## Phylogeny

RIOK2 is an atypical protein kinase-like enzyme of the RIO family that branches outside the canonical eukaryotic protein kinase clades (Manning et al., 2002). A single-copy ortholog is conserved from fungi (S. cerevisiae Rio2; S. pombe Rio2) through metazoa (D. melanogaster Rio2, C. elegans RIOK-2, G. gallus Riok2, D. rerio Riok2, M. musculus Riok2, H. sapiens RIOK2) (LaRonde-LeBlanc & Wlodawer, 2005). Homologues are also present in parasitic and free-living nematodes (Breugelmans et al., 2014) and are retained in the minimal kinome of the early-branching eukaryote Giardia lamblia (Manning et al., 2011), underscoring broad evolutionary conservation and essentiality.

## Reaction Catalyzed

Protein-Ser/Thr + ATP ⇌ Protein-O-phospho-Ser/Thr + ADP + H⁺ (Asquith et al., 2019).

## Cofactor Requirements

Catalytic activity requires divalent cations; Mg²⁺ is preferred and Mn²⁺ can substitute (Cerezo et al., 2021).

## Substrate Specificity

High-throughput peptide profiling classifies RIOK2 as having broad or weak sequence selectivity (Johnson et al., 2023). Confirmed substrates include its own autophosphorylation site Ser128 (LaRonde-LeBlanc & Wlodawer, 2005) and the ribosome-biogenesis factor DIM1 in vitro (Cerezo et al., 2021).

## Structure

The human protein contains an N-terminal winged-helix domain (residues 10–75), bipartite kinase lobes (76–190, 196–291) linked by a short hinge (191–195), and an extended acidic C-terminal tail (292–552) harboring regulatory Ser483 (Maurice et al., 2019; Cerezo et al., 2021).  
A 2.35 Å crystal structure of the kinase domain bound to an inhibitor (PDB 6HK6) shows classical N- and C-lobes with the winged-helix packed against the N-lobe (Wang et al., 2019). Key catalytic elements include the Lys123-rich glycine loop, an HGD triad with catalytic Asp228, and Asp246 that coordinates Mg²⁺ (Maurice et al., 2019). The hydrophobic spine and αC helix are conserved. Head-to-head homodimerisation through F- and C-helices locks the ATP pocket in an apo conformation (Maurice et al., 2019). A C-terminal αI helix present in fungal Rio2 is disordered in the human enzyme, and an extended β3-αC loop may contact rRNA (Wang et al., 2019).

## Regulation

• Ser483 is phosphorylated by RSK1/RSK2, promoting release from cytoplasmic pre-40S particles, nuclear re-import, 18S-E processing, global translation and cell proliferation (Cerezo et al., 2021).  
• Ser335 is phosphorylated by PLK1 and is required for timely metaphase-to-anaphase transition (Cerezo et al., 2021; Wang et al., 2019).  
• Ser128 undergoes intrinsic autophosphorylation (LaRonde-LeBlanc & Wlodawer, 2005).  
• Homodimerisation occludes the ATP site; dissociation is necessary for catalytic competence, providing an allosteric regulatory layer (Maurice et al., 2019).

## Function

RIOK2 is essential for late cytoplasmic maturation of 40S ribosomal subunits, releasing factors NOB1, PNO1, LTV1, ENP1 and DIM2 and enabling conversion of 18S-E pre-rRNA to mature 18S rRNA (Cerezo et al., 2021). Ser483-dependent shuttling allows export of pre-40S particles and nuclear recycling (Cerezo et al., 2021). PLK1-mediated Ser335 phosphorylation couples RIOK2 to metaphase–anaphase progression (Cerezo et al., 2021). Over-expression elevates AKT Ser473 phosphorylation and forms a feed-forward loop with active AKT (Asquith et al., 2019). Expression is ubiquitous but enriched in proliferative tissues and is markedly up-regulated in several cancers, including non-small-cell lung carcinoma and glioblastoma (Cerezo et al., 2021; Liu et al., 2016; Read et al., 2013).

## Inhibitors

The 2-aminopyridine amide “compound 9” binds the ATP pocket with Kd ≈ 160 nM, forming hinge hydrogen bonds to Ile191 and hydrophobic contacts with Met188, Ile109 and Ile245 (Wang et al., 2019).

## Other Comments

Pan-cancer multi-omics analyses reveal recurrent RIOK2 overexpression, altered phosphorylation and correlations with immune infiltration, highlighting its therapeutic potential (Li et al., 2022). Elevated RIOK2 levels correlate with poor prognosis in non-small-cell lung cancer and other malignancies (Liu et al., 2016).

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