## Phylogeny

• Member of the CMGC kinome, CLK/LAMMER subfamily, defined by the invariant EHLAMMERILG motif (moyano2020cdclikekinases(clks) pages 1-3).  
• One of four human paralogs (CLK1-4); CLK3 arose from duplication of a CLK2-like gene in the ancestor of lobe-finned fishes, making it vertebrate-restricted (ogle2024comparisonofthe pages 4-7).  
• Vertebrate orthologs reported in Mus musculus, Gallus gallus, Xenopus tropicalis and Danio rerio (ogle2024comparisonofthe pages 7-9).  
• LAMMER kinase orthologs exist throughout eukaryotes, including Drosophila DOA, yeast KNS1 and Arabidopsis AFC1-3 (rabinow2018clk pages 545-547).  
• No homologs detected in prokaryotes (ogle2024comparisonofthe pages 4-7).

## Reaction Catalyzed

ATP + [protein]-L-Ser/Thr/Tyr → ADP + [protein]-L-Ser/Thr/Tyr-phosphate (bullock2009kinasedomaininsertions pages 1-2).

## Cofactor Requirements

Mg²⁺ is required for ATP binding and catalysis (walter2018molecularstructuresof pages 15-16, moyano2020cdclikekinases(clks) pages 1-3).

## Substrate Specificity

• Prefers serine within an R-x-x-S consensus, with a compulsory Arg at –3 (rabinow2018clk pages 545-547).  
• Efficiently phosphorylates RS-repeat peptide GRSRSRSRSRSR (walter2018molecularstructuresof pages 15-16).  
• Helix-aH and β7/β8 hairpin insertions confer relaxed docking requirements compared with SRPK1 and MAPKs (bullock2009kinasedomaininsertions pages 1-2).

## Structure

• 638-residue protein with an intrinsically disordered N-terminus (~1-300) containing short SR-like motifs that promote substrate engagement (song2023cdc2likekinasesstructure pages 3-3).  
• C-terminal bilobed kinase domain solved in multiple structures (PDB 2EU9, 2EXE, 2WU7, 3RAW) (lindberg2021dualspecificitytyrosinephosphorylationregulated pages 4-7).  
• N-lobe features Lys248 at the ATP-site entrance (moyano2020cdclikekinases(clks) pages 3-6).  
• C-lobe carries MAPK-like insertion, extended β-hairpin (residues 440-462) and catalytic EHLAMMERILG segment (song2023cdc2likekinasesstructure pages 3-3).  
• Ala319 at the DFG-1 position enlarges the ATP pocket, reducing van-der-Waals contacts with inhibitors (kallen2018x‐raystructuresand pages 2-3, lee2019structuralbasisfor pages 7-9).  
• Helix-aH insertion and β7/β8 hairpin block canonical docking grooves (bullock2009kinasedomaininsertions pages 1-2).

## Regulation

• Undergoes Ser/Thr/Tyr autophosphorylation, altering nuclear localization and substrate selectivity (rabinow2018clk pages 545-547).  
• Catalytic activity increases at temperatures slightly below physiological norms (ogle2024comparisonofthe pages 1-4).  
• miR-144 binding to the 3′-UTR down-regulates CLK3 translation (song2023cdc2likekinasesstructure pages 12-15).

## Function

• Predominantly nuclear; redistributes to cytoplasmic stress granules in testes (moyano2020cdclikekinases(clks) pages 3-6).  
• Highly expressed in mature spermatozoa (bullock2009kinasedomaininsertions pages 1-2).  
• Essential for vertebrate neural development; knockdown in Xenopus tropicalis causes cranial defects and lethality (ogle2024comparisonofthe pages 7-9).  
• Phosphorylates SR splicing factors, including SRSF family members, regulating alternative splicing (moyano2020cdclikekinases(clks) pages 3-6, bullock2009kinasedomaininsertions pages 1-2).  
• Controls SRSF1-dependent HMGA2 exon skipping (song2023cdc2likekinasesstructure pages 3-3).  
• Over-expression stimulates Wnt3a transcription and Wnt/β-catenin signaling in cholangiocarcinoma and hepatocellular carcinoma, promoting proliferation, migration and invasion (song2023cdc2likekinasesstructure pages 12-15).  
• Q607R gain-of-function mutation enhances phosphorylation of USP13 at Tyr708, stabilizing c-Myc and up-regulating purine synthesis (song2023cdc2likekinasesstructure pages 12-15).

## Inhibitors

• SM08502: pan-CLK inhibitor, Ki ≈ 22 nM for CLK3; reduces SRSF phosphorylation and Wnt-related gene expression (song2023cdc2likekinasesstructure pages 15-15, moyano2020cdclikekinases(clks) pages 19-23).  
• CX-4945: ATP-competitive, IC₅₀ ≈ 90 nM; affinity limited by Ala319 in the ATP pocket (song2023cdc2likekinasesstructure pages 20-21, lee2019structuralbasisfor pages 1-2).  
• KH-CB19: IC₅₀ = 488 nM (song2023cdc2likekinasesstructure pages 20-21).  
• Benzobisthiazole scaffold compounds show selective biochemical inhibition (prak2016benzobisthiazolesrepresenta pages 1-5).  
• Leucettine L41: weak inhibitor, IC₅₀ > 10 µM (song2023cdc2likekinasesstructure pages 20-21).  
• MU1210 minimally inhibits CLK3, illustrating isoform selectivity (moyano2020cdclikekinases(clks) pages 19-23).

## Other Comments

• Up-regulation correlates with advanced stage and poor prognosis in colon adenocarcinoma and several other tumor types (song2023cdc2likekinasesstructure pages 15-15).  
• Q607R somatic mutation is recurrent in cholangiocarcinoma (song2023cdc2likekinasesstructure pages 12-15).  
• Gene retention across vertebrates and absence in prokaryotes underscore its essential eukaryotic role (ogle2024comparisonofthe pages 4-7).

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