1. Phylogeny  
   Dual specificity protein kinase CLK3 is a member of the CDC‐like kinase (CLK) family, which comprises four human isoforms (CLK1–CLK4) belonging to the CMGC group of serine/threonine kinases. CLK3 orthologs are conserved across eukaryotes, as inferred from analyses of kinase complements in yeast, plants, and animals. Within the kinome, CLK3—and its family members—share a common evolutionary origin with related dual specificity kinases that emerged early in eukaryotic evolution, a relationship well documented by comprehensive kinase surveys (moyano2020cdclikekinases(clks) pages 1-3, song2023cdc2likekinasesstructure pages 1-3, fedorov2011specificclkinhibitors pages 1-2).
2. Reaction Catalyzed  
   CLK3 catalyzes the phosphorylation reaction in which the γ-phosphate from ATP is transferred to protein substrates. The reaction is typically described as: ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺. In addition to phosphorylating exogenous substrates, CLK3 is capable of autophosphorylation—most notably on tyrosine residues—in accordance with its dual specificity nature (addova…2017novelclk1inhibitors pages 1-2, fedorov2011specificclkinhibitors pages 1-2).
3. Cofactor Requirements  
   The catalytic activity of CLK3 is dependent on ATP as the phosphate donor and requires the presence of Mg²⁺ ions as a cofactor. Magnesium is essential for proper ATP coordination and for maximizing the efficiency of the phosphorylation reaction catalyzed by this kinase (moyano2020cdclikekinases(clks) pages 1-3).
4. Substrate Specificity  
   CLK3 displays a marked substrate specificity for serine-/arginine-rich (SR) proteins, which are integral components of the spliceosomal complex. The kinase phosphorylates key splicing factors such as SRSF1 and SRSF3; these phosphorylation events influence splice site recognition and the dynamic distribution of SR proteins within the nucleus. Notably, CLK3 regulates the alternative splicing of tissue factor (F3) pre-mRNA in endothelial cells by modifying SR protein activity. Consequently, its substrate preference centers on RS domain-containing proteins, where the consensus sequence includes arginine/serine dipeptide repeats (addova…2017novelclk1inhibitors pages 1-2, fedorov2011specificclkinhibitors pages 5-7, song2023cdc2likekinasesstructure pages 15-15, moyano2020cdclikekinases(clks) pages 25-26).
5. Structure  
   CLK3 is composed of a central, highly conserved kinase domain that adopts the typical bilobal fold found in protein kinases. The N-terminal lobe is primarily formed by β-sheets while the C-terminal lobe is dominated by α-helices. A defining structural feature of CLK3 is the presence of the conserved “EHLAMMERILG” motif, which is the hallmark of LAMMER kinases and is located within a critical catalytic subdomain; this motif is essential for both substrate recognition and catalytic activity (song2023cdc2likekinasesstructure pages 3-3, addova…2017novelclk1inhibitors pages 1-2). In addition to the core kinase fold, CLK3 contains several insertions that are less conserved among the CLK isoforms. These include a MAPK-like insertion and a β-hairpin element that likely contribute to the specificity of substrate interaction and influence the binding of small molecule inhibitors. Furthermore, the N-terminal region of CLK3 is notably less conserved compared to those of other CLKs, potentially imparting unique regulatory characteristics to CLK3 (moyano2020cdclikekinases(clks) pages 3-6, song2023cdc2likekinasesstructure pages 8-9, addova…2017novelclk1inhibitors pages 1-2). Structural studies employing X-ray crystallography and supported by modeling efforts have elucidated these domains and highlighted key catalytic features such as the ATP-binding hinge region, the conserved lysine involved in phosphate transfer, and conformational elements like the C-helix and activation loop that are critical for full kinase activity (song2023cdc2likekinasesstructure pages 12-15).
6. Regulation  
   Regulatory mechanisms for CLK3 involve both intrinsic and extrinsic factors. Autophosphorylation is a prominent regulatory mechanism, with evidence indicating that CLK3 can autophosphorylate on tyrosine residues, which may modulate its activity in a manner distinct from phosphorylation of exogenous substrates. In addition, CLK3 activity is influenced by its interactions with splicing factors, where binding to SR proteins facilitates a redistribution from nuclear speckles to a more diffuse nucleoplasmic pattern. This spatial change is important for the proper control of pre-mRNA splicing. Post-transcriptional regulation via microRNAs, notably miR-144, has also been observed to impact CLK3 expression levels in certain cells, further integrating CLK3 into broader signaling networks. Moreover, small molecule inhibitors have been shown to induce conformational changes in CLK3’s kinase domain, underscoring the importance of structural dynamics in its regulation (addova…2017novelclk1inhibitors pages 1-2, fedorov2011specificclkinhibitors pages 4-5, prak2016benzobisthiazolesrepresenta pages 14-18, moyano2020cdclikekinases(clks) pages 28-30).
7. Function  
   The primary biological function of CLK3 is the regulation of alternative pre-mRNA splicing through the phosphorylation of serine/arginine-rich splicing factors. By phosphorylating substrates such as SRSF1 and SRSF3, CLK3 modulates spliceosome assembly and influences splice site selection, thereby affecting the diversity of protein isoforms generated from a single gene. In endothelial cells, CLK3 plays a specific role by regulating the alternative splicing of tissue factor (F3) pre-mRNA, a process that is critical for maintaining normal hemostasis and may have implications in coagulation disorders (addova…2017novelclk1inhibitors pages 8-8). In addition to its well‐established role in splicing regulation, expression studies indicate that CLK3 is differentially expressed in tissue contexts, with notably higher levels in testicular germ cells, suggesting a role in spermatogenesis. Altered CLK3 activity has also been linked to oncogenic processes; dysregulated phosphorylation of SR proteins can lead to aberrant splicing patterns that contribute to cancer progression, and thus CLK3 is emerging as a potential therapeutic target in oncology (song2023cdc2likekinasesstructure pages 15-16, moyano2020cdclikekinases(clks) pages 19-23, funnell2017clkdependentexonrecognition pages 1-2).
8. Other Comments  
   A variety of small molecule inhibitors have been developed to target the CLK family kinases, including CLK3. Inhibitors such as TG003, SM08502, and several benzobisthiazole-based compounds have been reported to inhibit CLK kinase activity with varying potency and selectivity profiles. For instance, benzobisthiazole scaffolds have demonstrated sub-micromolar inhibitory activity against CLK3, underscoring the feasibility of chemical modulation of this kinase (prak2016benzobisthiazolesrepresenta pages 14-18, prak2016benzobisthiazolesrepresenta pages 18-19). The therapeutic implications of such inhibitors are significant, as modulation of CLK3 activity has been associated with changes in alternative splicing that could be exploited in cancer therapy as well as in diseases linked to aberrant RNA processing. Furthermore, the specific role of CLK3 in endothelial cells—via the regulation of tissue factor splicing—suggests possible links to thrombotic disorders. Although direct clinical correlation remains under investigation, these findings have stimulated interest in further exploring CLK3 as a drug target (fedorov2011specificclkinhibitors pages 5-7, moyano2020cdclikekinases(clks) pages 28-30, song2023cdc2likekinasesstructure pages 15-16).
9. References
10. addova…2017novelclk1inhibitors pages 1-2
11. addova…2017novelclk1inhibitors pages 8-8
12. cabel2023identificationandcharacterization pages 31-36
13. fedorov2011specificclkinhibitors pages 1-2
14. fedorov2011specificclkinhibitors pages 4-5
15. fedorov2011specificclkinhibitors pages 5-7
16. funnell2017clkdependentexonrecognition pages 1-2
17. moyano2020cdclikekinases(clks) pages 1-3
18. moyano2020cdclikekinases(clks) pages 3-6
19. moyano2020cdclikekinases(clks) pages 19-23
20. moyano2020cdclikekinases(clks) pages 25-26
21. moyano2020cdclikekinases(clks) pages 28-30
22. moyano2020cdclikekinases(clks) pages 30-31
23. song2023cdc2likekinasesstructure pages 1-3
24. song2023cdc2likekinasesstructure pages 3-3
25. song2023cdc2likekinasesstructure pages 12-15
26. song2023cdc2likekinasesstructure pages 15-15
27. song2023cdc2likekinasesstructure pages 20-21
28. song2023cdc2likekinasesstructure pages 22-23
29. song2023cdc2likekinasesstructure pages 8-9
30. song2023cdc2likekinasesstructure pages 10-12
31. song2023cdc2likekinasesstructure pages 12-12
32. song2023cdc2likekinasesstructure pages 15-16
33. song2023cdc2likekinasesstructure pages 16-17
34. song2023cdc2likekinasesstructure pages 17-18
35. song2023cdc2likekinasesstructure pages 19-20
36. song2023cdc2likekinasesstructure pages 21-22
37. song2023cdc2likekinasesstructure pages 24-25
38. prak2016benzobisthiazolesrepresenta pages 1-5
39. prak2016benzobisthiazolesrepresenta pages 14-18
40. prak2016benzobisthiazolesrepresenta pages 18-19
41. huang2024insilicoidentification pages 5-7

References

1. (addova…2017novelclk1inhibitors pages 1-2): Novel CLK1 inhibitors based on N-aryloxazol-2-amine skeleton-A possible way to dual VEGFR2 TK/CLK ligands
2. (addova…2017novelclk1inhibitors pages 8-8): Novel CLK1 inhibitors based on N-aryloxazol-2-amine skeleton-A possible way to dual VEGFR2 TK/CLK ligands
3. (cabel2023identificationandcharacterization pages 31-36): CR Cabel. Identification and characterization of clk3 in the colonic epithelium as a regulator of the wnt pathway. Unknown journal, 2023.
4. (fedorov2011specificclkinhibitors pages 5-7): 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.
5. (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.
6. (moyano2020cdclikekinases(clks) pages 19-23): 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.
7. (moyano2020cdclikekinases(clks) pages 25-26): 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.
8. (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.
9. (song2023cdc2likekinasesstructure pages 12-15): 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.
10. (song2023cdc2likekinasesstructure pages 15-15): 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.
11. (song2023cdc2likekinasesstructure pages 20-21): 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.
12. (song2023cdc2likekinasesstructure pages 22-23): 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.
13. (song2023cdc2likekinasesstructure pages 3-3): 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.
14. (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.
15. (fedorov2011specificclkinhibitors pages 4-5): 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.
16. (funnell2017clkdependentexonrecognition pages 1-2): Tyler Funnell, S. Tasaki, A. Oloumi, Shinsuke Araki, Esther Kong, Damian Boon Siew Yap, Yusuke Nakayama, Christopher S. Hughes, S. G. Cheng, H. Tozaki, Misa Iwatani, S. Sasaki, T. Ohashi, T. Miyazaki, N. Morishita, D. Morishita, Mari Ogasawara-Shimizu, Momoko Ohori, Shoichi Nakao, Masatoshi Karashima, Masaya Sano, Aiko Murai, T. Nomura, N. Uchiyama, T. Kawamoto, R. Hara, O. Nakanishi, K. Shumansky, Jamie Rosner, A. Wan, S. McKinney, G. Morin, Atsushi Nakanishi, Sohrab P. Shah, Hiroyoshi Toyoshiba, and S. Aparicio. Clk-dependent exon recognition and conjoined gene formation revealed with a novel small molecule inhibitor. Nature Communications, Feb 2017. URL: https://doi.org/10.1038/s41467-016-0008-7, doi:10.1038/s41467-016-0008-7. This article has 101 citations and is from a highest quality peer-reviewed journal.
17. (huang2024insilicoidentification pages 5-7): Cheng‐Chiao Huang, Chia‐Ming Hsu, Min‐Wu Chao, Kai‐Cheng Hsu, Tony Eight Lin, Shih‐Chung Yen, Huang‐Ju Tu, and Shiow‐Lin Pan. In silico identification of a novel cdc2‐like kinase 2 (clk2) inhibitor in triple negative breast cancer. Protein Science, May 2024. URL: https://doi.org/10.1002/pro.5004, doi:10.1002/pro.5004. This article has 1 citations and is from a peer-reviewed journal.
18. (moyano2020cdclikekinases(clks) pages 28-30): 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.
19. (moyano2020cdclikekinases(clks) pages 3-6): 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.
20. (moyano2020cdclikekinases(clks) pages 30-31): 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.
21. (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.
22. (prak2016benzobisthiazolesrepresenta pages 14-18): 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.
23. (prak2016benzobisthiazolesrepresenta pages 18-19): 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.
24. (song2023cdc2likekinasesstructure pages 10-12): 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.
25. (song2023cdc2likekinasesstructure pages 12-12): 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.
26. (song2023cdc2likekinasesstructure pages 15-16): 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.
27. (song2023cdc2likekinasesstructure pages 16-17): 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.
28. (song2023cdc2likekinasesstructure pages 17-18): 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.
29. (song2023cdc2likekinasesstructure pages 19-20): 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.
30. (song2023cdc2likekinasesstructure pages 21-22): 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.
31. (song2023cdc2likekinasesstructure pages 24-25): 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.
32. (song2023cdc2likekinasesstructure pages 8-9): 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.