## Phylogeny

• Member of the CMGC kinase group and assigned to the CDC-like kinase / LAMMER subfamily characterized by the invariant EHLAMMERILG motif (bullock2009kinasedomaininsertions pages 3-5)  
• Human paralogs: CLK2, CLK3 and CLK4 share 67–93 % identity within the catalytic domain (lindberg2021dualspecificitytyrosinephosphorylationregulated pages 2-4)  
• Experimentally verified orthologs are present in Saccharomyces cerevisiae Clk1, Schizosaccharomyces pombe LAMMER kinase, Drosophila DOA, Arabidopsis AFC1-3, Nicotiana PK12, mouse and rat Clk1, underscoring broad eukaryotic conservation (rabinow2018clk pages 545-547)

## Reaction Catalyzed

ATP + [protein] ⇄ ADP + [protein]-P (lindberg2021dualspecificitytyrosinephosphorylationregulated pages 1-2)

## Cofactor Requirements

• Catalytic activity requires divalent Mg²⁺ as shown in in-vitro kinase assays performed with 10 mM MgCl₂ (walter2018molecularstructuresof pages 15-16)  
• Mn²⁺ can substitute for Mg²⁺ with comparable activity (melcher1996identificationandcharacterization pages 5-6)

## Substrate Specificity

• Prefers serine over threonine (~2-fold) and requires an arginine at position −3 within an R-x-x-S/T consensus motif (bullock2009kinasedomaininsertions pages 3-5)  
• Efficiently phosphorylates repetitive RS dipeptides in SR proteins (aubol2014nterminusofthe pages 1-3)  
• Uniquely accepts Ser-Pro motifs in addition to Arg-Ser sites, expanding substrate range beyond SRPK targets (aubol2014nterminusofthe pages 1-3)  
• Kinome-wide profiling confirms predominant recognition of Arg-Ser and Ser-Pro contexts within RS domains (song2023cdc2likekinasesstructure pages 17-18)

## Structure

• Domain organisation: intrinsically disordered N-terminal RS-rich segment (~aa 1–140) mediating substrate docking, followed by a bilobal kinase domain (~aa 141–484) (aubol2014nterminusofthe pages 1-3)  
• Catalytic domain contains a β-hairpin insertion, MAPK-like insertion and internally buried LAMMER sequence EHLAMMERILG that stabilises the activation segment (song2023cdc2likekinasesstructure pages 3-3)  
• Key motifs: Lys-72 / Glu-91 ion pair, HRD catalytic loop (His-319-Arg-320-Asp-321) and DFG motif (Asp-354-Phe-355-Gly-356) align for phosphotransfer (moyano2020cdclikekinases(clks) pages 3-6)  
• Crystal structure PDB 6I5H shows a narrow, negatively charged ATP pocket underpinning selectivity of ATP-competitive inhibitors (lee2019structuralbasisfor pages 1-2)  
• Additional structures (e.g., PDB 6FT8) reveal intact regulatory and catalytic spines and capture inhibitor binding modes (walter2018molecularstructuresof pages 15-16)  
• Activation loop harbours autophosphorylation sites that orient the αC-helix and complete the hydrophobic spine for full activity (lindberg2021dualspecificitytyrosinephosphorylationregulated pages 4-7)

## Regulation

• Autophosphorylates on Ser, Thr and Tyr residues within the activation segment to generate the active conformation (rabinow2018clk pages 545-547)  
• Activation-segment phospho-sites Ser341 and Thr342 modulate catalytic output and substrate recognition (unknownauthors2024characterizationofthermoregulatory pages 20-23)  
• AKT phosphorylates the N-terminal SR region at Ser36, Thr122 and Ser139, altering substrate engagement (song2023cdc2likekinasesstructure pages 3-6)  
• Enzyme activity is temperature-sensitive, reversibly decreasing at febrile temperatures and recovering at 35 °C (song2023cdc2likekinasesstructure pages 9-9)  
• The gene autoregulates its own mRNA by exon skipping and intron retention, coupling kinase levels to splicing feedback (lindberg2021dualspecificitytyrosinephosphorylationregulated pages 10-12)

## Function

• Predominantly nuclear kinase with expression peaking at the G2/M phase of the cell cycle (song2023cdc2likekinasesstructure pages 1-3)  
• Hyperphosphorylates SR splicing factors SRSF1–12, thereby promoting spliceosome assembly and alternative exon selection in hundreds of transcripts (song2023cdc2likekinasesstructure pages 1-3)  
• Directly phosphorylates SRSF10 at Ser129/Ser131/Ser133 and spliceosomal protein SPF45, influencing cell migration and invasion (song2023cdc2likekinasesstructure pages 3-6)  
• Phosphorylates Ser226 of U1-70K, facilitating its spliceosomal interactions (song2023cdc2likekinasesstructure pages 3-6)  
• Ser50 phosphorylation of the tyrosine phosphatase PTPN1 increases phosphatase activity, linking CLK1 to broader signalling networks (moeslein1999theclkfamily pages 1-2)  
• Acts as a host factor for influenza A virus by regulating viral RNA splicing through SRSF3 phosphorylation; inhibition suppresses viral replication (song2023cdc2likekinasesstructure pages 9-10)  
• Cooperates with hnRNP A1 to fine-tune exon recognition decisions during alternative splicing (song2023cdc2likekinasesstructure pages 6-7)

## Inhibitors

• TG003 – prototype ATP-competitive CLK1/4 inhibitor used to modulate SR-protein phosphorylation in cells (song2023cdc2likekinasesstructure pages 6-7)  
• TG693 – metabolically stable analogue retaining CLK selectivity and splicing modulation activity (song2023cdc2likekinasesstructure pages 6-7)  
• CX-4945 (Silmitasertib) – binds the ATP site; biochemical IC₅₀ ≈ 82 nM for CLK1 (lee2019structuralbasisfor pages 1-2)  
• KH-CB19 – low-nanomolar biochemical potency with demonstrated cellular activity (song2023cdc2likekinasesstructure pages 20-21)  
• SGC-CLK-1 – selective chemical probe; cellular IC₅₀ ≈ 165 nM against CLK1 with excellent kinome selectivity (moyano2020cdclikekinases(clks) pages 19-23)  
• Pyrido[3,4-g]quinazoline 9m – biochemical IC₅₀ = 18 nM for CLK1 (moyano2020cdclikekinases(clks) pages 19-23)  
• MU1210 – highly selective inhibitor, biochemical IC₅₀ = 8 nM and minimal off-target activity (moyano2020cdclikekinases(clks) pages 19-23)

## Other Comments

• Dysregulated CLK1-dependent splicing promotes tumorigenesis in pancreatic, gastric, colorectal, ovarian and breast cancers (song2023cdc2likekinasesstructure pages 10-12)  
• CLK1 inhibition facilitates exon 31 skipping in the dystrophin gene, a potential strategy for Duchenne muscular dystrophy therapy (lindberg2021dualspecificitytyrosinephosphorylationregulated pages 10-12)

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