

Separate Domains of Fission Yeast Cdk9 (P-TEFb) Are Required for Capping Enzyme Recruitment and Primed (Ser7-Phosphorylated) Rpb1 Carboxyl-Terminal Domain Substrate Recognition

Courtney V. St. Amour,^{a,b} Miriam Sansó,^a Christian A. Böskén,^c Karen M. Lee,^a Stéphane Larochelle,^a Chao Zhang,^{d*} Kevan M. Shokat,^d Matthias Geyer,^c and Robert P. Fisher^a

Department of Structural and Chemical Biology, Mount Sinai School of Medicine, New York, New York, USA^a; Programs in Biochemistry, Cell and Molecular Biology, Weill-Cornell Graduate School of Medical Sciences, New York, New York, USA^b; Max-Planck-Institut für Molekulare Physiologie, Abteilung Physikalische Biochemie, Dortmund, Germany^c; and Department of Cellular and Molecular Pharmacology and HHMI, University of California, San Francisco, San Francisco, California, USA^d

In fission yeast, discrete steps in mRNA maturation and synthesis depend on a complex containing the 5'-cap methyltransferase Pcm1 and Cdk9, which phosphorylates the RNA polymerase II (Pol II) carboxyl-terminal domain (CTD) and the processivity factor Spt5 to promote transcript elongation. Here we show that a Cdk9 carboxyl-terminal extension, distinct from the catalytic domain, mediates binding to both Pcm1 and the Pol II CTD. Removal of this segment diminishes Cdk9/Pcm1 chromatin recruitment and Spt5 phosphorylation *in vivo* and leads to slow growth and hypersensitivity to cold temperature, nutrient limitation, and the IMP dehydrogenase inhibitor mycophenolic acid (MPA). These phenotypes, and the Spt5 phosphorylation defect, are suppressed by Pcm1 overproduction, suggesting that normal transcript elongation and gene expression depend on physical linkage between Cdk9 and Pcm1. The extension is dispensable, however, for recognition of CTD substrates "primed" by Mcs6 (Cdk7). On defined peptide substrates *in vitro*, Cdk9 prefers CTD repeats phosphorylated at Ser7 over unmodified repeats. *In vivo*, Ser7 phosphorylation depends on Mcs6 activity, suggesting a conserved mechanism, independent of chromatin recruitment, to order transcriptional CDK functions. Therefore, fission yeast Cdk9 comprises a catalytic domain sufficient for primed substrate recognition and a multivalent recruitment module that couples transcription with capping.

In eukaryotes, proper regulation of gene expression depends on coordination between transcription and maturation of mRNA. The largest subunit of RNA polymerase II (Pol II), Rpb1, contains a carboxyl-terminal domain (CTD) that integrates cotranscriptional processes. This domain consists of tandem repeats of the heptad sequence YSPTSPS and is a target for posttranslational modifications that direct binding of proteins involved in chromatin modification and mRNA processing (5, 42).

The Pol II CTD is phosphorylated on serine residues at positions 2, 5, and 7 (Ser2, Ser5, and Ser7) (6, 9, 41) and on threonine at position 4 (Thr4) (18), within the repeat unit. Multiple cyclin-dependent kinases (CDKs) collaborate to deposit these marks. Early in transcription, Ser5 phosphorylation (Ser5-P) predominates over Ser2-P; Cdk7, associated with metazoan transcription initiation factor IIH (TFIIH), seems to prefer Ser5 to Ser2 as a substrate *in vitro*, as do its orthologs, budding yeast Kin28 and fission yeast Mcs6 (12, 17, 35, 44, 45, 54). Inactivation of analog-sensitive (AS) Kin28 or Mcs6, each sensitized to inhibition by bulky adenine analogs with a mutation that enlarges the ATP-binding pocket, diminished total Ser5-P signals in their respective organisms, but other kinases are implicated in Ser5 phosphorylation in both species (20, 29, 55). In human cells, moreover, selective inhibition of AS Cdk7 did not cause a general reduction in Ser5-P but rather caused its specific loss on genes where occupancy by Cdk9, the catalytic subunit of positive transcription elongation factor b (P-TEFb), was low, suggesting that both TFIIH and P-TEFb contribute to Ser5-P levels *in vivo* (14, 28).

The Ser2-P/Ser5-P ratio typically increases as Pol II traverses the coding region. P-TEFb was thought to generate the bulk of Ser2-P in metazoans, but that assignment was challenged by the discovery of Cdk12 and Cdk13 as Ser2 kinases recruited during

elongation (3, 4). This additional complexity might harmonize yeast and metazoan kinase networks; both the fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae* contain essential orthologs of Cdk7 and Cdk9, but Ser2-P *in vivo* seems to be due mostly to the nonessential CDKs, Lsk1 in *S. pombe* and Ctk1 in *S. cerevisiae* (7, 21, 55). Ser7-P was first observed on Pol II transcribing both small nuclear RNA (snRNA) and protein-coding genes in mammalian cells (6, 9, 14), where loss of Ser7-P *in vivo* led to reduced snRNA levels (9). Ser7-P has since been detected in both budding and fission yeast as well (1, 23, 24, 32, 49, 53). Cdk7 and Kin28 have been implicated in generating this mark, but as in the case of Ser5, other kinases appear to contribute to Ser7-P levels *in vivo* (1, 14, 53). A Ser7 kinase—or a function for Ser7-P—has yet to be identified in fission yeast.

Although different CDKs seem to act in sequence to generate intragenic patterns of Pol II phosphorylation, it is in most cases unknown how that order is established. It was recently shown in both budding and fission yeast that inhibition of the TFIIH-associated kinase impaired recruitment of P-TEFb to chromatin (43, 55). *In vitro*, the binding of *S. cerevisiae* Bur1 (ortholog of Cdk9) to

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Address correspondence to Robert P. Fisher, robert.fisher@mssm.edu.

* Present address: Department of Chemistry, University of Southern California, Los Angeles, California, USA.

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synthetic CTD peptides was enhanced when the Ser5 position was phosphorylated (43), and prior phosphorylation of a CTD array by Mcs6 stimulated its subsequent phosphorylation by *S. pombe* Cdk9 (55). Both enhanced recruitment and substrate “priming” may impose temporal order on CDK action during the transcription cycle. However, it remains unclear if these are distinct mechanisms or if stronger binding between P-TEFb and a partially phosphorylated substrate is the sole basis for enhanced phosphorylation.

CDKs also regulate the elongation phase of transcription through the heterodimeric Spt4/Spt5 complex. In metazoans, this complex is the 5,6-dichloro-1- β -D-ribofuranosyl-benzimidazole (DRB) sensitivity-inducing factor (DSIF), which acts to pause transcription by recruiting a negative elongation factor (NELF) not present in yeasts. In archaea, the Spt4/5 homolog promotes transcription processivity by binding to the clamp region of RNA polymerase (15, 25, 31). In eukaryotes, Spt5 has a carboxyl-terminal region phosphorylated at sites embedded within repetitive sequences, similar to (but not as well conserved as) the Pol II CTD; phosphorylation by P-TEFb in this region converts mammalian DSIF from a pausing factor into a processivity factor (56, 58, 59). *S. pombe* Spt5 contains a carboxyl-terminal array of nonamer repeats with the consensus sequence TPAWNSGSK (39), which is phosphorylated by Cdk9 at the Thr1 position *in vitro* (38). Interactions with components of transcription and mRNA-capping machineries suggest that the CTD of Spt5 might function analogously to that of Pol II, as a protein-binding module important for orchestrating cotranscriptional events (39). Removal of this array was epistatic to chemical inhibition of Cdk9 (55), suggesting that, like its metazoan counterpart, fission yeast Cdk9 phosphorylates Spt5 to promote Pol II processivity.

Soon after initiation, nascent pre-mRNA is capped in three conserved steps: (i) the 5' γ -phosphate is cleaved by an RNA triphosphatase (Pct1 in *S. pombe*), (ii) GMP is added in inverted 5'-5' triphosphate linkage by a guanylyltransferase (Pce1), and (iii) the cap is methylated at the N⁷ position by a methyltransferase (Pcm1) (51). Capping enzymes bind CTDs of both Pol II (26, 36, 48) and Spt5 (39, 57); there is genetic evidence in *S. pombe* for functional redundancy between the two domains (47) and for capping-enzyme recruitment being the essential function of Rpb1 Ser5 phosphorylation (49). Although Pcm1 has not been shown to interact directly with Pol II or Spt5, we isolated it in a constitutive, apparently stoichiometric complex with Cdk9 and its cyclin partner Pch1 (35, 55). These interactions suggest a quality control mechanism to ensure that capped transcripts are efficiently elongated (37). Recruitment of Pct1 and Pce1 may be coupled to elongation complex assembly through associations with Pol II and Spt5 (37, 39). Pcm1, which performs the final step in 5'-end formation, is then recruited in complex with Cdk9, which phosphorylates Spt5 and Pol II to promote elongation of the fully capped transcript.

Here we show that a carboxyl-terminal region of *S. pombe* Cdk9, distinct from its kinase domain, is required to bind the Pol II CTD *in vitro* and to form complexes with Pcm1 both *in vivo* and *in vitro*. Although this portion of Cdk9 was dispensable for viability and kinase activity, a *cdk9 Δ C* strain lacking this segment grew poorly on both rich and minimal media and was cold sensitive and hypersensitive to mycophenolic acid (MPA), phenotypes consistent with a transcription elongation defect. Chromatin immunoprecipitation (ChIP) analysis revealed impaired recruitment of

Cdk9 and Pcm1 to transcribed genes, and immunoblotting showed decreased phosphorylation of Spt5 in *cdk9 Δ C* cells. Overexpression of Pcm1 partially rescued *cdk9 Δ C* phenotypes and the Spt5 phosphorylation defect, which are therefore likely to be due in part to impaired recruitment of the capping enzyme to chromatin. The carboxyl-terminal region of Cdk9 was not necessary for preferential phosphorylation of a Pol II CTD primed by Mcs6, however, suggesting that the ability to recognize a primed substrate resides in the conserved catalytic domain. Both Ser5-P and Ser7-P depended on Mcs6 activity *in vivo*; *in vitro*, prior phosphorylation of defined peptide substrates at Ser7 but not at Ser5 stimulated phosphorylation by Cdk9. Therefore, phosphates placed by TFIIH on Ser7 may mark the CTD for recognition by P-TEFb.

MATERIALS AND METHODS

General yeast methods. *S. pombe* cell culture, transformation, tetrad dissection, and sporulation were performed by standard methods (33). Cells were grown in yeast extract medium with supplements (YES), pombe glutamate medium with supplements, excluding leucine (PMG–Leu), or Edinburgh minimal medium (EMM) with supplements. MPA (Sigma) was dissolved in methanol and added to cooled media before they were poured on plates. Strains used in this study are listed in Table 1. Mutagenesis and tagging were done by homology-directed gene targeting (2). The *cdk9 Δ C* strain was constructed by deleting *cdk9*⁺ nucleotides 1327 to 1947. Analog-sensitive (AS) kinases were inhibited in appropriate strains by adding 1-(*tert*-butyl)-3-(3-methylbenzyl)-¹H-pyrazolo[3,4-*d*]pyrimidin-4-amine (3-MB-PP1) to mid-log-phase cultures to a final concentration of 40 μ M, and cells were collected after 30 min. Cell lysates were prepared as described previously (55).

Protein production. Purified wild-type and truncated Cdk9/His-Pch1 complexes were derived from baculovirus-infected Sf9 insect cells and isolated by Ni-nitrilotriacetic acid (NTA) affinity as described previously (35). Csk1, Cdk7, and human Cdk9 were purified using a similar protocol (27). Cdk9 and Mcs6 complexes were isolated from *S. pombe* cell lysates using tandem-affinity purification (TAP)-tagged complex members (Pch1-TAP and Pmh1-TAP, respectively) as described previously (55). Proteins were also generated and [³⁵S]methionine labeled using the TNT T7 quick coupled transcription/translation system (Promega). Maltose binding protein (MBP)-tagged Pcm1 was purified using amylose resin (New England BioLabs), and glutathione S-transferase (GST)–Rpb1 CTD and GST–Spt5 CTD from amino acids 801 to 990 (Spt5 CTD[801-990]) were purified from bacteria using glutathione-Sepharose (GE Healthcare), according to the manufacturers' protocols.

Binding assays. Cdk9, Cdk9 Δ C (consisting of amino acids 1 to 385), or Cdk9 Δ N (consisting of amino acids 386 to 591) was incubated for 1 h at 25°C with either MBP-Pcm1 or GST-Rpb1 CTD. Binding was measured by precipitating MBP-Pcm1 or GST-Rpb1 CTD using amylose resin (New England BioLabs) or glutathione-Sepharose (GE Healthcare), respectively. Coprecipitating Cdk9 was detected by kinase assay, immunoblot analysis, or autoradiography.

Kinase assays. Kinase assays were performed either in solution with purified Cdk9 or on beads using Cdk9 coprecipitated with the binding partner indicated in the figures. Recombinant Cdk9 was first activated by Csk1 (35). Kinases were incubated in reaction mixtures (10 mM HEPES [pH 7.4], 1 mM dithiothreitol [DTT], 10 mM MgCl₂ [or 2.5 mM MnCl₂], 100 μ M ATP, and [γ -³²P]ATP) with \sim 3 μ g of the substrate indicated in the figures at 25°C for 15 min. Reactions were stopped by boiling the mixtures in SDS, and products were visualized by SDS-PAGE followed by autoradiography and quantified with a phosphorimager.

CTD peptide phosphorylation assay. CTD peptides (Biosynton; 95% purity) consisted of three consensus repeats followed by a polyethylene glycol (PEG) spacer and two arginine residues (to enhance transfer in the radioactive filter binding assay): YSPTSPS YSPTSPS YSPTSPS-[PEG]₂-

TABLE 1 Strains used in this study

Strain	Genotype	Source
JS78	<i>leu1-32 ura4-D18 his3-D1 ade6-M210 h⁺</i>	Saiz and Fisher (45a)
CS208	<i>cdk9ΔC::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X h⁺</i>	This work
HD8-6	<i>cdk9^{T212A}::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X h⁺</i>	This work
JS207	<i>mcs6^{S165A}3HA::KanMX4 leu1-32 ura4-D18 his3-D1 ade6-M216 h⁻</i>	Pei et al. (35)
CS110	<i>spt5-13myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M216 h⁻</i>	This work
LV216	<i>cdk9ΔC::KanMX6 spt5-13myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X h?</i>	This work
LV231	<i>cdk9^{T212A}::KanMX6 spt5-13myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X h?</i>	This work
HD6-51	<i>cdk9-13myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h⁺</i>	Pei et al. (35)
CS217	<i>cdk9ΔC-13myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h⁺</i>	This work
CS143	<i>pcm1-3HA::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M216 h⁻</i>	This work
CS184	<i>pch1-2XTAP::KanMX6 pcm1-13 myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X h⁺</i>	This work
CS245	<i>cdk9ΔC::KanMX6 pch1-2XTAP::KanMX6 pcm1-13 myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X h?</i>	This work
CS316	<i>cdk9^{T212A}::KanMX6 pch1-2XTAP::KanMX6 pcm1-13 myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X h?</i>	This work
CS177	<i>pch1-2XTAP::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M216 h⁻</i>	This work
CS146	<i>pcm1-13myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h⁺</i>	Viladevall et al. (55)
CS172	<i>pch1-13myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M216 h⁻</i>	This work
CS145	<i>pcm1-13myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M216 h⁻</i>	This work
CS275	<i>cdk9ΔC::KanMX6 pch1-13myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X h?</i>	This work
CS278	<i>cdk9ΔC::KanMX6 pcm1-13myc::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X h?</i>	This work
CS80	<i>pmh1-2XTAP::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h⁺</i>	Viladevall et al. (55)
CS223	<i>cdk9ΔC::KanMX6 pch1-2XTAP::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X h⁻</i>	This work
LV77	<i>mcs6^{L87G}::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M216 h⁻</i>	Viladevall et al. (55)
LV7	<i>cdk9^{T120G}::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h⁺</i>	Viladevall et al. (55)
LV46	<i>mcs6^{L87G}::KanMX6 cdk9^{T120G}::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X h⁻</i>	This work
CS333	<i>leu1-32 ura4-D18 his3-D1 ade6-M210 + pREP81X (empty vector) h⁺</i>	This work
CS336	<i>leu1-32 ura4-D18 his3-D1 ade6-M210 + pREP81X (pcm1⁺) h⁺</i>	This work
CS334	<i>leu1-32 ura4-D18 his3-D1 ade6-M210 + pREP81X (pct1⁺) h⁺</i>	This work
CS346	<i>leu1-32 ura4-D18 his3-D1 ade6-M210 + pREP81X (cdk9⁺) h⁺</i>	This work
CS339	<i>cdk9ΔC::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X + pREP81X (empty vector) h⁺</i>	This work
CS342	<i>cdk9ΔC::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X + pREP81X (pcm1⁺) h⁺</i>	This work
CS340	<i>cdk9ΔC::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X + pREP81X (pct1⁺) h⁺</i>	This work
CS348	<i>cdk9ΔC::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X + pREP81X (cdk9⁺) h⁺</i>	This work
CS349	<i>cdk9^{T212A}::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X + pREP81X (empty vector) h⁺</i>	This work
CS350	<i>cdk9^{T212A}::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X + pREP81X (pcm1⁺) h⁺</i>	This work
CS352	<i>cdk9^{T212A}::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X + pREP81X (pct1⁺) h⁺</i>	This work
CS351	<i>cdk9^{T212A}::KanMX6 leu1-32 ura4-D18 his3-D1 ade6-M21X + pREP81X (cdk9⁺) h⁺</i>	This work

RR, uniformly phosphorylated on residue Ser2, Ser5, or Ser7. Kinase assays were performed as described above, with 2 μg of one of the peptides in a 20-μl reaction volume, and reactions were stopped after 2 h by addition of EDTA. Samples were spotted on Optitrans BA-S 85 nitrocellulose membranes, washed three times with 0.75% phosphoric acid, and air dried. Phosphate incorporation was measured by scintillation counting, and background signals (reaction mixture without a substrate or kinase) were subtracted.

Priming assays. In the first step, *S. pombe* GST-Rpb1 CTD was phosphorylated by Mcs6 complexes derived from fission yeast cell lysates (55) or *S. cerevisiae* GST-Rpb1 CTD was phosphorylated by 300 ng purified Cdk7/cyclin H/MAT1 for 2 h at 25°C. Reactions were stopped by the addition of 20 mM EDTA, and substrates were isolated by glutathione-Sepharose pulldown. Cdk9, either isolated from *S. pombe* cells or purified from insect cells, was then used to phosphorylate GST-Rpb1 CTD in a second step with added [γ-³²P]ATP. Reactions were stopped by boiling mixtures in SDS, and products were visualized by SDS-PAGE followed by autoradiography and quantified with a phosphorimager.

Immunological methods. Immunoprecipitation (IP) was performed with monoclonal antibody 9E10 or 16B12 (Covance) and protein G-Sepharose (GE Healthcare) for Pcm1-Myc or Cdk9-HA, respectively, or IgG-Sepharose (GE Healthcare) for Pch1-TAP. Immunoprecipitates were washed with 10 mM HEPES (pH 7.4), 150 mM NaCl, and 0.1% (vol/vol) Triton X-100. Additional antibodies used for immunoblotting include

anti-α-tubulin (T5168, clone B-5-1-2; Sigma), anti-GST (Z-5; Santa Cruz Biotechnology), anti-6×His (Covance), anticalmodulin binding protein for the CBP portion of the TAP tag (C16T; Millipore), anti-Spt5-P (to be described elsewhere), 8WG16 (unphosphorylated CTD; Covance), CTD Ser2-P (Abcam), CTD Ser5-P (Bethyl), and CTD Ser7-P (4E12; Millipore).

ChIP. Chromatin IP (ChIP) was performed as described previously (46). The specific primers amplifying the 5' end, middle, or 3' end from the coding regions of the genes corresponded to the following positions with respect to the translation initiation sites: +134 to +244, +1099 to +1201, and +2631 to +2748 of the *eng1⁺* gene and -80 to +21 and +975 to +1072 of the *upf1⁺* gene.

RESULTS

Cdk9 carboxyl-terminal segment required to bind Pcm1 and the Pol II CTD *in vitro*. *S. pombe* Cdk9 was identified based on a yeast two-hybrid interaction with Pct1, which required the carboxyl-terminal region of Cdk9 (38). To determine if this region is also required to bind Pcm1, we incubated purified complexes, derived from baculovirus-infected insect cells, of Pch1 and Cdk9—full length or truncated (Cdk9ΔC, consisting of amino acids 1 to 385)—with a purified maltose-binding protein (MBP)-Pcm1 fusion protein, isolated MBP-Pcm1 on amylose beads, and mea-

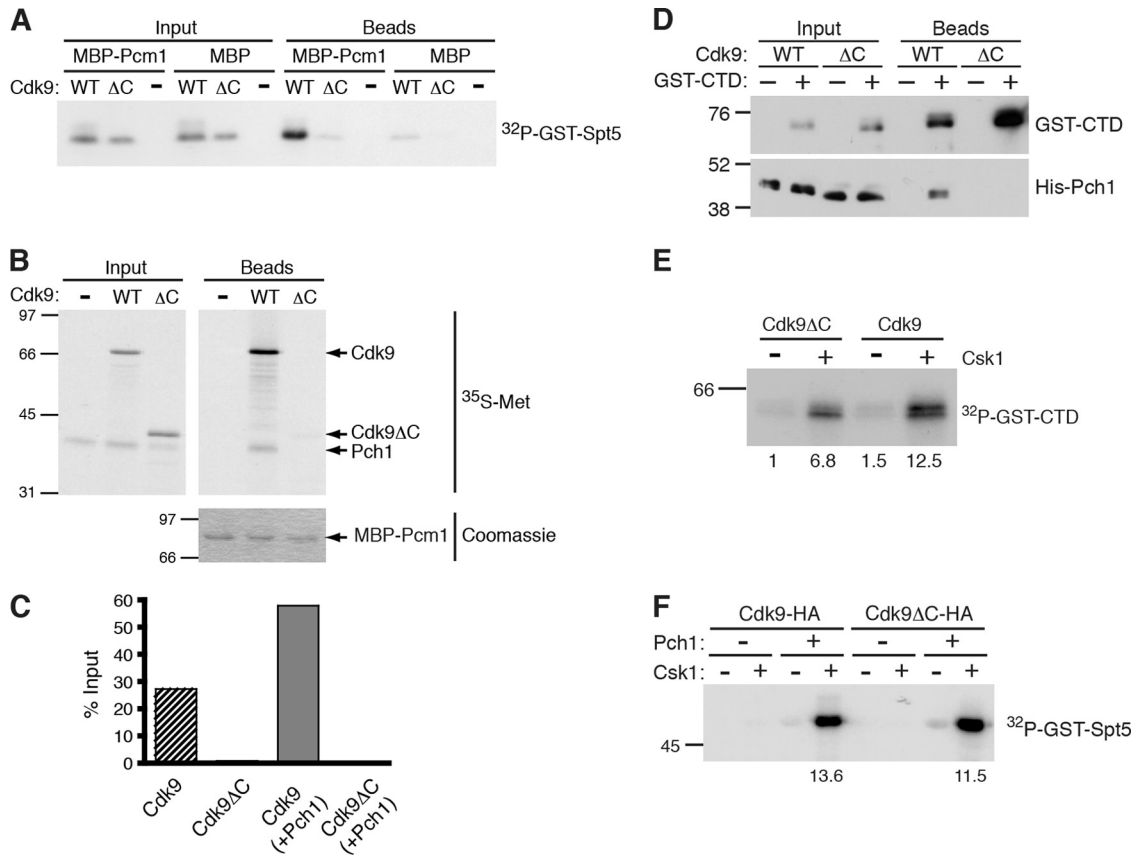


FIG 1 The Cdk9 carboxyl terminus is required to bind both Pcm1 and the Pol II CTD *in vitro*. (A) Purified Cdk9/His-Pch1 or Cdk9ΔC/His-Pch1 complexes were incubated with MBP-Pcm1 purified from bacteria (or MBP alone). Cdk9 activity associated with MBP-Pcm1 was detected by kinase assay after amylose pulldown, using GST-Spt5 CTD as the substrate. (B) Truncated or full-length Cdk9 and Pch1 were transcribed and translated in rabbit reticulocyte lysates in the presence of [³⁵S]methionine. The proteins were incubated with bacterially derived MBP-Pcm1. Binding of Cdk9 (full-length or ΔC) and Pch1 was detected by autoradiography after amylose pulldown of MBP-Pcm1. Coomassie blue staining shows that MBP-Pcm1 was pulled down in all cases. Numbers at the left are molecular masses (in kilodaltons). (C) Quantification of data from panel B and data not shown. Phosphorimager signals were adjusted to account for the relative number of methionine residues in each protein. (D) Purified Cdk9/His-Pch1 or Cdk9ΔC/His-Pch1 complexes were incubated with GST-CTD. The CTD was recovered on glutathione-agarose beads, and bound Pch1 was detected by immunoblot analysis of the 6×His tag. (E) Purified Cdk9 (full-length or ΔC)/Pch1 was activated by purified Csk1 (+) or mock treated (-) and incubated with GST-CTD and radiolabeled ATP. Kinase activity was measured by phosphorimaging. (F) Full-length and truncated Cdk9s tagged at their carboxyl termini with a hemagglutinin (HA) epitope were translated *in vitro*, with or without cotranslated Pch1, and immunoprecipitated with anti-HA antibodies. Kinase complexes were activated by purified Csk1 or mock treated, as indicated, and then incubated with GST-Spt5 and radiolabeled ATP to measure kinase activity. Results were quantified by phosphorimaging. WT, wild type.

sured precipitated kinase activity toward the Spt5 CTD (Fig. 1A). Although full-length and truncated Cdk9 had similar activities in the input samples and in more quantitative kinase assays (see below), active kinase coprecipitated with MBP-Pcm1 only when Cdk9 was full length. Therefore, productive binding of Pcm1 to a purified Cdk9/Pch1 complex requires the Cdk9 carboxyl-terminal segment.

To look directly at Cdk9-Pcm1 binding, we generated ³⁵S-labeled wild-type and truncated Cdk9, with or without Pch1, by translation *in vitro*. We incubated combinations of proteins with MBP-Pcm1 and detected binding by autoradiography after amylose pulldown. Although wild-type Cdk9 coprecipitated with MBP-Pcm1 in the presence or absence of Pch1, Cdk9ΔC was not pulled down efficiently under either condition (Fig. 1B and C). Pch1 alone was unable to bind MBP-Pcm1, but its presence increased the fraction of Cdk9 recovered by ~2-fold without affecting the amount of Cdk9 translated *in vitro* (Fig. 1C). There was no detectable binding of the isolated Cdk9 car-

boxyl-terminal segment to MBP-Pcm1 or of any *S. pombe* proteins to MBP alone (data not shown). We conclude that the Cdk9 carboxyl-terminal region is necessary but not sufficient for binding to Pcm1.

Binding of *S. cerevisiae* Bur1 to Pol II CTD peptides depended on the carboxyl terminus of Bur1 and was stimulated by Ser5 phosphorylation (43). We sought to determine the requirements for *S. pombe* Cdk9 to bind the Pol II CTD. We incubated purified Cdk9/Pch1 or Cdk9ΔC/Pch1 with a glutathione *S*-transferase-Rpb1-CTD fusion protein (GST-CTD), which we then recovered on glutathione-agarose; coprecipitating CDK complexes were detected with antibodies specific for a polyhistidine tag on Pch1. Pch1 associated with GST-CTD in reaction mixtures containing wild-type Cdk9 but not Cdk9ΔC (Fig. 1D). We did not detect an effect of prior CTD phosphorylation by Cdk7 or Mcs6 on this interaction (data not shown). These results nevertheless show that stable binding to both Pcm1 and the Pol II CTD requires an intact Cdk9 carboxyl-terminal segment.

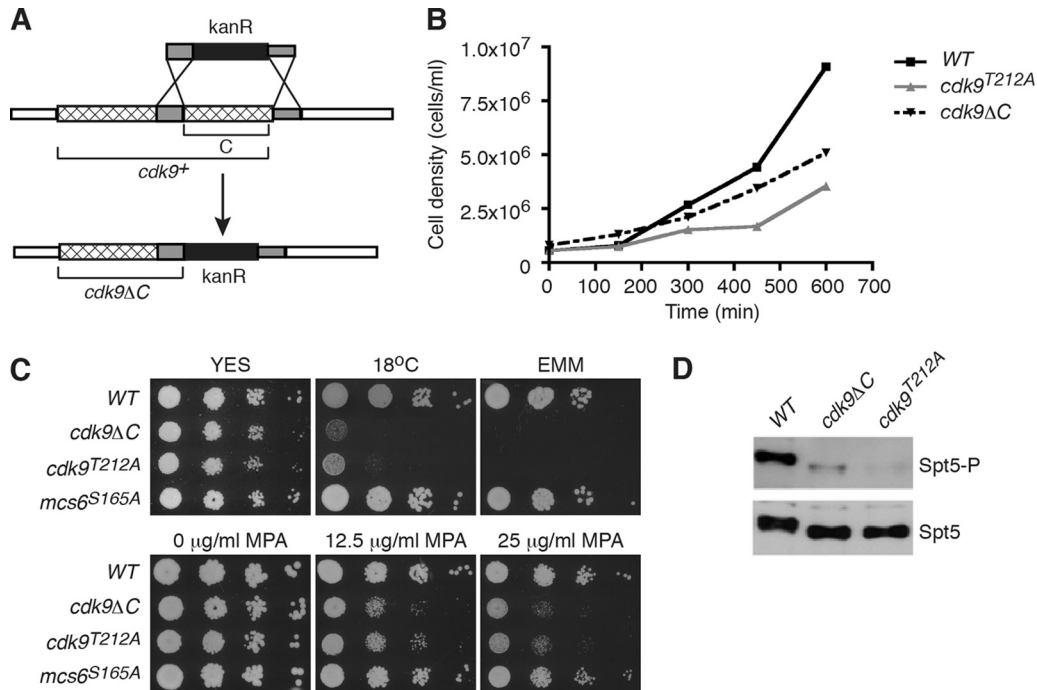


FIG 2 Cdk9 truncation impairs growth and transcript elongation *in vivo*. (A) The *cdk9ΔC* strain was constructed by transforming a wild-type diploid strain with a PCR product containing a kanamycin resistance marker flanked by regions of homology to the *cdk9⁺* genomic locus. Homologous recombination resulted in introduction of a stop codon and replacement of the region of *cdk9⁺* encoding the carboxyl terminus by the marker. The heterozygous diploid strain was sporulated to produce the *cdk9ΔC* haploid strain. (B) Growth curves were performed by growing cells in rich medium for 10 h. Cells were counted every 2.5 h. (C) Cultures were serially diluted 10-fold and spotted on plates of the indicated media. Plates were photographed after incubation for 2 days (YES), 3 days (MPA), 4 days (EMM), or 6 days (18°C). (D) Immunoblot analysis of lysates of the indicated strains. Total Spt5 was detected by an antibody recognizing its carboxyl-terminal Myc epitope tag. Phosphorylated Spt5 was visualized with an antibody raised against an Spt5 CTD peptide phosphorylated at the Thr1 position.

The Cdk9 carboxyl-terminal segment is dispensable for kinase activation. We next asked whether Cdk9 lacking the carboxyl-terminal segment could be fully activated by a CDK-activating kinase (CAK), as predicted by the similar basal activities of full-length Cdk9 and Cdk9ΔC (35). Cdk9ΔC/Pch1 complexes were preincubated with ATP, with or without the CAK Csk1, and then tested for activity toward GST-CTD (35). Both basal and Csk1-stimulated levels of Cdk9ΔC activity were similar (within 2-fold) to those of full-length wild-type kinase (Fig. 1E). We also compared the activities of kinase complexes generated by translation *in vitro* toward Spt5. By this measurement also, Cdk9 and Cdk9ΔC were stimulated to similar extents by Csk1 (Fig. 1F), indicating that the carboxyl-terminal segment is dispensable for normal kinase activation.

Cdk9 carboxyl-terminal truncation impairs growth and Spt5 phosphorylation. Cdk9ΔC was unable to interact stably with a partner protein and a substrate *in vitro*. To assess its function *in vivo*, we deleted nucleotides encoding the 206 carboxyl-terminal residues from one endogenous copy of the *cdk9* gene and replaced them with a termination codon linked to a kanamycin resistance marker in a diploid strain (Fig. 2A). Sporulation of the resulting heterozygote resulted in 2:2 segregation of kanamycin-resistant colonies, indicating that a *cdk9ΔC* haploid strain was viable (data not shown).

The *cdk9ΔC* mutant cells divided more slowly than did a *cdk9⁺* strain (doubling in ~3.7 and ~2.4 h, respectively) (Fig. 2B). By comparison, a *cdk9^{T212A}* strain, in which Cdk9 cannot be activated above basal levels by CAK, had a doubling time of ~3.9 h. We

previously observed that *cdk9^{T212A}* cells were hypersensitive to stress conditions, including growth on EMM and reduced temperature (35). A *cdk9ΔC* strain was also cold sensitive and grew poorly on EMM (Fig. 2C). In contrast, growth of *mcs6^{S165A}* cells, in which the Mcs6 activation loop is mutated to prevent phosphorylation by a CAK, was not impaired under either condition.

To obtain another measure of whether the Cdk9 carboxyl terminus is needed for normal gene expression, we tested *cdk9* mutants for sensitivity to MPA, a drug that inhibits IMP dehydrogenase and thus limits cellular GTP pools (10). MPA exacerbates growth impairment in mutants defective in transcript elongation (8, 11, 50), although the precise mechanism of this effect is unknown (34). Both *cdk9ΔC* and *cdk9^{T212A}* strains were hypersensitive to MPA, whereas *mcs6^{S165A}* cells had wild-type sensitivity to the drug (Fig. 2C).

Phosphorylation of Spt5 by P-TEFb promotes elongation in yeasts and metazoans (30, 47, 58). Although purified wild-type and truncated Cdk9 had similar activities *in vitro* (Fig. 1A), we reasoned that truncation might interfere with kinase targeting *in vivo*. To test this possibility, we measured levels of Spt5 phosphorylation with an antibody that specifically recognizes the Spt5 CTD nonapeptide repeat phosphorylated on the Thr1 residue (Spt5-T1P, to be described elsewhere). Spt5 phosphorylation was diminished in extracts from a *cdk9ΔC* strain, relative to that of a *cdk9⁺* strain (Fig. 2D, top panel). Spt5-T1P was also decreased, to a similar extent, by a *cdk9^{T212A}* mutation. Cdk9 truncation did not affect Spt5 expression, measured with antibodies to a Myc tag on endogenous Spt5 (Fig. 2D, bottom panel). Therefore, whereas

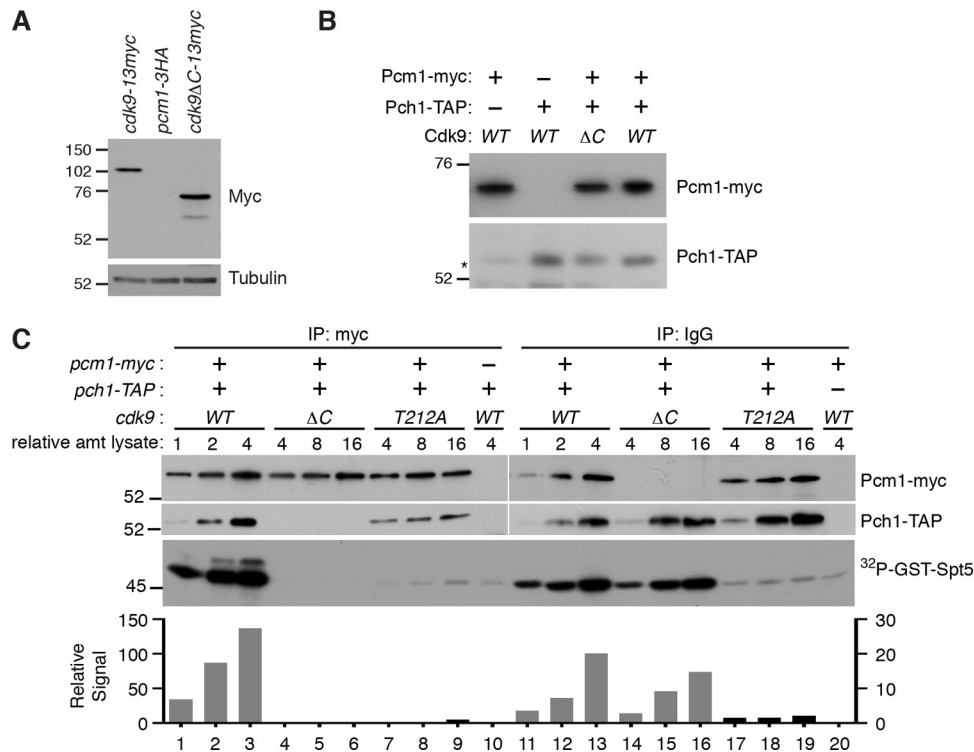


FIG 3 Cdk9 Δ C has normal kinase activity but cannot bind Pcm1 *in vivo*. (A) Lysates from the indicated tagged strains were probed with anti-Myc antibody to detect the expression of full-length and truncated Cdk9-Myc and with antitubulin antibody to ensure equal loadings. Numbers at the left are molecular masses (in kilodaltons). (B) Protein lysates from the indicated strains were subjected to immunoblot analysis to detect levels of Pcm1-Myc and Pch1-TAP. An asterisk denotes a background band running ahead of Pch1-TAP. (C) Lysates of the indicated strains were incubated with either anti-Myc-bound protein G beads (left) or IgG-agarose (right) to precipitate Pcm1-Myc or Pch1-TAP, respectively. Input amounts were normalized as indicated to give roughly equal amounts of recovered Pch1-TAP (75 μ g of lysate protein = 1) and thereby allow comparison of Cdk9 specific activities. Spt5 kinase assays were performed on immunoprecipitates, followed by immunoblot analysis to detect Pcm1 and Pch1 with anti-Myc and anti-CBP antibodies, respectively. Phosphorylation signals were visualized by autoradiography and quantified by phosphorimaging. Activity from strains harboring *cdk9*^{T212A} (lanes 7 to 9 and 17 to 19) is plotted on the y axis at the right, whereas signals from *cdk9*⁺ and *cdk9* Δ C cells (all other lanes) are plotted on the y axis at the left.

Cdk9 Δ C was fully active as an Spt5 kinase *in vitro*, levels of Spt5 phosphorylation were diminished in *cdk9* Δ C cells. This suggests that the carboxyl-terminal extension is required for proper targeting of Cdk9 to its substrates *in vivo*. Taken together, our results indicate that this segment of Cdk9, while not essential, might be important for efficient transcript elongation and normal gene expression.

Cdk9 Δ C has a Pcm1-binding defect *in vivo*. To ensure that *cdk9* Δ C mutant phenotypes and biochemical defects were not due to decreased Cdk9 expression, we measured levels of full-length and truncated Cdk9, both tagged at the carboxyl terminus and expressed from the endogenous promoter. A *cdk9* Δ C-13myc strain grew more slowly than did an untagged *cdk9* Δ C strain, indicating that the tag further interfered with mutant protein function (data not shown). For that reason, we did not use this strain for subsequent genetic or biochemical experiments; we were able to confirm, however, that full-length Cdk9-Myc and Cdk9 Δ C-Myc were expressed at similar levels (Fig. 3A).

We then asked if cellular Cdk9 Δ C also exhibited normal kinase activity. Importantly, truncation of Cdk9 did not affect expression levels of either Pch1 or Pcm1 (Fig. 3B). In cells expressing tandem-affinity purification (TAP)-tagged Pch1, endogenous Cdk9/Pch1-TAP complexes retaining or lacking the Cdk9 carboxyl-terminal segment had similar specific activities; when normalized to the

amount of Pch1-TAP, roughly equal amounts of activity were recovered in IgG pulldown assays with *cdk9*⁺ and *cdk9* Δ C extracts (Fig. 3C, compare lanes 11 to 13 to lanes 14 to 16). In contrast, Cdk9^{T212A} had diminished activity in *S. pombe* extracts (lanes 17 to 19), consistent with previous results (35).

Cdk9 Δ C failed to bind Pcm1 *in vitro* (Fig. 1B), so we looked for a similar defect *in vivo*. We performed reciprocal coprecipitations to detect an association between Pch1-TAP and Pcm1-Myc in the presence of either wild-type or mutant Cdk9. (Pch1 cannot directly bind Pcm1 [Fig. 1B].) The two proteins coprecipitated efficiently when cells expressed wild-type, but not truncated, Cdk9 (Fig. 3C, compare lanes 1 to 3 to lanes 4 to 6 and lanes 11 to 13 to lanes 14 to 16). The defect was specific to *cdk9* Δ C; Pch1-Pcm1 association was maintained in a *cdk9*^{T212A} strain (Fig. 3C, lanes 7 to 9 and 17 to 19). Similarly, kinase activity was recovered in Pcm1-containing complexes from *cdk9*⁺ but not *cdk9* Δ C cells. In contrast, Cdk9^{T212A} activity was detectable, but reduced relative to that of wild-type Cdk9, when Cdk9^{T212A} was coprecipitated with either Pch1 or Pcm1. Therefore, the carboxyl-terminal segment of Cdk9 is dispensable for kinase activity but required to bind Pcm1 *in vivo*, as it is *in vitro*.

Cdk9/Pch1/Pcm1 chromatin recruitment depends on the Cdk9 carboxyl terminus. Because the Cdk9 carboxyl-terminal segment also mediates Pol II CTD binding *in vitro*, we asked if it is

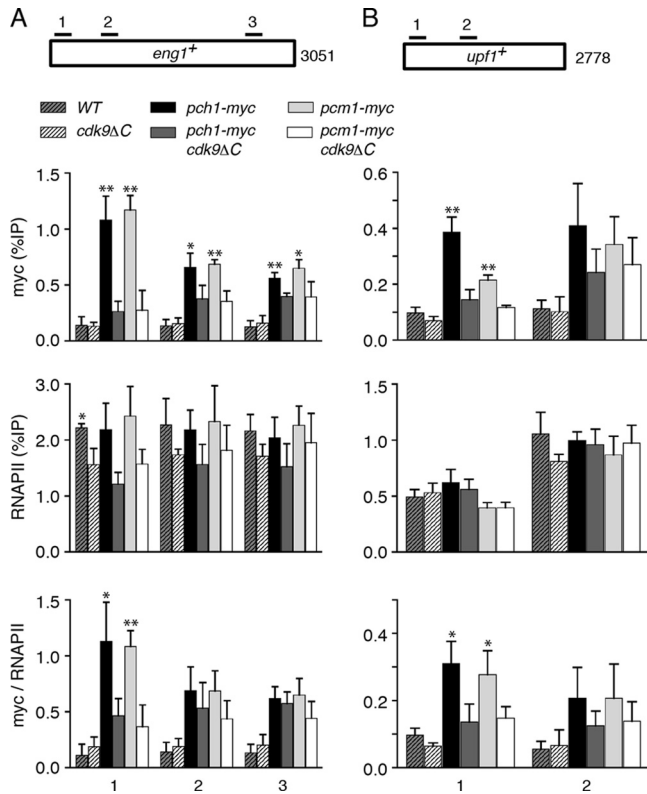


FIG 4 Chromatin recruitment of both Cdk9/Pch1 and Pcm1 is reduced in *cdk9ΔC* mutant cells. ChIP analysis was performed to monitor Cdk9/Pch1-Myc and Pcm1-Myc recruitment to *eng1*⁺ (A) and *upf1*⁺ (B) genes in the presence of full-length or truncated Cdk9 (untagged wild-type and *cdk9ΔC* strains served as controls). Occupancy was examined at the promoter region and within the gene body for both genes, as well as at the 3' end of *eng1*⁺, and is expressed as the percentage of input protein precipitated. In addition to Cdk9/Pch1 and Pcm1 recruitment (top graphs), cross-linking of Rpb1 was also measured using the 8WG16 antibody (middle graphs). The occupancy of Cdk9/Pch1 and Pcm1 normalized to Pol II is shown in the bottom graphs. Error bars were calculated using data from three independent experiments. Asterisks denote a significant difference between wild-type and mutant values (*, $P < 0.05$; **, $P < 0.01$; unpaired *t* test).

needed for proper recruitment of the Cdk9/Pch1/Pcm1 complex to chromatin. ChIP analysis at two Cdk9-dependent genes, *eng1*⁺ and *upf1*⁺ (55), revealed diminished Pch1 and Pcm1 recruitment in cells with truncated Cdk9 (Fig. 4A and B, top graphs). Cross-linking of Rpb1 was also decreased near the 5' end of *eng1*⁺ (Fig. 4A, middle graph). However, after normalization to Pol II, occupancy by both Pch1 and Pcm1 was decreased 2- to 3-fold at the 5' ends of *eng1*⁺ and *upf1*⁺, and these reductions were statistically significant (Fig. 4A and B, bottom graphs). Diminished Pch1 cross-linking is in agreement with decreased chromatin binding of Bur1 complexes in *bur1-Δ* budding yeast (22, 43). Although a previous report argued that chromatin recruitment of Pcm1 was Cdk9 independent in *S. pombe* (16), our results indicate impaired recruitment of both Cdk9/Pch1 and Pcm1 to promoter regions of *eng1*⁺ and *upf1*⁺ when physical linkage of the two enzymes is broken (see Discussion for possible explanations).

Pcm1 overexpression suppresses *cdk9ΔC* phenotypes. Loss of the physical association of Cdk9 and Pcm1 might thus disrupt coordination between transcription and mRNA capping. If that is the case, we reasoned that Pcm1 overexpression might restore

some capping function and suppress phenotypes due to a *cdk9ΔC* mutation. To test this prediction, we transformed wild-type and *cdk9ΔC* strains with multicopy plasmids encoding Pcm1, Pct1, or wild-type Cdk9. Ectopic expression of Pcm1 partially rescued the growth of a *cdk9ΔC* strain at low temperature, on EMM, and at two different concentrations of MPA (Fig. 5A and data not shown). In all cases, the growth of *cdk9ΔC* cells overexpressing Pcm1 was intermediate between that of cells harboring an empty vector or one encoding wild-type Cdk9 (Fig. 5A). In contrast, overexpression of the RNA triphosphatase Pct1 selectively impaired the growth of *cdk9ΔC* cells, implicating specific restoration of a function of the methyltransferase, rather than general hyperactivation of the capping pathway, as the mechanism of suppression.

Rescue was also *cdk9ΔC* specific; similar phenotypes due to *cdk9*^{T212A} were refractory to Pcm1 overexpression (Fig. 5B). This indicates that defects in *cdk9ΔC* cells are caused by impaired Pcm1 recruitment. Our results complement the recent observation that overexpression of Pct1 and Pce1 suppressed growth defects due to Spt5 truncation (47). Whereas the Spt5 CTD is important for recruiting Pct1 and Pce1, recruitment of Pcm1 to the transcription complex requires an intact Cdk9 carboxyl terminus. Taken together, both studies indicate that excess production of specific capping enzymes can correct defects due to mutations affecting specific elongation factors, such as Spt5 (47) or Cdk9 (this report). In support of this idea, Spt5-Thr1 phosphorylation, which was impaired in both *cdk9* mutants, was restored by overexpression of Pcm1 (but not Pct1) in *cdk9ΔC* but not *cdk9*^{T212A} mutant cells (Fig. 5C). Therefore, increased concentration of a specific capping enzyme can rescue a compromised proelongation function of truncated Cdk9.

Recognition of a primed CTD is an intrinsic property of the Cdk9 catalytic domain. We previously demonstrated that Cdk9 activity toward the Pol II CTD was stimulated when the substrate was first phosphorylated by Mcs6 (55). Stable binding of Cdk9 to the CTD suggested a possible mechanism: a partially phosphorylated CTD might tether Cdk9 through multiple rounds of catalysis. If that were the sole basis for the stimulatory effect, CTD binding, and thus the Cdk9 carboxyl-terminal region, would be required for priming. To test this requirement, we first incubated GST-CTD with unlabeled ATP in the presence or absence of Mcs6 complexes isolated from *S. pombe*. After recovering the substrate on beads, we radiolabeled it with Cdk9ΔC/Pch1-TAP complexes. Preincubation with Mcs6 stimulated phosphorylation by Cdk9ΔC ~6-fold relative to mock treatment (Fig. 6A), which is similar to the stimulation observed for wild-type Cdk9 complexes (55). Therefore, the Cdk9 carboxyl-terminal segment is not needed for preferential recognition of a primed CTD substrate.

Next, to ask if sensitivity to priming was an intrinsic property of the Cdk9/cyclin dimer, we tested the activity of Cdk9/Pch1 or Cdk9ΔC/Pch1 complexes purified from insect cells toward a Pol II CTD that was mock treated or prephosphorylated with human Cdk7 (Fig. 6B). Phosphorylation by full-length Cdk9 or Cdk9ΔC was stimulated to similar degrees by preincubation of the substrate with Cdk7. Therefore, priming does not depend on additional proteins that may copurify with Cdk9 from *S. pombe* lysates or on modifications of the Cdk9 complex that take place only in fission yeast cells. Taken together, our results suggest that a preference for CTD substrates primed by the TFIIF-associated kinase is an intrinsic property of the conserved Cdk9 catalytic domain.

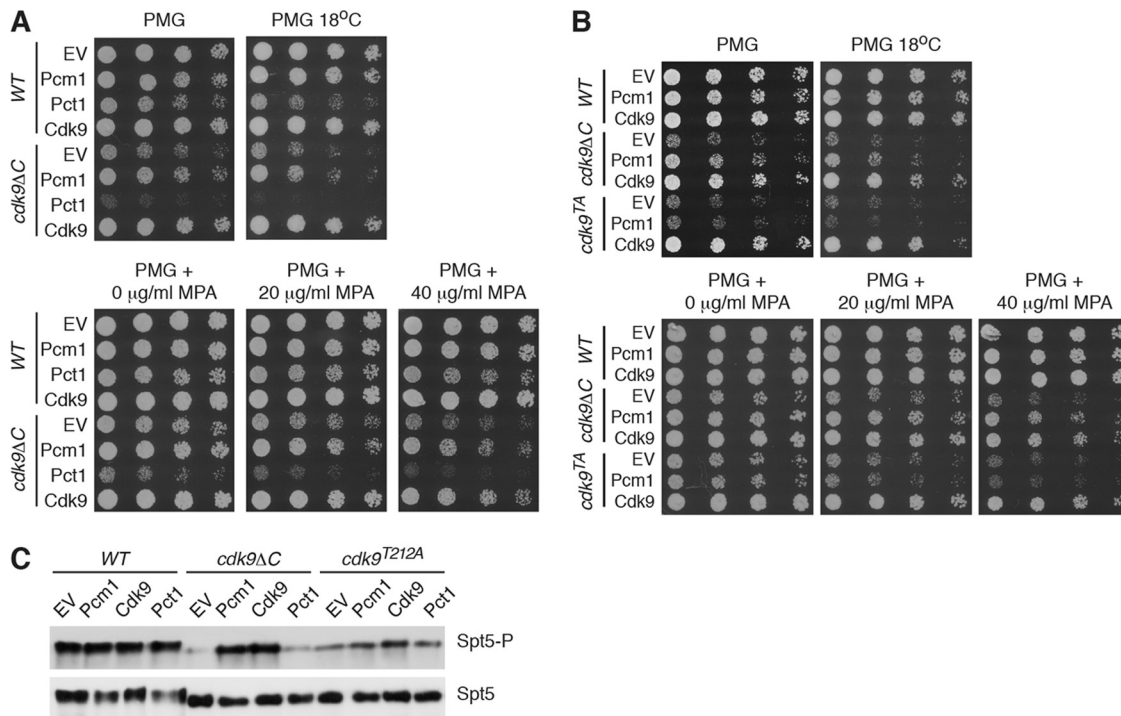


FIG 5 Overexpression of Pcm1 rescues *cdk9ΔC* phenotypes. (A, B) Wild-type or *cdk9* mutant strains were transformed with plasmids expressing the indicated proteins. Cultures were grown in the minimal medium PMG lacking leucine to select for the plasmid and serially diluted 3-fold before being spotted onto plates of the indicated medium. Plates were photographed after incubation for 3 days (PMG), 4 days (MPA), or 14 days (18°C). (B) *cdk9^{TA}* denotes the *cdk9^{T212A}* mutant strain. (C) Immunoblot analysis of lysates of the indicated transformed strains grown in PMG lacking leucine. Phosphorylated Spt5 was detected as described for Fig. 2D, and total Spt5 was visualized with an antibody that recognizes the Spt5 CTD irrespective of phosphorylation state. EV, empty vector.

Ser7-P is a potential determinant of priming. We next asked which feature(s) of a primed CTD Cdk9 might recognize. Mcs6 places Ser5-P marks, but its metazoan and *S. cerevisiae* orthologs also target Ser7 (1, 14, 24). We phosphorylated the Pol II CTD *in vitro* with Mcs6 or Cdk9 affinity purified from *S. pombe* lysates. Immunoblot analysis indicated that Mcs6 phosphorylates Ser2, Ser5, and Ser7, as did human Cdk7 (Fig. 6C). *S. pombe* Cdk9 also generated Ser2-P and Ser5-P signals but was relatively inefficient at phosphorylating Ser7. We next analyzed the requirements for specific CDKs in generating Ser7-P *in vivo* by selective inhibition of Mcs6, Cdk9, or both with the bulky adenine analog 3-MB-PP1 in the *mcs6^{ΔS}*, *cdk9^{ΔS}*, or *mcs6^{ΔS} cdk9^{ΔS}* mutant strain, respectively (55). Inhibition of Mcs6 alone or of both Mcs6 and Cdk9 reduced Ser7 phosphorylation, whereas inhibition of Cdk9 alone had little effect on Ser7-P levels *in vivo* (Fig. 6D). Cdk9 inhibition did cause a shift of all phosphorylated CTD signals to a faster-migrating form, however, consistent with a requirement for P-TEFb to deposit additional phosphates on Rpb1 molecules modified by Mcs6. This result suggests that a CTD-priming mechanism operates *in vivo*.

To ask which modification(s) of the Pol II CTD could directly stimulate phosphorylation by Cdk9, we performed kinase assays with purified recombinant Cdk9/Pch1 complexes (with full-length Cdk9 or Cdk9ΔC) and synthetic peptides containing three CTD repeats, either unmodified and invariant from the consensus sequence, YSPTSPS, or phosphorylated at each Ser2, Ser5, or Ser7 position. Prior phosphorylation at Ser7 enhanced phosphorylation by wild-type or truncated Cdk9 ~2-fold compared to that of an unmodified consensus peptide (Fig. 6E). In contrast, the same complex could not phosphorylate a peptide modified at all three

Ser5 positions above background levels, whereas Ser2-P inhibited but did not abolish phosphorylation by Cdk9. We observed a similar pattern—stimulation by Ser7-P, partial reduction by Ser2-P, and near-complete inhibition by Ser5-P—when we phosphorylated the same peptides with human Cdk9 in complexes with cyclin T1 (7a; data not shown). Therefore, priming phosphorylation of CTD Ser7 by the TFIIH-associated kinase may be a conserved mechanism to promote P-TEFb function.

DISCUSSION

The Cdk9 carboxyl-terminal extension couples transcription and capping. Cotranscriptional maturation of Pol II transcripts is thought to provide control over mRNA quality (13, 40), but the mechanisms ensuring this coordination are largely unknown. Here we have characterized a protein-protein interaction that couples 5'-end capping to the polymerase cycle. Whereas CTDs of Pol II and Spt5 might act redundantly to recruit the first two enzymes in the capping pathway (47), tight association between P-TEFb and the cap methyltransferase Pcm1 provides a unique mechanism to recruit the third and final enzyme.

Cdk9 lacking its carboxyl-terminal portion retained kinase activity but lost its interaction with Pcm1, whereas CAK-refractory Cdk9 had reduced activity but remained Pcm1 bound. Accordingly, Pcm1 overexpression suppressed conditional growth phenotypes and Spt5 phosphorylation impairment of *cdk9ΔC* cells but not similar defects due to a *cdk9^{T212A}* mutation. Stable binding of Cdk9 to the Pol II CTD likewise depends on the carboxyl-terminal region; loss of this interaction might explain reduced

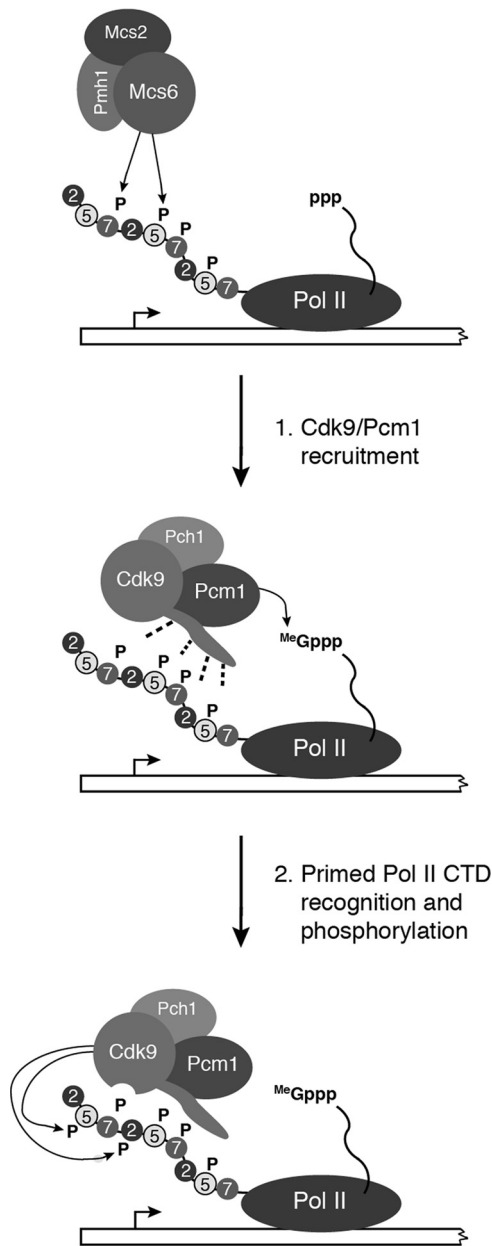


FIG 7 Sequential CDK action in transcription enforced by multiple mechanisms. The activity of the TFIIH-associated Mcs6/Mcs2/Pmh1 complex toward the Pol II CTD, and perhaps other substrates, promotes the subsequent function of the Cdk9/Pch1/Pcm1 complex (P-TEFb) through two distinct mechanisms. (i) The P-TEFb/Pcm1 complex is recruited dependent on both the carboxyl-terminal extension of Cdk9 and phosphorylations by Mcs6, possibly on Ser5, leading to completion of the mRNA 5'-cap structure. (ii) P-TEFb activity toward the CTD is stimulated by priming phosphorylation of Ser7, catalyzed by Mcs6 and recognized through direct interaction with the Cdk9 catalytic domain. Note that the inferred temporal order of P-TEFb recruitment (step 1) and stimulation (step 2) does not imply a kinetic difference in the rates of phosphorylation of Ser5 and Ser7 by Mcs6.

phatase Pct1, partially rescued phenotypes due to *cdk9ΔC*. Interestingly, Pct1 overexpression rescued similar growth and transcription phenotypes caused by mutation of the Spt5 CTD (47), indicating ligand specificity in the recruitment of different capping enzymes to the elongation complex.

Mechanism to order CDK functions in transcription independently of recruitment. Active Mcs6 promotes Cdk9 recruitment to chromatin and primes the Pol II CTD for subsequent phosphorylation by Cdk9 (55). Here we have shown that the two mechanisms can act independently: a portion of Cdk9 required for CTD binding *in vitro* is dispensable for priming. Because preference for a primed substrate resides in the conserved Cdk9 catalytic domain, rather than the yeast-specific carboxyl-terminal segment, metazoan P-TEFb might also be sensitive to priming. Consistently with this idea, fission yeast Cdk9 was stimulated to similar degrees when the CTD was preincubated with Mcs6 or human Cdk7, and human Cdk9 also preferred peptides containing Ser7-P (7a; data not shown). The effect of Ser7-P on CTD peptide phosphorylation by *S. pombe* Cdk9 was modest compared to the stimulation that we observed on the longer GST-CTD substrate. This may reflect amplification of the signal because of multiple independent priming events on the same molecule when more repeats are present. Moreover, because the peptides used in these studies were homogeneously phosphorylated in every repeat, it remains possible that Ser5-P also promotes phosphorylation within a different, unmodified repeat in the full-length CTD and thus contributes to priming on natural CTD arrays. Nevertheless, the data suggest a conserved role of Ser7-P in promoting P-TEFb activity.

Mcs6 phosphorylated Ser7 *in vitro* and was required for Ser7-P *in vivo*. Similarly, Cdk7 and Kin28 have been implicated in generating Ser7-P in their respective organisms (1, 14, 24), and levels of both Ser5-P and Ser7-P are high at the 5' ends of highly transcribed genes in *S. cerevisiae* (23, 32, 53). Based on results obtained in budding and fission yeast (43, 55), we propose a model in which the TFIIH-associated kinase phosphorylates Ser5 to promote the recruitment (43), and Ser7 to stimulate the activity, of P-TEFb (Fig. 7). The similar substrate preferences of human and fission yeast Cdk9 catalytic domains suggest that priming phosphorylation is a conserved mechanism to ensure that Pol II CTD kinases act in proper sequence. Moreover, the increase in the electrophoretic mobility of Ser7-P-containing Rpb1, with minimal loss of signal intensity, when Cdk9 was selectively inhibited suggests that this mechanism operates *in vivo*.

In summary, we have shown that *S. pombe* Cdk9 consists of two domains: an amino-terminal kinase domain necessary and sufficient for viability and recognition of a primed (Ser7-phosphorylated) Pol II CTD and a carboxyl-terminal protein-binding domain important for efficient substrate targeting, transcript elongation, and mRNA-capping enzyme recruitment. Growth defects and biochemical abnormalities of *cdk9ΔC* cells and their rescue by increased Pcm1 expression suggest that the union of these two enzymatic activities ensures coordination between elongation and capping. The preference of the conserved Cdk9 catalytic domain in fission yeast and metazoans for Ser7-modified CTD substrates, moreover, implies a conserved mechanism to impose order on the Pol II transcription cycle.

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