THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 284, NO. 39, pp. 26421–26426, September 25, 2009

Phosphorylation of the Yeast Rpb1 C-terminal Domain at Serines 2, 5, and 7*

Received for publication, June 2, 2009, and in revised form, July 23, 2009 Published, JBC Papers in Press, August 13, 2009, DOI 10.1074/jbc.M109.028993

Minkyu Kim[‡§1], **Hyunsuk Suh**[‡], **Eun-Jung Cho**[¶], **and Stephen Buratowski**[‡2]

From the [‡]*Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, the* [§]*Department of Biophysics and Chemical Biology, Seoul National University, Seoul 151-742, Korea, and the* [¶]*College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea*

The C-terminal domain (CTD) of Rpb1, the largest subunit of RNA polymerase II, acts as a binding platform for various mRNA processing and histone-modifying enzymes that act co-transcriptionally. These factors are targeted to specific phosphorylation states of the CTD that predominate at different stages of transcription. Within the repeating sequence YSPTSPS, serines 2 and 5 are major phosphorylation sites, but serine 7 phosphorylation was recently discovered in mammalian cells. Here we show that CTD serine 7 is also phosphorylated in yeast and that Ser-7(P) chromatin immunoprecipitation patterns resemble those of Ser-5(P). The basal factor TFIIH can phosphorylate Ser-7 *in vitro* and is necessary for Ser-7(P) *in vivo*. Interestingly, deletion of the CTD Ser-5(P) phosphatase Rtr1 leads to an increase in Ser-5(P) but not Ser-7(P).

Transcription by RNA polymerase II (RNA pol II)[3] is coupled to several other nuclear processes. RNA transcripts produced by RNA pol II are capped at their 5′ ends, often spliced, and processed at their 3′ ends. RNA pol II transcription also generates a stereotypical set of histone modifications, with high levels of methylation of histone H3 lysine 4 (H3K4) at active promoters and H3K36 methylation marking downstream transcribed regions. For both mRNA processing and histone methylation, the relevant enzymes directly bind to the C-terminal domain (CTD) of the RNA pol II largest subunit (1, 2). The CTD consists of multiple repeats of the sequence YSPTSPS. Different phosphorylation sites predominate at different stages of transcription, with various accessory proteins binding to specific CTD phosphorylation patterns (3). Through this mechanism, information about the location of the RNA pol II molecule on the gene is encoded in the CTD phosphorylation pattern and used to recruit the appropriate mRNA and histone-modifying enzymes at the proper time during the transcription cycle.

The current model is that CTD serine 5 is phosphorylated at the promoter by the basal transcription factor TFIIH. As RNA pol II proceeds into elongation, Ser-5(P) levels are reduced by the Rtr1 phosphatase (4). Meanwhile, Ser-2(P) levels increase as RNA pol II moves farther away from the promoter. The Ser-2 kinases in *Saccharomyces cerevisiae* are Bur1 and Ctk1, which correspond to P-TEFb/Cdk9 in higher eukaryotes (5–7). Levels of Ser-2(P) are modulated by the Ser-2(P) phosphatase Fcp1, which leads to a gradual increase in Ser-2(P) as RNA pol II moves farther away from the promoter (8). Yeast factors that are localized to promoters via the Ser-5(P) CTD include capping enzyme (3, 9, 10), the H3K4 methyltransferase complex Set1/COMPASS (11), and the Nrd1 protein that contributes to the early termination pathway used at snoRNAs and cryptic unstable transcripts (12). Factors that bind Ser-2(P) or doubly phosphorylated CTD include the H3K36 methyltransferase Set2 (13–18), the polyadenylation factor Pcf11 (19–21), and the Rtt103 protein that contributes to mRNA "torpedo" termination (22).

It is important to note that the Ser-5(P)/Ser-2(P) model is based almost entirely on experiments using the monoclonal IgM antibodies H14 and H5 (3, 23, 24). Although these antibodies clearly recognize distinct epitopes, the Ser-2(P)-recognizing antibody H5 shows some cross-reactivity with Ser-5(P) (8, 25). There have also been varying reports about whether Ser-5(P) is confined to promoters or persists throughout transcribed regions. Recently, Chapman *et al.* (26) generated a new set of monoclonal antibodies with strong specificity for Ser-5(P) (3E8) and Ser-2(P) (3E10), as well as an antibody that recognizes Ser-7(P) (4E12). Here we use these antibodies to confirm that Ser-5(P) levels are highest at promoters, whereas Ser-2(P) levels rise with increasing distance from the promoter. We also help explain why Ser-5(P) levels are usually seen to be highest at promoters but sometimes reported to remain high throughout elongation. Finally, in agreement with a recent report (27), we find that yeast CTD is also phosphorylated at Ser-7 and that this phosphorylation is dependent upon the kinase activity of basal transcription factor TFIIH. Ser-7(P) patterns are similar to those of Ser-5(P), and inhibition of Kin28 leads to loss of both phosphorylations. In contrast, deletion of the Ser-5(P) phosphatase Rtr1 (4) increases Ser-5(P) but not Ser-7(P).

* This work was supported, in whole or in part, by National Institutes of Health Grants GM46498 and GM56663 (to S. B.).
[1] Supported in part by the World Class University project (Project 305-20080089) from the Korean Ministry of Education, Science and Technology.
[2] To whom correspondence should be addressed: Harvard Medical School, 240 Longwood Ave., Boston, MA 02115. Tel.: 617-432-0696; Fax: 617-738-0516; E-mail: SteveB@hms.harvard.edu.
[3] The abbreviations used are: RNA pol II, RNA polymerase II; CTD, C-terminal domain; GST, glutathione *S*-transferase; ChIP, chromatin immunoprecipitation; Ser-5(P)/-2(P)/-7(P), phosphorylated serine 5, 2, or 7; DMSO, dimethyl sulfoxide; as, analog-sensitive; NA-PP1, 1-(1,1-dimethylethyl)-3-(1-naphthalenyl)-1*H*-pyrazolo[3,4-D]pyrimidin-4-amine; snoRNA, small nucleolar RNA; snRNA, small nuclear RNA.

EXPERIMENTAL PROCEDURES

Chromatin Immunoprecipitation (ChIP)—Chromatin solutions were prepared as described previously (28). Twenty or ten microliters of 4E12, 3E8, and 3E10 rat monoclonal antibodies (cell culture supernatant, a generous gift from Dirk Eick, Munich Center for Integrated Protein Science) were prebound to 15 μl of protein G-Sepharose (GE Healthcare) at room temperature for 2 h and then incubated overnight with 400 μl of chromatin solution (~800 μg of protein) at 4 °C. Beads were then washed sequentially with FA lysis buffer (50 mM HEPES-KOH (pH 7.5), 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS) plus 275 mM NaCl, FA lysis buffer plus 500 mM NaCl, ChIP wash buffer (10 mM Tris-HCl (pH 8.0), 0.25 M LiCl, 1 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate), and TE (10 mM Tris-HCl (pH 8.0), 1 mM EDTA). Immunoprecipitated chromatin was eluted from the beads by heating for 10 min at 65 °C in the presence of 50 mM Tris-HCl (pH 7.5), 10 mM EDTA, 1% SDS and then incubated with Pronase (Roche Applied Science, 1 mg/ml final concentration) for 1 h at 42 °C. Samples were then heated for 5 h at 65 °C to reverse the cross-links. For H14 and H5 ChIP, antibodies and anti-mouse IgM agarose (Sigma A4540) were added simultaneously to chromatin without preincubation. For monoclonal antibody H14, the first two washes were substituted with FA lysis buffer plus 750 mM NaCl. In contrast, H5-precipitated beads were washed with FA lysis buffer plus 150 mM NaCl. After cross-link reversal, samples were processed and assayed by PCR as described previously (28).

In Vitro CTD Kinase Assay—Native TFIIH and recombinant GST·CTD were purified as described previously (29, 30). To prepare dephosphorylated substrate, 600 μg of GST·CTD were dephosphorylated with 60 units of Antarctic phosphatase (New England Biolabs) in 500 μl of 1× Antarctic phosphatase buffer for 1 h at 37 °C. The protein was then repurified with glutathione-agarose (Sigma), dialyzed overnight in 20 mM HEPES-KOH (pH 7.0), 150 mM NaCl, 10% glycerol, 1 mM dithiothreitol at 4 °C, and stored at −80 °C until use.

Kinase reactions were carried out at room temperature in 25 μl of 20 mM HEPES-KOH (pH 7.5), 7.5 mM magnesium acetate, 2 mM dithiothreitol, 100 mM potassium acetate, 2% glycerol, 25 μM ATP, 200 ng of GST·CTD, and ~50 ng of purified TFIIH. Reaction products were resolved on a 6.5% SDS-polyacrylamide gel, transferred to an Optitran BA-S 83 reinforced nitrocellulose membrane (Whatman), and analyzed by immunoblotting. Primary antibodies that were used include rat anti-Ser-7(P) (4E12, 1:200), rat anti-Ser-5(P) (3E8, 1:200), rat anti-Ser-2(P) (3E10, 1:200), and mouse anti-GST (Santa Cruz Biotechnology, B-14, 1:1000) monoclonal antibodies. Peroxidase-conjugated secondary antibodies were anti-rat IgG (Sigma A9037, 1:10,000) or anti-mouse IgG (Sigma A2304, 1:10,000). Signals were developed with the SuperSignal West Pico chemiluminescent substrate (Thermo Scientific) for Ser-5(P) and GST signals and with the SuperSignal West Femto maximum sensitivity substrate (Thermo Scientific) for Ser-7(P) and Ser-2(P) signals.

NA-PP1 Treatment—Kin28-as (analog-sensitive) and isogenic wild-type cells (31) were a gift from S. Hahn, Fred Hutchinson Cancer Research Center, Seattle, WA. Cells were grown to an A_{595} of 0.3 in synthetic complete medium and treated with either NA-PP1 (Toronto Research Chemicals, final concentration 10 μM) or DMSO (mock) for 2 h.

RESULTS

Chromatin Immunoprecipitation Using New CTD Ser-5(P) and Ser-2(P) Antibodies—To verify our earlier results using the H5 and H14 antibodies, ChIP experiments were carried out using the new monoclonal antibodies created by Chapman *et al.* (26). On both the *PMA1* and the *ADH1* mRNA genes, the anti-Ser-2(P) antibody 3E10 showed a pattern very similar to that seen with H5, confirming that Ser-2(P) levels are low near the 5′ end of genes and progressively increase with distance from the promoter (Fig. 1, *A* and *B*). On the *snR33* snoRNA gene, neither 3E10 nor H5 showed appreciable reactivity, consistent with termination only a few hundred nucleotides downstream of the promoter, before Ser-2(P) reaches detectable levels (Fig. 1*C*).

The anti-Ser-5(P) antibodies H14 and 3E8 both showed strong reactivity at the 5′ ends of genes (Fig. 1, *A–C*). However, in initial experiments, the 3E8 pattern showed a much stronger signal persisting throughout the transcribed region of longer genes, essentially matching total RNA pol II (not shown). In contrast, H14 reactivity dropped soon after the promoter (Fig. 1, *A* and *B*). Although this could indicate that the two antibodies were recognizing different patterns of Ser-5(P), we instead suspected that the 3E8 was more sensitive to lower levels of Ser-5(P) that persist during elongation (3). To test this idea, the ChIPs were repeated with less 3E8 antibody (Fig. 2). As predicted, the 3E8 pattern now closely resembled that of H14, with a distinct peak of Ser-5(P) apparent at the promoter. It is also notable that the normalized signal was much higher due to a significant reduction in the non-transcribed background signal. The variation in Ser-5(P) pattern is readily explained by the multi-epitope nature of the CTD. At saturating levels of antibody, any RNA pol II molecules with at least one accessible Ser-5(P) epitope will be precipitated. The results under these conditions correctly lead to the conclusion that some level of Ser-5(P) persists throughout elongation. In contrast, at lower antibody levels, the RNA pol II molecules with the most Ser-5-phosphorylated repeats will be preferentially recognized, leading to the equally correct conclusion that Ser-5(P) levels are highest at promoters. Therefore, the different Ser-5(P) patterns reported are unlikely to be due to the antibodies specifically but rather to the affinity or titer of that specific antibody lot.

CTD Serine 7 Phosphorylation in S. cerevisiae Is Dependent upon TFIIH—ChIP of mammalian chromatin using the 4E12 antibody, which reacts with Ser-7(P), indicated that this modification predominated in downstream transcribed regions (26). Given that this modification was linked specifically to recruitment of snRNA processing factors that are not present in yeast (32), it was unclear whether to expect a Ser-7(P) signal in *S. cerevisiae*. In fact, ChIP in yeast cells with 4E12 gave a very clear signal (Figs. 1 and 2). Surprisingly, the 4E12 signal was strongest at the 5′ ends of genes and decreased with distance from the promoter, similar to the Ser-5(P) pattern. Ser-7(P) was also observed on the short, non-polyadenylated *snR33* gene (Fig. 1*C*). As observed with

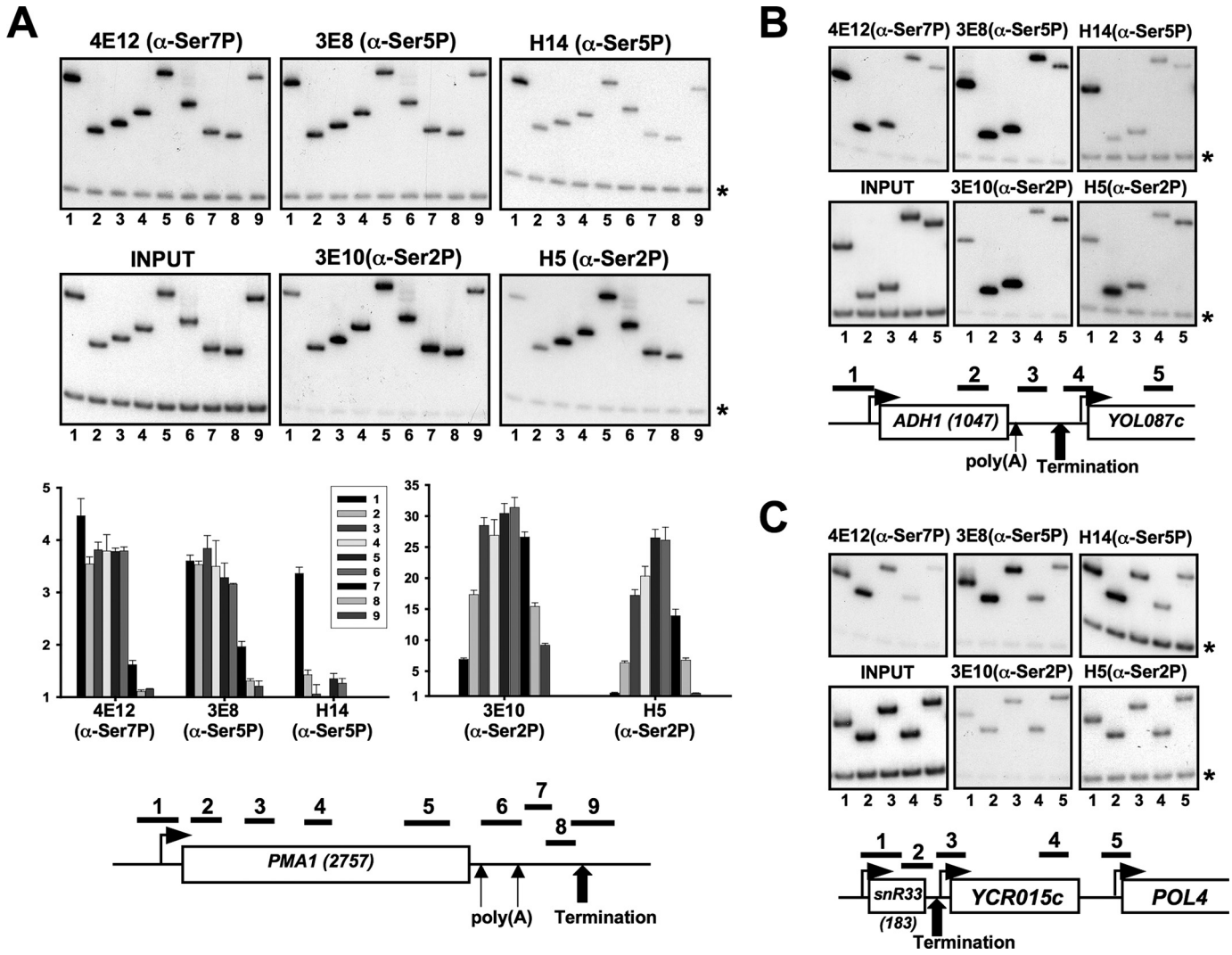

FIGURE 1. **CTD Ser-7 is phosphorylated at active genes in budding yeast.** Chromatin was immunoprecipitated with the indicated antibodies recognizing different CTD phosphorylation sites. *A*, precipitated chromatin was used for PCR amplification with *PMA1* primers as diagrammed in the *bottom panel*. The top band is the *PMA1*-specific band, whereas the common lower band (marked by an *asterisk*) is an internal background control from a non-transcribed region on chromosome VI. The *middle panel* shows quantification of ChIP data, where the *y* axis shows the -fold enrichment of specific signal over the background. *Ser7P*, Ser-7(P); *Ser5P*, Ser-5(P); *Ser2P*, Ser-2(P). *B* and *C*, a similar analysis was carried out on the *ADH1* and *snR33* genes. *Numbers in parentheses* signify the length of the open reading frame or processed snoRNA (represented as *rectangles*). The *bent arrow* signifies the transcription start site; the *straight arrows* mark the polyadenylation and termination sites.

3E8, using lower levels of 4E12 antibody accentuated the postpromoter decrease in Ser-7(P). However, a peak of reactivity was also now observed in the polyadenylation site of the *PMA1* (but not *ADH1*) gene (Fig. 2). The source for this reactivity is unclear but suggests that Ser-5(P) and Ser-7(P) are not necessarily tightly linked.

Given that the Ser-7(P) pattern resembles that of Ser-5(P), we tested whether the kinase activity of TFIIH subunit Kin28, which phosphorylates CTD Ser-5, might also be necessary for the Ser-7(P) signal. This was done using an altered substrate mutant of Kin28 (Kin28-as) that can be specifically inhibited by the chemical NA-PP1 (31). Treatment of cells with NA-PP1 followed by ChIP showed that Ser-5(P) levels were greatly reduced as expected. Furthermore, Ser-7(P) levels were also decreased to near background levels upon Kin28 inhibition (Fig. 3). In contrast, Ser-2(P) and Rpb3 levels were only decreased by about 50–70%, consistent with previous reports by us and others that loss of Kin28 kinase activity, as opposed to Kin28 protein, does not lead to loss of transcription (3, 6, 27, 33). Therefore, the drop in Ser-7(P) is not an indirect effect caused by cessation of transcription.

TFIIH Can Directly Phosphorylate CTD Ser-7—Because the dependence of Ser-7(P) on Kin28 could be direct or indirect, purified TFIIH was tested for the ability to phosphorylate the CTD on Ser-7 *in vitro*. TFIIH was purified from yeast extracts as described (29, 30) and incubated with recombinant GST·CTD produced in bacteria. After incubating the proteins with ATP, the reactions were probed with the new CTD monoclonal antibodies (Fig. 4). As expected, TFIIH produced a strong Ser-5(P) signal but little or no Ser-2(P). It was difficult to interpret the Ser-7(P) result because the GST·CTD produced a positive signal with 4E12 in the absence of any kinase, although a new band appeared that co-migrated with the shifted Ser-5(P) band (Fig. 4*A*). This

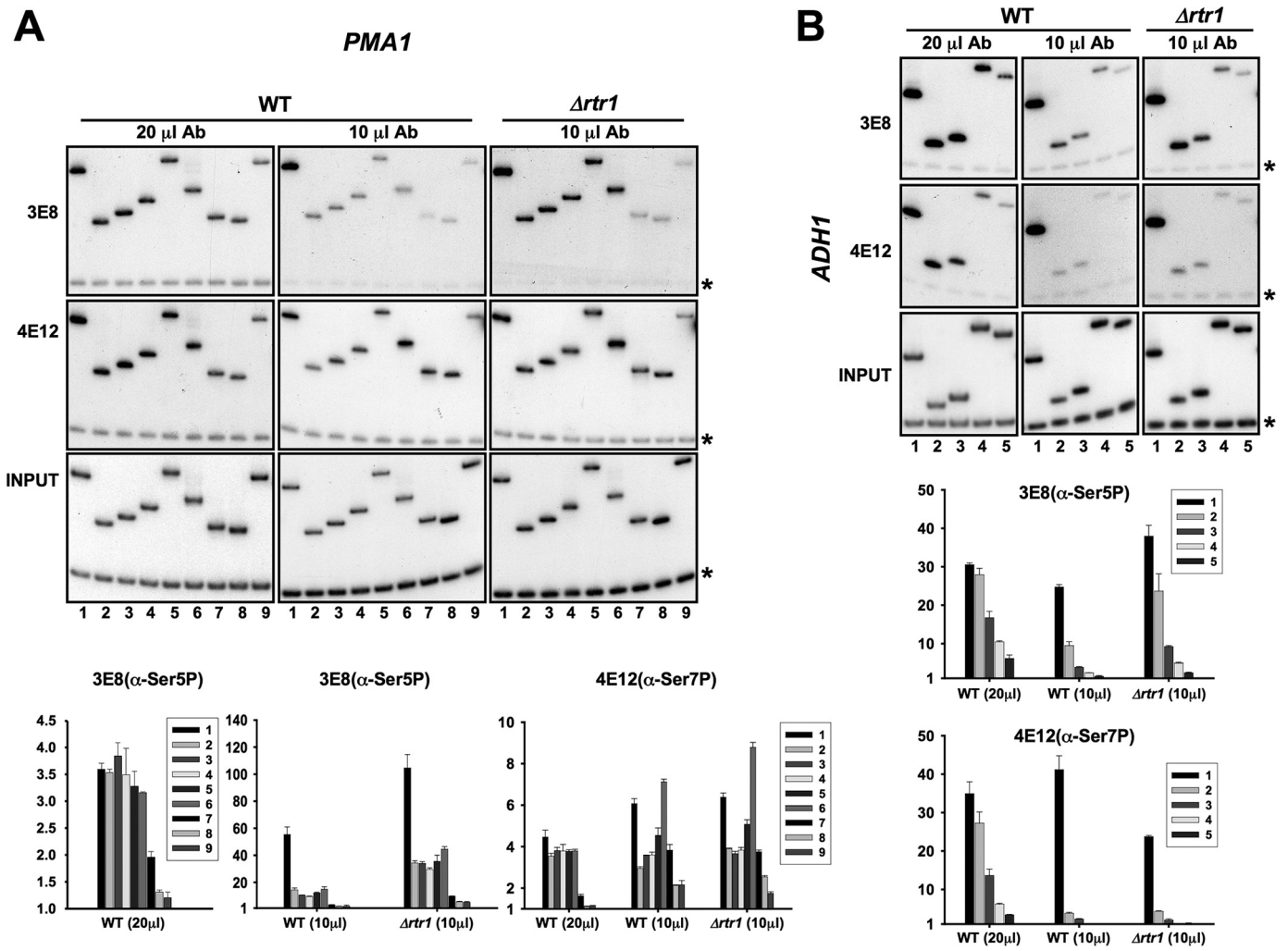

FIGURE 2. **CTD Ser-5(P) (*Ser5P*) and Ser-7(P) (*Ser7P*) ChIP patterns using different antibody (*Ab*) concentrations and cells lacking the Rtr1 CTD phosphatase.** Chromatin from wild-type (*WT*) cells was immunoprecipitated with 20 or 10 μl of 3E8 (α-Ser-5(P)) or 4E12 (α-Ser-7(P)) followed by PCR amplification with primers depicted in Fig. 1. The higher level of antibody shows Ser-5(P) and Ser-7(P) evenly throughout the gene, whereas reducing the amount of antibody accentuates higher phosphorylation levels near the promoter. In parallel, chromatin from an *rtr1*Δ mutant was immunoprecipitated with 10 μl of each antibody to test its effect on Ser-5(P) and Ser-7(P). *A*, ChIP analysis on the *PMA1* gene. Gels are shown in the *top panels*, and quantification of ChIP data appears below. *B*, ChIP analysis on *ADH1* gene as in *panel A*.

result could mean either that 4E12 cross-reacted with unphosphorylated CTD or that some bacterial kinase was phosphorylating the recombinant GST·CTD. To distinguish between these possibilities, the GST·CTD was incubated with phosphatase and the experiment was repeated (Fig. 4*B*). This treatment led to loss of the kinase-independent Ser-7(P) signal, indicating that GST·CTD produced in bacteria is already phosphorylated at Ser-7. However, dephosphorylated GST·CTD incubated with purified TFIIH and ATP clearly reacted with anti-Ser-7(P) antibody, indicating that TFIIH can directly phosphorylate this residue.

Loss of Rtr1 Increases Ser-5(P) but Not Ser-7(P)—The Rtr1 protein was recently shown to be responsible for dephosphorylation of Ser-5(P) during elongation, with Ser-5(P) being assayed by reactivity with the H14 antibody (4). An *rtr1*Δ strain was tested with 3E8 and 4E12 antibodies (Fig. 2). As reported previously, a large increase in Ser-5(P) levels was observed, particularly in downstream transcribed regions. In contrast, levels of Ser-7(P) did not increase. This suggests that Rtr1 may be specific for Ser-5(P) and that there could be a distinct phosphatase that targets Ser-7(P).

DISCUSSION

The proposal that Ser-5(P) marks active promoters, whereas Ser-2(P) gradually increases with distance from the promoter, was based on ChIP experiments using the H14 and H5 monoclonal antibodies (3). The Ser-5(P)/Ser-2(P) pattern has now been confirmed using an independently derived set of monoclonal antibodies (26). In the process of characterizing the new antibodies, some insight was gained into the variability that has been reported as to whether Ser-5(P) is limited to promoters or persists throughout elongation. We believe that this variability is due to the repetitive nature of the CTD. At saturating antibody titers, a single phosphorylated repeat should be sufficient to precipitate RNA pol II. Under these conditions, it becomes clear that some level of Ser-5(P) persists throughout elongation. However, because there is no distinction between RNA pol II molecules that are highly phosphorylated and those with low levels, the immunoprecipitation signal is not

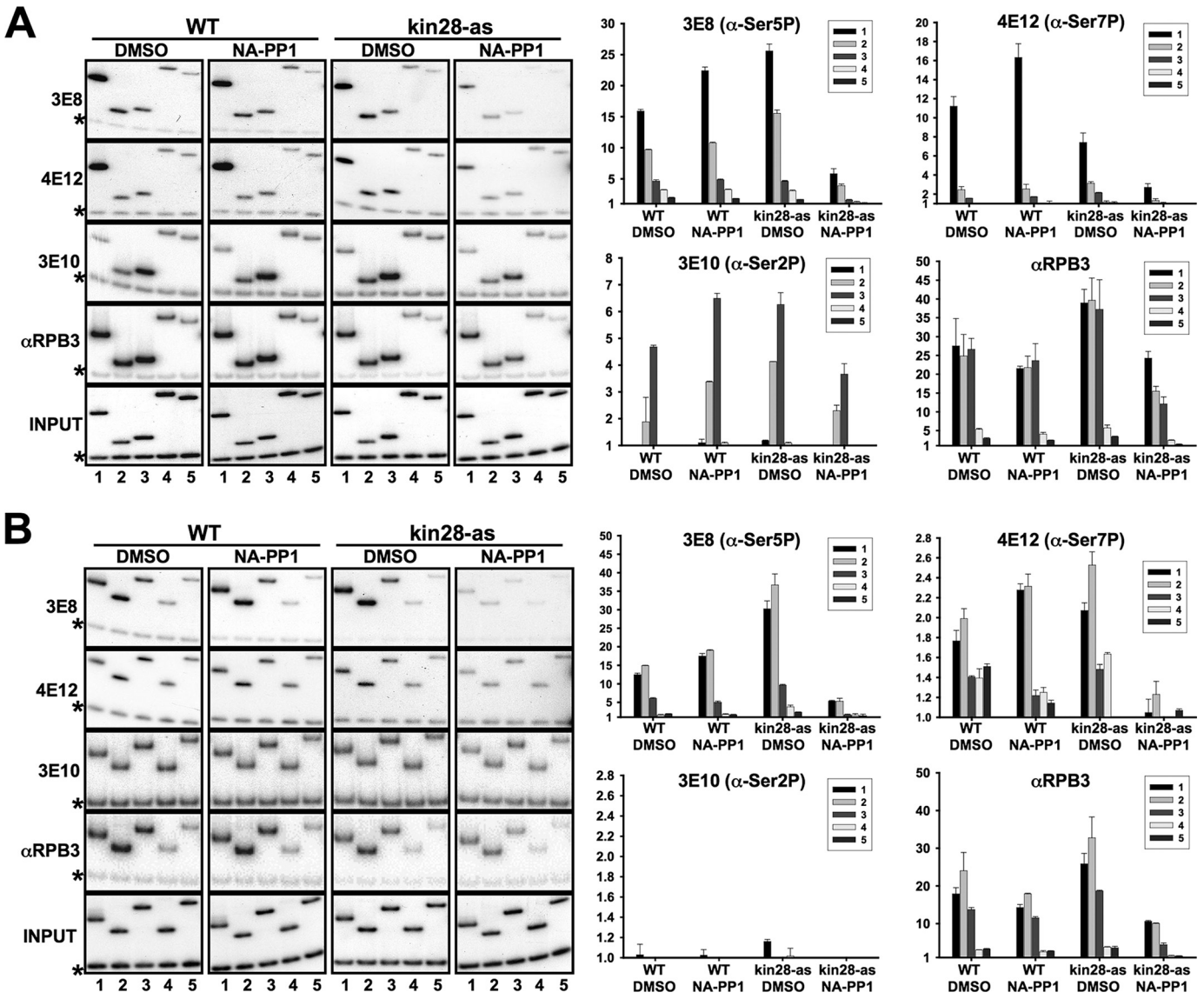

FIGURE 3. **Chemical inhibition of Kin28 kinase results in a decrease of both Ser-5(P) and Ser-7(P).** An analog-sensitive Kin28 mutant strain (kin28-as) and its isogenic wild-type (*WT*) counterpart were treated with DMSO (the solvent for NA-PP1) or 10 μM NA-PP1 for 2 h at 30 °C. Cells were then subjected to ChIP assays with 3E8 (α-Ser-5(P) (*α-Ser5P*)), 4E12 (α-Ser-7(P) (*α-Ser7P*)), 3E10 (α-Ser-2(P) (*α-Ser2P*)), and α-Rpb3 antibodies. *A*, ChIP analysis of the *ADH1* gene (*left panel*) and its quantification (*right panels*). *B*, similar ChIP analysis of the *snR33* gene.

linearly responsive to the number of epitopes. In contrast, at subsaturating antibody titers, there is clearly greater reactivity at promoters. This is presumably due to the increased probability of an antibody molecule reacting with a CTD that contains many phosphorylated repeats.

This hypothesis helps to explain several earlier observations. First, we have noted variability between different lots of H14 antibody, with only some batches showing appreciable signals in internal transcribed regions (3). Inconsistent antibody titers are likely to contribute to variability. H14 is an IgM, a class of multivalent antibodies, which may result in H14 binding being highly cooperative when multiple Ser-5(P) sites are encountered, further enhancing non-linearity of reactivity. Second, ChIPs in mammalian and *Drosophila* cells, which have a greater number of CTD repeats per polymerase molecule, often show a robust H14 signal in downstream transcribed regions. Third, deletion of the CTD phosphatase Rtr1 in yeast leads to a high H14 signal extending throughout the transcribed region, consistent with an increased number of Ser-5(P) epitopes being generated (4). In any case, the variation in ChIP patterns seen with different amounts of antibodies should be taken into consideration when comparing different reports and serves to underscore the need for additional approaches to characterize CTD phosphorylation patterns.

The existence of Ser-7(P) in *S. cerevisiae* is interesting and unexpected. The only role assigned to Ser-7(P) so far is in recruiting the mammalian snRNA 3′-processing complex Integrator (32). Given that *S. cerevisiae* does not contain an Integrator-like complex, it remains to be seen whether Ser-7(P) has an undiscovered function. It is interesting that the Ser-7(P) ChIP pattern is similar but not always identical to Ser-5(P), and Ser-7(P) does not appear to be targeted by the Ser-5(P) phosphatase Rtr1. We also observed Ser-7 phosphorylation on recombinant GST·CTD made in *Escherichia coli*, a finding that must be taken into consideration when using bacterially produced CTD for binding experiments.

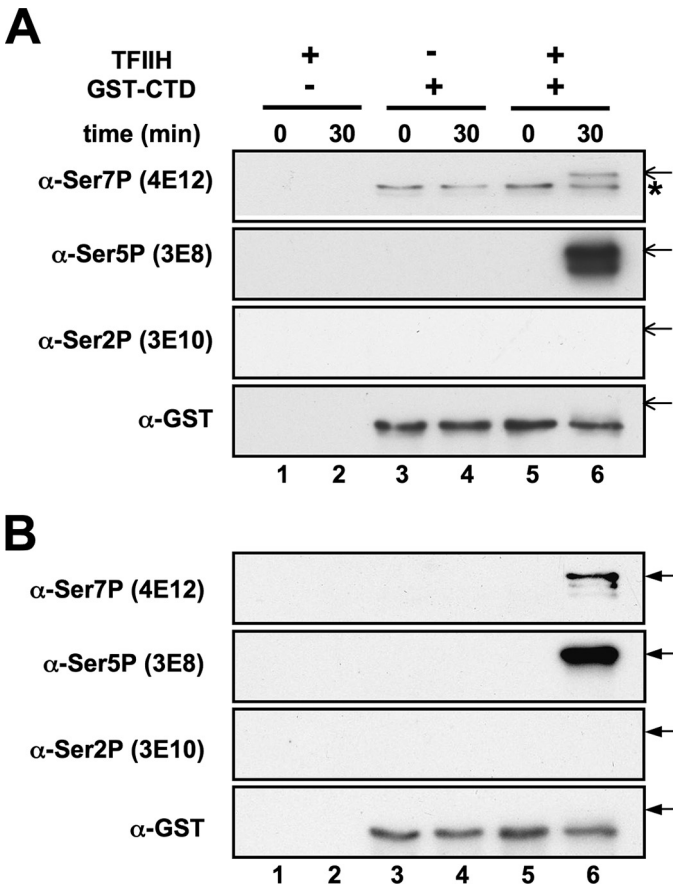

FIGURE 4. ***In vitro* phosphorylation of CTD Ser-7 and Ser-5 by TFIIH complex.** *A*, *in vitro* CTD kinase assays were carried out using recombinant GST·CTD and affinity-purified TFIIH, as indicated. Reactions were carried out at room temperature for 0 min or 30 min. GST·CTD or phosphorylated forms of CTD were detected by immunoblotting with α-GST, α-Ser-7(P) (*α-Ser7P (4E12)*), α-Ser-5(P) (*α-Ser5P (3E8)*), and α-Ser-2(P) (*α-Ser2P (3E10)*) antibodies. Unexpectedly, untreated recombinant GST·CTD reacted with α-Ser-7(P) antibody (*lanes 3* and *4*, marked by an *asterisk*), suggesting that a bacterial kinase phosphorylated Ser-7. *B*, the same assay as in *panel A* was carried out using dephosphorylated GST·CTD as a substrate. Ser-7(P) and Ser-5(P) signals were only detected in *lane 6*. *Arrows* indicate identical positions along the length of each blot.

Serine 7 is the most degenerate position of the CTD (34). Only about half of the human repeats, mostly in the N-terminal half of the CTD, has a serine at position 7. In *Drosophila*, the number is even less, with only 7 out of 44 repeats containing a Ser-7. The divergence at this position has led to speculation that the non-consensus repeats may have evolved specific functions not shared with consensus repeats. Further experiments will be required to discern its function, but the fact that Ser-7 phosphorylation appears widely conserved over evolution suggests that this modification could be important at some stage of the CTD cycle.

Acknowledgments—We thank Dirk Eick for CTD antibodies, Steve Hahn for the Kin28-as strain, Michael Keogh for GST·CTD, and Dirk Eick and Aseem Ansari for sharing information before publication.

REFERENCES

1. Buratowski, S. (2003) *Nat. Struct. Biol.* **10,** 679–680
2. Hampsey, M., and Reinberg, D. (2003) *Cell* **113,** 429–432
3. Komarnitsky, P., Cho, E. J., and Buratowski, S. (2000) *Genes Dev.* **14,** 2452–2460
4. Mosley, A. L., Pattenden, S. G., Carey, M., Venkatesh, S., Gilmore, J. M., Florens, L., Workman, J. L., and Washburn, M. P. (2009) *Mol. Cell* **34,** 168–178
5. Peterlin, B. M., and Price, D. H. (2006) *Mol. Cell* **23,** 297–305
6. Qiu, H., Hu, C., and Hinnebusch, A. G. (2009) *Mol. Cell* **33,** 752–762
7. Viladevall, L., St Amour, C. V., Rosebrock, A., Schneider, S., Zhang, C., Allen, J. J., Shokat, K. M., Schwer, B., Leatherwood, J. K., and Fisher, R. P. (2009) *Mol. Cell* **33,** 738–751
8. Cho, E. J., Kobor, M. S., Kim, M., Greenblatt, J., and Buratowski, S. (2001) *Genes Dev.* **15,** 3319–3329
9. Schroeder, S. C., Schwer, B., Shuman, S., and Bentley, D. (2000) *Genes Dev.* **14,** 2435–2440
10. Fabrega, C., Shen, V., Shuman, S., and Lima, C. D. (2003) *Mol. Cell* **11,** 1549–1561
11. Ng, H. H., Robert, F., Young, R. A., and Struhl, K. (2003) *Mol. Cell* **11,** 709–719
12. Vasiljeva, L., Kim, M., Mutschler, H., Buratowski, S., and Meinhart, A. (2008) *Nat. Struct. Mol. Biol.* **15,** 795–804
13. Gerber, M., and Shilatifard, A. (2003) *J. Biol. Chem.* **278,** 26303–26306
14. Krogan, N. J., Kim, M., Tong, A., Golshani, A., Cagney, G., Canadien, V., Richards, D. P., Beattie, B. K., Emili, A., Boone, C., Shilatifard, A., Buratowski, S., and Greenblatt, J. (2003) *Mol. Cell. Biol.* **23,** 4207–4218
15. Li, B., Howe, L., Anderson, S., Yates, J. R., 3rd, and Workman, J. L. (2003) *J. Biol. Chem.* **278,** 8897–8903
16. Li, J., Moazed, D., and Gygi, S. P. (2002) *J. Biol. Chem.* **277,** 49383–49388
17. Schaft, D., Roguev, A., Kotovic, K. M., Shevchenko, A., Sarov, M., Shevchenko, A., Neugebauer, K. M., and Stewart, A. F. (2003) *Nucleic Acids Res.* **31,** 2475–2482
18. Xiao, T., Hall, H., Kizer, K. O., Shibata, Y., Hall, M. C., Borchers, C. H., and Strahl, B. D. (2003) *Genes Dev.* **17,** 654–663
19. Licatalosi, D. D., Geiger, G., Minet, M., Schroeder, S., Cilli, K., McNeil, J. B., and Bentley, D. L. (2002) *Mol. Cell* **9,** 1101–1111
20. Meinhart, A., and Cramer, P. (2004) *Nature* **430,** 223–226
21. Noble, C. G., Hollingworth, D., Martin, S. R., Ennis-Adeniran, V., Smerdon, S. J., Kelly, G., Taylor, I. A., and Ramos, A. (2005) *Nat. Struct. Mol. Biol.* **12,** 144–151
22. Kim, M., Krogan, N. J., Vasiljeva, L., Rando, O. J., Nedea, E., Greenblatt, J. F., and Buratowski, S. (2004) *Nature* **432,** 517–522
23. Kim, E., Du, L., Bregman, D. B., and Warren, S. L. (1997) *J. Cell Biol.* **136,** 19–28
24. Patturajan, M., Schulte, R. J., Sefton, B. M., Berezney, R., Vincent, M., Bensaude, O., Warren, S. L., and Corden, J. L. (1998) *J. Biol. Chem.* **273,** 4689–4694
25. Jones, J. C., Phatnani, H. P., Haystead, T. A., MacDonald, J. A., Alam, S. M., and Greenleaf, A. L. (2004) *J. Biol. Chem.* **279,** 24957–24964
26. Chapman, R. D., Heidemann, M., Albert, T. K., Mailhammer, R., Flatley, A., Meisterernst, M., Kremmer, E., and Eick, D. (2007) *Science* **318,** 1780–1782
27. Akhtar, M. S., Heidemann, M., Tietjen, J. R., Zhang, D. W., Chapman, R. D., Eick, D., and Ansari, A. Z. (2009) *Mol. Cell* **34,** 387–393
28. Kim, M., Ahn, S. H., Krogan, N. J., Greenblatt, J. F., and Buratowski, S. (2004) *EMBO J.* **23,** 354–364
29. Keogh, M. C., Cho, E. J., Podolny, V., and Buratowski, S. (2002) *Mol. Cell. Biol.* **22,** 1288–1297
30. Svejstrup, J. Q., Feaver, W. J., LaPointe, J., and Kornberg, R. D. (1994) *J. Biol. Chem.* **269,** 28044–28048
31. Liu, Y., Kung, C., Fishburn, J., Ansari, A. Z., Shokat, K. M., and Hahn, S. (2004) *Mol. Cell. Biol.* **24,** 1721–1735
32. Egloff, S., O'Reilly, D., Chapman, R. D., Taylor, A., Tanzhaus, K., Pitts, L., Eick, D., and Murphy, S. (2007) *Science* **318,** 1777–1779
33. Kanin, E. I., Kipp, R. T., Kung, C., Slattery, M., Viale, A., Hahn, S., Shokat, K. M., and Ansari, A. Z. (2007) *Proc. Natl. Acad. Sci. U.S.A.* **104,** 5812–5817
34. Allison, L. A., Wong, J. K., Fitzpatrick, V. D., Moyle, M., and Ingles, C. J. (1988) *Mol. Cell. Biol.* **8,** 321–329