1. Phylogeny  
   RIOK3, also known as RIO kinase 3 or sudD homolog, is a member of the RIO kinase family of atypical serine/threonine protein kinases. Within this family, RIOK1 and RIOK2 are conserved from archaea through unicellular and multicellular eukaryotes, whereas RIOK3 is found exclusively in multicellular eukaryotic organisms, indicating an evolutionary divergence in which RIOK3 adopted specialized functions in higher organisms (baumas2012humanriok3is pages 1-2, larondeleblanc2005theriokinases pages 4-6). Comparative analyses based on kinase domain architectures and genomic studies have placed the RIO kinases into a distinct evolutionary branch of the eukaryotic kinome, one that diverges from canonical eukaryotic protein kinases in both sequence and structure (miranda‐saavedra2007classificationandfunctional pages 3-4, rangwala2022kinasesondouble pages 8-10). This sub‐family’s phylogenetic context is further supported by studies showing that the ancestral RIO kinase existed in the common ancestor of eukaryotes, with RIOK3 emerging later and exclusively in multicellular lineages, suggesting its involvement in regulatory processes linked to the complexity of tissue- and cell-type differentiation (pang2022roleofprotein pages 16-17, OpenTargets Search: -RIOK3).
2. Reaction Catalyzed  
   RIOK3 catalyzes the phosphorylation of serine/threonine residues on substrate proteins using ATP as a phosphate donor. The canonical reaction mechanism is as follows: ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺ (OpenTargets Search: -RIOK3, larondeleblanc2005theriokinases pages 1-2). In its functional roles, RIOK3 phosphorylates specific substrates such as the cytosolic innate immune receptor IFIH1 on Ser-828, a modification that interferes with IFIH1 filament assembly on long double-stranded RNA, and thereby modulates the downstream type I interferon signaling pathway (Information, feng2014riok3isan pages 7-9). Furthermore, RIOK3 is implicated in ribosome biogenesis, where its kinase activity is thought to contribute to the processing of 21S pre-rRNA into mature 18S rRNA, although the direct substrates in this context remain less clearly defined (baumas2012humanriok3is pages 6-8).
3. Cofactor Requirements  
   The kinase activity of RIOK3 is dependent on divalent metal ions. As with many serine/threonine kinases, RIOK3 requires Mg²⁺ as a cofactor to coordinate ATP binding within its catalytic site, enabling the phosphate transfer reaction (larondeleblanc2005theriokinases pages 7-9, ferreiracerca2014dominantrio1kinaseatpase pages 1-2). In some in vitro experiments, Mn²⁺ may also substitute to support enzymatic activity, but physiological conditions predominantly favor Mg²⁺ (larondeleblanc2005theriokinases pages 4-6).
4. Substrate Specificity  
   RIOK3 exhibits substrate specificity characteristic of serine/threonine-protein kinases. One clearly documented target is IFIH1, which is phosphorylated on Ser-828; this modification reduces the assembly of IFIH1 filaments on long dsRNA and attenuates its signaling, thereby serving a regulatory function in the type I interferon response (Information, feng2014riok3isan pages 7-9). While a strict consensus sequence for RIOK3 has not been firmly established, its activity towards substrates involved in both innate immunity and ribosomal maturation suggests that other substrates may be recognized by structural determinants within their three-dimensional conformation or by specific sequence motifs present in components of the pre-40S ribosomal particle (baumas2012humanriok3is pages 6-8, ferreiracerca2014dominantrio1kinaseatpase pages 1-2).
5. Structure  
   RIOK3 is defined by an atypical kinase fold that is distinct from classical eukaryotic protein kinases. Its central catalytic unit is the conserved RIO domain, which comprises an N-terminal lobe with a beta-sheet structure and a C-terminal lobe dominated by alpha-helices. This domain harbors the ATP-binding site, a flexible loop region, a hinge segment, and a metal-binding loop, all of which are critical for its catalytic activity (baumas2012humanriok3is pages 1-2, baumas2012humanriok3is pages 2-4). Unlike conventional kinases, RIOK3 lacks a typical activation loop and substrate-recognition loops, which results in a more streamlined domain architecture that nonetheless maintains enzymatic function (larondeleblanc2005theriokinases pages 4-6, ferreiracerca2014dominantrio1kinaseatpase pages 1-2). Additionally, RIOK3 contains a unique N-terminal region characterized by a helical structure that is highly conserved among multicellular eukaryotes but does not show significant homology to any other known protein domains (baumas2012humanriok3is pages 1-2, larondeleblanc2005theriokinases pages 9-10). Structural analyses and crystallographic studies of related RIO kinases suggest that conserved catalytic residues, including those forming the phosphate-binding P-loop—which in RIOK3 is noted to include two conserved serines—are essential for ligand (ATP) coordination and subsequent catalytic function (larondeleblanc2005theriokinases pages 6-7, widmann2012thekinaseactivity pages 1-2). The overall three-dimensional organization inferred from these comparative models indicates that RIOK3 retains the characteristic bilobal organization seen in other kinases, with dynamic flexible loops that may contribute to its adaptability in substrate recognition and regulatory control (larondeleblanc2005theriokinases pages 7-9, feng2014riok3isan pages 4-5).
6. Regulation  
   Regulatory mechanisms of RIOK3 include post-translational modifications and protein–protein interactions that influence its activity. Autophosphorylation events have been reported in related RIO kinases, and there is evidence that RIOK3 undergoes phosphorylation that modulates its catalytic activity and possibly its interaction with other proteins (larondeleblanc2005theriokinases pages 7-9, baumas2012humanriok3is pages 2-4). Within innate immune signaling, RIOK3 functions as an adaptor protein that facilitates the association between TBK1 and IRF3; this interaction is critical for the phosphorylation and activation of IRF3, leading to the production of type I interferons (feng2014riok3isan pages 6-7, feng2014riok3isan pages 7-9). In addition, RIOK3 has been shown to interfere with CASP10 isoform 7-mediated activation of the NF-kappaB signaling pathway, a function that underscores its ability to modulate multiple signaling cascades via differential phosphorylation events (Information, singleton2015hypoxicregulationof pages 3-4). In the context of ribosome biogenesis, regulatory phosphorylation of RIOK3 is implicated in the progression of 21S pre-rRNA processing into mature 18S rRNA, although the specific phosphorylation sites and mechanisms remain to be fully elucidated (baumas2012humanriok3is pages 6-8, widmann2012thekinaseactivity pages 2-3).
7. Function  
   RIOK3 plays dual roles in distinct cellular processes. In the innate immune system, it acts as an essential adaptor protein that bridges TBK1 and IRF3, thereby facilitating IRF3 phosphorylation and the subsequent induction of type I interferon expression in response to viral nucleic acids (feng2014riok3isan pages 6-7, feng2014riok3isan pages 7-9). This function is critical for mounting a rapid antiviral response and modulating IFN-dependent immune signaling (Information, feng2014riok3isan pages 2-4). Furthermore, RIOK3 phosphorylates IFIH1 on Ser-828, which interferes with the formation of IFIH1 filaments on long double-stranded RNA and attenuates IFIH1-mediated signaling, thus contributing to the fine regulation of the antiviral response (Information). Beyond its immunomodulatory role, RIOK3 also participates in the biogenesis of ribosomes. It is associated with cytoplasmic pre-40S ribosomal particles and is involved in the processing of the 21S pre-rRNA intermediate to generate mature 18S rRNA—a step that is fundamental for the production of functional 40S ribosomal subunits (baumas2012humanriok3is pages 1-2, baumas2012humanriok3is pages 6-8). Expression analyses have shown that RIOK3 is expressed at relatively low levels across many tissues, with higher expression observed in lymphoid and myeloid cells, which are key components of the immune system (feng2014riok3isan pages 4-5). Such distribution patterns reflect its dual roles in supporting both protein synthesis via ribosome maturation and innate immune signaling.
8. Other Comments  
   RIOK3 has garnered attention as a potential therapeutic target in both infectious diseases and oncology. Its central role in the activation of the type I interferon pathway—specifically through its function in bridging TBK1 and IRF3—positions it as an attractive candidate for modulating antiviral responses (feng2014riok3isan pages 7-9, sun2025theeconomicalrole pages 1-2). In addition, studies in hypoxic breast cancer models have demonstrated that RIOK3 expression is upregulated under low oxygen conditions, correlating with enhanced cell migration and invasion, thereby implicating it in cancer metastasis (singleton2015hypoxicregulationof pages 3-4, singleton2015hypoxicregulationof pages 4-6). Despite these connections, selective inhibitors for RIOK3 have not been developed for clinical use, and further research is needed to define inhibitor specificity and efficacy (rangwala2022kinasesondouble pages 10-11, pang2022roleofprotein pages 26-27). In genetic association studies, variations in the RIOK3 gene have been linked to blood-related traits, particularly red blood cell parameters, suggesting that RIOK3 might have broader physiological roles beyond immune regulation and ribosome biogenesis (OpenTargets Search: -RIOK3). Collectively, these findings underscore the bifunctional nature of RIOK3 as it integrates roles in cytoplasmic ribosome maturation and the regulation of innate immunity.
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