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 29. N. Netzer *et al.*, *Nature* **462**, 522 (2009).
 30. R. J. Jackson, S. Naphine, I. Brierley, *RNA* **7**, 765 (2001).
 31. M. Sisido, in *Chemical Biology: From Small Molecules to Systems Biology and Drug Design*, S. L. Schreiber, T. M. Kapoor, G. Wess, Eds. (Wiley-VCH Verlag, Weinheim, Germany, 2007), vol. 1, pp. 271–284.
 32. R. Geslain, T. Pan, *J. Mol. Biol.* **396**, 821 (2010).
 33. S. E. Dmitriev *et al.*, *J. Biol. Chem.* **285**, 26779 (2010).
 34. M. A. Skabkin *et al.*, *Genes Dev.* **24**, 1787 (2010).
 35. W. C. Merrick, W. F. Anderson, *J. Biol. Chem.* **250**, 1197 (1975).
 36. S. L. Adams, B. Safer, W. F. Anderson, W. C. Merrick, *J. Biol. Chem.* **250**, 9083 (1975).
 37. J. H. Kim, S. M. Park, J. H. Park, S. J. Keum, S. K. Jang, *EMBO J.* **30**, 2454 (2011).
 38. Materials and methods are available as supplementary materials on Science Online.

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Supplementary Materials

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Materials and Methods

Figs. S1 to S10

Table S1

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CTD Tyrosine Phosphorylation Impairs Termination Factor Recruitment to RNA Polymerase II

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In different phases of the transcription cycle, RNA polymerase (Pol) II recruits various factors via its C-terminal domain (CTD), which consists of conserved heptapeptide repeats with the sequence Tyr¹-Ser²-Pro³-Thr⁴-Ser⁵-Pro⁶-Ser⁷. We show that the CTD of transcribing yeast Pol II is phosphorylated at Tyr¹, in addition to Ser², Thr⁴, Ser⁵, and Ser⁷. Tyr¹ phosphorylation stimulates binding of elongation factor Spt6 and impairs recruitment of termination factors Nrd1, Pcf11, and Rtt103. Tyr¹ phosphorylation levels rise downstream of the transcription start site and decrease before the polyadenylation site, largely excluding termination factors from gene bodies. These results show that CTD modifications trigger and block factor recruitment and lead to an extended CTD code that explains transcription cycle coordination on the basis of differential phosphorylation of Tyr¹, Ser², and Ser⁷.

The C-terminal domain (CTD) is a flexible, tail-like extension of RNA polymerase (Pol) II and consists of 26 (yeast) or 52 (human) highly conserved heptapeptide repeats of the consensus sequence Tyr¹-Ser²-Pro³-Thr⁴-Ser⁵-Pro⁶-Ser⁷. During the transcription cycle, changes in CTD phosphorylation patterns coordinate the recruitment of transcription and mRNA processing factors to Pol II (1–3). During early transcription, Ser⁵ phosphorylation recruits the mRNA capping enzyme (4, 5). Ser² phosphorylation occurs during transcription elongation and functions in recruitment of RNA 3'-processing and termination factors (6). Phosphorylations at Ser⁷ (7–9) and Thr⁴ (10) have roles in processing of specific RNAs. Tyr¹ phosphorylation was described for human Pol II almost two decades ago (11), but whether this has

a functional role and whether it exists in other species are unknown.

We generated a monoclonal antibody against a Tyr¹-phosphorylated CTD peptide (3D12, Methods). Because the functional CTD unit is a pair of repeats (12), we determined antibody specificity by using di-heptapeptides bearing combinations of phosphorylations (Fig. 1A and fig. S1). This revealed a high affinity for the Tyr¹-phosphorylated CTD that was not impaired by adjacent Ser² phosphorylation and no affinity to other CTD peptides (Fig. 1A and fig. S1). The antibody immunoprecipitated Pol II from extracts of the yeast *Saccharomyces cerevisiae* (Fig. 1B), and the precipitated polymerases were also phosphorylated at Ser², Ser⁵, and Ser⁷ (fig. S2). The antibody also recognized Pol II that was purified from human cells with antibody 1C7 (fig. S1) and phosphorylated in vitro by the Tyr¹ kinase c-Abl (13) (Fig. 1C). Thus, antibody 3D12 specifically recognizes the Tyr¹-phosphorylated CTD, and Tyr¹ phosphorylation occurs in yeast.

To investigate whether genome-associated Pol II is phosphorylated at Tyr¹, we used high-resolution chromatin immunoprecipitation (ChIP) profiling in proliferating yeast (14). Data from two biological replicates ($R = 0.94$) were averaged and revealed strong signals over protein-coding and small nucleolar RNA genes (fig. S3). To test whether Tyr¹ phosphorylation occurs on

all transcribed protein-coding genes, we measured covariation in ChIP data for other CTD phosphorylations by singular value decomposition (14). The first singular vector explained 83.8% of the variance (fig. S4), indicating a similar occurrence of phosphorylations at Tyr¹,

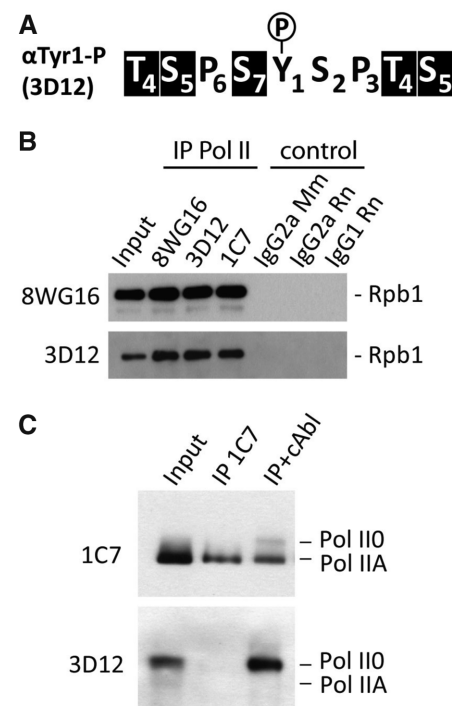


Fig. 1. Pol II CTD is phosphorylated at Tyr¹. (A) Part of the CTD sequence around phosphorylated Tyr¹ (Y₁). Residues Ser², Pro³, Thr⁴, Ser⁵, Pro⁶, and Ser⁷ are denoted S₂, P₃, T₄, S₅, P₆, and S₇, respectively. CTD residues that interfere with 3D12 antibody binding upon phosphorylation are highlighted in black. (B) Western blot analysis of whole-cell extract from proliferating yeast (Input). Pol II was immunoprecipitated with antibodies 8WG16, 3D12, and 1C7 (IP Pol II) and probed with 8WG16 or 3D12. Isotype controls are shown. Ig, immunoglobulin. (C) Antibody 3D12 detects CTD Tyr¹ phosphorylation in HeLa cells (Input). Pol II was immunoprecipitated with antibody 1C7 (IP 1C7, fig. S1) and incubated with cAbl kinase, leading to a 3D12 signal (IP+cAbl). The hyper- (II0) and hypophosphorylated forms (IIA) of Pol II are indicated.

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Ser², and Ser⁵. A correlation between levels of Tyr¹ phosphorylation and mRNA expression (15) (fig. S5A) further indicated that Tyr¹ phosphorylation is functionally relevant.

Gene-averaging of ChIP profiles (14) revealed Tyr¹ phosphorylation in the coding region (Fig. 2, A and B). Whereas Tyr¹ phosphorylation signals were low at promoters, they increased downstream of the transcription start site (TSS). The gene-averaged profile resembled that for Ser² phosphorylation, except that Ser² phosphorylation signals persist downstream of the polyadenylation (pA) site for ~200 nucleotides (nt), whereas Tyr¹ phosphorylation signals decrease already around 180 nt upstream of the pA site (Fig. 2, A, B, and D). The point of Tyr¹ phosphorylation signal increase was dependent on the TSS, whereas the point of decrease was dependent on the pA site but not on gene length or expression level (Fig. 2D and figs. S5B and S6). These results indicate that Tyr¹ phosphorylation marks are set and removed within the transcription cycle.

To investigate whether Tyr¹ phosphorylation influences factor recruitment to Pol II, we determined genomic occupancy profiles for termination factors Nrd1, Rtt103, and Pcf11, which contain a CTD-interacting domain (CID). The gene-averaged Nrd1 occupancy peaked at the beginning of the transcribed region, 193 ± 44 nt downstream of the TSS (Fig. 2C). This region also showed maximum signals in Ser⁵ phosphorylation, and genomic Nrd1 and Ser⁵ phosphorylation profiles correlate ($R = 0.6$), consistent with Nrd1 binding to the Ser⁵-phosphorylated CTD (16). The general presence of Nrd1 at protein-coding genes extends previous results (17, 18) and befits a role of Nrd1 in early transcription termination (2, 19–21). Rtt103 showed peak occupancy at the end of genes, 112 ± 27 nt downstream of the pA site, where peak levels of Pcf11 were also observed (14) (Fig. 2C and fig. S7). Because this region shows the maximum difference between Ser² and Tyr¹ phosphorylation signals, Tyr¹ phosphorylation may impair recruitment of Rtt103 and Pcf11 upstream of the pA site. Consistent with this, genome-wide occupancies of Rtt103 and Pcf11 do not correlate well with Ser² phosphorylation signals ($R = 0.4$, for both), although both proteins bind the Ser²-phosphorylated CTD (22, 23).

To test whether Tyr¹ phosphorylation impairs CTD binding of termination factors, we determined the affinity of purified recombinant CIDs of yeast Nrd1, Pcf11, and Rtt103 for various CTD diheptad phosphopeptides (table S1) by using fluorescence anisotropy (Fig. 3, A to C, and fig. S8). None of the CIDs bound to an unphosphorylated CTD peptide. Consistent with previous results (16, 23), Pcf11-CID and Rtt103-CID bound to the Ser²-phosphorylated CTD peptide [dissociation constants (K_D) = 54 ± 6 μ M ($\pm$ SD) and 12 ± 2 μ M, respectively; Methods], whereas the Nrd1-CID preferentially bound to a Ser⁵-phosphorylated CTD peptide ($K_D = 85 \pm$

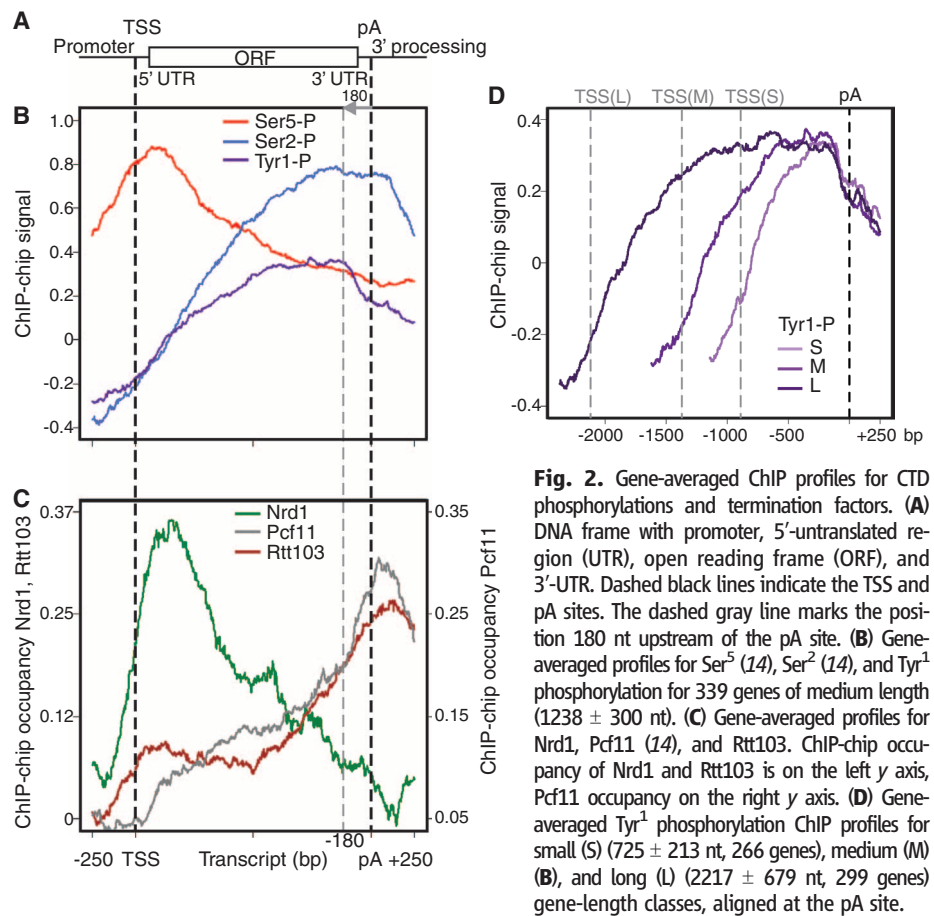


Fig. 2. Gene-averaged ChIP profiles for CTD phosphorylations and termination factors. (A) DNA frame with promoter, 5'-untranslated region (UTR), open reading frame (ORF), and 3'-UTR. Dashed black lines indicate the TSS and pA sites. The dashed gray line marks the position 180 nt upstream of the pA site. (B) Gene-averaged profiles for Ser⁵ (14), Ser² (14), and Tyr¹ phosphorylation for 339 genes of medium length (1238 ± 300 nt). (C) Gene-averaged profiles for Nrd1, Pcf11 (14), and Rtt103. ChIP-chip occupancy of Nrd1 and Rtt103 is on the left y axis, Pcf11 occupancy on the right y axis. (D) Gene-averaged Tyr¹ phosphorylation ChIP profiles for small (S) (725 ± 213 nt, 266 genes), medium (M) (B), and long (L) (2217 ± 679 nt, 299 genes) gene-length classes, aligned at the pA site.

25 μ M). In contrast, none of the CIDs bound Tyr¹-phosphorylated CTD peptides, regardless of whether additional phosphorylations were present or not. Thus, Tyr¹ phosphorylation blocks CID binding to the CTD in vitro, consistent with the hypothesis that it impairs termination factor recruitment in vivo.

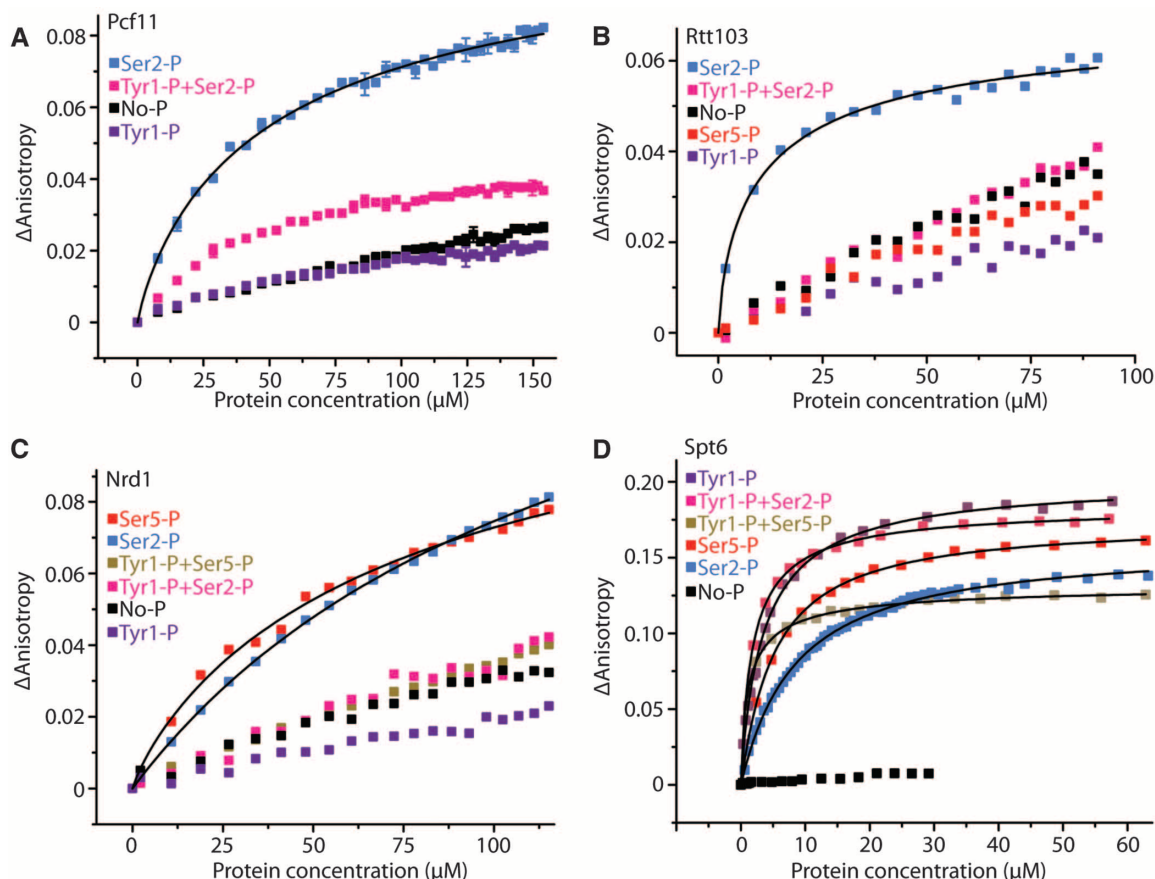
Structural modeling reveals how Tyr¹ phosphorylation blocks the CID-CTD interaction. Crystal structures of Ser²-phosphorylated CTD peptides bound to Pcf11-CID (22) and Rtt103-CID (23) are available, and the structure of the Nrd1-CID (16) is known. In the Pcf11-CTD structure, the Tyr¹ hydroxyl group forms a hydrogen bond with the Asp⁶⁸ side chain in the CID (22) (fig. S9A). This indicates that Tyr¹ phosphorylation blocks CTD binding because of electrostatic repulsion of two negatively charged groups, the Asp⁶⁸ side chain and the Tyr¹ phosphate. The CTD-binding aspartate residue is conserved in the Nrd1 CID (16). In the Rtt103-CTD structure, the corresponding residue, Asn⁶⁵, forms a hydrogen bond with the Tyr¹ hydroxyl group (23) that is incompatible with Tyr¹ phosphorylation. Generally, a Tyr¹ phosphate group modeled onto CTD peptides in CID complex structures results in steric clashes (fig. S9B).

Structural considerations also indicated that Thr⁴ phosphorylation (10) interferes with CID binding by destabilizing the bound CTD conformation (22). This predicted that Thr⁴ phospho-

rylation levels at the pA site are low, to enable recruitment of Pcf11 and Rtt103. Indeed, ChIP profiling revealed that Thr⁴ phosphorylation is limited to the transcribed region (fig. S10). Modeling further indicated that Ser⁷ phosphorylation is unlikely to interfere with CTD-CID binding, consistent with Nrd1 recruitment in the 5' region of genes where Ser⁷ phosphorylation levels are high (14). Thus, genome-wide signals of CTD phosphorylation at Thr⁴ and Ser⁷ are consistent with the function of Tyr¹ phosphorylation in impairing termination factor recruitment.

To investigate whether Tyr¹ phosphorylation also impairs CTD interactions of factors with other CTD-binding domains, we investigated the tandem Src homology 2 (SH2) domain of elongation factor Spt6. This domain binds the Ser²-phosphorylated CTD (24–27) and is required for high Spt6 occupancy on transcribed genes (14), suggesting that Tyr¹ phosphorylation does not interfere with its CTD binding. Indeed, the recombinant domain (residues 1250 to 1444) bound very well to CTD peptides phosphorylated at Tyr¹, Tyr¹ and Ser², or Tyr¹ and Ser⁵ but not to unphosphorylated CTD (Fig. 3D). These results were consistent with recent data (26, 27) and showed that interactions with Tyr¹-phosphorylated CTD peptides were even stronger than for peptides with phosphorylations at Ser² or Ser⁵ alone. This shows that Tyr¹ phosphorylation stimulates CTD binding of a bona fide elongation factor.

Fig. 3. CTD Tyr¹ phosphorylation blocks termination factor binding. Fluorescence anisotropy titration of CTD peptides with recombinant Pcf11-CID (A), Rtt103-CID (B), Nrd1-CID (C), and Spt6 tandem SH2 domain (D). When possible, binding affinity was determined as the protein concentration at half-maximum binding by nonlinear Hill fit (Origin). The remaining affinity of Pcf11-CID for the Tyr¹/Ser²-phosphorylated CTD peptide was not observed at higher salt concentration (fig. S7). Tyr¹-phosphorylated CTD peptides were not bound by CIDs [(A) to (C)], but by Spt6 tandem SH2 domain (for Tyr1-P, $K_D = 3.6 \pm 0.15 \mu\text{M}$; Tyr1-P+Ser2-P, $K_D = 1.9 \pm 0.04 \mu\text{M}$; Tyr1-P+Ser5-P, $K_D = 1.3 \pm 0.06 \mu\text{M}$; Ser2-P, $K_D = 8.4 \pm 0.19 \mu\text{M}$; Ser5-P, $K_D = 5.2 \pm 0.09 \mu\text{M}$).



We tested whether Tyr¹ phosphorylation depends on one of the yeast CTD kinases, Kin28, Srb10, Bur1, or Ctk1, which correspond to human Cdk7, Cdk8, Cdk9, and Cdk12, respectively. Inhibition of these kinases in vivo did not significantly affect Tyr¹ phosphorylation signals (table S2 and fig. S11). This indicates that Tyr¹ phosphorylation of the yeast CTD depends on a kinase other than the known CTD kinases. Consistent with this, Tyr¹ phosphorylation in human cells is achieved by c-Abl (13), a kinase that lacks a yeast homolog.

Our results extend the previously proposed CTD code (3, 28, 29), which was based on Ser² and Ser⁵ phosphorylation, leading to an extended CTD code for the coordination of the transcription cycle with factor recruitment (fig. S12). During initiation and early elongation, the CTD is phosphorylated on Ser⁵, which facilitates recruitment of the capping enzyme and Nrd1. Peak occupancy levels are reached for Nrd1 and Pol II 150 to 200 nt downstream of the TSS (14), likely marking a decision point where Pol II transiently pauses and either terminates or continues elongation (2). When Tyr¹ and Ser² phosphorylation levels rise, Pol II binds elongation factors stably and continues elongation. Tyr¹ phosphorylation releases Nrd1 and impairs recruitment of Rtt103 and Pcf11, suppressing termination during elongation. Before the pA site, Tyr¹ phosphorylation levels drop, whereas Ser² phosphorylation levels remain high. This enables recruitment of Rtt103 and Pcf11

that is enhanced by cooperative interactions between factors (23) and with nascent RNA (18), resulting in 3'-RNA processing and transcription termination. Our results indicate that Tyr¹ CTD phosphorylation is a target to activate transcription by suppressing Pol II termination and explain why mutation of Tyr¹ to phenylalanine, which lacks the oxygen atom required for phosphorylation, is lethal (30).

References and Notes

- R. D. Chapman, M. Heidemann, C. Hintermair, D. Eick, *Trends Genet.* **24**, 289 (2008).
- S. Buratowski, *Mol. Cell* **36**, 541 (2009).
- J. L. Corden, *Science* **318**, 1735 (2007).
- P. Komarnitsky, E.-J. Cho, S. Buratowski, *Genes Dev.* **14**, 2452 (2000).
- S. C. Schroeder, B. Schwer, S. Shuman, D. Bentley, *Genes Dev.* **14**, 2435 (2000).
- S. H. Ahn, M. Kim, S. Buratowski, *Mol. Cell* **13**, 67 (2004).
- R. D. Chapman *et al.*, *Science* **318**, 1780 (2007).
- M. Kim, H. Suh, E.-J. Cho, S. Buratowski, *J. Biol. Chem.* **284**, 26421 (2009).
- S. Egloff *et al.*, *Science* **318**, 1777 (2007).
- J.-P. Hsin, A. Sheth, J. L. Manley, *Science* **334**, 683 (2011).
- R. Baskaran, M. E. Dahmus, J. Y. Wang, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 11167 (1993).
- J. W. Stillier, M. S. Cook, *Eukaryot. Cell* **3**, 735 (2004).
- R. Baskaran, S. R. Escobar, J. Y. J. Wang, *Cell Growth Differ.* **10**, 387 (1999).
- A. Mayer *et al.*, *Nat. Struct. Mol. Biol.* **17**, 1272 (2010).
- S. Dengl, A. Mayer, M. Sun, P. Cramer, *J. Mol. Biol.* **389**, 211 (2009).
- L. Vasiljeva, M. Kim, H. Mutschler, S. Buratowski, A. Meinhardt, *Nat. Struct. Mol. Biol.* **15**, 795 (2008).
- H. Kim *et al.*, *Nat. Struct. Mol. Biol.* **17**, 1279 (2010).
- T. J. Creamer *et al.*, *PLoS Genet.* **7**, e1002329 (2011).
- K.-Y. Kim, D. E. Levin, *Cell* **144**, 745 (2011).
- E. J. Steinmetz, N. K. Conrad, D. A. Brow, J. L. Corden, *Nature* **413**, 327 (2001).
- A. G. Rondón, H. E. Mischo, J. Kawauchi, N. J. Proudfoot, *Mol. Cell* **36**, 88 (2009).
- A. Meinhardt, P. Cramer, *Nature* **430**, 223 (2004).
- B. M. Lunde *et al.*, *Nat. Struct. Mol. Biol.* **17**, 1195 (2010).
- M. Sun, L. Larivière, S. Dengl, A. Mayer, P. Cramer, *J. Biol. Chem.* **285**, 41597 (2010).
- M.-L. Diebold *et al.*, *J. Biol. Chem.* **285**, 38389 (2010).
- D. Close *et al.*, *J. Mol. Biol.* **408**, 697 (2011).
- J. Liu *et al.*, *J. Biol. Chem.* **286**, 29218 (2011).
- S. Buratowski, *Nat. Struct. Mol. Biol.* **10**, 679 (2003).
- S. Egloff, S. Murphy, *Trends Genet.* **24**, 280 (2008).
- M. L. West, J. L. Corden, *Genetics* **140**, 1223 (1995).

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Supplementary Materials

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