## Phylogeny

CLK4 is a member of the CMGC group, CDC-like kinase (CLK) subfamily that in vertebrates comprises four paralogs (CLK1-4) (walter2018molecularstructuresof pages 1-2).  
Comparative genomics indicates CLK4 originated from a duplication of an ancestral CLK2 gene early in vertebrate evolution (ogle2024comparisonofthe pages 4-7).  
Orthologous proteins are retained in Mus musculus Clk4, Xenopus tropicalis Clk4, Danio rerio Clk4, Caenorhabditis elegans MADD-3, Drosophila melanogaster DOA, Arabidopsis thaliana AFC2 and Schizosaccharomyces pombe Lkh1, illustrating conservation across animals, plants and fungi (ogle2024comparisonofthe pages 22-25, haltenhof2020cdc2likekinasesrepresent pages 24-28, rabinow2018clk pages 545-547).  
CLKs share approximately 55 % sequence similarity with DYRK kinases and cluster next to DYRK2 within the CMGC clade (walter2018molecularstructuresof pages 1-2).

## Reaction Catalyzed

ATP + [protein]-Ser/Thr/Tyr → ADP + [protein]-O-phospho-Ser/Thr/Tyr (song2023cdc2likekinasesstructure pages 1-3).

## Cofactor Requirements

Catalytic activity requires divalent cations; in vitro assays employ 10 mM Mg²⁺ to support phosphorylation of RS-peptide substrates (walter2018molecularstructuresof pages 15-16).

## Substrate Specificity

CLK4 preferentially phosphorylates Arg-X-X-Ser/Thr motifs and shows highest efficiency toward Ser-Arg di-peptides in SR protein RS domains (song2023cdc2likekinasesstructure pages 1-3).  
The kinase also accepts Ser-Lys and Ser-Pro contexts, reflecting broader specificity than SRPK family members (haltenhof2020cdc2likekinasesrepresent pages 24-28).  
Validated cellular substrates include the spliceosomal SR proteins SRSF1 and SRSF3 (fedorov2011specificclkinhibitors pages 1-2).

## Structure

CLK4 contains an intrinsically disordered N-terminal RS domain that mediates substrate docking and nuclear speckle localisation, followed by a C-terminal bilobal kinase domain (song2023cdc2likekinasesstructure pages 3-3).  
The kinase domain harbours canonical VAIK (Lys191), HRD and DFG motifs together with the CLK-defining “EHLAMMERILG” sequence in the activation segment (kallen2018x‐raystructuresand pages 2-3, haltenhof2020cdc2likekinasesrepresent pages 24-28).  
Crystal structure PDB 7O4P shows CLK4 bound to CX-4945; Lys191 engages the inhibitor core and gatekeeper Phe243 provides π-stacking within a closed αC-helix conformation (kallen2018x‐raystructuresand pages 2-3, unknownauthorsUnknownyearpřípravanovýchsloučenin pages 24-28).  
A MAPK-like insertion and an extended β-hairpin create a shallow groove that accommodates RS repeats (haltenhof2020cdc2likekinasesrepresent pages 24-28).  
Structures of CLK1/3 with KH-CB19 reveal glycine-rich loop and αC-helix displacement, underscoring active-site plasticity conserved in CLK4 (fedorov2011specificclkinhibitors pages 1-2).  
AlphaFold models confirm high fold conservation across human CLK1-4 (ogle2024comparisonofthe pages 22-25).

## Regulation

CLK4 undergoes extensive Ser/Thr/Tyr autophosphorylation required for full catalytic competence (prak2016benzobisthiazolesrepresenta pages 1-5).  
Conserved AKT1 sites (Ser34, Thr127, Thr344 equivalents) are phosphorylated by AKT kinases (prak2016benzobisthiazolesrepresenta pages 1-5).  
Oxidation of Met307 inhibits kinase activity and lowers protein stability in esophageal squamous cell carcinoma (song2023cdc2likekinasesstructure pages 15-15).  
The enzyme is temperature-sensitive, displaying reduced activity at 38 °C and recovery at 35 °C, thereby modulating heat-responsive splicing (song2023cdc2likekinasesstructure pages 9-10).  
Autophosphorylation on distinct residues modulates substrate preference toward SRSF1 or SRSF2 (unknownauthors2024characterizationofthermoregulatory pages 20-23).

## Function

CLK4 localises predominantly to nuclear speckles via interactions with SR proteins (unknownauthors2011aninhibitorof pages 1-6).  
Protein expression is widespread in mammalian tissues, with nuclear and occasional cytoplasmic detection in HeLa cells (rabinow2018clk pages 548-550).  
Phosphorylation of SRSF1 and SRSF3 by CLK4 regulates alternative splicing of MAPT/Tau pre-mRNA (fedorov2011specificclkinhibitors pages 1-2).  
CLK4 is required for correct splicing of tissue factor (F3) pre-mRNA in endothelial cells (unknownauthors2011aninhibitorof pages 1-6).  
The kinase phosphorylates the SR-like protein CLASP, modulating exon 4 exclusion in CLK1 transcripts (rabinow2018clk pages 548-550).  
In cardiomyocytes, CLK4 phosphorylates Nexilin at Ser437, mitigating pathological hypertrophy (song2023cdc2likekinasesstructure pages 9-10).  
CLK4 phosphorylates MITF at Tyr360, promoting its autophagic degradation (song2023cdc2likekinasesstructure pages 15-15).  
Elevated CLK4 expression enhances epithelial-mesenchymal transition in mesenchymal-like triple-negative breast cancer cells (song2023cdc2likekinasesstructure pages 15-15).  
Promoter methylation and oxidative inhibition reduce CLK4 levels in esophageal squamous cell carcinoma, facilitating tumorigenesis (song2023cdc2likekinasesstructure pages 15-15).  
Global CLK inhibition diminishes HIV-1 Gag expression, indicating a supportive role for CLK4 in viral replication (song2023cdc2likekinasesstructure pages 6-6).

## Inhibitors

TG003 is a low-nanomolar ATP-competitive inhibitor of CLK4 but displays broad kinase promiscuity (unknownauthors2011aninhibitorof pages 1-6).  
KH-CB19 is an ATP-non-mimetic inhibitor selective for CLK1/CLK4 with IC₅₀ ≈ 20 nM (fedorov2011specificclkinhibitors pages 1-2).  
ML167 inhibits CLK4 with IC₅₀ ≈ 136 nM and >10-fold kinome selectivity (unknownauthors2011aninhibitorof pages 1-6).  
CX-4945 binds the ATP pocket of CLK4 in PDB 7O4P, conferring sub-micromolar potency (kallen2018x-raystructuresand pages 2-3).  
The indol-8-one KuWal151 selectively targets CLK1/2/4 while sparing DYRKs (walter2018molecularstructuresof pages 1-2).  
Additional nanomolar inhibitors include CLK1-IN-1 (IC₅₀ 8 nM) and KUWal151 (IC₅₀ 28 nM) (song2023cdc2likekinasesstructure pages 16-17).  
Leucettine L41 inhibits CLK4 together with DYRK family kinases (song2023cdc2likekinasesstructure pages 20-21).  
The multikinase agent CC-671 encompasses CLK4 in its inhibitory profile (song2023cdc2likekinasesstructure pages 21-22).

## Other Comments

Elevated CLK4 correlates with poor prognosis in triple-negative breast cancer (song2023cdc2likekinasesstructure pages 15-15).  
Met307 oxidation constitutes a redox-sensitive lesion that impairs kinase function in esophageal squamous cell carcinoma (song2023cdc2likekinasesstructure pages 15-15).  
Clk4 deficiency accelerates heart failure in murine models of cardiac hypertrophy (song2023cdc2likekinasesstructure pages 9-10).  
Lineage-specific loss of CLK4 in Neobatrachia amphibians shows the gene is dispensable in certain vertebrate clades (ogle2024comparisonofthe pages 7-9).

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