thestrangecase pages 2-3).

1. Regulation  
   The regulation of PRKAA1/AMPK1 is characterized by a multi‑layered network of post‑translational modifications and nucleotide‑mediated allosteric interactions. Paramount among these regulatory events is the phosphorylation of Thr172 within the activation loop, a modification that increases the catalytic activity of AMPK by more than 100‑fold. This phosphorylation is predominantly executed by upstream kinases such as LKB1—thus serving as the major sensor of energy deficiency—along with contributions from Ca²⁺/calmodulin‑dependent protein kinase kinase β (CaMKKβ) and, in certain cellular contexts, transforming growth factor‑β‑activated kinase 1 (TAK1) (arad2007ampactivatedproteinkinase pages 3-4, dandapani2013theampksignalling pages 37-41). In parallel with this covalent modification, allosteric regulation occurs via the binding of AMP (and to a subordinate extent ADP) to conserved cystathionine‑β‑synthase (CBS) domains within the γ‑subunit. The binding of AMP results in three major consequences: (i) it directly allosterically activates the enzyme, (ii) it facilitates further phosphorylation of Thr172 by rendering the activation loop more accessible to kinase action, and (iii) it shields the phosphorylated Thr172 from dephosphorylation by phosphatases such as PP1, PP2A, and PP2C (dandapani2013theampksignalling pages 46-50, scanlon2021investigatingthecontribution pages 44-49). Conversely, ATP competes with AMP for binding at these sites and thus acts to attenuate AMPK activation. Additionally, inhibitory phosphorylation events have been reported, with specific serine residues (for example, Ser485 on the α‑subunit) serving to negatively modulate activity by interfering with the activation loop dynamics and subsequent conformational rearrangements (baskin2012regulationofprotein pages 49-53, russell2020ampactivatedproteinkinase pages 2-4). Collectively, these regulatory inputs enable PRKAA1/AMPK1 to function as a finely tuned sensor, rapidly adjusting its catalytic output in response to shifts in the intracellular energy landscape (faubert2015lkb1andampk pages 51-56, tamargogomez2018ampkregulationof pages 3-5).
2. Function  
   Functionally, PRKAA1/AMPK1 is central to the maintenance of cellular energy homeostasis, acting as a master metabolic sensor whose activation triggers a coordinated response to energy stress. PRKAA1 is ubiquitously expressed across a wide range of tissues, including liver, skeletal muscle, heart, brain, adipose tissue, and endothelial cells, as well as in immune cells such as macrophages. When intracellular ATP levels fall due to nutrient deprivation, hypoxia, or increased energy demand, AMPK becomes activated via Thr172 phosphorylation and AMP‑mediated allosteric mechanisms. Once activated, it orchestrates a shift in cellular metabolism by phosphorylating a wide array of substrates that regulate both anabolic and catabolic pathways. For instance, AMPK phosphorylates and inhibits key metabolic enzymes such as acetyl‑CoA carboxylase (ACACA/ACACB) to reduce fatty acid synthesis and promote fatty acid oxidation and simultaneously affects cholesterol metabolism by targeting hydroxymethylglutaryl‑CoA reductase (HMGCR) (altarejos2005molecularandhormonalc pages 43-48, russell2020ampactivatedproteinkinase pages 2-4). In terms of glucose metabolism, AMPK promotes glucose uptake through effects on the insulin signaling cascade and by regulating the translocation of glucose transporters, thus ensuring sufficient substrate availability for ATP production (herzig2018ampkguardianof pages 1-2, wang2012ampactivatedproteinkinase pages 1-2).

Beyond the immediate modulation of metabolic enzyme activities, PRKAA1/AMPK1 influences longer‑term cellular adaptations via phosphorylation of transcriptional regulators. It modulates gene expression programs by targeting substrates such as the transcriptional co‑activator CRTC2, the forkhead box protein FOXO3, and histone proteins like H2B—thereby impacting mitochondrial biogenesis, autophagy, and cell survival pathways. Moreover, through the phosphorylation of key signaling regulators such as TSC2 and RPTOR, AMPK attenuates the mammalian target of rapamycin complex 1 (mTORC1) pathway under conditions of nutrient limitation, thereby integrating energy sensing with control over cell growth and proliferation. This expansive range of substrates positions PRKAA1/AMPK1 as a central node that coordinates diverse physiological processes related to metabolism, stress response, and even cytoskeletal organization (altarejos2005molecularandhormonalc pages 43-48, mohanty2025rethinkingampka pages 1-2, rhein2021investigationofphysiological pages 24-27, zhu2014regulationofmacrophage pages 21-26).

1. Other Comments  
   Several pharmacological agents have been identified that modulate AMPK activity, reflecting its importance as a therapeutic target in metabolic disorders, cardiovascular diseases, and certain cancers. Activators such as metformin, AICAR, A-769662, and compound 991 have been extensively studied for their ability to elevate cellular AMP levels or mimic its binding at the γ‑subunit, thereby enhancing Thr172 phosphorylation and promoting a catabolic state that favors ATP generation. These compounds are instrumental in experimental models and clinical settings for their capacity to restore energy balance in conditions of metabolic stress (dandapani2013theampksignalling pages 46-50, scanlon2021investigatingthecontribution pages 44-49). In addition, dysregulation of the AMPK pathway has been linked to a variety of disease states including type 2 diabetes, obesity, and specific forms of cancer, thereby making PRKAA1 an important focus of drug discovery efforts. Although direct evidence for post‑translational modifications such as ubiquitination or acetylation on PRKAA1 itself is limited within the current literature, phosphorylation and nucleotide‐mediated regulation remain the principal mechanisms of control. Thus, PRKAA1/AMPK1 is central not only to acute metabolic control but also to long‑term adaptive responses to energetic stress, with its activity tightly modulated by a network of upstream kinases and allosteric effectors (altarejos2005molecularandhormonalc pages 43-48, russell2020ampactivatedproteinkinase pages 2-4).
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