1. Phylogeny  
   Dual specificity tyrosine-phosphorylation‑regulated kinase 4 (DYRK4) is a member of the DYRK family, which is part of the greater CMGC group of eukaryotic protein kinases. The DYRK family comprises several isoforms including DYRK1A, DYRK1B, DYRK2, DYRK3, and DYRK4, all of which share a conserved catalytic domain and a characteristic DYRK homology (DH) box. Comparative analyses of the human kinome have established that kinases within the CMGC group descend from early eukaryotic ancestors, and the DYRK subfamily is believed to have arisen by gene duplication events early in metazoan evolution (hanks1995theeukaryoticprotein pages 6-7, soundararajan2013structuresofdown pages 1-2). Although the literature devoted specifically to DYRK4 is limited relative to other family members, its phylogenetic positioning is supported by the conservation of key sequence motifs and structural features within the kinase core. In mammals, orthologous sequences for DYRK4 have been identified, with human DYRK4 (UniProt ID Q9NR20) and murine counterparts displaying divergence in non‑catalytic regions while preserving the conserved catalytic function. Moreover, DYRK4 is consistently assigned to the subclass of “class II” DYRK kinases based on distinctions in domain architecture and regulatory sequence elements that separate it from “class I” kinases such as DYRK1A and DYRK1B (papadopoulos2011identificationandcharacterization pages 5-7, thiriet2013dualspecificityproteinkinases pages 1-5). This phylogenetic classification is further reinforced through evolutionary studies that trace the emergence of the DYRK kinase family from a common ancestral kinase present in early eukaryotes, and subsequent evolutionary adaptations have paved the way for tissue‑specific expression dynamics observed in higher organisms. The available sequence comparisons illustrate that while the catalytic domains of DYRK kinases are highly conserved, the overall sequence divergence among paralogs suggests functional specialization; in the case of DYRK4, this specialization may be linked to its expression predominantly in testicular tissues (papadopoulos2011identificationandcharacterization pages 35-39). As such, DYRK4 is understood within the context of evolutionary conservation with other DYRK family members, yet it also exhibits features that signal a degree of species‑ and tissue‑specific adaptation.
2. Reaction Catalyzed  
   DYRK4 catalyzes the transfer of a phosphate group from ATP to specific amino acid residues on protein substrates. The standard chemical reaction can be represented as follows: ATP + [protein substrate] → ADP + [protein substrate]-phosphate + H⁺. This reaction involves the utilization of one molecule of ATP per phosphorylation event to yield ADP, a phosphorylated substrate, and a proton, thereby maintaining the overall stoichiometry of the reaction. In addition to phosphorylating serine and threonine residues on target proteins, DYRK4 undergoes an intramolecular autophosphorylation event on a conserved tyrosine residue located within its activation loop; this autophosphorylation is critical for achieving full catalytic activity and is characteristic of dual specificity kinases (soundararajan2013structuresofdown pages 7-8). The combination of substrate phosphorylation and autophosphorylation events underscores the dual functionality of DYRK4, which necessitates the transfer of the γ‑phosphate group from ATP in both self‑activation and downstream signaling processes.
3. Cofactor Requirements  
   The enzymatic activity of DYRK4 is dependent on the presence of divalent metal ions that assist in ATP binding and catalysis. As with most serine/threonine kinases, Mg²⁺ ions serve as a critical cofactor by coordinating with the phosphate groups of ATP and stabilizing the transition state during catalysis (soundararajan2013structuresofdown pages 7-8). This requirement for Mg²⁺ is common among kinases within the CMGC group and is essential not only for the phosphotransfer reaction but also for the proper alignment of substrates in the active site. Although direct kinetic analyses specific to DYRK4 have not been extensively detailed in the literature, the conservation of catalytic mechanisms across the DYRK family strongly indicates that Mg²⁺ fulfills a similar role for DYRK4 as it does for its homologs (hanks1995theeukaryoticprotein pages 6-7).
4. Substrate Specificity  
   The substrate specificity of DYRK4 is intricately determined by the architecture of its catalytic cleft and the sequence motifs present within its substrate proteins. Empirical data have demonstrated that DYRK4 is capable of phosphorylating the generic DYRK substrate “DYRKtide” in vitro, confirming its serine/threonine kinase activity (papadopoulos2011identificationandcharacterization pages 48-52). Moreover, studies have identified that DYRK4 phosphorylates the splicing factor SF3B1 at threonine 434 in living cells, establishing a precedent for its ability to modify proteins involved in RNA processing (papadopoulos2011identificationandcharacterization pages 48-52). In addition, peptide array screening has revealed that a particular peptide substrate, Pep285 — derived from sequences such as that in c-Jun — is selectively phosphorylated by DYRK4 but not by other kinases like DYRK1A or DYRK2, thereby underscoring a degree of substrate specificity that differentiates DYRK4 within the DYRK family (papadopoulos2011identificationandcharacterization pages 48-52). Although a clear consensus substrate motif for DYRK4 has not been rigorously defined, these studies indicate that its active site can recognize sequences that may include specific flanking residues; by contrast, studies on other DYRK kinases have demonstrated preferences for basic residues at certain positions and a proline residue immediately following the phosphorylation site (soundararajan2013structuresofdown pages 9-10). Given the structural conservation among DYRK family members, it is anticipated that DYRK4 likely uses a similar mode of substrate recognition, albeit with unique nuances that remain to be fully elucidated in a dedicated biochemical study.
5. Structure  
   DYRK4 is predicted to share the canonical structural features common to the DYRK family. Central to its structure is the kinase domain, which exhibits a bilobal configuration composed of a smaller N-terminal lobe rich in β‑strands and a larger C‑terminal lobe predominantly containing α‑helices. Within this catalytic core, several conserved motifs are present, including the DFG motif at the beginning of the activation loop, the catalytic loop with a key aspartate residue that functions as a proton acceptor, and a C‑helix that is important for maintaining the enzyme’s active conformation (soundararajan2013structuresofdown pages 2-3, soundararajan2013structuresofdown pages 7-8). The activation loop of DYRK4 harbors a conserved tyrosine residue that undergoes a “one‑off” autophosphorylation event during the nascent stages of protein folding; this phosphorylation is essential for locking the kinase into an active state (papadopoulos2011identificationandcharacterization pages 7-11). Adjacent to the catalytic domain is the DYRK homology box (DH box), which contributes to structural stabilization and is involved in the proper folding of the overall kinase architecture. Although no dedicated high‑resolution crystal structure for DYRK4 has been published, computational models such as those predicted by AlphaFold are consistent with the experimentally derived structures of related DYRK family members (soundararajan2013structuresofdown pages 1-2). Furthermore, evidence from studies on alternative splicing in the DYRK family suggests that different isoforms may exist; for instance, in other family members, longer isoforms often contain additional N‑terminal sequences with nuclear localization signals (NLS), whereas shorter isoforms remain predominantly cytoplasmic. While detailed reports of such alternative splicing for DYRK4 are limited, analogous mechanisms observed in the DYRK kinases imply that DYRK4 may also exist in variants with distinct subcellular localizations (papadopoulos2011identificationandcharacterization pages 93-96, thiriet2013dualspecificityproteinkinases pages 1-5). The overall structural conformation, marked by a conserved kinase fold and key catalytic motifs, underpins the enzyme’s ability to bind ATP, coordinate Mg²⁺, and engage substrates in a manner that is essential for its function.
6. Regulation  
   The regulation of DYRK4 is governed by mechanisms that are characteristic of dual specificity kinases. A primary regulatory event is the intramolecular autophosphorylation of a conserved tyrosine residue within the activation loop, an event that occurs cotranslationally and is required for full activation. This “one‑off” autophosphorylation ensures that the kinase adopts a stable active conformation capable of efficient substrate phosphorylation (papadopoulos2011identificationandcharacterization pages 7-11, soundararajan2013structuresofdown pages 7-8). In addition to this intrinsic mechanism, DYRK family kinases are known to be modulated by phosphorylation events carried out by other upstream cellular kinases; in the case of DYRK4, phosphorylation at residues outside the catalytic domain has been documented, although the precise upstream regulators remain to be fully characterized (papadopoulos2011identificationandcharacterization pages 93-96, papadopoulos2011identificationandcharacterization pages 106-109). Alternative splicing further contributes to the regulation of DYRK4 by generating isoforms with different N-terminal regions, which in turn dictate subcellular localization. For example, the long isoform of DYRK4 contains a nuclear localization signal (NLS) that enables partial nuclear retention, whereas the short isoform lacks such signals and remains predominantly in the cytosol (papadopoulos2011identificationandcharacterization pages 93-96, papadopoulos2011identificationandcharacterization pages 35-39). This differential localization is expected to influence substrate accessibility and, consequently, the downstream signaling events mediated by DYRK4. Although detailed studies on the dynamic regulation of DYRK4 via additional posttranslational modifications such as ubiquitination or acetylation are not available, the established regulatory paradigms for other DYRK family members indicate that the combination of autophosphorylation, alternative splicing, and potential phosphorylation by other kinases constitutes the central mechanism by which DYRK4’s activity is modulated (papadopoulos2011identificationandcharacterization pages 116-119, lindberg2021dualspecificitytyrosinephosphorylationregulated pages 2-4).
7. Function  
   DYRK4 is predominantly expressed in testicular tissues, and its expression profile suggests a role in the process of spermiogenesis. In mammalian systems, DYRK4 transcripts have been detected in specific stages of germ cell development, particularly in late spermatids. Although functional studies in murine models indicate that the complete absence of DYRK4 does not lead to infertility, this observation underscores its classification as having a non‑essential role in spermiogenesis while still potentially contributing to the fine tuning of germ cell differentiation (papadopoulos2011identificationandcharacterization pages 35-39, papadopoulos2011identificationandcharacterization pages 119-122). In addition to its involvement in testicular function, DYRK4 exhibits catalytic activity typical of its kinase family, phosphorylating serine/threonine residues on protein substrates. In cellular models, DYRK4 has been shown to phosphorylate the splicing factor SF3B1 at threonine 434, linking its activity to the regulation of RNA splicing events (papadopoulos2011identificationandcharacterization pages 48-52). Furthermore, substrate specificity studies using peptide arrays have identified the unique phosphorylation of peptide substrates such as Pep285, thereby highlighting a distinct substrate recognition profile compared to other DYRK family members (papadopoulos2011identificationandcharacterization pages 48-52). While the data available do not comprehensively delineate all downstream targets of DYRK4, the conservation of its kinase domain and catalytic properties suggest that it may participate in broader intracellular signaling pathways that govern cell cycle progression and stress response mechanisms. Expression analyses indicate that beyond its testis‑restricted expression, DYRK4 transcripts can also be detected at low levels in other cell types, although its expression outside the male gonad is less prominent (boni2020thedyrkfamily pages 11-13, izquierdo2023nuevosmecanismosde pages 34-40). This tissue‑specific expression pattern supports its functional role as a modulator of spermiogenesis with potential additional involvement in local cellular signaling processes.
8. Other Comments  
   DYRK4 remains one of the less extensively characterized members of the DYRK family, and accordingly, there is limited information regarding its pharmacological targeting and disease associations. No peer‑reviewed studies to date have reported selective inhibitors developed specifically for DYRK4, and there is an absence of well‑established pathogenic mutations that directly implicate DYRK4 in human disease. Its restricted expression pattern in testicular tissue, coupled with experimental evidence showing that knockout models do not display compromised fertility, suggests that DYRK4 may participate in redundant regulatory networks, with its role being potentially masked by compensatory mechanisms provided by other kinases within the DYRK family (izquierdo2023nuevosmecanismosde pages 167-169, papadopoulos2011identificationandcharacterization pages 116-119). Additionally, while other DYRK kinases such as DYRK1A have been associated with neurological disorders and oncogenic processes, similar associations for DYRK4 have not been definitively observed in the literature (lindberg2021dualspecificitytyrosinephosphorylationregulated pages 2-4, papadopoulos2011identificationandcharacterization pages 119-122). The current state of knowledge thus supports the view that DYRK4 primarily functions in the testis to modulate aspects of spermatogenesis, and further research in peer‑reviewed studies is needed to clarify whether it has additional roles in other cellular contexts or disease processes. This limited functional insight, combined with the lack of known inhibitors, underscores the need for additional biochemical and cell‑based studies that could expand our understanding of the unique activities and potential clinical relevance of DYRK4. Continued investigation into its substrate specificity, alternative splicing variants, and potential interactions with other key regulatory proteins will be critical for establishing a more comprehensive profile of this kinase within the broader chromosomal imprint of the DYRK family.

References

1. (papadopoulos2011identificationandcharacterization pages 5-7): C Papadopoulos. Identification and characterization of a new splice variant of the protein kinase dyrk4 and the role of dyrk1a during mitotic exit. Unknown journal, 2011.
2. (papadopoulos2011identificationandcharacterization pages 93-96): C Papadopoulos. Identification and characterization of a new splice variant of the protein kinase dyrk4 and the role of dyrk1a during mitotic exit. Unknown journal, 2011.
3. (izquierdo2023nuevosmecanismosde pages 167-169): RM Jiménez Izquierdo. Nuevos mecanismos de control de la proteína supresora de tumores fbxw7 mediados por la quinasa dyrk2. Unknown journal, 2023.
4. (izquierdo2023nuevosmecanismosde pages 34-40): RM Jiménez Izquierdo. Nuevos mecanismos de control de la proteína supresora de tumores fbxw7 mediados por la quinasa dyrk2. Unknown journal, 2023.
5. (lindberg2021dualspecificitytyrosinephosphorylationregulated pages 2-4): Mattias F. Lindberg and Laurent Meijer. Dual-specificity, tyrosine phosphorylation-regulated kinases (dyrks) and cdc2-like kinases (clks) in human disease, an overview. International Journal of Molecular Sciences, 22:6047, Jun 2021. URL: https://doi.org/10.3390/ijms22116047, doi:10.3390/ijms22116047. This article has 92 citations and is from a peer-reviewed journal.
6. (papadopoulos2011identificationandcharacterization pages 106-109): C Papadopoulos. Identification and characterization of a new splice variant of the protein kinase dyrk4 and the role of dyrk1a during mitotic exit. Unknown journal, 2011.
7. (papadopoulos2011identificationandcharacterization pages 116-119): C Papadopoulos. Identification and characterization of a new splice variant of the protein kinase dyrk4 and the role of dyrk1a during mitotic exit. Unknown journal, 2011.
8. (papadopoulos2011identificationandcharacterization pages 119-122): C Papadopoulos. Identification and characterization of a new splice variant of the protein kinase dyrk4 and the role of dyrk1a during mitotic exit. Unknown journal, 2011.
9. (papadopoulos2011identificationandcharacterization pages 35-39): C Papadopoulos. Identification and characterization of a new splice variant of the protein kinase dyrk4 and the role of dyrk1a during mitotic exit. Unknown journal, 2011.
10. (papadopoulos2011identificationandcharacterization pages 48-52): C Papadopoulos. Identification and characterization of a new splice variant of the protein kinase dyrk4 and the role of dyrk1a during mitotic exit. Unknown journal, 2011.
11. (papadopoulos2011identificationandcharacterization pages 7-11): C Papadopoulos. Identification and characterization of a new splice variant of the protein kinase dyrk4 and the role of dyrk1a during mitotic exit. Unknown journal, 2011.
12. (thiriet2013dualspecificityproteinkinases pages 1-5): M Thiriet M Thiriet. Dual-specificity protein kinases. Biomathematical and Biomechanical Modeling of the Circulatory and Ventilatory Systems, pages 379-386, Jul 2013. URL: https://doi.org/10.1007/978-1-4614-4370-4\_7, doi:10.1007/978-1-4614-4370-4\_7. This article has 4 citations.
13. (boni2020thedyrkfamily pages 11-13): Jacopo Boni, Carlota Rubio-Perez, Nuria López-Bigas, Cristina Fillat, and Susana de la Luna. The dyrk family of kinases in cancer: molecular functions and therapeutic opportunities. Cancers, 12:2106, Jul 2020. URL: https://doi.org/10.3390/cancers12082106, doi:10.3390/cancers12082106. This article has 88 citations and is from a peer-reviewed journal.
14. (hanks1995theeukaryoticprotein pages 6-7): Steven K. Hanks and Tony Hunter. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification 1. The FASEB Journal, 9:576-596, May 1995. URL: https://doi.org/10.1096/fasebj.9.8.7768349, doi:10.1096/fasebj.9.8.7768349. This article has 3994 citations.
15. (soundararajan2013structuresofdown pages 1-2): Meera Soundararajan, Annette K. Roos, Pavel Savitsky, Panagis Filippakopoulos, Arminja N. Kettenbach, Jesper V. Olsen, Scott A. Gerber, Jeyanthy Eswaran, Stefan Knapp, and Jonathan M. Elkins. Structures of down syndrome kinases, dyrks, reveal mechanisms of kinase activation and substrate recognition. Structure(London, England:1993), 21:986-996, Jun 2013. URL: https://doi.org/10.1016/j.str.2013.03.012, doi:10.1016/j.str.2013.03.012. This article has 182 citations.
16. (soundararajan2013structuresofdown pages 2-3): Meera Soundararajan, Annette K. Roos, Pavel Savitsky, Panagis Filippakopoulos, Arminja N. Kettenbach, Jesper V. Olsen, Scott A. Gerber, Jeyanthy Eswaran, Stefan Knapp, and Jonathan M. Elkins. Structures of down syndrome kinases, dyrks, reveal mechanisms of kinase activation and substrate recognition. Structure(London, England:1993), 21:986-996, Jun 2013. URL: https://doi.org/10.1016/j.str.2013.03.012, doi:10.1016/j.str.2013.03.012. This article has 182 citations.
17. (soundararajan2013structuresofdown pages 7-8): Meera Soundararajan, Annette K. Roos, Pavel Savitsky, Panagis Filippakopoulos, Arminja N. Kettenbach, Jesper V. Olsen, Scott A. Gerber, Jeyanthy Eswaran, Stefan Knapp, and Jonathan M. Elkins. Structures of down syndrome kinases, dyrks, reveal mechanisms of kinase activation and substrate recognition. Structure(London, England:1993), 21:986-996, Jun 2013. URL: https://doi.org/10.1016/j.str.2013.03.012, doi:10.1016/j.str.2013.03.012. This article has 182 citations.