1. Phylogeny  
   CLK1, also known as CDC‐like kinase 1 (Uniprot P49759), is a member of the CDC‐like kinase family that is nested within the broader CMGC group of serine/threonine kinases. The CMGC group comprises cyclin‐dependent kinases (CDKs), mitogen‐activated protein kinases (MAPKs), glycogen synthase kinases (GSKs), and dual‐specificity tyrosine phosphorylation‐regulated kinases (DYRKs). Detailed analyses of the conserved kinase domain have revealed that CLK1 and its paralogs contain a signature “EHLAMMERILG” motif, commonly referred to as the LAMMER motif, which is central to substrate recognition and helps demarcate the CLK subfamily from other related kinases (mott2009evaluationofsubstituted pages 4-5). Phylogenetic studies have shown that members of the CLK family are evolutionarily conserved across a wide range of eukaryotic organisms, including yeast, plants, invertebrates, and vertebrates. In mammals, four closely related isoforms (CLK1–CLK4) have been identified, and these isoforms are thought to have originated via gene duplication events early in vertebrate evolution. The conservation of the kinase domain, including key catalytic residues and secondary structure elements, indicates that CLK1 can be traced back to ancestral kinases that were already present in the Last Eukaryotic Common Ancestor (LECA) (hogg2023functionsofsrpkclkanddyrkkinasesin pages 2-4, moyano2020cdclikekinases(clks) pages 1-3).

In addition, sequence comparisons reveal that the catalytic domain of CLK1 shares high levels of identity with its paralog CLK4, with studies reporting over 85% sequence similarity in the core catalytic region (mott2009evaluationofsubstituted pages 4-5). Despite this high degree of conservation within the kinase domain, the N-terminal regions of CLK family members are more divergent, which is reflected in differences in substrate docking and regulatory interactions. This divergence in non-catalytic regions contributes to functional specialization among the isoforms while maintaining the overall conservation of catalytic activity. Thus, from an evolutionary perspective, CLK1 exemplifies how kinases can evolve subtle structural modifications to fulfill specialized roles in cellular regulation while retaining the hallmark features inherited from their ancient progenitors (hogg2023functionsofsrpkclkanddyrkkinasesin pages 2-4, moyano2020cdclikekinases(clks) pages 1-3).

1. Reaction Catalyzed  
   The fundamental reaction catalyzed by CLK1 is the transfer of a phosphate group from ATP to specific amino acid residues on substrate proteins. This phosphotransfer reaction can be summarized by the following chemical equation:  
     ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺.  
   CLK1 is a dual specificity kinase, meaning that while its exogenous substrates are phosphorylated exclusively on serine/threonine residues, the enzyme itself is capable of autophosphorylation on tyrosine residues. This dual-specificity characteristic is a critical aspect of its catalytic function. The phosphorylation event catalyzed by CLK1 modifies spliceosomal proteins that harbor serine/arginine-rich domains, thereby modulating their activity during the assembly and function of the spliceosome (elhady2017developmentofselective pages 13-14, keshwani2015nuclearproteinkinase pages 1-3, song2023cdc2likekinasesstructure pages 1-3).
2. Cofactor Requirements  
   The catalytic activity of CLK1 is dependent on the presence of divalent metal ions, specifically magnesium ions (Mg²⁺). Mg²⁺ is essential for coordinating the phosphate groups of ATP within the kinase active site, thereby facilitating the proper orientation and stabilization required for efficient phosphoryl transfer. The requirement for Mg²⁺ is common among serine/threonine kinases and is critical for the catalytic activity of CLK1, ensuring that ATP is bound in the proper conformation to allow for subsequent phosphorylation of substrate proteins (moyano2020cdclikekinases(clks) pages 1-3, song2023cdc2likekinasesstructure pages 1-3).
3. Substrate Specificity  
   CLK1 is primarily responsible for phosphorylating proteins involved in pre-mRNA splicing, most notably members of the serine/arginine-rich (SR) protein family. Its substrate specificity is defined by its ability to recognize and phosphorylate serine residues within RS domains—a feature that is critical for modulating spliceosome assembly and the regulation of alternative splicing events. In addition to its activity on generic RS motifs, CLK1 demonstrates the capacity to phosphorylate both serine-arginine and serine-proline dipeptides. While the enzyme is capable of autophosphorylation on tyrosine residues, phosphorylation of exogenous substrates occurs predominantly on serine/threonine residues (haltenhof2020cdc2likekinasesrepresent pages 24-28, hogg2023functionsofsrpkclkanddyrkkinasesin pages 2-4).

The substrate recognition by CLK1 is further facilitated by structural elements in its catalytic domain that extend the substrate binding site, allowing for broader specificity compared to other kinases such as SR protein kinases (SRPKs), which typically have a more constrained substrate recognition motif. This broader substrate specificity is important for CLK1’s role in phosphorylating a range of SR proteins (including SRSF1, SRSF3, and others) that are key regulators of alternative splicing. The ability of CLK1 to target multiple substrates underlines its central role in the regulation of RNA splicing and highlights the importance of its substrate selection in maintaining proper splicing dynamics (hogg2023functionsofsrpkclkanddyrkkinasesin pages 2-4).

1. Structure  
   At the structural level, CLK1 comprises a central kinase domain that exhibits the canonical bilobed architecture common among protein kinases. The N-terminal lobe is primarily composed of β-sheets, whereas the larger C-terminal lobe is predominantly α-helical. The two lobes are connected by a hinge region that forms part of the ATP binding pocket—a critical feature for the enzyme’s catalytic activity. Within this conserved kinase domain, the LAMMER motif (EHLAMMERILG) is located in a region that is essential for substrate recognition, and its presence serves as a defining feature of the CLK family (mott2009evaluationofsubstituted pages 4-5, song2023cdc2likekinasesstructure pages 1-3).

In addition to the conserved catalytic core, CLK1 contains unique insertions within the C-lobe, including a MAPK-like insertion that forms a shallow substrate-binding groove. This insertion is believed to contribute to the distinct substrate recognition properties of CLK1 by providing an extended interface for the interaction with RS domain-containing proteins. The kinase domain also harbors key regulatory elements such as the activation loop, which, despite its lack of extensive post-translational modifications, is maintained in a conformation favorable for catalysis (song2023cdc2likekinasesstructure pages 1-3, keshwani2015nuclearproteinkinase pages 11-13).

A notable structural feature of CLK1 is its long, intrinsically disordered N-terminal extension. Unlike many kinases that possess well-defined docking domains, CLK1’s N-terminus is characterized by its flexibility and high content of serine/arginine-like sequences. This intrinsically disordered region functions as a docking module that facilitates high-affinity interactions with SR protein substrates, thereby enhancing the efficiency of substrate phosphorylation. The overall structural arrangement of CLK1—combining a conserved, well-ordered catalytic domain with a flexible N-terminal regulatory region—underpins its ability to serve as a constitutively active kinase in the regulation of alternative splicing (aubol2014nterminusofthe pages 1-3, keshwani2015nuclearproteinkinase pages 1-3).

Furthermore, crystallographic and cryo-EM studies (supported by AlphaFold predictions) have provided insights into the active conformation of CLK1, revealing that the activation segment adopts a conformation compatible with catalysis without the need for external activating phosphorylation events. This constitutive activity is supported by the stabilization of key structural features such as the C-helix and the hydrophobic spine, which are essential for maintaining the active state of protein kinases. The combination of these structural characteristics not only informs the basic enzymatic mechanism of CLK1 but also aids in the design of selective inhibitors targeted against its unique active site architecture (song2023cdc2likekinasesstructure pages 1-3, mott2009evaluationofsubstituted pages 4-5).

1. Regulation  
   The regulation of CLK1 activity appears to be driven primarily by intrinsic properties rather than by extensive external modulation. CLK1 is known to be a constitutively active enzyme, a fact that is underscored by its ability to adopt an active conformation in the absence of additional activating phosphorylations. One of the key regulatory mechanisms for CLK1 is autophosphorylation; the kinase phosphorylates itself on serine, threonine, and tyrosine residues, and this autophosphorylation event is integral to its dual-specificity characteristic (elhady2017developmentofselective pages 13-14, keshwani2015nuclearproteinkinase pages 1-3).

The intrinsically disordered N-terminal region of CLK1 plays a significant role in modulating its activity. This flexible segment facilitates oligomerization and promotes substrate docking, thereby enhancing the enzyme’s capacity to phosphorylate SR proteins. The dynamic nature of this region allows CLK1 to adjust its conformation in response to binding interactions with its targets, which in turn contributes to the fine-tuned regulation of splicing factor phosphorylation. In several studies, alterations in the N-terminal domain have been shown to significantly affect both substrate affinity and overall catalytic efficiency, highlighting the importance of this segment in regulating kinase activity (aubol2014nterminusofthe pages 8-10, keshwani2015nuclearproteinkinase pages 11-13).

Pharmacological regulation of CLK1 has also been explored, with several small-molecule inhibitors developed to target its active site or interfere with its substrate docking mechanisms. Compounds identified in studies employing structure-based drug design have been shown to bind selectively to CLK1, thereby modulating its kinase activity and the subsequent phosphorylation of SR proteins. While these chemical inhibitors serve as useful tools for dissecting the functional roles of CLK1 in cellular systems, they also underscore the potential for therapeutic intervention in diseases associated with aberrant splicing regulation (elhady2017developmentofselective pages 13-14, fedorov2011specificclkinhibitors pages 1-2, prak2016benzobisthiazolesrepresenta pages 1-5).

1. Function  
   CLK1 is primarily involved in the regulation of pre-mRNA splicing, a complex process critical for the generation of mature mRNA transcripts in eukaryotic cells. The enzyme accomplishes this regulatory role by phosphorylating serine/arginine-rich (SR) proteins, which are essential components of the spliceosome. By modifying SR proteins through phosphorylation, CLK1 influences both the assembly of the spliceosomal machinery and the selection of alternative splice sites, thereby affecting gene expression patterns. Notably, CLK1-mediated phosphorylation has been directly linked to the regulation of tissue factor (F3) pre-mRNA splicing in endothelial cells, a process that is pivotal in maintaining normal cellular function (hogg2023functionsofsrpkclkanddyrkkinasesin pages 2-4).

CLK1 is predominantly localized within the nucleus, where it associates with nuclear speckles—subnuclear domains rich in splicing factors. The nuclear localization of CLK1 is largely attributed to its interactions with these splicing components, and its constitutive activity ensures that the phosphorylation state of SR proteins is maintained at levels sufficient to support dynamic splicing events. The tight regulation of alternative splicing by CLK1 means that the enzyme is positioned within a broader network of kinases, such as SR protein kinases (SRPKs) and certain members of the DYRK family, that collectively modulate splicing decisions in response to developmental cues and cellular stress (hogg2023functionsofsrpkclkanddyrkkinasesin pages 2-4, moyano2020cdclikekinases(clks) pages 1-3).

Beyond its canonical role in splicing, CLK1 has also been implicated in other cellular processes including cell cycle regulation, signal transduction, and transcriptional control. The phosphorylation events mediated by CLK1 can influence mRNA stability and processing, thus indirectly affecting protein synthesis and cellular metabolism. Although the majority of functional studies have focused on its role in RNA splicing, the broad substrate specificity and constitutive activity of CLK1 suggest that it may participate in additional regulatory pathways that are yet to be fully elucidated. The versatility in function and the ability to integrate multiple regulatory signals underscore the importance of CLK1 as a central hub in the post-transcriptional control of gene expression (hogg2023functionsofsrpkclkanddyrkkinasesin pages 2-4, moyano2020cdclikekinases(clks) pages 1-3).

1. Other Comments  
   CLK1 has emerged as a significant target of interest both from a biological standpoint and as a potential therapeutic target. Its central role in the regulation of alternative splicing has made it an attractive candidate for therapeutic intervention, especially in pathologies such as cancer and neurodegenerative disorders, where aberrant splicing patterns are frequently observed. Several studies have reported the discovery and characterization of selective inhibitors for CLK1. For example, work described by Fedorov et al. has resulted in the identification of novel chemotypes that demonstrate potent inhibitory activity against CLK isoforms, particularly CLK1 and CLK4, with promising selectivity profiles (fedorov2011specificclkinhibitors pages 1-2). In parallel, research employing benzobisthiazole scaffolds has further expanded the repertoire of small molecules capable of modulating CLK activity (prak2016benzobisthiazolesrepresenta pages 1-5).

Inhibitors of CLK1 have been shown to reduce the phosphorylation of SR proteins, thereby altering alternative splicing patterns. Such alterations hold therapeutic promise in cases where dysregulated splicing contributes to disease progression. In addition, the unique structural features of CLK1, including its intrinsically disordered N-terminal region and the specific configuration of its active site, have provided opportunities for the development of compounds that selectively target these features, potentially minimizing off-target effects. Studies focusing on the chemical biology of CLK1 have contributed valuable insights into the design of inhibitors that may ultimately be used to correct splicing defects in disease states (elhady2017developmentofselective pages 13-14, fedorov2011specificclkinhibitors pages 1-2, prak2016benzobisthiazolesrepresenta pages 1-5).

Furthermore, ongoing research into the functional roles of CLK1 in cellular physiology continues to reveal its involvement in multiple signaling cascades beyond splicing. For instance, modulation of CLK1 activity has been linked to alterations in cell cycle progression and stress responses, indicating that its inhibition could have broad implications for cellular homeostasis. The therapeutic targeting of CLK1 is therefore being actively explored both in preclinical models and through the development of chemical probes that enable a precise dissection of its biological functions. Overall, the continuous effort to characterize CLK1 at both the structural and functional levels reinforces its importance as a regulatory kinase and potential drug target in diseases where splicing dysregulation plays a critical role (elhady2017developmentofselective pages 13-14, fedorov2011specificclkinhibitors pages 1-2).

1. References
2. Hogg, E. K. J. & Findlay, G. M. Functions of srpk, clk and dyrk kinases in stem cells, development, and human developmental disorders. FEBS Letters, 597: 2375–2415, Sep 2023. (hogg2023functionsofsrpkclkanddyrkkinasesin pages 2-4)
3. Mott, B. et al. Evaluation of substituted 6-arylquinazolin-4-amines as potent and selective inhibitors of cdc2-like kinases (clk). Bioorganic & Medicinal Chemistry Letters, 19(23): 6700–6705, Dec 2009. (mott2009evaluationofsubstituted pages 4-5)
4. Moyano, P. M., Němec, V., & Paruch, K. Cdc-like kinases (clks): biology, chemical probes, and therapeutic potential. International Journal of Molecular Sciences, 21: 7549, Oct 2020. (moyano2020cdclikekinases(clks) pages 1-3, pages 25-26, pages 3-6, pages 11-13, pages 13-16, pages 30-31)
5. Talevich, E., Mirza, A., & Kannan, N. Structural and evolutionary divergence of eukaryotic protein kinases in apicomplexa. BMC Evolutionary Biology, 11: 321, Nov 2011. (talevich2011structuralandevolutionary pages 1-2, pages 4-5, pages 10-11, pages 14-15, pages 15-17)
6. Aubol, B. E. et al. N-terminus of the protein kinase clk1 induces sr protein hyperphosphorylation. The Biochemical Journal, 462: 143–152, Jul 2014. (aubol2014nterminusofthe pages 1-3, pages 8-10)
7. ElHady, A. K. et al. Development of selective clk1 and -4 inhibitors for cellular depletion of cancer-relevant proteins. Journal of Medicinal Chemistry, 60: 5377–5391, May 2017. (elhady2017developmentofselective pages 13-14)
8. Keshwani, M. M. et al. Nuclear protein kinase clk1 uses a non-traditional docking mechanism to select physiological substrates. The Biochemical Journal, 472(3): 329–338, Dec 2015. (keshwani2015nuclearproteinkinase pages 1-3, pages 11-13)
9. Fedorov, O. et al. Specific clk inhibitors from a novel chemotype for regulation of alternative splicing. Chemistry & Biology, 18: 67–76, Jan 2011. (fedorov2011specificclkinhibitors pages 1-2)
10. Prak, K. et al. Benzobisthiazoles represent a novel scaffold for kinase inhibitors of clk family members. Biochemistry, 55: 608–617, Jan 2016. (prak2016benzobisthiazolesrepresenta pages 1-5)
11. Song, M. et al. Cdc2-like kinases: structure, biological function and therapeutic targets for diseases. Signal Transduction and Targeted Therapy, Apr 2023. (song2023cdc2likekinasesstructure pages 1-3, pages 21-22, pages 3-3)
12. Chowdhury, I. et al. CMGC kinases in health and cancer. Cancers, Jul 2023. (chowdhury2023cmgckinasesin pages 18-19)

References

1. (haltenhof2020cdc2likekinasesrepresent pages 24-28): T Haltenhof. Cdc2-like kinases represent evolutionarily adapted temperature-sensors, which globally control alternative splicing and gene expression. Unknown journal, 2020. URL: https://doi.org/10.17169/refubium-26535, doi:10.17169/refubium-26535. This article has 2 citations.
2. (hogg2023functionsofsrpkclkanddyrkkinasesin pages 2-4): Elizabeth K. J. Hogg and Greg M. Findlay. Functions ofsrpk,clkanddyrkkinases in stem cells, development, and human developmental disorders. FEBS Letters, 597:2375-2415, Sep 2023. URL: https://doi.org/10.1002/1873-3468.14723, doi:10.1002/1873-3468.14723. This article has 7 citations and is from a peer-reviewed journal.
3. (mott2009evaluationofsubstituted pages 4-5): B. Mott, C. Tanega, M. Shen, D. Maloney, P. Shinn, W. Leister, J. Marugan, James Inglese, C. Austin, T. Misteli, D. Auld, and Craig J. Thomas. Evaluation of substituted 6-arylquinazolin-4-amines as potent and selective inhibitors of cdc2-like kinases (clk). Bioorganic & medicinal chemistry letters, 19 23:6700-5, Dec 2009. URL: https://doi.org/10.1016/j.bmcl.2009.09.121, doi:10.1016/j.bmcl.2009.09.121. This article has 83 citations.
4. (moyano2020cdclikekinases(clks) pages 1-3): Paula Martín Moyano, Václav Němec, and Kamil Paruch. Cdc-like kinases (clks): biology, chemical probes, and therapeutic potential. International Journal of Molecular Sciences, 21:7549, Oct 2020. URL: https://doi.org/10.3390/ijms21207549, doi:10.3390/ijms21207549. This article has 75 citations and is from a peer-reviewed journal.
5. (song2023cdc2likekinasesstructure pages 1-3): Mengqiu Song, Luping Pang, Mengmeng Zhang, Yingzi Qu, Kyle Vaughn Laster, and Zigang Dong. Cdc2-like kinases: structure, biological function and therapeutic targets for diseases. Signal Transduction and Targeted Therapy, Apr 2023. URL: https://doi.org/10.1038/s41392-023-01409-4, doi:10.1038/s41392-023-01409-4. This article has 47 citations and is from a peer-reviewed journal.
6. (aubol2014nterminusofthe pages 1-3): Brandon E. Aubol, Ryan M. Plocinik, Malik M. Keshwani, Maria L. McGlone, Jonathan C. Hagopian, Gourisankar Ghosh, Xiang-Dong Fu, and Joseph A. Adams. N-terminus of the protein kinase clk1 induces sr protein hyperphosphorylation. Biochemical Journal, 462:143-152, Jul 2014. URL: https://doi.org/10.1042/bj20140494, doi:10.1042/bj20140494. This article has 48 citations and is from a domain leading peer-reviewed journal.
7. (elhady2017developmentofselective pages 13-14): Ahmed K. ElHady, Mohammad Abdel-Halim, Ashraf H. Abadi, and Matthias Engel. Development of selective clk1 and -4 inhibitors for cellular depletion of cancer-relevant proteins. Journal of Medicinal Chemistry, 60:5377-5391, May 2017. URL: https://doi.org/10.1021/acs.jmedchem.6b01915, doi:10.1021/acs.jmedchem.6b01915. This article has 53 citations and is from a highest quality peer-reviewed journal.
8. (keshwani2015nuclearproteinkinase pages 1-3): Malik M. Keshwani, Kendra L. Hailey, Brandon E. Aubol, Laurent Fattet, Maria L. McGlone, Patricia A. Jennings, and Joseph A. Adams. Nuclear protein kinase clk1 uses a non-traditional docking mechanism to select physiological substrates. The Biochemical journal, 472 3:329-38, Dec 2015. URL: https://doi.org/10.1042/bj20150903, doi:10.1042/bj20150903. This article has 25 citations.
9. (keshwani2015nuclearproteinkinase pages 11-13): Malik M. Keshwani, Kendra L. Hailey, Brandon E. Aubol, Laurent Fattet, Maria L. McGlone, Patricia A. Jennings, and Joseph A. Adams. Nuclear protein kinase clk1 uses a non-traditional docking mechanism to select physiological substrates. The Biochemical journal, 472 3:329-38, Dec 2015. URL: https://doi.org/10.1042/bj20150903, doi:10.1042/bj20150903. This article has 25 citations.
10. (talevich2011structuralandevolutionary pages 1-2): Eric Talevich, Amar Mirza, and Natarajan Kannan. Structural and evolutionary divergence of eukaryotic protein kinases in apicomplexa. BMC Evolutionary Biology, 11:321-321, Nov 2011. URL: https://doi.org/10.1186/1471-2148-11-321, doi:10.1186/1471-2148-11-321. This article has 115 citations.
11. (aubol2014nterminusofthe pages 8-10): Brandon E. Aubol, Ryan M. Plocinik, Malik M. Keshwani, Maria L. McGlone, Jonathan C. Hagopian, Gourisankar Ghosh, Xiang-Dong Fu, and Joseph A. Adams. N-terminus of the protein kinase clk1 induces sr protein hyperphosphorylation. Biochemical Journal, 462:143-152, Jul 2014. URL: https://doi.org/10.1042/bj20140494, doi:10.1042/bj20140494. This article has 48 citations and is from a domain leading peer-reviewed journal.
12. (chowdhury2023cmgckinasesin pages 18-19): Iftekhar Chowdhury, Giovanna Dashi, and S. Keskitalo. Cmgc kinases in health and cancer. Cancers, Jul 2023. URL: https://doi.org/10.3390/cancers15153838, doi:10.3390/cancers15153838. This article has 19 citations and is from a peer-reviewed journal.
13. (fedorov2011specificclkinhibitors pages 1-2): O. Fedorov, K. Huber, A. Eisenreich, P. Filippakopoulos, Oliver N. F. King, A. Bullock, Damian Szklarczyk, L. Jensen, D. Fabbro, J. Trappe, U. Rauch, F. Bracher, and S. Knapp. Specific clk inhibitors from a novel chemotype for regulation of alternative splicing. Chemistry & Biology, 18:67-76, Jan 2011. URL: https://doi.org/10.1016/j.chembiol.2010.11.009, doi:10.1016/j.chembiol.2010.11.009. This article has 231 citations.
14. (prak2016benzobisthiazolesrepresenta pages 1-5): Krisna Prak, Janos Kriston-Vizi, A. W. Edith Chan, Christin Luft, Joana R. Costa, Niccolo Pengo, and Robin Ketteler. Benzobisthiazoles represent a novel scaffold for kinase inhibitors of clk family members. Biochemistry, 55:608-617, Jan 2016. URL: https://doi.org/10.1021/acs.biochem.5b01128, doi:10.1021/acs.biochem.5b01128. This article has 17 citations and is from a peer-reviewed journal.