## Phylogeny

PHKG2 is a paralog of PHKG1, with which it shares significant sequence homology (bali2014variabilityofdisease pages 12-12). Both are gamma subunits of phosphorylase kinase (manning2002theproteinkinase pages 7-8). The gamma subunit is highly homologous to the catalytic subunit of cAMP-dependent protein kinase (PKA) and is highly conserved across species (brushia1999phosphorylasekinasethe pages 2-3). PHKG2 belongs to the eukaryotic protein kinase (ePK) superfamily (brushia1999phosphorylasekinasethe pages 18-19, hanks2003genomicanalysisof pages 1-2). Phylogenetic classification based on Manning et al. (2002) is conflicting in the provided sources, with PHKG2 assigned to the AGC kinase group (brushia1999phosphorylasekinasethe pages 2-3, hanks2003genomicanalysisof pages 6-7), the calcium/calmodulin-dependent protein kinase (CAMK) group (bali2014variabilityofdisease pages 12-12, burwinkel2003muscleglycogenosiswith pages 1-2), and the CMGC kinase group (bali2014variabilityofdisease pages 12-12, manning2011theminimalkinome pages 5-6).

## Reaction Catalyzed

Phosphorylase kinase catalyzes the transfer of the gamma-phosphate from ATP to serine residues on its substrate, glycogen phosphorylase b, converting it to the active phosphorylase a form (bali2014variabilityofdisease pages 12-12). This reaction activates glycogen phosphorylase to catalyze the conversion of glycogen to glucose-1-phosphate (albash2014novelphkg2mutation pages 5-6, bali2014variabilityofdisease pages 12-12). The reaction is: phosphorylase b + ATP → phosphorylase a + ADP (bali2014variabilityofdisease pages 12-12). Other substrates phosphorylated in vitro include glycogen synthase, troponin I/T, casein, and the neuronal proteins B-50 and neurogranin (brushia1999phosphorylasekinasethe pages 16-17).

## Cofactor Requirements

Catalytic activity requires divalent metal ion cofactors, primarily Mg²⁺ or Mn²⁺, which are essential for coordinating ATP binding and the phosphoryl transfer reaction (bali2014variabilityofdisease pages 12-12, brushia1999phosphorylasekinasethe pages 18-19). The enzyme is also dependent on Ca²⁺, which binds to the integral delta subunit (calmodulin) to mediate activation (brushia1999phosphorylasekinasethe pages 1-2, bali2014variabilityofdisease pages 12-12).

## Substrate Specificity

The substrate specificity of PHKG2 was determined as part of a comprehensive analysis of the human serine/threonine kinome using positional scoring matrices (PSSMs) to define amino acid preferences at positions -5 to +5 relative to the phosphorylation site (johnson2023anatlasof pages 4-5, johnson2023anatlasof pages 9-10). The enzyme shows specificity for serine residues (brushia1999phosphorylasekinasethe pages 16-17). Substrate recognition involves a consensus motif favoring positively charged residues at positions P-3 and P-4, a hydrophobic residue at P+1, and a basic residue at P+2 relative to the phosphorylated serine (position 0) (brushia1999phosphorylasekinasethe pages 15-16). Substrate binding is influenced by both the linear peptide sequence and the tertiary/quaternary structure of the native substrate (brushia1999phosphorylasekinasethe pages 15-16).

## Structure

PHKG2 is the gamma (γ) catalytic subunit of the large (αβγδ)₄ phosphorylase kinase holoenzyme, which has a mass of ~1.3 MDa and a ‘butterfly-like’ shape with D2 symmetry (brushia1999phosphorylasekinasethe pages 2-3, brushia1999phosphorylasekinasethe pages 8-9). The N-terminal two-thirds of the gamma subunit (residues 1-298) forms the catalytic domain, which has a bilobal architecture homologous to the PKA catalytic subunit (brushia1999phosphorylasekinasethe pages 2-3, brushia1999phosphorylasekinasethe pages 3-4). The C-terminal third (residues 299-386) is a unique regulatory domain that functions as an autoinhibitor and contains two calmodulin-binding domains (residues 301-327 and 332-371) (brushia1999phosphorylasekinasethe pages 3-4). The catalytic domain contains key conserved structural features, including a C-helix, a catalytic spine (C-spine), and a regulatory spine (R-spine), which assemble to form the active kinase core (taylor2011proteinkinasesevolution pages 4-5, taylor2012evolutionofthe pages 3-4). The activation segment, which is part of the R-spine, lacks phosphorylatable residues; instead, a conserved glutamate (Glu182) may mimic a phosphoserine to help orient the substrate for catalysis (brushia1999phosphorylasekinasethe pages 16-17, johnson1996activeandinactive pages 3-4).

## Regulation

The catalytic activity of the gamma subunit (PHKG2) is inhibited by the alpha and beta regulatory subunits and its own C-terminal autoinhibitory domain (brushia1999phosphorylasekinasethe pages 1-2, brushia1999phosphorylasekinasethe pages 3-4). Activation occurs through cooperative deinhibition via two mechanisms: Ca²⁺ binding to the delta subunit (calmodulin) and phosphorylation of the alpha and beta subunits by cAMP-dependent protein kinase (PKA) (brushia1999phosphorylasekinasethe pages 1-2). PKA phosphorylates the alpha subunit at multiple sites, with Ser1018 being a major regulatory site (brushia1999phosphorylasekinasethe pages 2-3, brushia1999phosphorylasekinasethe pages 6-7). The beta subunit is phosphorylated by PKA at Ser26, which is also a major regulatory site (brushia1999phosphorylasekinasethe pages 2-3, brushia1999phosphorylasekinasethe pages 6-7). The alpha and beta subunits are also subject to farnesylation at their C-termini (brushia1999phosphorylasekinasethe pages 3-4, brushia1999phosphorylasekinasethe pages 5-6). Activity is also allosterically stimulated by ADP, which binds to the beta subunit (brushia1999phosphorylasekinasethe pages 15-16).

## Function

PHKG2 encodes the liver-specific isoform of the phosphorylase kinase gamma subunit and is primarily expressed in hepatic tissue, with a testis/liver variant also described (albash2014novelphkg2mutation pages 5-6, bali2014variabilityofdisease pages 12-12, burwinkel2003muscleglycogenosiswith pages 9-10). It is a key enzyme in glycogenolysis, the pathway for glycogen breakdown to glucose, and is critical for maintaining glucose homeostasis (albash2014novelphkg2mutation pages 5-6). PHKG2 functions as the catalytic core of the phosphorylase kinase holoenzyme, interacting with the alpha (PHKA2), beta (PHKB), and delta (calmodulin) subunits (bali2014variabilityofdisease pages 12-12). In the glycogenolytic pathway, it acts downstream of glycogen synthesis enzymes and upstream of its primary substrate, glycogen phosphorylase b, which it activates through phosphorylation (albash2014novelphkg2mutation pages 5-6, bali2014variabilityofdisease pages 12-12).

## Inhibitors

The catalytic activity of the PHKG2-containing holoenzyme is inhibited by its regulatory alpha and beta subunits (brushia1999phosphorylasekinasethe pages 1-2). Experimentally, synthetic peptides that mimic the calmodulin-binding domains of the alpha and beta subunits can partially inhibit activated phosphorylase kinase (brushia1999phosphorylasekinasethe pages 13-14).

## Other Comments

Mutations in PHKG2 cause Glycogen Storage Disease type IXd (GSD IXd), an autosomal recessive disorder resulting from deficient phosphorylase kinase activity in the liver (albash2014novelphkg2mutation pages 5-6, bali2014variabilityofdisease pages 12-12). This leads to glycogen accumulation in hepatocytes and a clinical phenotype that includes hepatomegaly, hypoglycemia, elevated liver transaminases, growth retardation, and potential progression to hepatic fibrosis and cirrhosis (albash2014novelphkg2mutation pages 5-6, burwinkel2003muscleglycogenosiswith pages 1-2). Pathogenic mutations reported in patients include the homozygous missense mutation c.659G>A (p.G220E), splice site mutations (c.647+5G>T, c.96-11G>A), nonsense mutations (p.Gln83*, p.Trp300*), missense mutations (p.Tyr358Cys), and small deletions (p.Lys53del) (albash2014novelphkg2mutation pages 5-6, bali2014variabilityofdisease pages 12-12). These mutations lead to defective or completely abolished enzyme activity (albash2014novelphkg2mutation pages 5-6, brushia1999phosphorylasekinasethe pages 17-18).

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