## Phylogeny

Human RIOK3 is an atypical Ser/Thr kinase belonging to the RIO family, Rio3 sub-family, positioned outside the conventional ePK groups in the Manning kinome tree (mammalian “other atypical kinases”) (manning2002theproteinkinase pages 3-3).  
Orthologs are detected only in multicellular eukaryotes and include Drosophila RIOK3, Caenorhabditis and Strongyloides RIOK3, Xenopus, Danio rerio and Mus musculus RIOK3; unicellular models possess only Rio1/Rio2 paralogs (baumas2012humanriok3is pages 1-2, yuan2014exploringfeaturesand pages 1-2).  
Within the kinase superfamily RIOK3 clusters with Rio1 and Rio2 but bears a unique N-terminal helical extension that defines the Rio3 lineage (larondeleblanc2005theriokinases pages 4-6).

## Reaction Catalyzed

Protein-L-Ser/Thr + ATP → Protein-L-Ser/Thr-phosphate + ADP + H⁺ (larondeleblanc2005theriokinases pages 4-6).

## Cofactor Requirements

Catalysis is strictly divalent-cation dependent; Mg²⁺ is essential and Mn²⁺ can substitute (baumas2012humanriok3is pages 13-13, ferreiracerca2014dominantrio1kinaseatpase pages 3-4).

## Substrate Specificity

No consensus phosphorylation motif or positional scoring matrix for RIOK3 is currently available; the comprehensive Ser/Thr kinome atlas did not report substrate preferences for this kinase (johnson2023anatlasof pages 3-4, johnson2023anatlasof pages 4-4).

## Structure

Domain organization  
• N-terminal Rio3-specific α-helical domain (~200 aa) required for functions unique to metazoan RIOKs (larondeleblanc2005theriokinases pages 6-7).  
• Central RIO kinase domain: reduced ePK fold with N-lobe five-stranded β-sheet, C-helix, hinge, and C-lobe three α-helices plus β-hairpin; lacks classical activation loop (larondeleblanc2005theriokinases pages 6-7).  
• C-terminal lysine-rich segment corresponding to truncated ePK subdomains X–XI (larondeleblanc2005theriokinases pages 9-10).

Catalytic motifs  
– P-loop (STGKES), catalytic loop Asp/Asn, and metal-binding loop Asp are invariant; Asp in m-loop forms transient phosphoaspartate intermediate (ferreiracerca2014dominantrio1kinaseatpase pages 4-5).  
– Active site adopts P-loop ATPase geometry; two Mg²⁺ ions stabilize α/β and β/γ phosphates, as shown for Rio1/2 homologs, and these features are conserved in RIOK3 (ferreiracerca2014dominantrio1kinaseatpase pages 5-6).

Unique elements  
– Flexible loop between β3 and αC is sub-family specific and often disordered, proposed to modulate substrate or particle binding (larondeleblanc2005theriokinases pages 7-9).  
No experimentally determined RIOK3 crystal structure is yet published; homology models rely on Rio1/Rio2 templates exhibiting “active” and “inactive” conformational states (ferreiracerca2014dominantrio1kinaseatpase pages 4-5).

## Regulation

Post-translational modifications  
– Autophosphorylation on the catalytic Asp generates a phosphoaspartate intermediate, coupling ATP hydrolysis to conformational cycling (ferreiracerca2014dominantrio1kinaseatpase pages 5-6).  
– Additional PTMs on RIOK3 itself are not yet defined; however, RIOK3 regulates TRIM40-mediated K27/K48 ubiquitination of RIG-I/MDA5, thereby influencing downstream signaling (shen2021riok3inhibitsthe pages 1-3).

Conformational/allosteric control  
– Metal ion occupancy and hinge/β-hairpin movements toggle active vs. inactive states in RIO family structures, a mechanism inferred for RIOK3 (ferreiracerca2014dominantrio1kinaseatpase pages 4-5).

## Function

Expression and cellular context  
Highly expressed in erythroid, lymphoid and myeloid lineages; associates with cytoplasmic pre-40S ribosomal particles (feng2014riok3isan pages 9-10, baumas2012humanriok3is pages 1-2).

Innate immunity  
• Acts downstream of TBK1 and upstream of IRF3; bridges TBK1–IRF3 interaction enabling IRF3 phosphorylation and type I IFN-β transcription (feng2014riok3isan pages 6-7).  
• Phosphorylates IFIH1 (MDA5) at Ser828 to dampen filament assembly and signaling (Protein information section; primary literature not in supplied context).  
• Facilitates TRIM40-dependent degradation of RIG-I/MDA5, serving as negative feedback to limit excessive IFN responses (shen2021riok3inhibitsthe pages 1-3).

Ribosome biogenesis  
Participates in late cytoplasmic maturation of pre-40S ribosomal subunits, contributing to 21S→18S rRNA processing (baumas2012humanriok3is pages 1-2).

Signaling cross-talk  
Can inhibit CASP10 isoform-7-driven NF-κB activation and has been linked to hedgehog pathway modulation (Protein information section; underlying references not provided in context).

Interacting partners  
TBK1, IRF3, IFIH1, RIG-I, MDA5, TRIM40, and multiple pre-40S assembly factors (feng2014riok3isan pages 6-7, shen2021riok3inhibitsthe pages 1-3, baumas2012humanriok3is pages 1-2).

## Inhibitors

No selective small-molecule inhibitors of RIOK3 have been reported in the literature provided.

## Other Comments

Loss-of-function enhances resistance to RNA virus infection in mice, highlighting potential therapeutic interest in antiviral modulation (shen2021riok3inhibitsthe pages 1-3).

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