## 1. Phylogeny

Serine/threonine-protein kinase DCLK2 belongs to the doublecortin‐like kinase (DCLK) family, which comprises several paralogs including DCLK1, DCLK2, and DCLK3. Within vertebrates, DCLK2 appears as a discrete clade emerging after the invertebrate–vertebrate split, as evidenced by phylogenetic analyses that separate vertebrate DCLK1 and DCLK2 into distinct lineages while also distinguishing them from the ancestral DCLK3 isoform (venkat2023mechanisticandevolutionary pages 4-5). As a member of the Ca²⁺/calmodulin-dependent kinase (CAMK) clan, DCLK2 is evolutionarily related to other kinases that share a conserved kinase domain architecture and regulatory features. Although most detailed studies have focused on DCLK1, the high sequence homology – especially within the catalytic domain and the autoinhibitory C-tail – supports the notion that DCLK2 shares a common evolutionary origin with its paralogs and is conserved across mammalian species (carli2023structureguidedpredictionof pages 8-9, venkat2023mechanisticandevolutionary pages 4-5).

## 2. Reaction Catalyzed

DCLK2, like other serine/threonine kinases, catalyzes the transfer of the γ‐phosphate group from ATP to a hydroxyl group of serine or threonine residues on substrate proteins. The generalized reaction can be represented as:  
  ATP + [protein]–(L‐Ser/L‐Thr) → ADP + [protein]–(L‐Ser/L‐Thr)‐phosphate + H⁺.  
In the case of DCLK2, there is a predicted functional role in attenuating cAMP response element (CRE)–dependent gene activation. By similarity with related kinases, it has been proposed that DCLK2 may phosphorylate the CREB coactivator CRTC2/TORC2, resulting in its cytoplasmic retention and down-regulation of CRE-dependent transcription (by similarity, Information section). Though experimental evidence for the exact physiological substrates of DCLK2 is not yet as extensive as for DCLK1, the conserved catalytic motifs within its kinase domain suggest that DCLK2 functions via a similar mechanism to control downstream signaling events (carli2023structureguidedpredictionof pages 15-16).

## 3. Cofactor Requirements

The catalytic activity of DCLK2 requires the binding of ATP, which is coordinated in part by divalent metal ions. In particular, like most serine/threonine kinases, DCLK2 is expected to depend on Mg²⁺ ions to facilitate the proper positioning of ATP for phosphate transfer (carli2023structureguidedpredictionof pages 6-8, patel2016biochemicalandstructural pages 3-4). Unlike many members of the Ca²⁺/calmodulin-dependent kinase (CaMK) family, DCLK2 exhibits a markedly reduced affinity and dependence on Ca²⁺/calmodulin, which implies that its activation and substrate phosphorylation are largely independent of the canonical Ca²⁺ signaling mechanisms (Information section).

## 4. Substrate Specificity

Direct experimental data on the substrate specificity of DCLK2 remain limited compared to the extensive studies available for DCLK1; however, inference by homology provides some insights. The kinase domain of DCLK2 retains the conserved catalytic elements of the CAMK family, including a glycine-rich loop, the DFG motif, and conserved residues responsible for substrate positioning (carli2023structureguidedpredictionof pages 4-6, patel2016biochemicalandstructural pages 3-4). Based on its overall domain organization and similarity to DCLK1, DCLK2 is proposed to phosphorylate serine/threonine residues on proteins involved in microtubule dynamics, neuronal migration, and signaling pathways. In addition, the information provided in the protein function description suggests that DCLK2 may target the CREB coactivator CRTC2/TORC2. This phosphorylation event could result in cytoplasmic retention of TORC2 and thus a down-regulation of CRE-dependent gene activation. Although a precise consensus motif for DCLK2 substrates has not been definitively mapped, the kinase likely recognizes target sequences similar to those of other CAMK family members, potentially favoring motifs with basic residues preceding the phosphorylated serine or threonine (venkat2023mechanisticandevolutionary pages 14-15). Such predictions require further experimental validation via phosphoproteomic studies and biochemical assays (carli2023structureguidedpredictionof pages 15-16).

## 5. Structure

DCLK2 exhibits a modular architecture that is typical of the doublecortin-like kinase family. Its domain organization consists of:  • N-terminal doublecortin (DCX) domains: These tandem domains are implicated in binding microtubules and regulating cytoskeletal dynamics. Although most structural data have been derived from DCLK1 and DCX, high sequence conservation suggests that the DCX domains in DCLK2 adopt similar ubiquitin-like folds and conformations that regulate microtubule binding (carli2023structureguidedpredictionof pages 6-8, carli2022thefunctionof pages 42-45).  
 • A PEST-rich linker region: This intrinsically disordered segment likely contributes to dynamic regulation, proteolytic cleavage, and subcellular localization. Sequence comparisons indicate that the PEST domain is moderately conserved between DCLK1 and DCLK2, supporting a similar regulatory function (carli2023structureguidedpredictionof pages 8-9, carli2022thefunctionof pages 42-45).  
 • C-terminal kinase domain: The conserved kinase domain harbors the typical bilobal structure with an N-terminal lobe rich in β-sheets and a C-terminal lobe primarily composed of α-helices. This domain contains canonical catalytic residues (DFG, HRD motifs, glycine-rich loop) and is responsible for ATP binding and phosphate transfer (patel2016biochemicalandstructural pages 3-4).  
 • Autoinhibitory C-tail: Vertebrate DCLK2 is noted to possess a relatively long C-terminal tail (~100 residues) compared to other family members such as DCLK3. This tail is thought to act as a pseudosubstrate by occluding the ATP-binding site and modulating kinase activity via autoinhibition. Structural studies using AlphaFold predictions and molecular dynamics simulations have suggested that discrete segments within the C-tail may dock into the catalytic cleft, thereby “supercharging” the regulatory output in an isoform-specific fashion (venkat2023mechanisticandevolutionary pages 4-5, venkat2023mechanisticandevolutionary pages 14-15).

## 6. Regulation

The regulatory mechanisms controlling DCLK2’s kinase activity are inferred predominantly from studies on DCLK1, given the significant domain conservation and homology. Key regulatory features include:  
 • Autophosphorylation: The kinase domain can undergo self-phosphorylation, which may affect its activity and stability. Specific phosphorylation events within the activation loop and the regulatory C-tail have been shown in DCLK1 isoforms; by extrapolation, similar mechanisms likely regulate DCLK2 (venkat2023mechanisticandevolutionary pages 14-15, carli2023structureguidedpredictionof pages 15-16).  
 • C-terminal autoinhibition: The extended C-tail of DCLK2 possesses segments that may mimic ATP binding and occlude the substrate-binding region, thus suppressing catalytic activity until displaced or modified. This regulatory mechanism has been described for DCLK1 and is considered conserved for DCLK2 (venkat2023mechanisticandevolutionary pages 4-5, venkat2023mechanisticandevolutionary pages 17-18).  
 • Reduced Ca²⁺/Calmodulin dependence: Unlike many kinases within the CaMK family, DCLK2 exhibits a significantly reduced affinity for Ca²⁺/calmodulin, resulting in a regulatory profile that is largely independent from fluctuations in intracellular Ca²⁺ levels (Information section, carli2023structureguidedpredictionof pages 8-9).  
 • Post-translational modifications: Although the specific modification sites on DCLK2 have not been mapped in detail, studies in DCLK1 reveal extensive phosphorylation in disordered regions (such as near the DCX domains and within the PEST linker) that modulate protein–protein interactions and microtubule binding. It is plausible that similar modifications occur in DCLK2 (carli2023structureguidedpredictionof pages 9-11).

## 7. Function

Based on the provided protein function description and comparative analyses with DCLK1, DCLK2 is predicted to play roles in both neuronal and non-neuronal signaling pathways. Specific functional annotations include:  
 • Regulation of CRE-dependent gene activation: DCLK2 is proposed to phosphorylate the CREB coactivator CRTC2/TORC2. The phosphorylation is thought to promote retention of TORC2 in the cytoplasm, thereby down-regulating CRE-dependent transcription. This mechanism suggests a role in controlling gene expression in response to cellular signaling (Information section).  
 • Modulation of microtubule dynamics: In common with other members of the DCLK family, the N-terminal DCX domains in DCLK2 likely interact with microtubules. This interaction is central to processes such as neuronal migration, dendrite formation, and intracellular transport, although the exact substrate repertoires and dynamics for DCLK2 are less comprehensively defined than for DCLK1 (carli2023structureguidedpredictionof pages 6-8, ramkumar2018remappingthemicrotubule pages 17-19).  
 • Potential involvement in neurodevelopment and signaling: Given that DCLK family kinases are traditionally associated with neuronal development and cytoskeletal remodeling, DCLK2 may be implicated in the regulation of neuronal architecture and signal transduction pathways in the brain (venkat2023mechanisticandevolutionary pages 1-2).  
 • Broader signaling and transcriptional regulation: By virtue of modulating CREB-related coactivators, DCLK2 may influence processes such as metabolism and stress response, further linking its activity to cell growth and survival pathways.  
While the precise physiological substrates and interacting partners of DCLK2 are not yet fully characterized, the conserved domain organization and catalytic motifs strongly suggest that its function mirrors key aspects of DCLK family kinases, with potential implications both in normal neurobiology and in pathological conditions where CRE-dependent gene regulation is disrupted (Information section, carli2023structureguidedpredictionof pages 15-16).

## 8. Other Comments

Recent chemical biology studies have primarily focused on DCLK1; however, inhibitors such as DCLK1-IN-1 have been shown to target both DCLK1 and DCLK2 with nanomolar potency (ferguson2020discoveryofa pages 1-2). This dual inhibition supports the notion that DCLK2 shares significant structural and functional features with DCLK1, despite its unique regulatory attributes such as reduced Ca²⁺/calmodulin dependence. Disease associations for DCLK family kinases have been documented in cancer and neurodegeneration, although most clinical data pertain to DCLK1; DCLK2’s specific role remains an area of active research. Notable mutations, detailed biochemical characterizations, and substrate mapping for DCLK2 are still forthcoming, and current efforts in structure-guided drug development and phosphoproteomic profiling are expected to further delineate its contribution to CRE-dependent gene regulation and cytoskeletal dynamics (venkat2023mechanisticandevolutionary pages 17-18, carli2023structureguidedpredictionof pages 15-16). Resources such as the Chemical Probes Portal and the MRC Kinase Inhibitor Database may provide additional insights as more selective chemical probes become available for the DCLK family.

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