1. Phylogeny  
   Dual specificity protein kinase CLK2, also known as CDC‐like kinase 2, is a member of the CDC‐like kinase (CLK) family that comprises four human isoforms – CLK1, CLK2, CLK3, and CLK4 – all of which belong to the CMGC group of kinases. This family is evolutionarily conserved across eukaryotes and can be traced back to the common ancestral kinases present in metazoans; orthologs of CLK2 have been identified in all major mammalian species as well as in lower eukaryotes, confirming its ancient origin and critical regulatory role (moyano2020cdclikekinases(clks) pages 1-3, song2023cdc2likekinasesstructure pages 1-3). Phylogenetic analyses reveal that while all CLK family members share a common catalytic core, distinct sequence differences – particularly in regions dictating substrate recognition and inhibitor sensitivity – help differentiate CLK2 from its paralogs, such as CLK1 and CLK4, with CLK3 often exhibiting further divergence. In addition, comparative studies including those performed on apicomplexan kinases indicate lineage‐specific adaptations in substrate docking sites; however, the mammalian CLK2 maintains high conservation in its catalytic and regulatory domains, underscoring its essential role in spliceosomal control (talevich2011structuralandevolutionary pages 10-11).
2. Reaction Catalyzed  
   CLK2 catalyzes the phosphorylation of protein substrates by transferring a phosphate group from ATP to specific amino acid residues. The general reaction can be represented as follows:  
     ATP + [protein]–OH → ADP + [protein]–O–phosphate + H⁺  
   In this reaction, CLK2 utilizes ATP as the phosphate donor to modify serine, threonine, and, to a lesser extent, tyrosine residues on target proteins mainly involved in RNA splicing. This phosphorylation reaction is central to the regulation of the substrate’s function, as the addition of a phosphate group can induce changes in protein conformation, subcellular localization, and the ability to interact with other macromolecules (moyano2020cdclikekinases(clks) pages 19-23, song2023cdc2likekinasesstructure pages 1-3).
3. Cofactor Requirements  
   The kinase activity of CLK2 is dependent on the binding of ATP, which serves as the phosphate donor for the phosphorylation reaction. In addition to ATP, CLK2, like many serine/threonine kinases, requires divalent metal ions – most commonly Mg²⁺ – to facilitate ATP binding and stabilize the transition state during catalysis. Although specific experimental details for CLK2’s metal ion preference are not provided in every study, the requirement for Mg²⁺ is well established among kinases of the CMGC family (moyano2020cdclikekinases(clks) pages 8-10).
4. Substrate Specificity  
   CLK2 exhibits a marked substrate specificity for serine/arginine‐rich (SR) proteins, which are essential components of the spliceosome responsible for regulating alternative and constitutive pre‐mRNA splicing. The kinase phosphorylates residues located within RS dipeptide repeats present in these SR proteins, a modification that is crucial for modulating their activity, nuclear localization, and interaction with other spliceosomal components. Among the key substrates of CLK2 are SRSF1 and SRSF3, as well as other SR proteins that contain conserved RS domains. Beyond the classical splicing factors, CLK2 has also been reported to phosphorylate additional targets including the protein tyrosine phosphatase PTPN1 and PPP2R5B, which in turn is involved in forming a phosphatase complex with AKT1. Furthermore, CLK2 phosphorylates PAGE4 on several serine and threonine residues, thereby attenuating PAGE4’s ability to potentiate the transcriptional activator activity of JUN. The consensus phosphorylation motif for CLK2 is defined by the presence of arginine and serine residues within the target region, although subtle differences in substrate recognition may occur among the CLK isoforms (moyano2020cdclikekinases(clks) pages 16-19, song2023cdc2likekinasesstructure pages 3-3).
5. Structure  
   CLK2 contains a central catalytic domain that conforms to the canonical kinase fold characteristic of the CMGC family. This catalytic core is composed of two major lobes: an N-terminal lobe predominantly formed by beta strands and a C-terminal lobe that is largely helical. The two lobes are connected by a hinge region which forms part of the ATP-binding pocket. Crystallographic studies (for example, as exemplified by PDB entry 3NR9) have resolved the structure of CLK2 and reveal key structural features such as the DFG motif in the activation segment, which is essential for coordinating divalent metal ion binding and positioning ATP for catalysis (moyano2020cdclikekinases(clks) pages 25-26, song2023cdc2likekinasesstructure pages 3-3).

Unique to CLK2 and other CLK family members is an insertion within the kinase domain that contributes to substrate recognition – a feature that distinguishes the catalytic properties of CLK2 from those of related kinases like CLK3. Additionally, the activation loop – a flexible segment that undergoes phosphorylation – plays a pivotal role in modulating the kinase’s activity through conformational changes that either permit or restrict substrate access. The conserved catalytic residues, including those forming the hydrophobic spine and the C-helix, are crucial for maintaining the active conformation of CLK2. Moreover, regulatory regions outside the catalytic domain contribute to subcellular targeting and interactions with splicing factors; these regions include intrinsically disordered segments that facilitate transient binding to SR proteins and possibly determine the redistribution of these proteins from nuclear speckles to a diffuse nucleoplasmic localization upon phosphorylation (song2023cdc2likekinasesstructure pages 16-17, moyano2020cdclikekinases(clks) pages 6-8, song2023cdc2likekinasesstructure pages 23-24).

1. Regulation  
   The activity of CLK2 is modulated by multiple regulatory mechanisms, which include post-translational modifications and dynamic protein–protein interactions. Phosphorylation of the activation loop and other regulatory segments is a central mechanism controlling CLK2 activity. In particular, phosphorylation by upstream kinases such as AKT at specific residues (for example, serine 34 and threonine 127 as reported in some studies) is associated with enhanced CLK2 stability and activity, especially under conditions of cellular stress or DNA damage (moyano2020cdclikekinases(clks) pages 8-10, song2023cdc2likekinasesstructure pages 17-18).

In addition to phosphorylation mediated by external kinases, CLK2 also undergoes autophosphorylation, a process that can fine-tune its catalytic efficiency and substrate binding capability. This autophosphorylation may alter the conformation of the activation loop, thereby influencing the overall turnover rate of substrate phosphorylation. Furthermore, CLK2 phosphorylates regulatory proteins such as PPP2R5B; this phosphorylation event facilitates the assembly of the PP2A phosphatase complex with the PPP2R5B-AKT1 subunits, ultimately leading to downstream dephosphorylation of AKT1 and modulation of insulin signaling pathways. Expression levels of CLK2 are differentially regulated across various tissues, and its overexpression has been observed in several cancer cell lines, where it contributes to oncogenic signaling by promoting cell proliferation and resistance to apoptosis. The subcellular localization of CLK2 also varies in response to regulatory cues, with its redistribution from defined nuclear speckles to a more diffuse nucleoplasmic pattern correlating with alterations in splicing activity (moyano2020cdclikekinases(clks) pages 30-31, song2023cdc2likekinasesstructure pages 7-7).

1. Function  
   CLK2 is central to the regulation of pre‐mRNA splicing, exerting its effects by phosphorylating serine/arginine-rich (SR) splicing factors. This post‐translational modification is critical for modulating the assembly, activity, and subnuclear localization of spliceosomal complexes. In its role as a splicing regulator, CLK2 influences alternative splicing decisions that determine the diversity of the proteome. Altered CLK2 activity can lead to changes in splice site selection that impact the expression of genes involved in cell cycle regulation, apoptosis, and metabolism (moyano2020cdclikekinases(clks) pages 16-19, song2023cdc2likekinasesstructure pages 3-6).

Beyond its canonical role in RNA processing, CLK2 has significant functions in metabolic regulation. In hepatocytes, CLK2 acts as a suppressor of hepatic gluconeogenesis by repressing the transcriptional activity of PPARGC1A (PGC-1α) on genes that drive glucose production. This regulatory function is mediated by CLK2-induced phosphorylation events that ultimately reduce hepatic glucose output, thereby linking CLK2 activity with insulin sensitivity and overall metabolic homeostasis (moyano2020cdclikekinases(clks) pages 26-28, Information).

Additional substrates of CLK2 include proteins involved in signal transduction pathways. For example, the phosphorylation of PTPN1 and PAGE4 by CLK2 affects cellular stress responses and modulates transcription factor activity (such as JUN), which, in turn, impacts cell growth and survival. In endothelial cells, CLK2 regulates the alternative splicing of tissue factor (F3) pre-mRNA, thereby influencing coagulation pathways and vascular function. In cancer, overexpression of CLK2 has been linked to oncogenic splicing programs that promote cell proliferation, resistance to apoptosis, and enhanced migratory capabilities. These diverse functions underscore the importance of CLK2 not only as a key regulator of splicing but also as an integrative node in metabolic and signaling networks (moyano2020cdclikekinases(clks) pages 8-10, song2023cdc2likekinasesstructure pages 12-12).

1. Other Comments  
   Several small-molecule inhibitors have been developed to target CLK2 activity. Notable among these compounds are CX-4945 (Silmitasertib), TG003, T-025, CC-671, and SM08502; each of these inhibitors has been shown to modulate CLK2 function with varying degrees of selectivity and potency in both cellular and in vivo models (moyano2020cdclikekinases(clks) pages 23-25, song2023cdc2likekinasesstructure pages 15-16). Inhibitor studies have demonstrated that attenuation of CLK2 activity can lead to significant changes in alternative splicing patterns and reduced proliferation in cancer cell lines, supporting its candidacy as a therapeutic target in oncology as well as in metabolic disorders characterized by dysregulated gluconeogenesis.

Disease associations with CLK2 include its upregulation in several cancers such as breast cancer, non-small cell lung cancer, and others, where enhanced CLK2 activity correlates with aggressive tumor growth and resistance to conventional therapies. Beyond oncology, CLK2 has been implicated in metabolic disorders, as its role in suppressing hepatic gluconeogenesis directly impacts blood glucose levels and the pathogenesis of type 2 diabetes. Although specific disease mutations in CLK2 have not been extensively documented in the currently available peer-reviewed literature, altered expression and aberrant splicing regulation due to dysregulated CLK2 activity are recognized as contributing factors in the progression of various disease states (moyano2020cdclikekinases(clks) pages 8-10, moyano2020cdclikekinases(clks) pages 28-30, song2023cdc2likekinasesstructure pages 12-15).

Furthermore, CLK2’s interactions with other signaling molecules – for instance, its role in stimulating the PP2A phosphatase complex via phosphorylation of PPP2R5B – highlight its function in orchestrating broader cellular responses, including those related to insulin signaling and cell survival pathways. The breadth of CLK2’s regulatory influence and the availability of selective inhibitors underscore its potential as a target for therapeutic intervention in diseases where aberrant RNA splicing and metabolic dysregulation are central pathogenic mechanisms (moyano2020cdclikekinases(clks) pages 13-16, song2023cdc2likekinasesstructure pages 15-16).

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