## Phylogeny

Member of the AGC serine/threonine kinase superfamily and clustered within the PKN/PRK branch of the kinome (Manning scheme) (Hutchinson et al., 2013). The catalytic domain shares > 80 % identity with paralogs PKN2 and PKN3 and ~ 34 % identity with ROCK1/2, highlighting proximity to PKC-related clades (Unknown Authors, 2020). Orthologs are present across all vertebrate classes and in Drosophila, consistent with a conserved Rho-effector role throughout metazoan evolution (Arencibia et al., 2013).

## Reaction Catalyzed

ATP + [protein] Ser/Thr ⇌ ADP + [protein]-phospho-Ser/Thr (Arencibia et al., 2013).

## Cofactor Requirements

Mg²⁺ is required for phosphoryl-transfer activity, as for other AGC kinases (Sophocleous et al., 2021).

## Substrate Specificity

Phospho-proteomics defined a preference for basic residues (Arg/Lys) at –3/–2 and a hydrophobic residue at +1 relative to the target Ser/Thr (Sophocleous et al., 2021; Johnson et al., 2023, cited therein). Confirmed cellular substrates include vimentin, neurofilament heavy/medium/light chains, MAPT/Tau (Ser575/637/669) and histone H3 (Thr11) (Arencibia et al., 2013; Ostrovskyi et al., 2016).

## Structure

Domain organisation comprises:  
• N-terminal HR1a-c antiparallel coiled-coil repeats that bind Rho-family GTPases.  
• A C2-like lipid-regulated autoinhibitory segment.  
• A C-terminal serine/threonine kinase domain (Mukai, 2003).

Crystal structures:  
– Isolated kinase domain adopts the canonical AGC fold with an ordered activation segment (PDB 4CRS) (Arencibia et al., 2013).  
– HR1a in complex with RhoA illustrates the effector interface (PDB 4OTC) (Hutchinson et al., 2013).

Catalytic landmarks: Lys644-Glu663 salt bridge, DFG Asp711 coordinating Mg²⁺–ATP, activation-loop Thr774, and a hydrophobic-motif Ser916 that docks to the N-lobe hydrophobic groove to align the regulatory spine (Sophocleous et al., 2021). Upstream of HR1, a leucine-zipper-like ACC segment promotes oligomerisation and partner binding (Mukai, 2003).

## Regulation

• Phosphorylation: Thr774 is activated by PDK1; Ser916 and Ser533/537/562 are phosphorylated by CDK1 during mitosis to boost activity independently of Thr774 (Hutchinson et al., 2013; Unknown Authors, 2022).  
• Lipid binding: PI(4,5)P₂, PI(3,4,5)P₃, cardiolipin and unsaturated fatty acids relieve C2-domain autoinhibition (Lin & Yuan, 2024).  
• Small-GTPase interaction: RhoA, RhoB and Rac1 bind HR1 domains, triggering conformational activation and membrane recruitment (Hutchinson et al., 2013).  
• Proteolysis: Limited proteolysis or caspase-3 cleavage produces constitutively active fragments under stress (Sophocleous et al., 2021).

## Function

Expression is ubiquitous, with high levels in spleen, thymus, testes and neurons; over-expressed in prostate and ovarian carcinomas (Mukai, 2003; Unknown Authors, 2022).  
Key roles include:  
– Cytoskeletal regulation via phosphorylation of vimentin and neurofilaments, affecting filament assembly and cell migration (Arencibia et al., 2013).  
– Control of microtubule dynamics through Tau phosphorylation, reducing microtubule binding (Arencibia et al., 2013).  
– Transcriptional co-activation: histone H3 Thr11 phosphorylation promotes androgen-receptor-dependent gene expression (Ostrovskyi et al., 2016).  
– Signal transduction downstream of ADRA1B to activate MAPK14/p38 (Arencibia et al., 2013).  
– Neuronal survival under hypoxic stress by enhancing neurite stability and anti-apoptotic signalling (Thauerer et al., 2014).

## Inhibitors

Lestaurtinib (low-µM IC₅₀), Ro318220 (nanomolar), Tofacitinib (Kd ≈ 96 nM; EC₅₀ ≈ 122 nM) and derivative compound 25 (three-fold selectivity improvement) inhibit the kinase domain (Arencibia et al., 2013; Ostrovskyi et al., 2016).

## Other Comments

Enhanced activity drives prostate cancer migration/metastasis and contributes to ovarian cancer aggressiveness (Sophocleous et al., 2021; Unknown Authors, 2022). Dysregulated phosphorylation of neurofilaments and Tau links the kinase to ALS and Alzheimer’s disease pathways (Thauerer et al., 2014). Clinical interest in the PKN axis is illustrated by the siRNA nanotherapeutic Atu027 targeting PKN3; similar strategies are being explored for this paralog (Asquith et al., 2022).

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