## Phylogeny

PRKAA1, the catalytic α1 subunit of AMP-activated protein kinase (AMPK), is widely conserved throughout eukaryotes with the yeast SNF1 kinase representing the ancestral ortholog (Steinberg & Hardie, 2023; Carling et al., 2012; Witczak et al., 2008). Mammalian α1 and α2 isoforms share ~90 % sequence identity across the kinase domain (Kurumbail & Calabrese, 2016). Within the protein kinase super-family, PRKAA1 is classified in the Ca2+/calmodulin-dependent protein kinase (CAMK)-like group and further assigned to the RD (Arg-Asp) sub-family (Steinberg & Hardie, 2023; Yan et al., 2018).

## Reaction Catalyzed

ATP + protein ⇌ ADP + phosphoprotein (Yan et al., 2018; Gu et al., 2017).

## Cofactor Requirements

Catalysis requires a divalent metal ion, most commonly Mg2+ (also Mn2+), which coordinates ATP in the active site (Steinberg & Hardie, 2023; Yan et al., 2018; Woods et al., 2003).

## Substrate Specificity

PRKAA1 phosphorylates Ser/Thr residues located within basic, hydrophobic motifs. Optimal peptide features include a hydrophobic residue at –5 (Met preferred), Arg/Lys at –3, and a hydrophobic residue at +4, yielding the consensus ϕ-X-Arg/Lys-X-X-Ser/Thr-X-ϕ (Johnson et al., 2023; Dale et al., 1995; Steinberg & Hardie, 2023). Large-scale profiling confirms a general preference for basic residues N-terminal to the phospho-acceptor (Johnson et al., 2023). An illustrative minimal motif is L-X-R-X-X-S/T (Ducommun et al., 2015).

## Structure

The AMPK holo-enzyme is a heterotrimer comprising the catalytic α (PRKAA1), scaffolding β and regulatory γ subunits (Steinberg & Hardie, 2023; Carling et al., 2012).  
• α1 architecture: N-terminal kinase domain (KD), autoinhibitory domain (AID), and C-terminal region that contacts β/γ (Kurumbail & Calabrese, 2016; Russell & Hardie, 2020).  
• Key KD elements: ATP-binding cleft, activation loop (Thr172), and a mobile αC helix that shifts from an autoinhibitory to an active position during activation (Yan et al., 2018; Xiao et al., 2013).  
• β subunit: contains a carbohydrate-binding module (CBM) that docks onto the KD N-lobe (Kurumbail & Calabrese, 2016).  
• γ subunit: houses four cystathionine-β-synthase (CBS) motifs forming adenine nucleotide–binding sites that sense cellular energy status (Carling et al., 2012).

## Regulation

Allosteric and covalent mechanisms cooperate to modulate PRKAA1 activity (Steinberg & Hardie, 2023).  
• Allosteric control: AMP binding to the γ subunit promotes an active conformation, enhances Thr172 phosphorylation and protects this site from phosphatases; ADP affords partial protection, whereas ATP antagonizes activation. The α-subunit AID suppresses basal activity (Kurumbail & Calabrese, 2016; Carling et al., 2012).  
• Phosphorylation: Full activation requires Thr172 phosphorylation by upstream kinases LKB1 and CAMKK2 (Steinberg & Hardie, 2023; Russell & Hardie, 2020). Inhibitory Ser487 phosphorylation by AKT and others reduces Thr172 phosphorylation (Russell & Hardie, 2020).  
• Dephosphorylation: PP2C family enzymes, PP1 and PP2A remove the Thr172 phosphate (Carling et al., 2012; Gu et al., 2017).  
• Ubiquitination: E3 ligase WWP1 can target AMPK for proteasomal degradation (Yan et al., 2018).

## Function

AMPK incorporating PRKAA1 is a master sensor of cellular energy, highly expressed in skeletal muscle, liver and heart (Steinberg & Hardie, 2023; Witczak et al., 2008).  
• Upstream/Downstream signaling: Activated by LKB1 and CAMKK2, PRKAA1 phosphorylates metabolic enzymes (acetyl-CoA carboxylase, HMG-CoA reductase, hormone-sensitive lipase) and regulators of autophagy and transcription (ULK1, TFEB, FOXO3) to restore ATP balance (Steinberg & Hardie, 2023).  
• Cellular roles: Inhibits anabolic pathways (lipid and carbohydrate synthesis) while stimulating catabolic processes (fatty-acid oxidation, glucose uptake). Also governs cell proliferation, autophagy, mitochondrial homeostasis and lysosomal biogenesis (Steinberg & Hardie, 2023; Kurumbail & Calabrese, 2016).

## Inhibitors

Compound C is a widely used experimental ATP-competitive inhibitor of AMPK activity (Steinberg & Hardie, 2023; Witczak et al., 2008).

## Other Comments

Genetic or functional disruption of AMPK subunits contributes to metabolic disorders, cancer and cardiovascular disease (Steinberg & Hardie, 2023; Kurumbail & Calabrese, 2016). Mutations in PRKAG2 (γ2) cause glycogen-storage cardiomyopathies (Gu et al., 2017). Loss-of-function mutations in the tumor-suppressor kinase LKB1 impair AMPK activation in many cancers (Russell & Hardie, 2020).

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