## Phylogeny

• Orthologous genes are experimentally confirmed in mouse, rat, zebrafish, chicken and Xenopus, demonstrating conservation across vertebrates (sato2015theevolvingimpact pages 2-3).  
• The closest characterized invertebrate homolog is Drosophila Gprk2, which clusters with the vertebrate GRK4/5/6 branch (benovic2021historicalperspectiveof pages 7-8).  
• Within the human kinome GRK4, GRK5 and GRK6 constitute the GRK4 sub-family of the AGC Ser/Thr kinase group, distinct from the GRK1/7 and GRK2/3 branches (gurevich2012gproteincoupledreceptor pages 2-4).  
• Pair-wise sequence identity: GRK4 shares ~81 % identity with GRK5, ~79 % with GRK6 and ~36 % with GRK2/3, underscoring internal coherence of the GRK4 sub-family (benovic2021historicalperspectiveof pages 4-6).

## Reaction Catalyzed

ATP + [GPCR Ser/Thr] → ADP + [GPCR O-phospho-Ser/Thr] (allen2015structureandfunction pages 9-10).

## Cofactor Requirements

Catalytic activity is Mg²⁺-dependent; in vitro assays employ 10 mM MgCl₂ (allen2015structureandfunction pages 4-5).

## Substrate Specificity

• A consensus phosphorylation motif has not been formally defined for GRK4; the crystal structure reveals a neutral-to-basic substrate channel that accommodates substrates containing acidic residues C-terminal to the phosphoacceptor (allen2015structureandfunction pages 9-10).  
• Positional-scanning arrays on the close homolog GRK5 show preference for acidic residues at +1/+3 and an aromatic residue at –2, suggesting a sub-family trend (komolov2021structureofa pages 7-9).

Validated protein substrates  
– Rhodopsin (phosphorylated by the α-isoform only) (unknownauthors2009identificationofa pages 22-26).  
– Dopamine receptor D3 (α and γ isoforms) (unknownauthors2009identificationofa pages 22-26).  
– β₂-Adrenergic receptor (all isoforms) (unknownauthors2009identificationofa pages 22-26).  
– Dopamine receptor D1, angiotensin II receptors AT1R/AT2R, endothelin-B receptor, thromboxane receptor and adiponectin receptor 1 (yang2022comprehensiveinsightsin pages 9-11, yang2022comprehensiveinsightsin pages 11-13).

## Structure

Domain organisation  
• N-terminal amphipathic αN helix followed by a basic segment (residues 20-39) that binds calmodulin and anionic lipids (allen2015structureandfunction pages 11-12).  
• Regulator of G-protein Signalling (RH) domain (four-helix bundle and terminal subdomain) contiguous with an inserted bilobal AGC kinase domain (allen2015structureandfunction pages 1-2).  
• C-terminal extension bearing palmitoylation sites Cys563/Cys578 and splice-variant inserts (allen2015structureandfunction pages 11-12).

3-D structural information  
• Crystal structures: wild-type GRK4 (PDB 4YFK, 2.6 Å) and hypertension variant A486V (PDB 4YHJ, 2.6 Å) resolve residues 25-525 (allen2015structureandfunction pages 1-2, allen2015structureandfunction pages 11-12).  
• AlphaFold full-length prediction AF-Q9NQT5-F1 complements missing termini (allen2015structureandfunction pages 11-12).

Catalytic and regulatory elements  
– Catalytic motifs: VAIK Lys216, HRD Asp331, DLG Asp456 adopt the active DFG-in configuration (allen2015structureandfunction pages 10-11).  
– Activation-loop boundaries have been reported as 351-366 in crystal structures; earlier biochemical mapping placed the segment at 325-343 (allen2015structureandfunction pages 11-12, allen2015structureandfunction pages 2-3).  
– The kinase domain is captured in a semi-open state requiring ~12° further lobe closure for full activation (allen2015structureandfunction pages 6-7).  
– An AST-loop hydrogen bond Asp469–Tyr474 secures the C-tail over the active site, a feature distinguishing GRK4 from GRK6 (allen2015structureandfunction pages 5-6).  
– Crystallographic dimer interface buries 4 945 Å², yet analytical ultracentrifugation indicates a monomeric enzyme (allen2015structureandfunction pages 7-9).

## Regulation

Post-translational modifications  
• Autophosphorylation sites: Ser139, Ser244, Ser249, Thr256, Ser485; Ser485 phosphorylation is markedly enhanced in variant A486V (allen2015structureandfunction pages 11-12, allen2015structureandfunction pages 7-9).  
• Palmitoylation: Cys563 and Cys578 mediate membrane anchoring (allen2015structureandfunction pages 2-3).  
• Heterologous phosphorylation: protein kinase C-ε phosphorylates GRK4, increasing activity (yang2022comprehensiveinsightsin pages 9-11).  
• Ubiquitination has been documented, but target lysines remain undefined (allen2015structureandfunction pages 11-12).

Allosteric and conformational control  
• Calmodulin binds residues 20-39 of the α-isoform and inhibits rhodopsin phosphorylation; other isoforms display negligible binding (allen2015structureandfunction pages 11-12).  
• Autophosphorylation abolishes the initial lag in substrate phosphorylation seen with wild-type GRK4, whereas A486V is constitutively rapid, indicating partial pre-activation (allen2015structureandfunction pages 7-9).

Transcriptional and trafficking regulation  
• GRK4 expression is up-regulated by c-Myc and C/EBP family members and down-regulated by microRNAs miR-430a and miR-218a (yang2022comprehensiveinsightsin pages 11-13).  
• Reactive oxygen species elevate GRK4 levels; antioxidants reduce expression (yang2022comprehensiveinsightsin pages 11-13).  
• Sorting nexin-5 controls intracellular trafficking of GRK4 (yang2022comprehensiveinsightsin pages 11-13).

## Function

Expression pattern  
• Highest expression in testis, renal proximal tubule and cerebellum; additional expression in brain and uterus myometrium (gurevich2012gproteincoupledreceptor pages 1-2, allen2015structureandfunction pages 1-2).

Biological roles  
• Phosphorylates activated GPCRs to promote β-arrestin-mediated desensitisation (pitcher1998gprotein–coupledreceptor pages 3-5).  
• In renal proximal tubule cells, GRK4-mediated hyper-phosphorylation of D1R, AT1R and AdipoR1 disrupts natriuretic signalling and enhances sodium reabsorption, contributing to blood-pressure control (yang2022comprehensiveinsightsin pages 9-11, yang2022comprehensiveinsightsin pages 11-13).  
• Upstream modulators include PKC-ε, Ca²⁺/calmodulin (isoform-specific) and PIP₂ binding via the basic N-terminal segment (yang2022comprehensiveinsightsin pages 9-11, allen2015structureandfunction pages 11-12).  
• GRK4 over-expression induces cellular senescence, linking kinase activity to age-related hypertension (yang2022comprehensiveinsightsin pages 11-13).

## Other Comments

• Gain-of-function variants A142V and A486V accelerate autophosphorylation and kinase activity; A142V elevates blood pressure on a normal-salt diet, whereas A486V requires high-salt intake (yang2022comprehensiveinsightsin pages 9-11).  
• The A486V crystal structure (PDB 4YHJ) shows a partially closed active site and enhanced Ser485 phosphorylation, explaining its hyperactivity (allen2015structureandfunction pages 1-2, allen2015structureandfunction pages 7-9).  
• Additional polymorphism R65L maps to the RH domain four-helix bundle implicated in Gαq binding (allen2015structureandfunction pages 6-7).  
• Four splice isoforms (α, β, γ, δ) differ in RH and C-terminal regions; only the α-isoform retains high-affinity calmodulin binding (unknownauthors2007nuclearlocalisationand pages 108-114).

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