## Phylogeny

Human RIOK3 is an atypical Ser/Thr kinase of the RIO family (Rio3 sub-family) that sits outside the conventional eukaryotic protein kinase (ePK) groups in the Manning kinome tree (Manning et al., 2002). Rio3 orthologues are confined to multicellular animals (e.g., Drosophila, Caenorhabditis, Strongyloides, Xenopus, Danio rerio, Mus musculus); unicellular eukaryotes contain only Rio1/Rio2 paralogues (Baumas et al., 2012; Yuan et al., 2014). A distinctive N-terminal α-helical extension defines the Rio3 lineage and separates it from Rio1/2 within the RIO superfamily (LaRonde-LeBlanc & Wlodawer, 2005).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + H⁺ + O-phospho-L-seryl/threonyl-[protein] (LaRonde-LeBlanc & Wlodawer, 2005).

## Cofactor Requirements

Catalysis is strictly divalent-cation dependent: Mg²⁺ is essential, while Mn²⁺ can substitute (Baumas et al., 2012; Ferreira-Cerca et al., 2014).

## Substrate Specificity

No consensus phosphorylation motif or positional scoring matrix has been defined, and large-scale peptide profiling failed to detect clear preferences for RIOK3 (Johnson et al., 2023).

## Structure

• N-terminal Rio3-specific α-helical domain (~200 aa).  
• Central RIO kinase core: reduced ePK fold (β-sheet N-lobe, short αC, minimal C-lobe with three α-helices and β-hairpin) lacking a classical activation loop (LaRonde-LeBlanc & Wlodawer, 2005).  
• C-terminal lysine-rich tail corresponding to truncated ePK sub-domains X–XI.  
Conserved catalytic signatures include an STGKES P-loop, catalytic Asp/Asn residues, and a metal-binding Asp that forms a transient phosphoaspartate (Ferreira-Cerca et al., 2014). Two Mg²⁺ ions coordinate ATP in a P-loop ATPase-like active site.  
A flexible β3-αC loop is sub-family specific and presumed to modulate substrate or particle binding (LaRonde-LeBlanc & Wlodawer, 2005). No crystal structure is available; homology models based on Rio1/2 reveal “active” and “inactive” conformations (Ferreira-Cerca et al., 2014).

## Regulation

Autophosphorylation of the catalytic Asp generates a phosphoaspartate intermediate that couples ATP hydrolysis to conformational cycling (Ferreira-Cerca et al., 2014). Metal-ion occupancy and hinge/β-hairpin movements toggle active and inactive states, as inferred from other RIO kinases (Ferreira-Cerca et al., 2014). Additional post-translational modifications remain undefined, but RIOK3 promotes TRIM40-mediated K27/K48 ubiquitination of RIG-I and MDA5, thereby modulating antiviral signalling (Shen et al., 2021).

## Function

Expression: highly expressed in erythroid, lymphoid and myeloid lineages; associates with cytoplasmic pre-40S ribosomal particles (Baumas et al., 2012; Feng et al., 2014).  
Ribosome biogenesis: participates in late cytoplasmic maturation of pre-40S subunits and 21S → 18S rRNA processing (Baumas et al., 2012).  
Innate immunity: acts downstream of TBK1 and upstream of IRF3 to facilitate IRF3 phosphorylation and type I IFN-β transcription (Feng et al., 2014). Phosphorylates MDA5 (Ser828) to limit filament assembly; promotes TRIM40-dependent degradation of RIG-I/MDA5 as negative feedback (Shen et al., 2021).  
Interacting partners: TBK1, IRF3, IFIH1/MDA5, RIG-I, TRIM40 and multiple pre-40S assembly factors (Baumas et al., 2012; Feng et al., 2014; Shen et al., 2021).  
Additional signalling links include inhibition of CASP10 isoform-7-driven NF-κB and modulation of Hedgehog pathway components (information contained in the provided context).

## Other Comments

Genetic loss-of-function enhances resistance to RNA virus infection in mice, underscoring therapeutic interest in antiviral modulation (Shen et al., 2021). No selective small-molecule inhibitors have been reported in the material provided.

## 9. References

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