A Cdk9-PP1 kinase-phosphatase switch regulates the elongation-termination

transition of RNA polymerase II

Pabitra K. Parua[1], Gregory T. Booth[2], Miriam Sansó[1], Bradley Benjamin[1], Jason C.

Tanny[3], John T. Lis[2] and Robert P. Fisher[1*]

[1]Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai,

New York, NY, USA

[2]Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY,

USA

[3]Department of Pharmacology and Therapeutics, McGill University, Montreal,

Canada

*Corresponding author:

Robert P. Fisher, Department of Oncological Sciences, Box 1130, Icahn School of

Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029, USA.

Phone: (212) 659-8677. Email: robert.fisher@mssm.edu

The end of the RNA polymerase II (Pol II) transcription cycle is strictly regulated to ensure proper mRNA maturation and prevent interference between neighboring genes[1]. Pol II slowing downstream of the cleavage and polyadenylation signal (CPS) leads to recruitment of cleavage and polyadenylation factors and termination[2], but how this chain of events is initiated remains unclear. In a chemical-genetic screen, we identified protein phosphatase 1 (PP1) isoforms as substrates of human positive transcription elongation factor b (P-TEFb), the cyclin-dependent kinase 9 (Cdk9)-cyclin T1 complex[3]. Here we show that Cdk9 and PP1 govern phosphorylation of the conserved transcription factor Spt5 in the fission yeast *Schizosaccharomyces pombe*. Cdk9 phosphorylates both Spt5 and a negative regulatory site on the PP1 isoform Dis2[4]. Sites phosphorylated by Cdk9 in the Spt5 carboxy-terminal domain (CTD) are dephosphorylated by Dis2 *in vitro*, and Cdk9 inhibition *in vivo* leads to rapid Spt5 dephosphorylation that is retarded by concurrent Dis2 inactivation. Chromatin immunoprecipitation and sequencing (ChIP-seq) analysis indicates that Spt5 is dephosphorylated as transcription complexes traverse the CPS, prior to or concomitant with slowing of Pol II[5]. A Dis2-inactivating mutation stabilizes Spt5 phosphorylation (pSpt5) on chromatin, promotes transcription beyond the normal termination zone detected by precision run-on transcription and sequencing (PRO-seq)[6], and is suppressed by ablation of Cdk9 target sites in Spt5. These results support a model whereby the transition of Pol II from elongation to termination is regulated by opposing activities of Cdk9 and Dis2 towards their common substrate Spt5—a bistable switch analogous to a Cdk1-PP1 module that controls mitotic progression[4].

In metazoans and fission yeast, commitment to and exit from mitosis depend on inhibitory phosphorylation of PP1 by Cdk1 and its reversal, respectively[4]. In human

extracts, analogue-sensitive (AS) Cdk9 modifies two isoforms of PP1, PP1β and PP1γ,

on conserved, carboxy-terminal sites analogous to the PP1α residue labeled by Cdk1[3,7].

Of the two PP1 isoforms in fission yeast, Dis2 and Sds21, only Dis2 has the potential for

inhibition by CDKs through phosphorylation of its Thr316 residue (Fig. 1a)[8,9]. Purified *S.*

pombe Cdk9 phosphorylated Dis2 but not Sds21 *in vitro* (Fig. 1b); labeling was

diminished by a *T316A* mutation changing Thr316 to alanine, but not by a Dis2-

inactivating mutation[10]. Moreover, treatment of asynchronously growing *cdk9[as]* but not

cdk9^{+} cells with the bulky adenine analogue 3-MB-PP1, a selective inhibitor of AS

Cdk9[11], decreased Thr316 phosphorylation of chromatin-associated Dis2 (Fig. 1c),

indicating that Dis2 is indeed regulated by Cdk9 *in vivo*.

The previously identified target of fission yeast Cdk9 is Thr1 in the CTD

nonapeptide repeat $T_1P_2A_3W_4N_5S_6G_7S_8K_9$ of Spt5[12,13]. A phosphopeptide containing this

sequence was dephosphorylated by PP1s purified from bacteria (Fig. 1d) or isolated

from *S. pombe* (Fig. 1e, Extended Data Fig. 1). We recovered similar amounts of Dis2

by immunoprecipitation from *dis2[+]* and *dis2-11* cold-sensitive mutant cell extracts, but

detected activity only in the former, consistent with the previous observation that the

enzyme encoded by *dis2-11* has diminished activity even at permissive temperatures[10].

Finally, the amount of Dis2 we recovered from *sds21Δ* cells was similar to that from wild-

type or *dis2-11* cells, but its activity was reduced, possibly suggesting a contribution by

Sds21 to Dis2 activation. Together, these results indicate that Dis2 is a target of

negative regulation by Cdk9 and a potential Spt5 phosphatase—an arrangement that

predicts switch-like responses of pSpt5 to fluctuations in Cdk9 activity *in vivo* (Fig. 1f).

Consistent with this prediction, pSpt5 was rapidly lost after 3-MB-PP1 addition to

exponentially growing *cdk9[as]* cells ($T_{1/2} \approx 20$ sec) (Fig. 2a, and accompanying paper by

Booth *et al*.). A comparison of Spt5 dephosphorylation kinetics after Cdk9 inhibition in

dis2$^+$ versus *dis2-11* cells revealed an ~2-fold decrease in the dephosphorylation rate

due to the *dis2-11* mutation at a permissive temperature of 30°C, and an ~4-fold

decrease in *cdk9*^{as} *dis2-11* cells shifted to 18°C prior to 3-MB-PP1 addition (Fig. 2a,

Extended Data Fig. 2a). Rapid decay at 18°C was restored by ectopic expression of

active Dis2 in *dis2-11* cells (Fig. 2b). These results indicate that pSpt5 turnover is

dependent on Dis2 activity in vivo.

Inhibition of the Cdk12 orthologue Lsk1 by 3-MB-PP1 treatment of *lsk1*^{as} cells[11]

had no effect on pSpt5 (Extended Data Fig. 2b) but rapidly diminished phosphorylation

on Ser2 (pS2) of the Pol II CTD heptad repeat $Y_1S_2P_3T_4S_5P_6S_7$. This mark became

refractory to Lsk1 inhibition at 37°C in cells that harbored a temperature-sensitive

mutation in *fcp1*[14], which encodes a conserved pS2-specific phosphatase[15,16] (Fig. 2c).

The rate of pS2 decay in *lsk1*^{as} cells was unaffected by the *dis2-11* mutation (Extended

Data Fig. 2c) and, conversely, Fcp1 inactivation had no effect on pSpt5 stability in *cdk9*^{as}

strains (Extended Data Fig. 2d). Therefore, orthogonal CDK-phosphatase pairs govern

pS2 and pSpt5, possibly to allow independent regulation of the two modifications, both of

which are implicated in transcription elongation[17].

Dis2 also influences pSpt5 turnover on chromatin; by ChIP-quantitative PCR

(ChIP-qPCR) analysis, pSpt5 became nearly undetectable on *eng1*$^+$ and *aro1*$^+$ genes

within 2 min of 3-MB-PP1 addition to *cdk9*^{as} *dis2*$^+$ cells (Fig. 2d, Extended Data Fig. 3a,

b), but persisted in *cdk9*^{as} *dis2-11* cells at 18°C (Fig. 2e, Extended Data Fig. 3c, d). Cdk9

inhibition stimulated GFP-Dis2 recruitment to regions where pSpt5 was stabilized by

Dis2 inactivation (Fig. 2f). Dis2 chromatin association was also enhanced by *cdk9ΔC*,

which removes a carboxy-terminal region of Cdk9 needed for its efficient recruitment to

chromatin[18], and by *cdk9*^{T212A}, which prevents Cdk9-activating phosphorylation[19]

(Extended Data Fig. 4a). Therefore, Cdk9 restricts Dis2 recruitment to chromatin in

addition to its ability to inhibit Dis2 activity; both functions would promote switch-like

changes in pSpt5 levels on genes in response to changes in Cdk9 activity.

97   ChIP-qPCR analysis of *hxk2*[+] and *rps17a*[+] genes, in *cdk9*^{+} cells with different *dis2*

alleles at 30°C, revealed statistically significant increases in pSpt5 in the loss-of-function

mutants *dis2Δ*, *dis2-11* and *dis2*[T316D] (aspartic acid substitution to mimic constitutive

Thr316 phosphorylation[4]), relative to *dis2*^{+} cells and cells harboring a CDK-refractory,

dis2[T316A] allele (Fig. 2g, Extended Data Fig. 4b). Stabilization of pSpt5 was more

prominent in *dis2-11* cells shifted to 18°C, and occurred preferentially in regions

downstream of the CPS (compare Fig. 2h to 2g, Extended Data Fig. 4c to 4b). The

relative increases in chromatin-associated pSpt5 in *dis2-11* and *dis2Δ* cells at 30°C

correlated with the degree of bulk pSpt5 stabilization after Cdk9 inactivation (Fig. 2a,

Extended Data Fig. 5a). We suspect that *dis2-11* is more severely affected than *dis2Δ*

because loss of Dis2 protein allows more effective compensation by other phosphatases

such as Sds21. An *sds21Δ* mutation, however, did not delay pSpt5 decay, and Cdk9

inhibition did not increase chromatin recruitment of Sds21 (Extended Data Fig. 4d, 5b),

indicating that, in wild-type cells, Dis2 is the major PP1 isoform that regulates pSpt5 in

opposition to Cdk9.

112   In the accompanying paper, PRO-seq analysis uncovered a rate-limiting role for

Cdk9 in Pol II elongation (Booth *et al.*). That function is likely to depend on Spt5,

depletion of which slowed elongation and caused Pol II accumulation in upstream gene

regions in fission yeast[20], and disrupted coupling between 3'-processing and termination

in budding yeast[21]. In ChIP-seq analysis, we detected total Spt5 and pSpt5 in the bodies

of Pol II-transcribed genes (Fig. 3a), with the phosphorylated form accumulating further

downstream of the transcription start site (TSS) (Fig. 3b). The patterns diverged again

downstream of the CPS; a 3' peak of apparently unphosphorylated Spt5 is prominent in

metagene profiles (Fig. 3c) and individual gene tracks (Fig. 3d), and correlates with a

peak of paused, Ser2-phosphorylated Pol II detected by Winston and co-workers[20]

(Extended Data Fig. 6a-f).

The Spt5-Myc metagene profile features a V-shaped depression centered just

upstream of the CPS (Fig. 3c), which is also seen in the Pol II pattern derived from

published ChIP-seq data[20] (Extended Data Fig. 6c), and in a ChIP-seq analysis of Spt5

in budding yeast, where it corresponds to a peak of Spt5 cross-linking to the nascent

transcript[21]. Although exchange of phosphorylated for unphosphorylated Spt5 is

possible, Spt5's tight association with the Pol II clamp[22] favors active dephosphorylation

as a more likely explanation for the divergence between pSpt5 and Spt5-Myc occupancy

downstream of the CPS—the same region where pSpt5 was preferentially stabilized by

Dis2 inactivation. Moreover, Spt5 and Dis2 interact genetically; replacement of Thr1 with

alanine in a truncated, seven-repeat Spt5 CTD—$spt5\text{-}(T1A)_7$, which by itself imparts

cold-sensitivity[23]—partially suppressed cold-sensitive lethality due to $dis2\text{-}11$, whereas a

phosphomimetic $spt5\text{-}(T1E)_7$ mutation (which did not affect growth on its own[23])

exacerbated this phenotype (Fig. 3e, Extended Data Fig. 7).

We next performed PRO-seq analysis[6] to uncover effects of Dis2 inactivation on

the distribution of transcriptionally engaged Pol II (Fig. 4, Extended Data Fig. 8). On

individual genes, transcribing Pol II decreased dramatically within a narrowly defined

zone following the CPS in $dis2^+$ cells, but this zone extended ~500 bp further

downstream in $dis2\text{-}11$ cells at both 18°C and 30°C. Alignment of PRO-seq and ChIP-

seq read distributions suggested correlation between the zone of Dis2-dependent

termination and Spt5 dephosphorylation (Fig. 4a). Metagene analysis of PRO-seq data

(Fig. 4b, c, Extended Data Fig. 9a, b) revealed pleiotropic effects of the $dis2\text{-}11$

mutation: 1) attenuated pausing in promoter-proximal regions[5]; 2) decreased density of

transcribing Pol II throughout gene bodies; and 3) increased transcription beyond the

CPS, both in absolute terms and relative to transcription of upstream regions. All three

defects were apparent at both 18°C and 30°C, indicating that the effects of Dis2

inactivation on Pol II distribution are constitutive. Loss of viability occurs only at low

temperatures, however, and is likely due in part to inappropriate persistence of pSpt5

(Fig. 2a), as suggested by the partial rescue achieved with the *spt5-(T1A)₇* mutation (Fig.

3e).

To quantify termination defects due to Dis2 impairment, we defined two metrics

based on PRO-seq read distribution: Termination Index (T.I.), the ratio of signals in the

regions 500 bp downstream and upstream of the CPS; and Termination Elongation

Index (T.E.I.), the signal ratio in the regions 250-750 bp downstream and 500 bp

upstream of the CPS (Fig. 4d). The *dis2-11* mutation caused statistically significant

increases in T.I. in *cdk9⁺* cells, and in T.E.I. in both *cdk9⁺* and *cdk9ᵃˢ* backgrounds (Fig.

4e). A heatmap analysis of genes ranked by T.E.I. revealed termination-zone expansion

consistent with decreased termination efficiency upon loss of Dis2 function (Fig. 4f,

Extended Data Fig. 9c). In the accompanying paper, Booth *et al*. show that Cdk9

inhibition has the opposite effect—decreasing both T.I. and T.E.I.—consistent with

functional antagonism between the kinase and phosphatase.

Recent studies suggest an ordered series of events at 3'-ends of protein-coding

genes: 1) slowing of elongation, 2) increased pS2 as a consequence of this pause, 3)

cleavage and polyadenylation factor (CPF) recruitment to the pS2-containing CTD, 4)

CPF-dependent cleavage and 5) termination facilitated by the 5'→3' exoribonuclease

XRN2/Rat1[2,3,21,24,25]. This sequence can be initiated ectopically by a block to transcription

imposed *in cis*[2], and pS2 is elevated in 5' gene regions by mutations that decrease the

intrinsic elongation rate of Pol II[26], but a physiologic trigger remains unknown. Both Spt5

and PP1 are implicated in this transition—the former as a regulator of Pol II processivity

and rate[20,21,27], the latter as a CPF component[28,29]. We now show that Spt5 and the PP1

isoform Dis2 are both substrates of Cdk9, a positive regulator of elongation[30]. The

enzyme-substrate relationships we define among Cdk9, Dis2 and Spt5 recapitulate an

important cell-cycle regulatory module[4], and suggest a model of "transcriptional exit"

(Fig. 4g): Dis2-dependent Spt5 dephosphorylation upon reversal of a Cdk9-PP1 switch,

leading to slowing of Pol II to facilitate its capture and dissociation by XRN2/Rat1.

1   Proudfoot, N. J. Transcriptional termination in mammals: Stopping the RNA
polymerase II juggernaut. *Science* **352**, aad9926, doi:10.1126/science.aad9926
(2016).
2   Davidson, L., Muniz, L. & West, S. 3' end formation of pre-mRNA and
phosphorylation of Ser2 on the RNA polymerase II CTD are reciprocally coupled
in human cells. *Genes Dev* **28**, 342-356, doi:10.1101/gad.231274.113 (2014).
3   Sanso, M. *et al.* P-TEFb regulation of transcription termination factor Xrn2
revealed by a chemical genetic screen for Cdk9 substrates. *Genes Dev* **30**, 117-
131, doi:10.1101/gad.269589.115 (2016).
4   Grallert, A. *et al.* A PP1-PP2A phosphatase relay controls mitotic progression.
Nature **517**, 94-98, doi:10.1038/nature14019 (2015).
5   Booth, G. T., Wang, I. X., Cheung, V. G. & Lis, J. T. Divergence of a conserved
elongation factor and transcription regulation in budding and fission yeast.
Genome Res **26**, 799-811, doi:10.1101/gr.204578.116 (2016).
6   Kwak, H., Fuda, N. J., Core, L. J. & Lis, J. T. Precise maps of RNA polymerase
reveal how promoters direct initiation and pausing. *Science* **339**, 950-953,
doi:10.1126/science.1229386 (2013).
7   Blethrow, J. D., Glavy, J. S., Morgan, D. O. & Shokat, K. M. Covalent capture of
kinase-specific phosphopeptides reveals Cdk1-cyclin B substrates. *Proc Natl*
Acad Sci U S A **105**, 1442-1447 (2008).
8   Ohkura, H., Kinoshita, N., Miyatani, S., Toda, T. & Yanagida, M. The fission
yeast dis2+ gene required for chromosome disjoining encodes one of two
putative type 1 protein phosphatases. *Cell* **57**, 997-1007 (1989).
9   Yamano, H., Ishii, K. & Yanagida, M. Phosphorylation of dis2 protein
phosphatase at the C-terminal cdc2 consensus and its potential role in cell cycle
regulation. *EMBO J* **13**, 5310-5318 (1994).
10   Kinoshita, N., Ohkura, H. & Yanagida, M. Distinct, essential roles of type 1 and
2A protein phosphatases in the control of the fission yeast cell division cycle. *Cell*
63, 405-415 (1990).
11   Viladevall, L. *et al.* TFIIH and P-TEFb coordinate transcription with capping
enzyme recruitment at specific genes in fission yeast. *Mol Cell* **33**, 738-751
(2009).
12   Pei, Y. & Shuman, S. Characterization of the Schizosaccharomyces pombe
Cdk9/Pch1 protein kinase: Spt5 phosphorylation, autophosphorylation, and
mutational analysis. *J Biol Chem* **278**, 43346-43356 (2003).
13   Sansó, M. *et al.* A Positive Feedback Loop Links Opposing Functions of P-
TEFb/Cdk9 and Histone H2B Ubiquitylation to Regulate Transcript Elongation in
Fission Yeast. *PLoS Genet* **8**, e1002822 (2012).

216 14 Sajiki, K. *et al.* Genetic control of cellular quiescence in S. pombe. *J Cell Sci* **122**,
217   1418-1429, doi:10.1242/jcs.046466 (2009).
218 15 Cho, E. J., Kobor, M. S., Kim, M., Greenblatt, J. & Buratowski, S. Opposing
219   effects of Ctk1 kinase and Fcp1 phosphatase at Ser 2 of the RNA polymerase II
220   C-terminal domain. *Genes Dev* **15**, 3319-3329 (2001).
221 16 Hausmann, S. & Shuman, S. Characterization of the CTD phosphatase Fcp1
222   from fission yeast. Preferential dephosphorylation of serine 2 versus serine 5. *J*
223   *Biol Chem* **277**, 21213-21220 (2002).
224 17 Sansó, M. & Fisher, R. P. Pause, Play, Repeat: CDKs Push RNAP II's Buttons.
225   *Transcription* **4** (2013).
226 18 St Amour, C. V. *et al.* Separate Domains of Fission Yeast Cdk9 (P-TEFb) Are
227   Required for Capping Enzyme Recruitment and Primed (Ser7-Phosphorylated)
228   Rpb1 Carboxyl-Terminal Domain Substrate Recognition. *Mol Cell Biol* **32**, 2372-
229   2383 (2012).
230 19 Pei, Y. *et al.* Cyclin-dependent kinase 9 (Cdk9) of fission yeast is activated by the
231   CDK-activating kinase Csk1, overlaps functionally with the TFIIH-associated
232   kinase Mcs6, and associates with the mRNA cap methyltransferase Pcm1 in
233   vivo. *Mol Cell Biol* **26**, 777-788 (2006).
234 20 Shetty, A. *et al.* Spt5 Plays Vital Roles in the Control of Sense and Antisense
235   Transcription Elongation. *Mol Cell* **66**, 77-88 e75,
236   doi:10.1016/j.molcel.2017.02.023 (2017).
237 21 Baejen, C. *et al.* Genome-wide Analysis of RNA Polymerase II Termination at
238   Protein-Coding Genes. *Mol Cell* **66**, 38-49 e36, doi:10.1016/j.molcel.2017.02.009
239   (2017).
240 22 Bernecky, C., Herzog, F., Baumeister, W., Plitzko, J. M. & Cramer, P. Structure
241   of transcribing mammalian RNA polymerase II. *Nature* **529**, 551-554,
242   doi:10.1038/nature16482 (2016).
243 23 Schneider, S., Pei, Y., Shuman, S. & Schwer, B. Separable functions of the
244   fission yeast Spt5 carboxyl-terminal domain (CTD) in capping enzyme binding
245   and transcription elongation overlap with those of the RNA polymerase II CTD.
246   *Mol Cell Biol* **30**, 2353-2364 (2010).
247 24 Fong, N. *et al.* Effects of Transcription Elongation Rate and Xrn2 Exonuclease
248   Activity on RNA Polymerase II Termination Suggest Widespread Kinetic
249   Competition. *Mol Cell* **60**, 256-267, doi:10.1016/j.molcel.2015.09.026 (2015).
250 25 Glover-Cutter, K., Kim, S., Espinosa, J. & Bentley, D. L. RNA polymerase II
251   pauses and associates with pre-mRNA processing factors at both ends of genes.
252   *Nat Struct Mol Biol* **15**, 71-78 (2008).
253 26 Fong, N., Saldi, T., Sheridan, R. M., Cortazar, M. A. & Bentley, D. L. RNA Pol II
254   Dynamics Modulate Co-transcriptional Chromatin Modification, CTD
255   Phosphorylation, and Transcriptional Direction. *Mol Cell*,
256   doi:10.1016/j.molcel.2017.04.016 (2017).
257 27 Yamada, T. *et al.* P-TEFb-mediated phosphorylation of hSpt5 C-terminal repeats
258   is critical for processive transcription elongation. *Mol Cell* **21**, 227-237 (2006).
259 28 Schreieck, A. *et al.* RNA polymerase II termination involves C-terminal-domain
260   tyrosine dephosphorylation by CPF subunit Glc7. *Nat Struct Mol Biol* **21**, 175-
261   179, doi:10.1038/nsmb.2753 (2014).
262 29 Vanoosthuyse, V. *et al.* CPF-associated phosphatase activity opposes
263   condensin-mediated chromosome condensation. *PLoS Genet* **10**, e1004415,
264   doi:10.1371/journal.pgen.1004415 (2014).
265 30 Peterlin, B. M. & Price, D. H. Controlling the elongation phase of transcription
266   with P-TEFb. *Mol Cell* **23**, 297-305 (2006).

Acknowledgements We thank I.M. Hagan, B. Schwer, S. Shuman, M. Yanagida and

M.J. O'Connell for providing yeast strains and antibodies; K. M. Shokat for providing 3-

MB-PP1; C. Zhang for guidance in AS-allele optimization; and N. Steinbach and R.

Parsons for advice and assistance in phosphatase activity measurements. J.C.T. was

supported by Canadian Institutes of Health Research grant MOP-130362 and by a

fellowship from Fond de recherche Quebec Santé (3315). This work was supported by

National Institutes of Health grants GM25232 to G.T.B. and J.T.L., and GM104291 to

R.P.F.

Author Contributions P.P., G.T.B. and M.S. designed and performed experiments and

performed data analysis. B.B. and J.C.T. performed data analysis. P.P., G.T.B., J.T.L.

and R.P.F. prepared the manuscript.

Author Information Correspondence and requests for materials should be addressed to

robert.fisher@mssm.edu

METHODS

Yeast strains and standard methods. Fission yeast strains used in this study are listed

in Extended Data Table 1. New strains were generated by standard techniques[31]. Cells

were grown in YES medium at 30°C unless otherwise specified.

Immunological methods. Antibodies used in this study recognized pSpt5 or total

Spt5[13], Dis2-pT316[4] or total Dis2[32], Myc epitope (EMD Millipore, 05-724), total Pol II

(BioLegend, MMS-126R), Pol II pSer2 (Abcam, ab5095), α-tubulin (Sigma, T-5168) and

green fluorescent protein (GFP) (Invitrogen, rabbit polyclonal (A11122) or Santa Cruz,

mouse monoclonal (sc-9996)). Proteins were visualized in immunoblot analysis either by

enhanced chemiluminescence (ECL, HyGLO HRP detection kit, Denville Scientific,

E250) or with Odyssey Imaging System (LI-COR Biosciences).

Kinase and phosphatase assays. Kinase assays were performed with purified proteins

(Cdk9/Pch1 complex and GST-Spt5[801-990]) and [γ-^{32}P] ATP (PerkinElmer,

BLU002A250UC), as described previously[19]. GST-PP1s were expressed in *E. coli* at

16°C for 16 h and purified with Glutathione Sepharose 4 Fast Flow beads[33]. To measure

protein phosphatase activity, PP1 isoforms (GST-Dis2 or GST-Sds21) purified from *E.*

coli (2 µg) or immunoprecipitated from fission yeast extracts (4 mg total protein) were

incubated with 50 µM peptide (Spt5-NP, Spt5-pT1 or H3pS10) at 37°C for 1 h.

Colorimetric assays were performed in triplicate using BioMOL Green (Enzo Life

Sciences, BML-AK111) in 25 mM HEPES (pH 7.5), 100 mM NaCl, 1 mM $MnCl_2$ and 1

mM DTT, in 96-well plates as described in manufacturer's protocol.

Chromatin immunoprecipitation analysis. Immunoprecipitation and chromatin

immunoprecipitation (ChIP) were carried out by published methods[18,34,35]. Quantitative

PCR was performed with USB VeriQuest SYBR Green qPCR Master Mix (2x)

(Affymetrix, 75600) in 386-well plates. To measure the dependency of Dis2-Thr316

phosphorylation on Cdk9 *in vivo*, cells expressing GFP-Dis2 from the chromosomal *dis2*

locus in either *cdk9*[+] or *cdk9*[as] background were grown at 30°C to a density of ~ 1.5 x

10^7 ml^{-1}, treated with either DMSO or 10 µM 3-MB-PP1 for 10 min, and crosslinked with

1% formaldehyde for 15 min at 25°C. Chromatin extracts were prepared according to a

standard protocol for ChIP sample preparation[18,34,35] and 5 mg total protein was

subjected to immunoprecipitation with anti-GFP antibody (sc-9996) and immunoblot

analysis with either anti-GFP or anti-Dis2-pT316 antibody[4].

Chemical genetics. To measure rates of Spt5 and Rpb1 dephosphorylation after CDK

inhibition, cells were grown at 30°C in YES medium to a density of ~1.2 x10^7 cells/ml,

harvested by centrifugation at 25°C, resuspended in fresh YES (preincubated at desired

temperature) and incubated for 10 min before addition of DMSO or 3-MB-PP1. At

intervals after addition of DMSO or 3-MB-PP1, cells (~0.6 x 10^8) were transferred directly

to tubes containing 500 µl 100% (w/v) trichloroacetic acid (TCA) and harvested by

centrifugation. Protein extracts were prepared in the presence of 20% (w/v) TCA[36] and

processed for immunoblot analysis with appropriate antibodies.

ChIP-seq. Multiplexed ChIP-seq libraries were prepared using the Illumina TruSeq DNA

Sample Preparation kit v2 with 75 ng of input or IP DNA and barcode adaptors. Paired-

end sequencing (50-nt reads) was performed on an Illumina HiSeq 2000 (Genome

Quebec Innovation Centre, McGill University). After adaptor trimming and quality control,

processed FASTQ files were aligned to the *S. pombe* genome using Bowtie2[37] (Galaxy

Version 2.2.6.2). Aligned sequences of each biological replicate were fed into MACS2[38]

(Galaxy Version 2.1.1.20160309.0) to call peaks from alignment results. Generated

"bedgraph treatment" files were concatenated (Galaxy Version 1.0.1) to combine

replicates of each sample, converted into bigwig using "Wig/BedGraph-to-bigWig

converter" (Galaxy Version 1.1.0) and processed using computeMatrix (Galaxy Version

2.3.6.0) in DeepTools[39] to prepare data for plotting a heatmap and/ or a profile of given

regions. Heatmap and Metagene plots were generated using "plotHeatmap" (Galaxy

Version 2.5.0.0) and "plotProfile" (Galaxy Version 2.5.0.0) tools, respectively.

PRO-seq. For PRO-seq analysis, all samples, with the exception of the wild-type (*wt*)

strain (prepared separately), were prepared simultaneously and in biological replicates.

A detailed description of PRO-seq libraries can be found in the methods of the

accompanying manuscript (Booth *et al*.). Briefly, biological replicates for each strain

were derived from separately picked colonies. Cultures were grown in YES medium at

30°C overnight and diluted to an OD_{600} = 0.2. After reaching $OD_{600} \approx$ 0.5, an equal

number of cells (based on OD) was set aside for all treatments (~10 ml culture). At this

point, a fixed amount of thawed *S. cerevisiae* (50 µl, OD = 0.68) was spiked into each

sample. Cells were spun down and resuspended in fresh YES medium, pre-conditioned

at the desired temperature (30°C or 18°C) and allowed to incubate for 10 min. Samples

were immediately spun down at 4 °C and subjected to permeabilization and library

preparation as described by Booth *et al*., using the standard 5' and 3' RNA adaptors[40].

Sequencing, alignment and processing of reads were conducted as described in the

accompanying paper.

Data Access

The raw and processed sequencing files have been submitted to the NCBI Gene

Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) (Accession number

pending).

31   Moreno, S., Klar, A. & Nurse, P. Molecular genetic analysis of fission yeast
364   *Schizosaccharomyces pombe*. *Methods Enzymol.* **194**, 795-823 (1991).
365   32   Stone, E. M., Yamano, H., Kinoshita, N. & Yanagida, M. Mitotic regulation of
366   protein phosphatases by the fission yeast sds22 protein. *Curr Biol* **3**, 13-26
367   (1993).
368   33   Parua, P. K., Mondal, A. & Parrack, P. HflD, an Escherichia coli protein involved
369   in the lambda lysis-lysogeny switch, impairs transcription activation by
370   lambdaCII. *Arch Biochem Biophys* **493**, 175-183, doi:10.1016/j.abb.2009.10.010
371   (2010).
372   34   Sanso, M. *et al.* Gcn5 facilitates Pol II progression, rather than recruitment to
373   nucleosome-depleted stress promoters, in Schizosaccharomyces pombe.
374   *Nucleic Acids Res* **39**, 6369-6379 (2011).
375   35   Tanny, J. C., Erdjument-Bromage, H., Tempst, P. & Allis, C. D. Ubiquitylation of
376   histone H2B controls RNA polymerase II transcription elongation independently
377   of histone H3 methylation. *Genes Dev* **21**, 835-847 (2007).

36   Kao, C. F. & Osley, M. A. In vivo assays to study histone ubiquitylation. *Methods*
31, 59-66 (2003).
37   Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat*
Methods **9**, 357-359, doi:10.1038/nmeth.1923 (2012).
38   Feng, J., Liu, T., Qin, B., Zhang, Y. & Liu, X. S. Identifying ChIP-seq enrichment
using MACS. *Nat Protoc* **7**, 1728-1740, doi:10.1038/nprot.2012.101 (2012).
39   Ramirez, F. *et al.* deepTools2: a next generation web server for deep-sequencing
data analysis. *Nucleic Acids Res* **44**, W160-165, doi:10.1093/nar/gkw257 (2016).
40   Mahat, D. B. *et al.* Base-pair-resolution genome-wide mapping of active RNA
polymerases using precision nuclear run-on (PRO-seq). *Nat Protoc* **11**, 1455-
1476, doi:10.1038/nprot.2016.086 (2016).
389

Figures

Figure 1 | A Cdk9-Dis2-Spt5 circuit. a, Alignment of C-termini of human and fungal

PP1 isoforms. In chemical-genetic screens, Thr320 in PP1α was identified as a potential

target of Cdk1, and analogous residues in PP1β and PP1γ were identified as potential

targets of Cdk9. This site of phospho-regulation (indicated by a red dot) is conserved in

one of the two PP1 isoforms in fission yeast (Dis2-T316), but not in the other (Sds21), or

in the budding yeast PP1 catalytic subunit Glc7. **b,** Phosphorylation of Dis2-T316 by

Cdk9 *in vitro*. Purified, insect-cell derived Cdk9/Pch1 complexes were incubated at

indicated molar ratios with purified, bacterially expressed GST-PP1 or GST-Spt5[801-990]

(containing the CTD), after activation by the CDK-activating kinase Csk1 (incubated

alone in indicated lanes). In addition to wild-type Dis2, we tested Dis2[T316A] and Dis2[R245Q],

the variant encoded by *dis2-11*. Autophosphorylation occurs on both Cdk9 and Pch1.

(*Top*: autoradiogram; *bottom:* Coomassie-stained gel to confirm equal loading.) **c,** Cdk9-

dependence of Dis2-T316 phosphorylation *in vivo*. Cells of *cdk9^{as}* strains, with or without

GFP-tagged Dis2 expressed from the chromosomal *dis2^{+}* locus, were treated for 10 min

with 20 μM 3-MB-PP1 or mock-treated, as indicated. Chromatin extracts were

immunoprecipitated with anti-GFP antibodies and probed with antibodies specific for

Dis2 phosphorylated at Thr316 (Dis2-T316P) or GFP. **d**, Spt5 dephosphorylation by

purified PP1 *in vitro*. Purified GST-Dis2 and GST-Sds21 were incubated with a control

phosphopeptide derived from histone H3 ("H3pS10"), an Spt5 CTD consensus

phosphopeptide ("Spt5-pT1"), or a non-phosphorylated peptide of the same sequence

("Spt5-NP"). **e**, Spt5 dephosphorylation by PP1 isolated from fission yeast. A polyclonal

anti-Dis2 antibody immunoprecipitates pSpt5 phosphatase activity from extracts of *dis2^{+}*

but not *dis2* mutant cells. Note: the antibody cross-reacts with Sds21 in immunoblots but

does not efficiently immunoprecipitate Sds21. For **d**, **e**, *n* = 3 biological replicates; error

bars show standard deviation (s.d.). **f**, A Cdk9-Dis2-Spt5 circuit diagram.

Figure 2 | Cdk9 and Dis2 regulate Spt5 phosphorylation *in vivo*. a, Dis2 inactivation

stabilizes pSpt5 after Cdk9 inhibition. Fission yeast strains—*cdk9^as spt5-13Myc dis2^+* or

cdk9^as spt5-13Myc dis2-11—were grown to mid-log phase at 30°C and shifted to 18°C

(bottom) or not shifted (top) for 10 min, before addition of 10 μM 3-MB-PP1, after which

cultures were sampled at indicated times and subjected to immunoblot analysis with

anti-pSpt5 or anti-Myc antibodies. **b**, Ectopic expression of wild-type Dis2 restores rapid

Spt5 dephosphorylation kinetics in a *dis2* mutant. Anti-pSpt5 immunoblots from *cdk9^as^*

dis2-11 cells shifted to 18°C and treated with 10 μM 3-MB-PP1 for indicated times,

without or with expression of Myc-Dis2 from a plasmid. **c**, Fcp1 inactivation stabilizes

Rpb1 Ser2 phosphorylation after Lsk1 inhibition. Fission yeast strains—*lsk1^as^* or *lsk1^as^*

fcp1-452—were shifted to 37°C (or not shifted), treated for the indicated time with 20 μM

3-MB-PP1, and analyzed by immunoblotting for Rpb1-Ser2 phosphorylation. Note: CTD

dephosphorylation leads to increased reactivity with the 8WG16 antibody used to detect

total Pol II. **d**, Rapid pSpt5 turnover on chromatin. ChIP-qPCR analysis of pSpt5 versus

total Spt5 crosslinking at the *eng1^+^* gene after 3-MB-PP1 treatment for various times

(expressed as a percentage of signal in the absence of inhibitor). **e**, Dis2 inactivation

stabilizes chromatin-associated pSpt5. Either *cdk9^as^ spt5-13myc* or *cdk9^as^ spt5-13myc*

dis2-11 cells were shifted to 18°C and treated with 10 μM 3-MB-PP1 or mock-treated

with DMSO for 2 min and subjected to ChIP-qPCR analysis at *eng1^+^* for pSpt5 and total

Spt5 (anti-Myc). The signal ratios between the two treatments were plotted for each

condition. (Note: higher residual pSpt5 in *cdk9^as^ dis2^+^* cells, compared to those analyzed

in Fig. 2d, may reflect less efficient dephosphorylation at 18°C, relative to 30°C.) **f**,

Suppression of Dis2 recruitment to transcribed chromatin by Cdk9. ChIP-qPCR analysis

of GFP-Dis2 crosslinking at *eng1^+^* in *cdk9^as^ GFP-dis2* cells treated for 10 min with 10 μM

3-MB-PP1. **g**, pSpt5 on chromatin in *dis2* mutants. ChIP-qPCR analysis of indicated

strains upstream and downstream of CPS on *hxk2^+^* gene at 30°C. **h**, Comparison of

pSpt5:Spt5 ratio upstream and downstream of CPS on *hxk2^+^* gene in *dis2^+^* and *dis2-11*

cells at 18°C. For **d-h**, n = 4 biological replicates; error bars show standard deviation

(s.d.); asterisks indicate p-values (Student's t-test: [∗] $p < 0.05$; [∗∗] $p < 0.001$; [NS] not

significant) between wild-type (*dis2^+^*) and mutant (*dis2Δ*, *dis2-11*, *dis2^T316A^* or *dis2^T316D^*)

cells (**e**, **g**, **h**), or between 3-MB-PP1 and DMSO treatment (**f**).

Figure 3 | The Spt5 CTD is phosphorylated in gene bodies and unphosphorylated

in the termination zone. a, pSpt5 distribution on transcribed genes. Heatmaps (top)

and metagene analysis (bottom) reveal the pattern of Spt5-Myc and pSpt5 occupancy

across transcribed genes, filtered to exclude those with neighboring genes closer than 1

kb. (Note: In this representation, the regions between +300 bp relative to the TSS and -

300 bp relative to the CPS have been scaled to allow comparisons among genes of

different lengths.) **b**, pSpt5 distribution around the TSS. An unscaled, TSS-centered

metagene analysis reveals a broad peak of pSpt5 downstream of TSS. **c**, Spt5

dephosphorylation in the termination zone. Unscaled metagene analysis centered at the

CPS reveals a monotonic drop in pSpt5, but "twin peaks" of total, Myc-tagged Spt5. **d**,

Spt5 dephosphorylation downstream of the CPS. Individual gene tracks show

accumulation of Spt5-myc in the region downstream of the CPS with no concomitant

peak of pSpt5. **e**, Suppression of *dis2-11* by an Spt5 CTD mutant that cannot be

phosphorylated by Cdk9. Serial dilutions of indicated strains grown at 30°C, 20°C and

18°C. The *spt5* mutant alleles tested were: *spt5-(wt)$_7$*, in which the CTD is truncated from

18 to seven wild-type, nonapeptide repeats; *spt5-(T1A)$_7$*, in which each Thr1 position in

the seven repeats is changed to alanine; and *spt5-(T1E)$_7$*, in which each Thr1 is

changed to glutamic acid.

Figure 4 | Loss of Dis2 function impairs termination. a, Transcription beyond normal

termination zone in *dis2-11* cells under multiple conditions. Representative gene browser

tracks where transcription terminates within a narrowly defined zone in *dis2*+ cells, but

extends ~500 bp further downstream in *dis2-11* cells at both 18°C and 30°C. Alignment

with ChIP-seq tracks reveals correlation between loss of pSpt5 and Dis2-dependent

termination. **b**, Pleiotropic, genome-wide effects on Pol II dynamics due to *dis2-11*

mutation. Metagene analysis of PRO-seq read distributions in *cdk9^{as} dis2$^+$* and *cdk9^{as}*

dis2-11 strains reveals multiple differences in *dis2-11*, relative to *dis2$^+$* cells. These

analyses were performed in the absence of 3-MB-PP1 treatment; under this condition,

PRO-seq read distributions were not significantly different between *cdk9$^+$* and *cdk9^{as}*

cells, as shown in the accompanying paper by Booth *et al*. Note: To compare genes of

different lengths, regions between +300 bp relative to TSS and -300 bp relative to CPS

are scaled. **c**, CPS-centered metagene analysis comparing PRO-seq read distributions

in wild-type and *dis2-11* cells reveals relative increase in transcription downstream of the

CPS in the mutant at 30°C. Peak heights (*y* axis) were scaled as a fraction of maximum

signal in each condition, whereas position along the gene (*x* axis) was unscaled. **d**, Loss

of Dis2 function affects two metrics of termination efficiency, shown schematically:

Termination Index (T.I.), the ratio of signals in the regions 500 bp downstream and

upstream of the CPS (b/a); and Termination Elongation Index (T.E.I.), the ratio of signals

in the region between +250 bp and +750 bp relative to CPS to that in the region 500 bp

upstream of CPS (c/a). **e**, Box plots show that *dis2-11* mutation causes a significant

increase in T.E.I. in both *cdk9^{as}* (left) and *cdk9$^+$* (WT, right) backgrounds, and in T.I. in

cdk9$^+$ cells (Student's *t*-test, *p* < 0.01). **f**, Heatmaps showing change in PRO-seq read

distribution due to *dis2-11* mutation. Genes were ranked by decreasing T.E.I. in *cdk9^{as}*

dis2-11 at 18°C, a measure of termination-window size. Dis2 impairment expands this

zone relative to *cdk9^{as}* at 18°C. All genes in **b**-**f** were required to be active and at least 1

kb from nearest genes on the same strand to eliminate effects of nearby initiation and

run-through transcription (*n* = 919). **g**, A transcription exit network comprising Cdk9, Dis2

and Spt5. At or near CPS, Dis2 becomes active due to a drop in Cdk9 activity and

triggers Spt5 dephosphorylation, to facilitate 3'-pausing and termination.

Extended Data

a

b

504 **Extended Data Figure 1 | Dis2 expressed in fission yeast dephosphorylates Spt5-**

505 **T1P** *in vitro*. **a**, Anti-Myc immunoprecipitates from extracts of Myc-Dis2-expressing cells

506 were tested for phosphatase activity towards the Spt5 CTD-derived phosphopeptide. n =

507 3 biological replicates; error bars show standard deviation (s.d.). **b**, Immunoblot to verify

508 expression and immunoprecipitation of Myc-Dis2.

Extended Data Figure 2 | Distinct kinase-phosphatase circuits regulate

phosphorylation of Spt5-Thr1 and Rpb1-Ser2 *in vivo*. a, Rapid kinetics of Spt5

dephosphorylation after Cdk9 inhibition, and stabilization of pSpt5 by Dis2 inactivation

occur in *spt5+* strain and do not depend on the C-terminal Myc-epitope tag. **b**, Spt5

dephosphorylation kinetics in single, pairwise and triple *cdkas* mutants treated with 3-MB-

PP1 indicate that Cdk9 is the sole kinase needed to phosphorylate this site *in vivo*. **c**,

Dis2 activity is dispensable for Pol II CTD Ser2 dephosphorylation. As in **Fig. 2a** except

that experiment was performed in *lsk1as* cells, 20 μM 3-MB-PP1 was added, and extracts

were probed for phosphorylation of the Ser2 position of the Rpb1 CTD (or tubulin as a

loading control). **d**, Fcp1 activity is dispensable for Spt5 Thr1 dephosphorylation. As in

Fig. 2c except that strains carried a *cdk9as* allele and were tested for both pSpt5

(unaffected by Fcp1 inactivation) and pS2 (unaffected by Cdk9 inhibition).

Extended Data Figure 3 | Rapid dephosphorylation of chromatin-associated Spt5 upon Cdk9 inhibition. a, Dephosphorylation of Spt5 on chromatin of *eng1*[+] gene after Cdk9 inhibition (raw data for phospho- and total Spt5 from which ratios in **Fig. 2d** were calculated). **b**, ChIP-qPCR analysis of phospho- versus total Spt5 crosslinking at the

aro1$^+$ gene after 3-MB-PP1 treatment for various times. Left: absolute ChIP signals for

anti-Myc; middle: absolute signals for anti-pSpt5; right: ratio of phospho- to total Spt5,

expressed as a percentage of ratio in the absence of the inhibitor. **c**, Dis2 inactivation

stabilizes pSpt5 on chromatin. Either *cdk9*^{as} *spt5-13Myc dis2*$^+$ or *cdk9*^{as} *spt5-13Myc*

dis2-11 cells were shifted to 18°C and treated with 10 μM 3-MB-PP1 or mock-treated

with DMSO for 2 min and subjected to ChIP-qPCR analysis at the *eng1*$^+$ locus for pSpt5

and Spt5-Myc (raw data for phospho- and total Spt5 from which ratios in **Fig. 2e** were

calculated). **d,** Same as (**c**) at *aro1*$^+$ gene: ChIP-qPCR analysis of pSpt5 (left) and Spt5-

Myc (middle) and the signal ratios in 3-MB-PP1- versus DMSO-treated samples (right).

For **a-d**, *n* = 4 biological replicates; error bars show standard deviation (s.d.).

538
Extended Data Figure 4 | Cdk9 regulates PP1 recruitment to chromatin in isoform-

specific fashion. a, Constitutive *cdk9* loss-of-function mutations increase GFP-Dis2

recruitment to chromatin. Dis2 occupancy at *eng1⁺* locus analyzed in *cdk9⁺* ("wt") cells, a

cdk9ΔC mutant and a *cdk9^T212A* mutant. **b**, Spt5 phosphorylation on chromatin in *dis2*

mutants. ChIP-qPCR analysis of indicated strains upstream and downstream of CPS on

rps17a⁺ gene at 30°C. **c**, Comparison of pSpt5:Spt5 ratio upstream and downstream of

CPS on *rps17a⁺* gene in *dis2⁺* and *dis2-11* cells at 18°C. **d**, Anti-GFP ChIP at *eng1⁺* in a

cdk9^as GFP-sds21 strain treated for 10 min with 10 μM 3-MB-PP1 reveals unchanged or

slightly decreased Sds21 occupancy when Cdk9 is inhibited. For **a-d**, *n* = 4 biological

replicates; error bars show standard deviation (s.d.); asterisks indicate *p*-values

(Student's *t*-test: [∗] $p < 0.05$; [∗∗] $p < 0.001$; [NS] not significant) (**a**) between wild-type

(*cdk9⁺*) and mutant (*cdk9 Δ C* or *cdk9^T212A*), (**b**, **c**) between wild-type (*dis2⁺*) and mutant

(*dis2Δ, dis2-11, dis2^T316A* or *dis2^T316D*), or (**d**) between 3-MB-PP1 and DMSO.

Extended Data Figure 5 | Spt5 dephosporylation kinetics in cells with a single PP1

isoform. a, Dephosphorylation of Spt5 after Cdk9 inhibition is retarded in *dis2Δ* strain,

relative to a *dis2⁺* strain. **b**, Spt5 dephosphorylation kinetics after Cdk9 inhibition are

unaffected by *sds21* deletion in a *dis2⁺* strain.

Extended Data Figure 6 | Pol II and pSpt5 distribution on chromatin. a-c, Heatmap

(top) and metagene (bottom) analyses, scaled as in **Fig. 3a-c**, of Pol II ChIP-seq data

compared with pSpt5 over entire gene (**a**), and in TSS (**b**) and CPS (**c**) regions. **d**,

Genome browser tracks of representative genes, showing occupancy of pSpt5 and Pol

II. **e**, Metagene analysis comparing distribution of pSpt5 and pS2 over entire gene,

scaled as in (**a**). **f**, Metagene analysis comparing distribution of pSpt5 and pS2 centered

around CPS, without scaling, as in (**c**).

Extended Data Figure 7 | A non-phosphorylatable Spt5 suppresses conditional

lethality of *dis2-11*. Growth kinetics in liquid culture of indicated strains after shift to

18°C.

Extended Data Figure 8 | PRO-seq experiments are reproducible. Scatter plots

comparing PRO-seq libraries from two biological replicates for each experiment. Values

represent $\log_{10}$ (normalized reads) within the gene body (TSS + 200 bp to CPS) of all

filtered genes (n = 3330). Colors indicate the number of genes represented by each

point. Spike-in based normalization should center scatter about the diagonal line x = y

(magenta, dotted). Correlation values represent Spearman's rank correlation.

Extended Data Figure 9 | The *dis2-11* mutation affects global transcription

properties independent of temperature. a, Comparison of composite PRO-seq

profiles of the *dis2-11* mutant alone (top panels) or *cdk9^as dis2-11* (bottom panels) at

18°C and 30°C. Profiles are either centered on the TSS (left) or CPS (right). Shaded

areas on composite profiles represent the 12.5 and 87.5% quantiles at each position.

Each panel represents data from filtered genes that are at least 1 kb from neighboring

genes on the same strand (*n* = 919). **b,** Composite PRO-seq profiles comparing a *dis2*⁺

strain (*cdk9^as*) with the *dis2-11* strain, both at 18°C. Genes were scaled to a common

589   length by fixing the middle gene body (TSS + 300 bp to CPS – 300 bp) region to 60

590   windows. **c,** Heat maps of spike-in normalized PRO-seq signal (log_{10}) within 10 bp

591   windows relative to the CPS (-250 to +1000) for *cdk9[as]* (left) and *cdk9[as] dis2-11* (right)

592   strains at 18°C. Genes were ranked by decreasing T.E.I. in *cdk9[as] dis2-11* at 18°C, a

593   measure of termination-window size.

Extended Data Table 1 | Yeast Strains

Strain	Alias	Genotype	Reference
JS78	*wt*	*leu1-32 ura4-D18 his3-D1 ade6-M210 h$^+$*	Saiz & Fisher 2002
LV7	*cdk9^{as}*	*cdk9^{T120G}::kanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h$^+$*	Viladevall et al. 2009
MS340	*mcs6^{as5}*	*mcs6$^{N84T-L87G}$::kanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h$^+$*	This study
CS118	*lsk1^{as}*	*lsk1^{F353G}::kanMX6 leu1-32 ura4-D18 his3-D1 ade6-M216 h$^-$*	Viladevall et al. 2009
CS111	*spt5-13myc*	*spt5-13myc::kanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h$^+$*	Viladevall et al. 2009
CS112	*cdk9^{as} spt5-13myc*	*cdk9^{T120G}::kanMX6 spt5-13myc::kanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h$^+$*	Viladevall et al. 2009
FY10092	*dis2-11*	*dis2-11 leu1-32 ura4-D18 h$^+$*	NBRP, Japan
CS310	*spt5-(WT)$_7$*	*spt5-(WT)$_7$::ura4$^+$ leu1-32 ura4-D18 his3-D1 ade6-M216 h$^-$*	Schneider et al. 2010
CS311	*spt5-(T1A)$_7$*	*spt5-(T1A)$_7$::ura4$^+$ leu1-32 ura4-D18 his3-D1 ade6-M216 h$^-$*	Schneider et al. 2010
CS312	*spt5-(T1E)$_7$*	*spt5-(T1E)$_7$::ura4$^+$ leu1-32 ura4-D18 his3-D1 ade6-M216 h$^-$*	Schneider et al. 2010
LV223	*cdk9^{as} spt5-13myc fcp1-452*	*cdk9^{T120G}::kanMX6 spt5-13myc::kanMX6 fcp1-452 leu1-32 ura4-D18 his3-D1 ade6-M210*	This study
PP14	*spt5-13Myc dis2Δ*	*spt5-13myc::kanMX6 dis2Δ::ura4$^+$ ura4-D18 leu1-32 h$^-$*	This study
PP17	*cdk9as spt5-13Myc dis2Δ*	*cdk9^{T120G}::kanMX6 spt5-13myc::kanMX6 dis2Δ::ura4$^+$ ura4-D18 leu1-33 h$^+$*	This study
PP20	*cdk9^{as} lsk1^{as}*	*cdk9^{T120G}::hphMX6 lsk1^{F353G}::kanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h$^+$*	This study
PP23	*cdk9^{as} mcs6^{as5}*	*cdk9^{T120G}::hphMX6 mcs6$^{N84T-L87G}$::kanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h$^-$*	This study
PP26	*mcs6^{as5} lsk1^{as}*	*mcs6$^{N84T-L87G}$::kanMX6 lsk1^{F353G}::kanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h$^-$*	This study
PP29	*cdk9^{as} mcs6^{as5} lsk1^{as}*	*cdk9^{T120G}::hphMX6 mcs6$^{N84T-L87G}$::kanMX6 lsk1^{F353G}::kanMX6 leu1-32 ura4-D18 his3-D1 ade6-M210 h$^-$*	This study
PP38	*spt5-13Myc dis2-11*	*spt5-13myc::kanMX6 dis2-11 ura4-D18 leu1-32 h$^+$*	This study
PP40	*cdk9^{as} spt5-13Myc dis2-11*	*cdk9^{T120G}::kanMX6 spt5-13myc::kanMX6 dis2-11 ura4-D18 leu1-32 h$^-$*	This study
PP43	*cdk9^{as} spt5-13Myc sds21Δ*	*cdk9^{T120G}::kanMX6 spt5-13myc::kanMX6 sds21Δ::ura4$^+$ ura4-D18 leu1-32*	This study
PP55	*cdk9^{as} spt5-13Myc GFP-dis2*	*cdk9^{T120G}::kanMx spt5-13Myc::kanMx GFP-dis2::ura4$^+$ ura4-D18 leu1-32*	This study
PP69	*spt5-13Myc dis2^{T316D}*	*spt5-13Myc::kanMx dis2^{T316D}::ura4$^+$ ura4-D18 leu1-32 h$^-$*	This study
PP75	*spt5-13Myc dis2^{T316A}*	*spt5-13Myc::kanMx dis2^{T316A}::ura4$^+$ ura4-D18 leu1-32 h$^+$*	This study
PP84	*cdk9ΔC GFP-dis2*	*cdk9ΔC::kanMx GFP-dis2::ura4$^+$ ura4-D18 leu1-32*	This study
PP88	*cdk9^{T212A} GFP-dis2*	*cdk9^{T212A}::kanMx GFP-dis2::ura4$^+$ ura4-D18 leu1-32 h$^-$*	This study
PP100	*spt5-(WT)$_7$ dis2-11*	*spt5-(WT)$_7$::ura4$^+$ dis2-11 ura4-D18 leu1-32*	This study
PP103	*spt5-(T1A)$_7$ dis2-11*	*spt5-(T1A)$_7$::ura4$^+$ dis2-11 ura4-D18 leu1-32*	This study
PP105	*spt5-(T1E)$_7$ dis2-11*	*spt5-(T1E)$_7$::ura4$^+$ dis2-11 ura4-D18 leu1-32*	This study
PP125	*cdk9^{as} spt5-13Myc GFP-sds21*	*cdk9^{T120G}::kanMx spt5-13Myc::kanMx GFP-sds21::ura4$^+$*	This study
PP185	*cdk9^{as} dis2-11*	*cdk9^{T120G}::natMx dis2-11 ura4-D18 leu1-32*	This study
PP217	*lsk1^{as} dis2-11*	*lsk1^{F353G}::kanMX dis2-11 ura4-D18 leu1-32*	This study
PP220	*lsk1^{as} fcp1-452*	*lsk1^{F353G}::kanMX fcp1-452 ura4-D18 leu1-32*	This study