## Phylogeny

• Member of the CMGC group, transcriptional CDK subfamily, with sequence conservation of the activation-loop Thr186 across Homo sapiens, Mus musculus, Gallus gallus, Xenopus laevis and Caenorhabditis elegans (baumli2008thestructureof pages 8-10).  
• Functional orthologs include Bur1 in Saccharomyces cerevisiae, Cdk9/Pch1 in Schizosaccharomyces pombe, Drosophila Cdk9, Danio rerio cdk9 and Arabidopsis CDKC, all retaining the signature PITALRE motif (albert2014characterizationofmolecular pages 1-2).

## Reaction Catalyzed

ATP + [protein]-Ser/Thr → ADP + [protein]-O-phospho-Ser/Thr (baumli2008thestructureof pages 8-10).

## Cofactor Requirements

Requires divalent Mg²⁺ coordinated by the conserved Asp167 of the DFG motif for phosphotransfer (baumli2008thestructureof pages 8-10).

## Substrate Specificity

• Recognises Ser/Thr-Pro dipeptides embedded in the Y¹S²P³T⁴S⁵P⁶S⁷ heptad repeat of the RNA polymerase II CTD and in SPT5 C-terminal repeats (baumli2008thestructureof pages 8-10).  
• P-TEFb preferentially phosphorylates CTD substrates pre-modified at Ser7 and containing a Lys7 substitution, showing highest activity toward Ser5 and Ser2 positions depending on prior phosphorylation state (itzen2014brd4activatesptefb pages 7-8).

## Structure

• Bilobal kinase fold: N-lobe with Gly-rich loop and αC helix; C-lobe containing HRD and DFG catalytic motifs and a 20-residue activation loop centred on Thr186 (baumli2008thestructureof pages 8-10).  
• Crystal structure of the active CDK9–Cyclin T1 complex (PDB 3BLQ) reveals an atypical rotation of Cyclin T1 and an additional N-terminal helix (HN) that stabilises the interface (baumli2008thestructureof pages 8-10).  
• The C-terminal tail (residues 330-372) folds over the ATP pocket; Phe336 and Glu337 enforce an ordered mechanism in which ATP binds before substrate and ADP is released last (paparidis2017theemergingpicture pages 10-12).  
• Flavopiridol occupies the ATP site and anchors to the hinge in the inhibitor-bound structure, validating the druggable pocket (baumli2008thestructureof pages 12-12).

## Regulation

Post-translational modifications  
– Thr186 autophosphorylation is obligatory for catalytic competence; the T186A mutant abolishes CTD phosphorylation (baumli2008thestructureof pages 8-10).  
– Ser175 phosphorylation and Lys44 acetylation modulate kinase output and complex assembly, while PP1 and PP2A dephosphorylate the activation loop to down-regulate activity (albert2014characterizationofmolecular pages 1-2).

Complex control  
– Approximately half of cellular CDK9 is sequestered in an inactive 7SK snRNP with HEXIM1; Brd4 PID binds directly to CDK9, displaces HEXIM1 inhibition and doubles CTD kinase activity (itzen2014brd4activatesptefb pages 1-2).  
– HIV-1 Tat engages Cyclin T1 via an RxL and arginine-rich motif, further stimulating CDK9 toward CTD substrates (itzen2014brd4activatesptefb pages 7-8).

## Function

• Forms the core of Positive Transcription Elongation Factor-b (P-TEFb) with Cyclin T1/T2, phosphorylating POLR2A CTD, DSIF (SPT5) and NELF to release promoter-proximal pausing and drive productive elongation (albert2014characterizationofmolecular pages 1-2, itzen2014brd4activatesptefb pages 1-2).  
• Two isoforms generated from alternative promoters: CDK9p42 localises to nucleoplasm; CDK9p55, extended by 117 aa at the N-terminus, accumulates in nucleoli (mandal2021targetingcdk9for pages 2-4).  
• Essential for viability; inhibition diminishes transcription of short-lived anti-apoptotic transcripts and triggers apoptosis in tumour cells (albert2014characterizationofmolecular pages 1-2).

## Inhibitors

• Flavopiridol – broad-spectrum CDK inhibitor captured in complex with CDK9/Cyclin T1 at 2.8 Å resolution (baumli2008thestructureof pages 12-12).  
• LDC000067 – ATP-competitive compound with >30-fold selectivity for CDK9 versus other CDKs and nanomolar cellular potency (albert2014characterizationofmolecular pages 1-2).  
• Zotiraciclib – multi-kinase agent that suppresses CDK9-dependent CTD phosphorylation and induces cytotoxicity in glioblastoma models (ranjan2021targetingcdk9for pages 4-5).

## Other Comments

• Over-expression or hyper-activation supports survival of glioblastoma, leukaemia and solid tumours by sustaining MCL-1 and MYC transcription; pharmacological blockade prompts apoptosis and metabolic stress (ranjan2021targetingcdk9for pages 4-5).  
• Conservation of Thr186 across metazoans and plants underscores an ancient requirement for activation-loop phosphorylation in transcriptional CDKs (baumli2008thestructureof pages 8-10).

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