## Phylogeny

• Member of the atypical protein kinase–like (PKL) RIO family positioned outside canonical ePK clades (manning2002theproteinkinase pages 3-3).  
• Single-copy orthologs are conserved from yeast (Saccharomyces cerevisiae Rio2, Schizosaccharomyces pombe Rio2) to metazoa including Drosophila melanogaster Rio2, Caenorhabditis elegans RIOK-2, Gallus gallus Riok2, Danio rerio Riok2, Mus musculus Riok2, and Homo sapiens RIOK2 (larondeleblanc2005theriokinases pages 7-9).  
• Orthologs extend to parasitic and free-living nematodes, confirming broad metazoan conservation (breugelmans2014bioinformaticexplorationof pages 10-10).  
• Retained in the minimal kinome of Giardia lamblia, underscoring essentiality in early‐branching eukaryotes (manning2011theminimalkinome pages 5-6).

## Reaction Catalyzed

Protein-Ser/Thr + ATP ⇌ Protein-O-phospho-Ser/Thr + ADP + H⁺ (asquith2019riok2straddlingthe pages 1-1).

## Cofactor Requirements

Catalytic activity requires divalent cations; Mg²⁺ is preferred and Mn²⁺ can substitute (cerezo2021riok2phosphorylationby pages 34-39).

## Substrate Specificity

• High-throughput profiling indicates very low positional preference, classifying RIOK2 among kinases with broad or weak motif selectivity (johnson2023anatlasof pages 1-2).  
• Validated autophosphorylation occurs at Ser128 situated in a flexible loop (larondeleblanc2005theriokinases pages 7-9).  
• Demonstrated in vitro phosphorylation of the ribosome biogenesis factor DIM1 (cerezo2021riok2phosphorylationby pages 8-11).

## Structure

• Domain organisation: N-terminal winged-helix domain (residues 10–75) implicated in nucleic acid binding; bipartite kinase lobes (76–190 and 196–291) connected by a short hinge (191–195); extended C-terminal acidic tail (292–552) harbouring regulatory Ser483 (maurice2019dimerizationofhuman pages 4-7, cerezo2021riok2phosphorylationby pages 14-17).  
• Crystal structure of human kinase domain with inhibitor (PDB 6HK6, 2.35 Å) reveals typical N- and C-lobes forming the ATP pocket and an ordered winged-helix closely packed against the N-lobe (wang2019crystalstructureof pages 2-3).  
• Catalytic motifs: Lys123-Gly loop coordinates ATP; HGD catalytic triad with Asp228 as base; Asp246 in the activation segment chelates Mg²⁺ (maurice2019dimerizationofhuman pages 4-7).  
• Hydrophobic spine and αC helix are conserved; head-to-head homodimer formed through F- and C-helices occludes the ATP site, locking an apo conformation (maurice2019invitrodimerization pages 24-29).  
• C-terminal αI helix present in fungal ctRio2 (PDB 4GYI) is disordered in human RIOK2, and an extended β3-αC loop is proposed to contact rRNA within pre-40S particles (wang2019crystalstructureof pages 3-4).

## Regulation

Post-translational modifications  
• Ser483: phosphorylated by RSK1/RSK2 within an RXRXXpS motif; promotes release from cytoplasmic pre-40S particles, nuclear re-import, 18S-E processing, global translation and proliferation (cerezo2021riok2phosphorylationby pages 11-14, cerezo2021riok2phosphorylationby pages 14-17, cerezo2021riok2phosphorylationby pages 47-49).  
• Ser335: phosphorylated by PLK1; required for proper metaphase-to-anaphase timing during mitosis (cerezo2021riok2phosphorylationby pages 34-39, wang2019crystalstructureof pages 2-3).  
• Ser128: intrinsic autophosphorylation site; conserved among eukaryotes (larondeleblanc2005theriokinases pages 7-9).

Allosteric and conformational regulation  
• Homodimerisation clamps the ATP pocket; catalytic competence requires dissociation, providing an additional regulatory layer (maurice2019invitrodimerization pages 24-29).

## Function

• Ribosome biogenesis: essential for late cytoplasmic maturation of 40S subunits by releasing NOB1, PNO1, LTV1, ENP1 and DIM2 and enabling conversion of 18S-E pre-rRNA to mature 18S rRNA (cerezo2021riok2phosphorylationby pages 47-49, cerezo2021riok2phosphorylationby pages 11-14).  
• Nuclear-cytoplasmic shuttling: escorts pre-40S export and recycles to the nucleus following Ser483 phosphorylation (cerezo2021riok2phosphorylationby pages 14-17).  
• Cell-cycle control: PLK1-dependent Ser335 phosphorylation modulates metaphase–anaphase progression (cerezo2021riok2phosphorylationby pages 34-39).  
• Signalling interactions: overexpression elevates AKT Ser473 phosphorylation and is stabilised by active AKT, forming a feed-forward loop (asquith2019riok2straddlingthe pages 1-1).  
• Expression: ubiquitous with enrichment in proliferative tissues; markedly overexpressed across numerous cancers including non-small-cell lung carcinoma and glioblastoma (cerezo2021riok2phosphorylationby pages 34-39, liu2016highexpressionof pages 7-8, read2013akinomewidernai pages 4-6).

## Inhibitors

• 2-Aminopyridine amide “compound 9” binds in the ATP pocket (Kd ≈ 160 nM); crystal structure defines hydrogen bonding to hinge residue Ile191 and hydrophobic contacts with Met188, Ile109 and Ile245 (wang2019crystalstructureof pages 1-2, wang2019crystalstructureof pages 3-4).

## Other Comments

• Pan-cancer multi-omics analyses reveal recurrent overexpression, phosphorylation changes and correlations with immune infiltration, highlighting therapeutic potential (li2022pancanceranalysesreveal pages 12-16, li2022pancanceranalysesreveal pages 2-3).  
• High RIOK2 levels correlate with poor prognosis in non-small-cell lung cancer and other malignancies (liu2016highexpressionof pages 7-8).

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