## Phylogeny

• Member of the AGC serine/threonine kinase group; classified within the cAMP-dependent protein kinase (PKA) catalytic subfamily yet forms a branch distinct from canonical Cα/Cβ/Cγ isoforms (huang2016prkxanovel pages 1-2, pearce2010thenutsand pages 1-2).  
• Kinome‐wide surveys place PRKX in the PKA family on human and multi-species kinome maps that derive from the Manning 2002 framework (martin2009kinomerv.1.0 pages 3-4, li2005profilesofprkx pages 7-7).  
• Experimentally verified vertebrate orthologs: Homo sapiens PRKX; Mus musculus Pkare (85.9 % identity); Rattus norvegicus Prkx; Danio rerio prkx-like; Xenopus laevis prkx-like (li2002prkxaphylogenetically pages 3-4, huang2016prkxanovel pages 1-2).  
• Invertebrate orthologs: Drosophila melanogaster DC2 kinase and Dictyostelium discoideum KAPC (li2002prkxaphylogenetically pages 2-3).  
• Paralog: human Y-chromosome PRKY shares 94 % sequence similarity but is truncated (huang2016prkxanovel pages 1-2, pearce2010thenutsand pages 1-2).

## Reaction Catalyzed

ATP + [protein]-Ser/Thr ⇌ ADP + [protein]-O-phospho-Ser/Thr (glesne2006smad6isa pages 3-4).

## Cofactor Requirements

Catalytic activity is strictly divalent-cation dependent; Mg²⁺ or Mn²⁺ support phosphorylation, whereas chelation abolishes activity (zimmermann1999prkxisa pages 3-3, glesne2006smad6isa pages 10-10).

## Substrate Specificity

• Kinome-wide peptide library profiling assigns PRKX a preference for basic residues at –3/–2 and a hydrophobic residue at +1, consensus R-R/K-X-S/T-Φ (johnson2023anatlasof pages 4-5).  
• Biochemical assays confirm optimal phosphorylation of Arg-Arg/Lys-X-Ser/Thr motifs (huang2016prkxanovel pages 2-4).  
• Validated site: Smad6 Ser435 within a PPX/VYSI motif required for myeloid differentiation (glesne2006smad6isa pages 2-3).  
• Additional reported substrates and sites include tau protein Ser214 and polycystin-1 cytoplasmic tail serines conforming to the basic consensus (huang2016prkxanovel pages 2-4).

## Structure

• Single 358-aa catalytic subunit; full-length sequence encodes the entire kinase without separate regulatory gene products (huang2016prkxanovel pages 1-2).  
• Canonical bilobal kinase fold: Lys72 (β3) anchors ATP, Glu91 (αC) forms the Lys-Glu salt bridge, catalytic loop Asp166 and Asp184 constitute the catalytic dyad, activation-segment Thr197 serves as the autophosphorylation site (zimmermann1999prkxisa pages 9-10).  
• αC helix orientation and hydrophobic regulatory spine align with other AGC kinases, supporting cAMP-dependent allostery (huang2016prkxanovel pages 1-2).  
• N-terminal extension harbours two WW-domain binding motifs (phospho-SP/TP and PPxY) that mediate interactions with Pin-1, Bag-3 and Magi-1 (huang2016prkxanovel pages 4-6).  
• Regulatory interface uniquely accommodates the PKA RIα subunit, explaining selective holoenzyme formation (huang2016prkxanovel pages 1-2).  
• No crystal structure reported; AlphaFold model AF-P51817 provides a high-confidence full-length prediction (huang2016prkxanovel pages 1-2).

## Regulation

Post-translational modifications  
– Autophosphorylation at Thr197 is obligatory for catalytic competence (zimmermann1999prkxisa pages 9-10).  
– Additional autophosphorylation events detected in vitro (glesne2006smad6isa pages 10-10).

Allosteric and conformational control  
– Inactive RIα–PRKX holoenzyme dissociates upon cAMP binding, releasing active PRKX (huang2016prkxanovel pages 1-2).  
– Heat-stable PKI peptide binds the catalytic cleft and competitively inhibits kinase activity (zimmermann1999prkxisa pages 9-10).  
– Adeno-associated virus 2 Rep78 protein binds PRKX and suppresses CRE-dependent transcription (huang2016prkxanovel pages 6-7).

Transcriptional regulation  
– PKCβ activity up-regulates PRKX expression during hematopoietic maturation (thiriet2013cytoplasmicproteinserinethreonine pages 27-30).

## Function

Expression  
– High mRNA and protein levels in human fetal kidney, brain, lung and heart; markedly reduced in adult counterparts (huang2016prkxanovel pages 4-4).  
– Detected in developing mouse neurons and vascular endothelial cells with both cytoplasmic and nuclear localisation (huang2016prkxanovel pages 4-4).

Biological roles  
– Nephrogenesis: drives ureteric-bud branching, epithelial migration and tubulogenesis (li2002prkxaphylogenetically pages 2-3).  
– Myeloid differentiation: Smad6 phosphorylation by PRKX promotes macrophage lineage commitment (glesne2006smad6isa pages 2-3).  
– Angiogenesis: essential for endothelial proliferation, migration and vascular-like network formation (li2011prkxcriticallyregulates pages 11-11).  
– Interacts with polycystin-1 and rescues PKD1 deficiency–induced defects (huang2016prkxanovel pages 7-7).  
– Enhances CREB-dependent transcription and regulates partners such as Pin-1, Bag-3 and Magi-1 via WW-binding motifs (huang2016prkxanovel pages 4-6).

## Inhibitors

• Heat-stable PKI peptide inhibits PRKX with KD ≈ 15 nM; competitive at the active site (zimmermann1999prkxisa pages 4-4).  
• Broad-spectrum ATP-site inhibitor H89 reduces PRKX activity in cellular assays (li2002prkxaphylogenetically pages 3-4).  
• No PRKX-selective small-molecule inhibitors reported (thiriet2013cytoplasmicproteinserinethreonine pages 27-30).

## Other Comments

• Autosomal dominant polycystic kidney disease: PRKX up-regulated in cyst epithelium and functionally connected to polycystin-1 (li2002prkxaphylogenetically pages 3-4).  
• Xp;Yp translocations involving PRKX and PRKY cause disorders of sex development (huang2016prkxanovel pages 4-6).  
• Over-expression promotes renal carcinoma resistance to sunitinib via CREB-MITF signalling (huang2016prkxanovel pages 4-6).  
• Gene resides in the Xp22.3 region linked to chondrodysplasia punctata (huang2016prkxanovel pages 1-2).

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