## Phylogeny

Cyclin-dependent kinase 9 (CDK9) belongs to the transcriptional CDK branch and is deeply conserved across eukaryotes, with orthologues in mammals, basal metazoans, fungi and several unicellular lineages—implying an origin that predates the last eukaryotic common ancestor (Cao et al., 2014; Gorman et al., 2020; Malumbres, 2014). Phylogenetic clustering places CDK9 closest to CDK12 and CDK13 and highlights its relationship to the yeast kinase Bur1, indicating conservation before the animal–yeast split (Paparidis et al., 2017; Napolitano et al., 2002).

## Reaction Catalyzed

ATP + [protein]-Ser/Thr → ADP + [protein]-Ser/Thr-phosphate + H⁺ (Adderley & Doerig, 2022).

## Cofactor Requirements

Mg²⁺ is required for catalysis (Baumli et al., 2008; Paparidis et al., 2017).

## Substrate Specificity

CDK9 preferentially phosphorylates Ser/Thr residues followed by Pro (S/T-P motif). A hydrophobic pocket in the catalytic cleft accommodates the +1 Pro, mirroring other CDKs, and additional recognition is provided by substrate structural elements or post-translational marks that bias the kinase toward transcription-associated targets such as the RNA polymerase II CTD (Echalier et al., 2010; Anshabo et al., 2021; Bacon & D’Orso, 2019).

## Structure

The 250–300 aa catalytic domain adopts the canonical bilobal kinase fold.  
• N-lobe: β-sheet–rich with an αC helix; binds cyclin T1 through the conserved PITALRE motif.  
• C-lobe: α-helix–rich; ATP binds in the inter-lobe cleft where hinge residues Asp104/Cys106 and the DFG-Asp167 coordinate nucleotide and Mg²⁺ (Baumli et al., 2008; Anshabo et al., 2021).  
Activation requires Thr186 phosphorylation (auto- or CDK7-mediated), which orders the activation loop and strengthens cyclin association (Mandal et al., 2021; Baumli et al., 2008). Compared with cell-cycle CDKs, the cyclin partner in P-TEFb is rotated, creating a smaller interface that may contribute to transcription-specific regulation (Bacon & D’Orso, 2019).

## Regulation

• T-loop phosphorylation on Thr186 is essential for full activity (Mandal et al., 2021; Anshabo et al., 2021).  
• Inactive sequestration: CDK9–cyclin T1 can be trapped in the 7SK snRNP with HEXIM1; release converts it to active P-TEFb (Adderley & Doerig, 2022; Brasier, 2008).  
• Additional modulators: phosphorylation at Ser175 and acetylation of cyclin T1 fine-tune complex assembly and substrate interactions (Anshabo et al., 2021).

## Function

As the catalytic core of P-TEFb, CDK9 phosphorylates Ser2 within the heptad repeats of the RNA polymerase II CTD, promoting the transition from initiation to productive elongation and recruiting RNA-processing and chromatin-remodelling factors (Adderley & Doerig, 2022; Napolitano et al., 2002). CDK9 also phosphorylates DSIF and NELF (relieving pausing), and non-CTD substrates such as EP300, MYOD1, and the androgen receptor, influencing cell growth, differentiation and viral transcription (Bacon & D’Orso, 2019; Anshabo et al., 2021). In complex with cyclin K, CDK9 supports genome stability after replication stress and couples elongation with histone modifications (Adderley & Doerig, 2022).

## Inhibitors

ATP-competitive inhibitors include flavopiridol, BAY 1251152, VIP152 and SNS-032; several agents are in clinical or pre-clinical evaluation for cancer therapy (Huang et al., 2022; Mandal et al., 2021; Boffo et al., 2018; Krystof et al., 2010).

## Other Comments

Aberrant or hijacked CDK9 activity is linked to oncogenesis, inflammatory disorders, cardiac hypertrophy and viral infections (e.g., HIV Tat-mediated recruitment of P-TEFb) (Bacon & D’Orso, 2019; Mandal et al., 2021). These disease associations underpin continued interest in selective CDK9 inhibition.

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