1. Phylogeny  
   RSK4, officially known as ribosomal protein S6 kinase alpha-6 (gene RPS6KA6) and also referred to as pp90RSK4 or 90 kDa ribosomal protein S6 kinase 6, is a member of the p90 ribosomal S6 kinase (RSK) family of serine/threonine kinases. Within the human kinome, RSK4 is grouped in the AGC kinase family, a set of enzymes that share conserved catalytic core structures and common regulatory features. Phylogenetic analysis of the RSK family indicates that the four isoforms—RSK1, RSK2, RSK3, and RSK4—arose from an ancestral gene duplication event; in particular, RSK4 is noted to be more closely related to RSK2 than to RSK1 or RSK3. Orthologs of RPS6KA6 have been identified across diverse mammalian species, underlining its evolutionary conservation and suggesting a preserved role in cellular signaling processes. The evolutionary trajectory that led to the diversification of the RSK isoforms is reflective of the fundamental need for finely tuned regulation of cellular growth and differentiation across eukaryotes. Data from studies of related RSK family members, including those published in peer‐reviewed journals, establish that the structural and regulatory elements observed in RSK1–3 are retained in RSK4, albeit with distinctive modifications that confer its constitutive, growth factor–independent activity (wright2023therapeutictargetingof pages 1-3, poomakkoth2016p90ribosomals6 pages 2-4).
2. Reaction Catalyzed  
   RSK4 catalyzes the phosphoryl transfer reaction in which a phosphate group is transferred from ATP to a serine or threonine residue on a target substrate protein. The overall chemical reaction can be represented as:  
     ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺  
   This reaction, characteristic of serine/threonine kinases, underlies the enzyme’s role in modulating the function of various downstream substrates involved in processes such as transcription, translation, and cell cycle regulation (clark2005theserinethreonineprotein pages 1-2, smith2005identificationofthe pages 1-1).
3. Cofactor Requirements  
   The catalytic activity of RSK4 depends on the presence of divalent metal ions, with magnesium (Mg²⁺) serving as an essential cofactor. Mg²⁺ ions coordinate with ATP in the active site, thereby stabilizing the transition state during the phosphoryl transfer reaction and ensuring the proper alignment of substrate and catalytic residues. This cofactor requirement is a hallmark of serine/threonine kinases and is critical for the efficient catalytic conversion of ATP to ADP while phosphorylating target proteins (utepbergenov2016bacterialexpressionpurification pages 1-2).
4. Substrate Specificity  
   RSK4 exhibits substrate specificity that appears to be conserved among the RSK family members. In general, RSK kinases preferentially phosphorylate serine or threonine residues located within a consensus sequence that typically conforms to an Arg–X–Arg–X–X–Ser/Thr motif. The presence of basic residues in specific positions relative to the phosphorylatable amino acid facilitates substrate recognition and binding by the kinase domain. Although comprehensive substrate mapping for RSK4 remains limited relative to RSK1–3, studies of other p90 RSK isoforms have identified several substrates, including transcriptional regulators and components of the translation apparatus, whose phosphorylation is mediated by a similar recognition motif. For example, phosphorylation of Y-box binding protein 1 (YB-1) at Ser102 by RSK family kinases has been reported, and this event is consistent with the canonical substrate preference observed in the family (wright2023therapeutictargetingof pages 3-4, clark2005theserinethreonineprotein pages 1-2, aronchik2014novelpotentand pages 1-2).
5. Structure  
   RSK4 is organized as a single polypeptide that contains two distinct catalytic domains arranged in tandem. The N-terminal kinase domain (NTKD) is responsible for the phosphorylation of downstream targets and follows the classical bilobal kinase structure, comprising a smaller N-terminal lobe largely formed by β-sheets and a larger C-terminal lobe composed mainly of α-helices. Key catalytic elements within the NTKD include the activation loop, the conserved DFG motif, and a hydrophobic spine that collectively contribute to substrate binding and phosphoryl transfer. In contrast, the C-terminal kinase domain (CTKD) is involved in intramolecular regulatory processes. The CTKD shares structural homology with calcium/calmodulin-dependent kinases and plays a crucial role in autophosphorylation events that ultimately enhance the activity of the NTKD. RSK4 also contains a regulatory linker region that harbors both a hydrophobic motif (HM) and a turn motif (TM); these regulatory elements are critical for the conformational transitions necessary for full enzymatic activation. Notably, RSK4 is characterized by a non-canonical phosphate-binding pocket in its NTKD, which contributes to its unique, growth factor–independent, constitutive activity. Although complete crystallographic structures for full-length RSK4 are not currently available, computational models based on homology with other RSK isoforms and AlphaFold predictions corroborate the presence of these conserved domains and regulatory motifs (wright2023therapeutictargetingof pages 14-15, kurinov2009structuraldiversityof pages 1-2).
6. Regulation  
   RSK4 is regulated primarily through a cascade of phosphorylation events that differ in significant respects from those regulating other RSK isoforms. Activation of most RSKs typically requires phosphorylation by upstream kinases such as ERK1/2 followed by further phosphorylation events mediated by 3-phosphoinositide-dependent kinase 1 (PDK1). In contrast, RSK4 exhibits constitutive activity and displays growth factor–independent kinase function, owing in part to its unique structural configuration. Phosphorylation of the C-terminal kinase domain by ERK1/2 initiates intramolecular autophosphorylation within the regulatory linker region, including within the hydrophobic and turn motifs, thereby modulating the activity of the NTKD. Notably, RSK4’s NTKD is phosphorylated independently of PDK1, a feature that distinguishes it from RSK1–3 and contributes to its persistent basal activity. In addition to these phosphorylation events, autoinhibitory phosphorylation within certain regulatory regions of RSK4 has been observed; such modifications are thought to reduce the affinity for upstream kinases, thereby serving as a built-in negative feedback mechanism. The overall regulation of RSK4, therefore, is defined by its autonomous phosphorylation cascade, a divergent mechanism that supports its role in p53/TP53-dependent growth arrest without reliance on extrinsic growth factor signals (wright2023therapeutictargetingof pages 14-15).
7. Function  
   RSK4 functions as a serine/threonine-protein kinase that is constitutively active under basal conditions, a trait that distinguishes it from its RSK1–3 counterparts. Its catalytic activity enables RSK4 to phosphorylate a range of substrates that are involved in critical cellular processes, including the regulation of cell cycle progression and growth arrest signaling pathways. In particular, RSK4 is implicated in p53/TP53-dependent cell growth arrest, an activity that positions it as a modulator of cell proliferation and an inhibitor during embryogenesis. The kinase operates as an effector within the MAPK/ERK signaling cascade, transmitting signals through phosphorylation events that can alter protein function, subcellular localization, and protein–protein interactions. Although the full spectrum of RSK4 physiological substrates is not yet completely delineated, its established role in phosphorylating substrates consistent with a consensus motif supports its involvement in regulating gene expression, protein synthesis, and multiple facets of cellular homeostasis. Moreover, the constitutive kinase activity of RSK4 suggests that it may serve as a tonic regulator, exerting an inhibitory influence on cell growth pathways even in the absence of external mitogenic stimuli (wright2023therapeutictargetingof pages 1-3, clark2005theserinethreonineprotein pages 1-2).
8. Other Comments  
   Several small molecule inhibitors have been developed to target the RSK family, and some of these compounds have been evaluated for their ability to modulate RSK4 activity in cellular models. For instance, LJH685 and LJI308 are novel inhibitors reported to be highly potent and selective for RSK isoforms, while BI-D1870 and TAS0612 have also demonstrated inhibitory activity against RSKs in preclinical studies. These inhibitors have been used to interrogate the functional roles of RSKs in oncogenic signaling and cell proliferation. In the context of disease, alterations in RSK4 expression and activity have been associated with various cancer types, and its involvement in p53-dependent cell growth arrest signaling suggests a potential role in tumor suppression. Although a definitive profile of disease-causing mutations specific to RSK4 has not been fully established, its unique regulatory and constitutive activity profile mark it as a protein of interest for further investigation in the development of targeted therapeutic strategies (aronchik2014novelpotentand pages 1-2, wright2023therapeutictargetingof pages 15-15).
9. References
10. Aronchik, I., Appleton, B. A., Basham, S. E., Crawford, K., Del Rosario, M., Doyle, L. V., Estacio, W. F., Lan, J., Lindvall, M. K., Luu, C. A., Ornelas, E., Venetsanakos, E., Shafer, C. M., & Jefferson, A. B. Novel potent and selective inhibitors of p90 ribosomal S6 kinase reveal the heterogeneity of RSK function in MAPK-driven cancers. Molecular Cancer Research, 12:803-812, May 2014 (aronchik2014novelpotentand pages 1-2).
11. Clark, D. E., Errington, T. M., Smith, J. A., Frierson, H. F., Weber, M. J., & Lannigan, D. A. The serine/threonine protein kinase, p90 ribosomal S6 kinase, is an important regulator of prostate cancer cell proliferation. Cancer Research, 65:3108-3116, Apr 2005 (clark2005theserinethreonineprotein pages 1-2).
12. Kurinov, I. Structural diversity of the active conformation of the N-terminal kinase domain of p90 ribosomal S6 kinase 2. Worldwide Protein Data Bank, Feb 2009 (kurinov2009structuraldiversityof pages 1-2).
13. Magnuson, B., Ekim, B., & Fingar, D. C. Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. Biochemical Journal, 441:1-21, Dec 2012 (magnuson2012regulationandfunction pages 1-2).
14. Moore, C. E., Xie, J., Gomez, E., & Herbert, T. P. Identification of cAMP-dependent kinase as a third in vivo ribosomal protein S6 kinase in pancreatic β-cells. Journal of Molecular Biology, 389:480-494, Jun 2009 (moore2009identificationofcampdependent pages 9-12).
15. Poomakkoth, N., Issa, A., Abdulrahman, N., Abdelaziz, S. G., & Mraiche, F. M. P90 ribosomal S6 kinase: A potential therapeutic target in lung cancer. Journal of Translational Medicine, Jan 2016 (poomakkoth2016p90ribosomals6 pages 2-4).
16. Smith, J. A., Poteet-Smith, C. E., Xu, Y., Errington, T. M., Hecht, S. M., & Lannigan, D. A. Identification of the first specific inhibitor of p90 ribosomal S6 kinase (RSK) reveals an unexpected role for RSK in cancer cell proliferation. Cancer Research, 65:1027-1034, Feb 2005 (smith2005identificationofthe pages 1-1).
17. Utepbergenov, D., Hennig, P. M., Derewenda, U., Artamonov, M. V., Somlyo, A. V., & Derewenda, Z. S. Bacterial expression, purification and in vitro phosphorylation of full-length ribosomal S6 kinase 2 (RSK2). PLOS ONE, 11:e0164343, Oct 2016 (utepbergenov2016bacterialexpressionpurification pages 1-2).
18. Wright, E. B., & Lannigan, D. A. Therapeutic targeting of p90 ribosomal S6 kinase. Frontiers in Cell and Developmental Biology, Dec 2023 (wright2023therapeutictargetingof pages 1-3, pages 3-4, pages 14-15, pages 15-15).
19. Yi, Y. W., You, K. S., Park, J.-S., Lee, S.-G., & Seong, Y.-S. Ribosomal protein S6: a potential therapeutic target against cancer? International Journal of Molecular Sciences, 23:48, Dec 2021 (yi2021ribosomalproteins6 pages 41-43).

References

1. (aronchik2014novelpotentand pages 1-2): Ida Aronchik, Brent A. Appleton, Stephen E. Basham, Kenneth Crawford, Mercedita Del Rosario, Laura V. Doyle, William F. Estacio, Jiong Lan, Mika K. Lindvall, Catherine A. Luu, Elizabeth Ornelas, Eleni Venetsanakos, Cynthia M. Shafer, and Anne B. Jefferson. Novel potent and selective inhibitors of p90 ribosomal s6 kinase reveal the heterogeneity of rsk function in mapk-driven cancers. Molecular Cancer Research, 12:803-812, May 2014. URL: https://doi.org/10.1158/1541-7786.mcr-13-0595, doi:10.1158/1541-7786.mcr-13-0595. This article has 89 citations and is from a peer-reviewed journal.
2. (wright2023therapeutictargetingof pages 1-3): Eric B. Wright and Deborah A. Lannigan. Therapeutic targeting of p90 ribosomal s6 kinase. Frontiers in Cell and Developmental Biology, Dec 2023. URL: https://doi.org/10.3389/fcell.2023.1297292, doi:10.3389/fcell.2023.1297292. This article has 11 citations and is from a peer-reviewed journal.
3. (wright2023therapeutictargetingof pages 14-15): Eric B. Wright and Deborah A. Lannigan. Therapeutic targeting of p90 ribosomal s6 kinase. Frontiers in Cell and Developmental Biology, Dec 2023. URL: https://doi.org/10.3389/fcell.2023.1297292, doi:10.3389/fcell.2023.1297292. This article has 11 citations and is from a peer-reviewed journal.
4. (clark2005theserinethreonineprotein pages 1-2): D.E. Clark, T.M. Errington, J.A. Smith, H.F. Frierson, M.J. Weber, and D.A. Lannigan. The serine/threonine protein kinase, p90 ribosomal s6 kinase, is an important regulator of prostate cancer cell proliferation. Cancer Research, 65:3108-3116, Apr 2005. URL: https://doi.org/10.1158/0008-5472.can-04-3151, doi:10.1158/0008-5472.can-04-3151. This article has 241 citations and is from a highest quality peer-reviewed journal.
5. (moore2009identificationofcampdependent pages 9-12): Claire E.J. Moore, Jianling Xie, Edith Gomez, and Terence P. Herbert. Identification of camp-dependent kinase as a third in vivo ribosomal protein s6 kinase in pancreatic β-cells. Journal of Molecular Biology, 389:480-494, Jun 2009. URL: https://doi.org/10.1016/j.jmb.2009.04.020, doi:10.1016/j.jmb.2009.04.020. This article has 62 citations and is from a domain leading peer-reviewed journal.
6. (poomakkoth2016p90ribosomals6 pages 2-4): Noufira Poomakkoth, Aya Issa, Nabeel Abdulrahman, Somaia Gamal Abdelaziz, and Fatima Mraiche. P90 ribosomal s6 kinase: a potential therapeutic target in lung cancer. Journal of Translational Medicine, Jan 2016. URL: https://doi.org/10.1186/s12967-016-0768-1, doi:10.1186/s12967-016-0768-1. This article has 45 citations and is from a peer-reviewed journal.
7. (wright2023therapeutictargetingof pages 3-4): Eric B. Wright and Deborah A. Lannigan. Therapeutic targeting of p90 ribosomal s6 kinase. Frontiers in Cell and Developmental Biology, Dec 2023. URL: https://doi.org/10.3389/fcell.2023.1297292, doi:10.3389/fcell.2023.1297292. This article has 11 citations and is from a peer-reviewed journal.
8. (smith2005identificationofthe pages 1-1): Jeffrey A. Smith, Celeste E. Poteet-Smith, Yaming Xu, Timothy M. Errington, Sidney M. Hecht, and Deborah A. Lannigan. Identification of the first specific inhibitor of p90 ribosomal s6 kinase (rsk) reveals an unexpected role for rsk in cancer cell proliferation. Cancer Research, 65:1027-1034, Feb 2005. URL: https://doi.org/10.1158/0008-5472.1027.65.3, doi:10.1158/0008-5472.1027.65.3. This article has 351 citations and is from a highest quality peer-reviewed journal.
9. (yi2021ribosomalproteins6 pages 41-43): Yong Weon Yi, Kyu Sic You, Jeong-Soo Park, Seok-Geun Lee, and Yeon-Sun Seong. Ribosomal protein s6: a potential therapeutic target against cancer? International Journal of Molecular Sciences, 23:48, Dec 2021. URL: https://doi.org/10.3390/ijms23010048, doi:10.3390/ijms23010048. This article has 96 citations and is from a peer-reviewed journal.
10. (kurinov2009structuraldiversityof pages 1-2): I. Kurinov. Structural diversity of the active conformation of the n-terminal kinase domain of p90 ribosomal s6 kinase 2. Worldwide Protein Data Bank, Feb 2009. URL: https://doi.org/10.2210/pdb3g51/pdb, doi:10.2210/pdb3g51/pdb. This article has 39 citations.
11. (utepbergenov2016bacterialexpressionpurification pages 1-2): Darkhan Utepbergenov, Paulina M. Hennig, Urszula Derewenda, Mykhaylo V. Artamonov, Avril V. Somlyo, and Zygmunt S. Derewenda. Bacterial expression, purification and in vitro phosphorylation of full-length ribosomal s6 kinase 2 (rsk2). PLOS ONE, 11:e0164343, Oct 2016. URL: https://doi.org/10.1371/journal.pone.0164343, doi:10.1371/journal.pone.0164343. This article has 10 citations and is from a peer-reviewed journal.
12. (wright2023therapeutictargetingof pages 15-15): Eric B. Wright and Deborah A. Lannigan. Therapeutic targeting of p90 ribosomal s6 kinase. Frontiers in Cell and Developmental Biology, Dec 2023. URL: https://doi.org/10.3389/fcell.2023.1297292, doi:10.3389/fcell.2023.1297292. This article has 11 citations and is from a peer-reviewed journal.
13. (magnuson2012regulationandfunction pages 1-2): Brian Magnuson, Bilgen Ekim, and Diane C. Fingar. Regulation and function of ribosomal protein s6 kinase (s6k) within mtor signalling networks. Biochemical Journal, 441:1-21, Dec 2012. URL: https://doi.org/10.1042/bj20110892, doi:10.1042/bj20110892. This article has 1235 citations and is from a domain leading peer-reviewed journal.