## Phylogeny

CDKL5 is a serine/threonine protein kinase classified within the CMGC kinase group, which also includes cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAPKs), glycogen synthase kinases (GSKs), and Cdc2-like kinases (CLKs) (bergen2022cdkl5deficiencydisorder pages 1-2, bergen2022cdkl5deficiencydisorder pages 2-4). This classification is consistent with the kinome classification established by Manning et al. (bergen2022cdkl5deficiencydisorder pages 1-2, johnson2023anatlasof pages 4-4). The CDKL family comprises five members in humans (CDKL1-5) (martincarrascosa2025aphylogeneticanalysis pages 1-2). Phylogenetic analysis suggests an ancestral CDKL5-like kinase existed in the last eukaryotic common ancestor (LECA), and the initial split in the CDKL family is between the CDKL5 branch and the other CDKL proteins (martincarrascosa2025aphylogeneticanalysis pages 8-10). Orthologs are highly conserved and exist in key model organisms, including mouse (Mus musculus), zebrafish (Danio rerio), and Drosophila (bergen2022cdkl5deficiencydisorder pages 2-4, martincarrascosa2025aphylogeneticanalysis pages 1-2).

## Reaction Catalyzed

CDKL5 catalyzes the transfer of the gamma-phosphate group from ATP to the hydroxyl group of serine or threonine residues on substrate proteins (bergen2022cdkl5deficiencydisorder pages 1-2, martincarrascosa2025aphylogeneticanalysis pages 8-10, katayama2020cyclindependentkinaselike5 pages 1-2).

Protein + ATP → Phosphoprotein + ADP

## Cofactor Requirements

The kinase activity of CDKL5 requires ATP as a phosphate donor cofactor (bergen2022cdkl5deficiencydisorder pages 1-2, katayama2020cyclindependentkinaselike5 pages 1-2). The reaction also requires divalent metal ions, such as Mg²⁺ or Mn²⁺, as essential cofactors to facilitate ATP binding and catalysis (martincarrascosa2025aphylogeneticanalysis pages 8-10).

## Substrate Specificity

CDKL5 is a proline-directed kinase with a strong preference for a proline residue at the +1 position relative to the phosphorylation site (johnson2023anatlasof pages 4-4, medici2019newinsightinto pages 35-39). The experimentally defined consensus substrate motif is Arg-Pro-X-Ser/Thr-Ala/Pro/Gly/Ser (bergen2022cdkl5deficiencydisorder pages 4-5, katayama2020cyclindependentkinaselike5 pages 4-6). The amino acids immediately C-terminal to the phosphorylation site are typically small, uncharged residues like alanine, proline, glycine, or serine (bergen2022cdkl5deficiencydisorder pages 4-5).

## Structure

The CDKL5 protein is composed of an N-terminal catalytic kinase domain and a long C-terminal regulatory domain (bergen2022cdkl5deficiencydisorder pages 2-4, medici2019newinsightinto pages 25-30). The kinase domain (amino acids 1-297) contains the ATP-binding site and the serine/threonine kinase active site (unknownauthors2022regolazionedellespressionedi pages 46-50). Its structure includes key catalytic and regulatory features of eukaryotic kinases such as the C-helix, the catalytic spine, and the hydrophobic spine (bergen2022cdkl5deficiencydisorder pages 2-4). A Thr-Glu-Tyr (TEY) motif is located in the activation loop, which is essential for regulating kinase activity (bergen2022cdkl5deficiencydisorder pages 2-4). The C-terminal tail contains nuclear localization signals (NLS) and a nuclear export signal (NES) that mediate its subcellular localization (bergen2022cdkl5deficiencydisorder pages 2-4). Structural data are available from the crystal structure of the human kinase domain (PDB ID: 4BGQ) and from AlphaFold models (UniProt ID O76039) (bergen2022cdkl5deficiencydisorder pages 2-4, rout2019structuralbioinformaticsinsights pages 2-4).

## Regulation

CDKL5 activity is regulated by post-translational modifications, subcellular localization, and protein stability.

**Autophosphorylation:** CDKL5 self-regulates its kinase activity via autophosphorylation within its kinase domain (bergen2022cdkl5deficiencydisorder pages 2-4). Tyr175 and Ser178, located within the TEY activation loop, are critical autophosphorylation sites essential for modulating catalytic function (bergen2022cdkl5deficiencydisorder pages 2-4, rout2019structuralbioinformaticsinsights pages 1-2).

**Trans-phosphorylation:** DYRK1A kinase phosphorylates CDKL5 at Serine 308, which promotes its cytosolic retention (medici2019newinsightinto pages 30-32, unknownauthors2018novelcdkl5substrates pages 26-28).

**Subcellular Localization:** CDKL5 shuttles between the nucleus and cytoplasm, a process mediated by its NLS and NES motifs and regulated developmentally (bergen2022cdkl5deficiencydisorder pages 2-4, medici2019newinsightinto pages 30-32).

**Protein Degradation:** Prolonged activation of extrasynaptic NMDA receptors leads to CDKL5 dephosphorylation and degradation by the proteasome (unknownauthors2018novelcdkl5substrates pages 26-28).

## Function

**Expression:** CDKL5 is highly expressed in the brain, particularly in glutamatergic and GABAergic neurons of the cortex, hippocampus, and cerebellum, with low expression in glial cells (bergen2022cdkl5deficiencydisorder pages 1-2, medici2019newinsightinto pages 30-32, unknownauthors2021caratterizzazionediun pages 14-17). Its expression increases significantly after birth and remains high throughout adulthood (unknownauthors2024roleofneuroinflammation pages 22-26).

**Substrates and Interacting Partners:** CDKL5 phosphorylates numerous nuclear and cytoplasmic substrates. - **Nuclear Substrates:** MeCP2, DNMT1, HDAC4, SMAD3, SOX9, EP400, and TTDN1 (bergen2022cdkl5deficiencydisorder pages 12-14, zhu2019molecularandsynaptic pages 7-9, unknownauthors2021caratterizzazionediun pages 14-17). - **Cytoplasmic Substrates:** NGL-1, PSD-95, Amphiphysin 1 (Amph1), Shootin1, MAP1S, EB2, ARHGEF2, and CEP131 (unknownauthors2021caratterizzazionediun pages 14-17, unknownauthors2024roleofneuroinflammation pages 22-26, zhu2019molecularandsynaptic pages 7-9). - **Interacting Partners:** CDKL5 forms complexes with proteins such as IQGAP1 (bergen2022cdkl5deficiencydisorder pages 11-12).

**Signaling Pathways and Biological Roles:** CDKL5 is a critical regulator of neuronal development and function (bergen2022cdkl5deficiencydisorder pages 1-2). It is involved in transcription, RNA processing, dendritic morphology, synaptic plasticity, ciliogenesis, and cell cycle regulation (bergen2022cdkl5deficiencydisorder pages 1-2, bergen2022cdkl5deficiencydisorder pages 11-12, katayama2020cyclindependentkinaselike5 pages 1-2). CDKL5 influences the BDNF-Rac1, Akt/GSK3β, and Akt/mTOR signaling pathways (medici2019newinsightinto pages 30-32, zhu2019molecularandsynaptic pages 7-9).

## Other Comments

Pathogenic variants in the X-linked CDKL5 gene cause CDKL5 Deficiency Disorder (CDD), a severe neurodevelopmental encephalopathy (bergen2022cdkl5deficiencydisorder pages 1-2). Clinical features include early-onset, drug-resistant seizures (usually starting before 3 months of age), profound developmental delay, and motor impairments (bergen2022cdkl5deficiencydisorder pages 1-2). Most disease-causing mutations are de novo and result in a partial (hypomorphic) or total loss of kinase catalytic activity, disrupting the phosphorylation of its downstream targets (bergen2022cdkl5deficiencydisorder pages 1-2).

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