Phosphatase Rtr1 Regulates Global Levels of Serine 5 RNA Polymerase II C-Terminal Domain Phosphorylation and Cotranscriptional Histone Methylation

Gerald O. Hunter,a Melanie J. Fox,a Whitney R. Smith-Kinnaman,a Madelaine Gogol,ab Brian Fleharty,b and Amber L. Mosleya,c

Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana, USAa; Stowers Institute for Medical Research, Kansas City, Missouri, USAb; Center for Computational Biology and Bioinformatics, Indiana University School of Medicine, Indianapolis, Indiana, USAc

In eukaryotes, the C-terminal domain (CTD) of Rpb1 contains a heptapeptide repeat sequence of (Y1S2P3T4S5P6S7)n that undergoes reversible phosphorylation through the opposing action of kinases and phosphatases. Rtr1 is a conserved protein that colocalizes with RNA polymerase II (RNAPII) and has been shown to be important for the transition from elongation to termination during transcription by removing RNAPII CTD serine 5 phosphorylation (Ser5-P) at a selection of target genes. In this study, we show that Rtr1 is a global regulator of the CTD code with deletion of RTR1 causing genome-wide changes in Ser5-P CTD phosphorylation and cotranscriptional histone H3 lysine 36 trimethylation (H3K36me3). Using chromatin immunoprecipitation and high-resolution microarrays, we show that RTR1 deletion results in global changes in RNAPII Ser5-P levels on genes with different lengths and transcription rates consistent with its role as a CTD phosphatase. Although Ser5-P levels increase, the overall occupancy of RNAPII either decreases or stays the same in the absence of RTR1. Additionally, the loss of Rtr1 in vivo leads to increases in H3K36me3 levels genome-wide, while total histone H3 levels remain relatively constant within coding regions. Overall, these findings suggest that Rtr1 regulates H3K36me3 levels through changes in the number of binding sites for the histone methyltransferase Set2, thereby influencing both the CTD and histone codes.

Reversible phosphorylation of the C-terminal domain (CTD) of the largest subunit of RNA polymerase II (RNAPII) controls transcription elongation and termination through the orderly recruitment of transcription regulatory factors. These factors are involved in various processes including mRNA processing and cotranscriptional histone modifications (1–3). The CTD is comprised of the heptapeptide repeat of Y1S2P3T4S5P6S7 that is highly conserved with 26 repeats in Saccharomyces cerevisiae, 42 repeats in Drosophila melanogaster, and 52 repeats in humans and is essential for viability (4–10). The CTD is an unstructured domain that is unique to RNAPII and contains some degenerative repeats that deviate from the canonical sequence (11). Multiple residues within the CTD repeats are phosphorylated (P) at different stages of the transcription cycle; these residues include Ser2, Ser5, Ser7, Tyr1, and Thr4, which thereby serve as a signaling scaffold for RNAPII transcription machinery (12). The phosphorylation of Ser2, Ser5, and Ser7 in S. cerevisiae has been well characterized by several laboratories (13, 14). Phosphorylation of Tyr1 and Thr4 are less characterized but have also been implicated in the regulation of RNAPII transcription (12, 15). Additionally, the proline at position six in the CTD repeats is subject to cis-trans isomerization by Ess1 (16, 17). Despite a great deal of research into these modifications, the extent to which these modifications coexist in a phosphorylated and nonphosphorylated state on a single heptapeptide repeat in vivo remains enigmatic.

The reversible phosphorylation of the CTD through the action of kinases and phosphatases generates a “CTD code” that has been shown to correlate with RNAPII location on the gene (13, 18–20). At the beginning of transcription, RNAPII is in a hypophosphorylated state as it is recruited to the preinitiation complex at the promoter of a target gene (21). Promoter escape correlates with a rise in the levels of Ser5 and Ser7 phosphorylation (Ser5-P and Ser7-P, respectively), which are modified through the action of the transcription factor IIA (TFIIA) subunit Kin28 (14, 18, 19, 22, 23). Once RNAPII enters into productive transcript elongation, Ser5-P levels decrease while Ser7-P and Ser2-P levels continue to accumulate (13, 19). These two modifications are significant for the recruitment of factors involved in transcription elongation, termination, and 3′-end processing (24–26). Chromatin immunoprecipitation (ChIP) studies investigating Ser5-P using the H14 antibody (shown to detect CTD sequences phosphorylated at both Ser5 and Ser2 [27]) suggest that Ser5 is dephosphorylated within the first few hundred base pairs of transcription elongation at a small number of RNAPII target genes (22). However, a more-recent global study with the 3E8 antibody found that Ser5-P levels begin to decrease within 500 bp after the transcription start site (TSS), but approximately half of the detectable signal remains throughout the gene body (18). Concurrent with Ser5 dephosphorylation, Ser2-P increases through the action of the kinase complex CTDK-I and remains phosphorylated through the transcription termination site (TTS), during which this modification has been shown to recruit transcription termination factors (25, 28). The phosphatase Fcp1 removes the Ser2-P mark at the end of transcription factor IIB (TFIIB) subunit Kin28 (14, 18, 19, 22, 23). Once RNAPII enters into productive transcript elongation, Ser5-P levels decrease while Ser7-P and Ser2-P levels continue to accumulate (13, 19). These two modifications are significant for the recruitment of factors involved in transcription elongation, termination, and 3′-end processing (24–26). Chromatin immunoprecipitation (ChIP) studies investigating Ser5-P using the H14 antibody (shown to detect CTD sequences phosphorylated at both Ser5 and Ser2 [27]) suggest that Ser5 is dephosphorylated within the first few hundred base pairs of transcription elongation at a small number of RNAPII target genes (22). However, a more-recent global study with the 3E8 antibody found that Ser5-P levels begin to decrease within 500 bp after the transcription start site (TSS), but approximately half of the detectable signal remains throughout the gene body (18). Concurrent with Ser5 dephosphorylation, Ser2-P increases through the action of the kinase complex CTDK-I and remains phosphorylated through the transcription termination site (TTS), during which this modification has been shown to recruit transcription termination factors (25, 28). The phosphatase Fcp1 removes the Ser2-P mark at the end of transcription factor IIB (TFIIB) subunit Kin28 (14, 18, 19, 22, 23). Once RNAPII enters into productive transcript elongation, Ser5-P levels decrease while Ser7-P and Ser2-P levels continue to accumulate (13, 19). These two modifications are significant for the recruitment of factors involved in transcription elongation, termination, and 3′-end processing (24–26). Chromatin immunoprecipitation (ChIP) studies investigating Ser5-P using the H14 antibody (shown to detect CTD sequences phosphorylated at both Ser5 and Ser2 [27]) suggest that Ser5 is dephosphorylated within the first few hundred base pairs of transcription elongation at a small number of RNAPII target genes (22). However, a more-recent global study with the 3E8 antibody found that Ser5-P levels begin to decrease within 500 bp after the transcription start site (TSS), but approximately half of the detectable signal remains throughout the gene body (18). Concurrent with Ser5 dephosphorylation, Ser2-P increases through the action of the kinase complex CTDK-I and remains phosphorylated through the transcription termination site (TTS), during which this modification has been shown to recruit transcription termination factors (25, 28). The phosphatase Fcp1 removes the Ser2-P mark at the end of transcription factor IIB (TFIIB) subunit Kin28 (14, 18, 19, 22, 23). Once RNAPII enters into productive transcript elongation, Ser5-P levels decrease while Ser7-P and Ser2-P levels continue to accumulate (13, 19). These two modifications are significant for the recruitment of factors involved in transcription elongation, termination, and 3′-end processing (24–26). Chromatin immunoprecipitation (ChIP) studies investigating Ser5-P using the H14 antibody (shown to detect CTD sequences phosphorylated at both Ser5 and Ser2 [27]) suggest that Ser5 is dephosphorylated within the first few hundred base pairs of transcription elongation at a small number of RNAPII target genes (22). However, a more-recent global study with the 3E8 antibody found that Ser5-P levels begin to decrease within 500 bp after the transcription start site (TSS), but approximately half of the detectable signal remains throughout the gene body (18). Concurrent with Ser5 dephosphorylation, Ser2-P increases through the action of the kinase complex CTDK-I and remains phosphorylated through the transcription termination site (TTS), during which this modification has been shown to recruit transcription termination factors (25, 28). The phosphatase Fcp1 removes the Ser2-P mark at the end of transcription factor IIB (TFIIB) subunit Kin28 (14, 18, 19, 22, 23). Once RNAPII enters into productive transcript elongation, Ser5-P levels decrease while Ser7-P and Ser2-P levels continue to accumulate (13, 19). These two modifications are significant for the recruitment of factors involved in transcription elongation, termination, and 3′-end processing (24–26). Chromatin immunoprecipitation (ChIP) studies investigating Ser5-P using the H14 antibody (shown to detect CTD sequences phosphorylated at both Ser5 and Ser2 [27]) suggest that Ser5 is dephosphorylated within the first few hundred base pairs of transcription elongation at a small number of RNAPII target genes (22). However, a more-recent global study with the 3E8 antibody found that Ser5-P levels begin to decrease within 500 bp after the transcription start site (TSS), but approximately half of the detectable signal remains throughout the gene body (18). Concurrent with Ser5 dephosphorylation, Ser2-P increases through the action of the kinase complex CTDK-I and remains phosphorylated through the transcription termination site (TTS), during which this modification has been shown to recruit transcription termination factors (25, 28). The phosphatase Fcp1 removes the Ser2-P mark at the end of
transcription, thereby making hypophosphorylated RNAPII available for a subsequent round of transcription (29, 30).

The transition of RNAPII from Ser5-P to Ser2-P is an important step in the transcription cycle and is regulated by multiple layers of regulation. The dual-specificity phosphatase family member Ssu72 has been shown to remove Ser5-P from the CTD (31). Interestingly, Ssu72 localizes primarily at the 3' end of the gene as a subunit of the cleavage and polyadenylation factor (CPF) and is involved in mRNA processing events and control of RNAPII termination (32–36). Ssu72 also participates in gene looping and the suppression of divergent transcription (37, 38). Rtr1 is a conserved protein that has been reported by both in vitro and in vivo experimental studies to be a phosphatase responsible for the removal of Ser5-P from the CTD (14, 39–42). ChIP assays found that the localization of Rtr1 is predominant between the peaks of Ser5-P and Ser2-P. Analysis of RNAPII in RTR1 deletion cells found that Ser5-P RNAPII accumulates in whole-cell extracts in the absence of RTR1 activity (39). In addition, the human homologue of Rtr1, RPAP2, also displays phosphatase activity that is specific for Ser5-P (40, 43). Overall, these studies have implicated both Ssu72 and Rtr1 in the removal of Ser5-P during the RNAPII transcription cycle.

Set2 is a histone lysine methyltransferase that modifies coding region histones on histone H3 at K36 and is the sole histone H3 lysine 36 (H3K36) methyltransferase in Saccharomyces cerevisiae. Set2 has been shown to interact with the Ser2 and Ser5 diphosphorylated form of transcribing RNAPII through the Set2-Rpb1-interacting (SRI) domain in a Ctk1-dependent manner (44–48). We have previously found that the Rtr1 interaction with RNAPII is dependent on Ctk1 activity and that Rtr1 can interact with RNAPII phosphorylated at Ser2, Ser5, and/or Ser7 (49). In fact, multiple lines of evidence suggest that Rtr1 binds to a multiphosphorylated form of the RNAPII CTD (40–42, 49). These data suggest that Rtr1 can act as both a reader and an eraser for the CTD code and could function as a potential regulator of Set2 targeting to RNAPII target genes through regulation of Ser5-P RNAPII levels. The recruitment of Set2 by Ser2 and Ser5 CTD phosphorylation directs H3K36 methylation across transcribed RNAPII genes (47, 50). However, the role of Rtr1-dependent Ser5-P CTD dephosphorylation in Set2 recruitment and histone H3K36 methylation has not been investigated.

In this study, we show that S. cerevisiae Rtr1 is a regulator of Ser5 phosphorylation in the RNAPII CTD in agreement with previous findings (14, 39, 41, 49, 51, 52). We show that deletion of RTR1 leads to global increases in Ser5-P CTD occupancy at RNAPII target genes, suggesting that Rtr1 is a general regulator of RNAPII phosphorylation during transcription elongation. Chromatin immunoprecipitation experiments in an RTR1 deletion strain (rtr1Δ) show global accumulation of Ser5-P RNAPII primarily at the 3' ends of target genes regardless of gene length. This finding is specific for Rtr1, since the RTR2 deletion strain (rtr2Δ) does not appear to affect Ser5-P levels at PMA1. Furthermore, we provide genome-wide evidence that Rtr1 activity regulates H3K36 trimethylation (H3K36me3) levels across RNAPII target genes. Overall, this study clearly shows that Rtr1 is an important global regulator of the CTD code during RNAPII transcription elongation. Changes in Rtr1-mediated dephosphorylation of RNAPII lead to increased Ser5-P levels at the 3' ends of mRNA-encoding genes, which causes a coordinate increase in histone H3K36me3 levels within the coding region.

**RESULTS**

**RTR1 deletion causes a shift in the distribution of Ser5-P.** To capture a global picture of the distribution changes of Ser5-P RNAPII in wild-type (WT) and rtr1Δ cells, we performed average gene analysis from biological replicate ChIP-chip experiments (Fig. 1A, n = 2). For illustration purposes, target genes were separated into quartiles according to gene length, since shorter genes (such as RPS5), show high levels of Ser5-P RNAPII occupancy across their entire coding regions (Fig. 1A. WT 1st quartile). In the first quartile that contains the shortest set of RNAPII target genes, we observe a 3' shift in Ser5-P RNAPII intensity in strains lacking Rtr1 activity (Fig. 1A, compare the 1st quartiles in WT and rtr1Δ cells). A similar 3' shift in the Ser5-P intensity can be seen for each subsequent quartile, with the 1st and 2nd quartile in rtr1Δ cells displaying the highest intensity of Ser5-P at the 3' end of an average gene. In addition, we observed a higher intensity of Ser5-P downstream of the 3' end in rtr1Δ cells than in WT cells. The increase occurs in each of the four quartiles. These results, in con-
junction with our previous findings, show that Rtr1 maintains the distribution of RNAPII Ser5-P levels in WT cells. Ser5-P levels peak at the 5’ ends of RNAPII target genes in yeast, and this mark has been shown to play a critical role in early transcription events, namely, mRNA capping (58). Changes in the Ser5-P distribution throughout the genome could lead to changes in specific CTD-interacting proteins that act as readers of the CTD code. To illustrate that the changes we observe in Ser5-P distribution following deletion of \( \text{RTR1} \) are not caused by global changes in total RNAPII occupancy across target genes, we have measured total RNAPII occupancy using Rpb3-FLAG ChIP-exo (\( \text{Fig. 1B} \)).

For ease of comparison, gene averaging was performed as for the Ser5-P RNAPII data set and is shown as quartiles of gene length (see Supplemental Methods in the supplemental material for more details). These data clearly show that the average total RNAPII occupancy across the genome either decreases (quartiles 1, 2, and 3) or stays relatively unchanged (quartile 4 in \( \text{Fig. 1B} \)). Overall, these data indicate that the increased levels of Ser5-P RNAPII are due to decreased Ser5-P CTD dephosphorylation in \( \text{rtr1}\Delta \) cells and not due to increases in overall RNAPII occupancy at the 3’ ends of genes.

Deletion of \( \text{RTR1} \) causes a 3’ prime shift in the distribution of Ser5-P, which was independent of gene length (\( \text{Fig. 1} \)). To determine the general classes of RNAPII Ser5-P distribution changes in \( \text{RTR1} \) deletion cells, we performed unsupervised k-means clustering analysis of differential enrichment data [(\( \text{rtr1}\Delta \text{ IP} - \text{WT IP} \)/input values] for annotated RNAPII target genes. We obtained five clusters from the k-means clustering, the majority of which show increased Ser5-P in the 3’ end consistent with our length analysis (\( \text{Fig. 2} \), clusters 2, 3, and 4). In \( \text{Fig. 2A} \), cluster 1 genes display an increase in Ser5-P RNAPII enrichment at the 3’ end of genes in the absence of Rtr1. Although cluster 3 genes show a pattern similar to the pattern of cluster 1 genes, on average, they show a higher enrichment of Ser5-P levels in WT samples relative to data from \( \text{rtr1}\Delta \) strains. The average differential Ser5-P RNAPII enrichment across cluster 4 genes is elevated in \( \text{rtr1}\Delta \) cells than in WT cells throughout the transcribed region (\( \text{Fig. 2B} \)), whereas the genes in cluster 2 show Ser5-P in the \( \text{rtr1}\Delta \) enrichment primarily at the 3’ end of the gene. Cluster 5 genes show a distinct differential enrichment pattern of Ser5-P RNAPII in \( \text{rtr1}\Delta \) cells primarily at the 5’ ends of genes (\( \text{Fig. 2C} \)).
term enrichment analysis and determined the top overrepresented terms ($P < 0.01$) within our clusters in order to better understand the classes of cellular functions that are represented by the individual gene clusters (see Table S1 in the supplemental material). Interestingly, cluster 4, in which Ser5-P RNAPII is enriched in $rtr1\Delta$ cells throughout the gene body, had GO term enrichment in genes related to ribosomal protein-coding genes: ribonucleoprotein complex biogenesis and assembly ($P$ value of $1.46 \times 10^{-36}$), rRNA metabolic process ($P$ value of $1.31 \times 10^{-30}$), and rRNA processing ($P$ value of $5.48 \times 10^{-30}$). In fact, additional analysis of cluster 4 genes found many highly expressed genes, including $PMA1$, $ADH1$, and the ribosomal protein-coding genes.

Analysis of the role of Rtr1 in the regulation of histone H3K36me3 levels in vivo. Once RNAPII escapes the promoter, the CTD tail becomes phosphorylated at Ser5 and Ser2 by the action of TFIIF and CTDK-I, respectively. The Ser2-P Ser5-P doubly modified RNAPII CTD has been shown to recruit the histone methyltransferase Set2 (45, 47). In yeast, Set2 catalyzes mono-, di-, and tri-methylation of K36 distributed over the coding region of the gene with H3K36 trimethylation enrichment at the 3’ ends of protein-coding genes (59). Set2-facilitated H3K36me3 coordinates the recruitment of the Rpd3S deacetylase complex to the coding regions of genes. Rpd3S is responsible for the deacetylation of coding region histones after the passage of RNAPII to maintain hypoacetylated chromatin architecture, which prevents cryptic transcripts (60–62). Taken together, we hypothesize that changes in Ser5-P distribution caused by loss of Rtr1 function will impact H3K36me3 levels by increasing the number of Set2-CTD-interacting sites during transcription elongation. To test our hypothesis and to better understand the role of Rtr1 in regulating the CTD binding protein Set2, we performed ChIP-chip experiments on H3K36me3 in WT and $rtr1\Delta$ strains using high-density Agilent 1×244k (1 × 244,000) arrays with an average probe resolution of 50 nucleotides (Fig. 3). The levels of H3K36me3 throughout RNAPII target genes was normalized by the levels of total histone H3. Average gene enrichment analysis was performed for RNAPII targets and separated into quartiles for transcription rate, since the total levels of histone H3K36me3 have been shown to correlate with RNAPII transcription rate (59, 63). The first quartile contains the most rarely transcribed set of genes, and the fourth quartile contains the most frequently transcribed group of genes. In agreement with previous results, we observed increasing levels of H3K36me3 for both WT and $rtr1\Delta$ data sets from the first quartile through the fourth quartile, showing that the total levels of H3K36me3 correlate with transcription rate (Fig. 3A). In addition, we can see increased levels of histone H3K36me3 in $rtr1\Delta$ cells relative to WT cells in all four quartiles. Our analysis of total RNAPII levels from Fig. 1B suggests that total RNAPII transcription does not increase in $rtr1\Delta$ cells, although we do see dramatic increases in Ser5-P CTD levels. The relative levels of H3K36me3 (adjusted for total histone H3 levels) are shown together for the first and fourth quartile gene sets in Fig. 3B. These data show that the largest differences in H3K36me3 levels occur at the 3’ ends of the RNAPII target genes. Together, these data suggest that Ser5-P CTD dephosphorylation by $rtr1\Delta$ regulates H3K36me3 levels by decreasing the number of Ser5-P Ser2-P binding sites available to Set2 during transcription elongation.

To clearly illustrate the changes we observed in both Ser5-P RNAPII and histone H3K36me3 levels, we have shown the patterns of Ser5-P RNAPII and histone H3K36me3 across a small region of chromosome 7 in Fig. 4. Deletion of $RTR1$ leads to large increases in total RNAPII Ser5-P (normalized for total RNAPII occupancy) across the coding region of $PMA1$ (Fig. 4A, left). At the downstream gene $LEU1$, increased levels of Ser5-P RNAPII are also observed at the 3’ end, although the changes are smaller than those observed at $PMA1$ (Fig. 4A, right). The pattern of
H3K36me3 in WT Ser5-P RNAPII across the PMA1 gene is what would be expected for a typical protein-coding gene, where the H3K36me3 signal peaks in the coding region of the gene and begins to decrease toward the 3' end just prior to the annotated polyadenylation sites (Fig. 4B). H3K36me3 levels (normalized for total histone H3 occupancy) increase in rtr1Δ/H9004 cells relative to WT cells (Fig. 4B, left). The largest differences in H3K36me3 levels at PMA1 are observed near the 3' end similar to the largest differences observed in Ser5-P RNAPII levels (compare the top and bottom representations in Fig. 5). At the downstream gene LEU1, histone H3K36me3 levels are also increased in rtr1Δ cells at the 3' end of the gene in the same region in which increased levels of Ser5-P RNAPII were also observed (compare the right portions of the top and bottom representations in Fig. 5). We propose that altered histone H3K36me3 enrichment at the 3' ends of genes occurs as a consequence of RTR1 deletion through increased Set2 recruitment to RNAPII during transcription elongation as a consequence of elevated Ser5-P CTD modification (Fig. 5).

**DISCUSSION**

Multiple reports have shown that deletion of RTR1 in yeast results in elevated Ser5-P at the 3' ends of a selection of model genes (14, 39, 52). Given that Rtr2 is an orthologue of Rtr1, it was reasonable to assume that Rtr2 may also have phosphatase activity. We tested this hypothesis by ChIP-quantitative PCR (qPCR) on the PMA1 gene and found that RTR2 deletion does not cause significant changes in Ser5-P RNAPII levels (see Fig. S3 in the supplemental material). These data suggest that Rtr2 is not a primary regulator of Ser5-P removal in vivo. We expanded our previous work (39) by addressing the role of Rtr1 on global levels of RNAPII phosphorylation in vivo. We performed ChIP-chip experiments to measure the changes in the Ser5-P levels in RTR1 deletion cells. Using this approach, we clearly demonstrate that Rtr1 is a global regulator of Ser5-P CTD modification, and Rtr1 appears to regulate Ser5-P levels regardless of gene length and independent of total RNAPII occupancy (Fig. 1). These findings are significant because they...
suggest that even at short genes, Rtr1 works to dephosphorylate RNAPII during the early phases of transcription elongation. Together, these findings indicate that Rtr1 is a Ser5-P CTD phosphatase that regulates RNAPII modification status during transcription elongation.

Using unsupervised k-means clustering, we grouped RNAPII target genes into five clusters based on the pattern of Ser5-P occupancy changes in rtr1Δ cells versus WT cells. We found that most genes, regardless of the cluster, have increased Ser5-P enrichment at the 3′ end (Fig. 2). Moreover, cluster four, in particular, displayed enriched Ser5-P throughout RNAPII target genes. Upon closer inspection of this cluster, we find a high percentage of the genes to be highly expressed yeast genes, including the prototypical yeast model genes, PMA1 and ADH1. We speculate that since highly expressed yeast genes will by definition have higher occupancy of RNAPII, this leads to a larger change in overall Ser5-P levels in the absence of Rtr1 phosphatase activity. Our finding that Rtr1 regulates global Ser5-P RNAPII levels at the 3′ ends of genes bears some similarity to data obtained from global analysis of Ser7-P RNAPII levels in an Ssu72 temperature-sensitive degron strain (Ssu72-td strain) (64). These data are intriguing, since Ssu72 has also been implicated in the removal of Ser5-P following cis isomerization of Pro6 in the RNAPII CTD regulated by Ess1 (65). However, Bataille et al. and Zhang et al. did not observe significant changes in global Ser5-P levels in the Ssu72-td strain at RNAPII target genes by ChIP-chip (18, 64). Recent work by Rosado-Lugo and Hampsey suggests that specific inactivating mutations in Ssu72, such as R129A and C15S, may block Rtr1 recruitment to the CTD during transcription elongation, whereas the loss of all Ssu72 protein in Ssu72-td strains allows Rtr1 to be recruited normally, resulting in near wild-type levels of Ser5-P RNAPII in the Ssu72-td strain (52). Our findings suggest that Rtr1 is a major regulator of Ser5-P RNAPII levels in vivo and that the wild-type Ssu72 in RTR1 deletion strains is not able to compensate for Rtr1. These data suggest that Rtr1 and Ssu72 may target different populations of RNAPII for Ser5-P dephosphorylation in vivo.

By examining the effects of RTR1 deletion on cotranscriptional histone modifications, we were able to test the concept of a CTD code. If precise timing of kinase-mediated CTD phosphorylation and phosphatase-mediated CTD dephosphorylation is needed to maintain CTD-interacting partner recruitment, loss of a CTD phosphatase should result in altered localization or amount of interacting partner function. In Fig. 4 and 5, it is clearly shown that loss of the early CTD phosphatase Rtr1 results in a 3′ spread of histone H3K36me3 by Set2, a well-characterized Ser2-P Ser5-P CTD-interacting protein and the sole H3K36me3 methyltransferase in yeast (66, 67). The changes in H3K36me3 are not caused by changes in overall histone occupancy (Fig. 4 and 5). These data show that Rtr1 has the greatest effect on H3K36me3 at the 3′ end of the gene, and these results directly correlate with the changes we observed in Ser5-P (Fig. 4). Since we observed global increases in H3K36me3 levels rather than spread of the 3′ modification in rtr1Δ cells, we propose that the methyltransferase Set2 is not mislocalized on the gene but likely remains in contact with the CTD.
for a longer period of time, thus increasing its modification output (Fig. 5). These data suggest that at some genes, Set2 may stay associated with RNAPII further downstream as a result of RTR1 deletion. This could occur as a result of Set2 mislocalization and/or termination defects in RNAPII, resulting in increased occupancy downstream.

On the basis of our observations and previous research from our group and others, we propose a model in which Rtr1 acts upstream of Set2 to regulate the levels of the SRI sites along the RNAPII CTD (Fig. 5). We have previously shown that Rtr1 recruitment to RNAPII occurs in a manner that is dependent upon the Ser2-P kinase Ctk1 (49). In fact, the increased levels of histone
H3K36me3 in rtr1Δ cells is additional indirect evidence that could suggest that Rtr1 targets Ser2-P Ser5-P modified RNAII for dephosphorylation in agreement with studies on RPAP2, the human homologue of Rtr1 (40). We propose the following model: as polymerase enters productive elongation, Ser2-P begins to increase, which leads to the recruitment of Set2 to RNAII. Rtr1 is recruited to the multiply phosphorylated CTD and starts to remove Ser5-P, which in turn will lower the affinity of Set2 for the CTD, thereby leading to less H3K36me3 by the time RNAPII moves Ser5-P, which in turn will lower the affinity of Set2 for the CTD. We also thank Swaminathan Venkatesh and Jerry Workman for manuscript and technical contributions during the course of the project. We thank the members of the Mosley lab for critical reading of the manuscript and technical contributions during the course of the project. We also thank Swaminathan Venkatesh and Jerry Workman for helpful discussions. We thank Michael Washburn and Laurence Flores for their support during the early stages of this project. Finally, we thank Yunlong Liu and Hongyu Gao for average gene analysis of total RNAPII occupancy.

ACKNOWLEDGMENTS

We thank the members of the Mosley lab for critical reading of the manuscript and technical contributions during the course of the project. We also thank Swaminathan Venkatesh and Jerry Workman for helpful discussions. We thank Michael Washburn and Laurence Flores for their support during the early stages of this project. Finally, we thank Yunlong Liu and Hongyu Gao for average gene analysis of total RNAPII occupancy.

FUNDING INFORMATION

This work, including the efforts of Amber L. Mosley, was funded by Stowers Institute for Medical Research (SIMR). L. Mosley, Gerald O. Hunter, and Melanie J. Fox, was funded by HHS | Showalter Young Investigator Award. This work, including the efforts of Amber L. Mosley, Gerald O. Hunter, and Melanie J. Fox, was funded by HHS | National Institutes of Health (NIH) (R01 GM099714). This work, including the efforts of Madelaine Gogol, Brian Flehart, and Amber L. Mosley, was funded by Stowers Institute for Medical Research (SIMR).

REFERENCES


64. Zhang DW, Mosley AL, Ramisetty SR, Rodriguez-Molina JH, Wash-


