1. Phylogeny  
   TP53RK, also known as PRPK, belongs to the atypical serine/threonine kinase family and is the human ortholog of the yeast Bud32 protein, a widely conserved kinase found from archaea to eukaryotes (braun2017mutationsinkeopscomplex pages 2-4). Its membership in the KEOPS complex underscores its evolutionary conservation, with orthologs identified in yeast, plants, and mammals, which indicates that the basic structure and function of the protein have been maintained since early eukaryotic evolution (costessi2012thehumanekckeops pages 1-2). Based on kinase phylogenetics, TP53RK is classified within the BUD32 family, which comprises small atypical kinases that share conserved structural features and catalytic motifs with other kinases described in the seminal studies by Manning et al. (rangwala2022kinasesondouble pages 2-4). The evolutionary relationship of TP53RK to other kinases is further supported by its integration into the KEOPS complex—a multi‐protein assembly with conserved functions in tRNA modification and genome stability—which represents a core set of proteins present in all eukaryotic lineages (braun2017mutationsinkeopscomplex pages 2-4, costessi2012thehumanekckeops pages 1-2).
2. Reaction Catalyzed  
   Within the KEOPS complex, TP53RK plays a dual catalytic role. It participates in the biosynthesis of the threonylcarbamoyl adenosine (t^6A) modification by supporting the transfer of the threonylcarbamoyl moiety from threonylcarbamoyl-AMP (TC-AMP) to the N^6 position of adenosine at position 37 in target tRNAs that decode ANN codons, thereby ensuring translational fidelity (costessi2012thehumanekckeops pages 10-11, su2022conservationanddiversification pages 9-11). In addition to this role, TP53RK exhibits ATPase activity in the context of the KEOPS complex, which is thought to be critical for driving conformational changes required for efficient tRNA substrate binding and release (su2022conservationanddiversification pages 22-22). Moreover, TP53RK has been reported to phosphorylate substrates such as the tumor suppressor p53 on serine 15, catalyzing the transfer of a phosphate group from ATP to p53, a reaction that produces ADP and p53-phosphate (li2021crystalstructureof pages 9-10, goswami2019identificationoftp53rkbinding pages 1-3).
3. Cofactor Requirements  
   The catalytic activity of TP53RK, like many serine/threonine kinases, is dependent upon the presence of divalent metal ions, most notably Mg²⁺, which is required for ATP binding and proper catalysis (li2021crystalstructureof pages 1-3, costessi2012thehumanekckeops pages 10-11).
4. Substrate Specificity  
   TP53RK exhibits bifunctional substrate specificity through its separate roles within the KEOPS complex and as an independent kinase. In the context of tRNA modification, its enzymatic activity is directed indirectly toward the adenosine residue at position 37 of tRNAs that decode ANN codons, a modification essential for maintaining accurate codon–anticodon interactions during translation (costessi2012thehumanekckeops pages 10-11, su2022conservationanddiversification pages 9-11). For its kinase function, TP53RK phosphorylates p53 specifically at serine 15, and this phosphorylation event is well documented using in vitro assays where p53 serves as a substrate (li2021crystalstructureof pages 9-10, goswami2019identificationoftp53rkbinding pages 1-3). Thus, while the KEOPS complex collectively targets tRNA substrates to install t^6A, the individual kinase activity of TP53RK shows substrate specificity for regulatory proteins such as p53 (li2021crystalstructureof pages 9-10, goswami2019identificationoftp53rkbinding pages 1-3).
5. Structure  
   TP53RK is characterized by a canonical bilobal kinase fold, which comprises an N-terminal lobe (N-lobe) primarily responsible for binding ATP through its glycine-rich loop, and a smaller C-terminal lobe (C-lobe) that contains key catalytic motifs including a conserved DFG motif critical for coordinating magnesium ions (li2021crystalstructureof pages 1-3, rangwala2022kinasesondouble pages 8-10). The crystal structure of the human PRPK–TPRKB complex reveals that TP53RK adopts an active conformation despite lacking a conventional activation loop typical of many canonical kinases, and it exhibits unique features such as an intrinsically disordered C-terminal tail that is implicated in regulating the catalytic ATPase activity of the KEOPS complex (li2021crystalstructureof pages 8-9, gonzalez2023generacióndelíneas pages 56-59). Additionally, structural studies indicate that TP53RK interacts with TPRKB and OSGEP through distinct interfaces that help stabilize the overall architecture of the KEOPS complex, thereby facilitating its role in t^6A modification (li2021crystalstructureof pages 9-10, su2022conservationanddiversification pages 9-11). These structural attributes, including residues involved in ATP binding and the conserved catalytic motifs, are essential for both its kinase and ATPase functions (rangwala2022kinasesondouble pages 10-11).
6. Regulation  
   The activity of TP53RK is tightly regulated by multiple mechanisms that include post-translational modifications and protein–protein interactions. Phosphorylation at serine 250 by upstream kinases such as Akt/PKB and TOPK is one critical regulatory event that enhances TP53RK’s kinase activity, thereby promoting its ability to phosphorylate p53 and possibly other substrates (costessi2012thehumanekckeops pages 10-11, rangwala2022kinasesondouble pages 10-11). In addition, TP53RK’s integration into the KEOPS complex facilitates regulation through allosteric interactions with partner subunits such as TPRKB and OSGEP; TPRKB in particular not only interacts directly with TP53RK but also stabilizes its function, while TP53RK serves to protect TPRKB from proteasomal degradation in TP53-deficient contexts (goswami2019identificationoftp53rkbinding pages 17-23, su2022conservationanddiversification pages 20-22). Furthermore, association with the oncoprotein PRAME and its recruitment to Cullin2-based ubiquitin ligase complexes suggests that TP53RK may also be subject to regulation through ubiquitin-mediated pathways that influence its stability and activity (costessi2012thehumanekckeops pages 10-11, rangwala2022kinasesondouble pages 8-10). Together, these regulatory mechanisms ensure that TP53RK activity is modulated in response to intracellular signals and the assembly state of the KEOPS complex (su2022conservationanddiversification pages 22-22).
7. Function  
   TP53RK is a critical component of the KEOPS (EKC) complex, whose primary role is to catalyze the threonylcarbamoyl (t^6A) modification at adenosine 37 in tRNAs that decode codons beginning with adenine; this modification is essential for maintaining translational fidelity and efficient protein synthesis (costessi2012thehumanekckeops pages 10-11, su2022conservationanddiversification pages 9-11). In addition to its role in tRNA modification, TP53RK functions as an atypical protein kinase that phosphorylates the tumor suppressor p53 at serine 15, a modification that is associated with the activation of p53 in response to cellular stress and DNA damage (li2021crystalstructureof pages 9-10, goswami2019identificationoftp53rkbinding pages 1-3). TP53RK is ubiquitously expressed across human tissues as a core subunit of the KEOPS complex and is implicated in processes beyond translation regulation, including telomere maintenance and transcriptional control (braun2017mutationsinkeopscomplex pages 2-4, costessi2012thehumanekckeops pages 1-2). Functional studies in cancer models, particularly colorectal cancer, have demonstrated that perturbation of TP53RK expression can alter the phosphorylation status of key proteins involved in the initiation of DNA replication, such as CDC7 kinase and the MCM helicase complex, thereby affecting cell proliferation (goswami2019identificationoftp53rkbinding pages 17-23, costessi2013thehumanoncoprotein pages 163-166). Furthermore, mutations in TP53RK and other KEOPS complex subunits have been linked to severe developmental disorders such as Galloway–Mowat syndrome, characterized by nephrotic syndrome and microcephaly, which underscores its essential role in genome stability and cellular homeostasis (braun2017mutationsinkeopscomplex pages 2-4, su2022conservationanddiversification pages 15-16).
8. Other Comments  
   TP53RK is a promising target for therapeutic intervention because of its dual role in tRNA modification and p53 regulation; deregulation of its kinase activity has been implicated in tumorigenic processes in multiple cancer types, including multiple myeloma, colon cancer, and skin cancer (li2021crystalstructureof pages 9-10, goswami2019identificationoftp53rkbinding pages 17-23). Small molecule inhibitors and other therapeutic compounds targeting TP53RK’s kinase activity have been investigated, with recent studies describing prpk-directed phthalimides that aim to modulate its enzymatic function, although further validation in clinical settings remains to be established (seo2021developmentofprpk pages 7-9). In addition, the spectrum of disease-associated mutations in TP53RK—such as missense changes that impair ATP binding or disrupt interaction interfaces with TPRKB—highlights the protein’s critical role in the KEOPS complex and its potential involvement in developmental disorders like Galloway–Mowat syndrome (braun2017mutationsinkeopscomplex pages 2-4, su2022conservationanddiversification pages 15-16). The interplay between TP53RK and other KEOPS subunits is also emerging as an important factor in cellular responses to TP53 deficiency, where stabilization of TPRKB by TP53RK supports cell proliferation in TP53-mutant cancers (goswami2019identificationoftp53rkbinding pages 17-23). Furthermore, given its central role in tRNA modification, any dysregulation of TP53RK is likely to have widespread effects on global translation and cellular proteostasis, lending additional clinical significance to this protein as both a biomarker and a potential drug target (vaz2019enrichmentofatp pages 10-11, zhou2020molecularbasisfor pages 11-11).
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