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Interplay of positive and negative effectors in function of the C-terminal repeat domain of RNA polymerase II

(yeast/transcription/protein kinase/protein kinase inhibitor)

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ABSTRACT **RNA polymerase II lacking a C-terminal domain (CTD) was active in transcription with purified proteins from yeast but failed to support transcription in a yeast extract. CTD dependence could be reconstituted in the purified system by addition of two fractions from the extract. An inhibitory fraction abolished transcription by both wild-type and CTD-less RNA polymerases; a stimulatory fraction restored activity of the wild-type polymerase but had a much lesser effect on the CTD-less enzyme. Parallel results were obtained with the use of a kinase inhibitor that prevents phosphorylation of the CTD by RNA polymerase II initiation factor b. The kinase inhibitor abolished transcription by wild-type polymerase in yeast extract but had no significant effect in the purified system. The requirement for both the CTD and kinase action for transcription in an extract indicates that CTD phosphorylation is involved in opposing the negative effector in the extract. Factor b must play a role(s) in addition to phosphorylation of the CTD because it was still required for transcription with polymerase lacking a CTD in the purified system.**

RNA polymerase II, the enzyme responsible for mRNA synthesis in eukaryotes, has much in common with other eukaryotic and bacterial RNA polymerases but is distinctive in possessing a repeated heptapeptide at the carboxy terminus of the largest subunit (1, 2). Although the size of this C-terminal domain (CTD) varies from 26 repeats in yeast to 52 in mammals, some number of repeats is essential (a minimum of ≈10 in yeast), and the consensus sequence of the repeat has been conserved across species. The CTD becomes extensively phosphorylated during the initiation of transcription (3), and the protein kinase involved has been identified as essential initiation factor b in yeast (4–6) and its homologs δ in the rat (7, 8) and BTF2/TFIIH (9, 10) in humans. Genetic studies have indicated possible interactions of the CTD with other proteins as well (11–13). Functions considered for the CTD include involvement in transcriptional activation (2), a role in the transition from initiation to elongation (14), and interaction with DNA (15), possibly leading to the displacement of histones or of other DNA-binding proteins (2, 12).

Biochemical studies to date have failed to clarify the role of the CTD in transcription. Some, but not all, promoters require the CTD for initiation (16–20), and some, but not all, activators require the CTD for stimulation of the process (19, 21, 22). Anti-CTD antibodies inhibit initiation both from promoters that require the CTD and from those that do not (7, 16, 23–25). The CTD kinase is essential for initiation, but a kinase inhibitor fails to block the process (26). Finally, cell extracts lacking SRB2 protein, an allele-specific suppressor of CTD truncations, are deficient in transcription (27), although SRB2 protein is not required for reconstitution of the process (28).

We report here on biochemical studies of CTD function showing that some of the complexity and seeming anomalies in previous work may have to do with the transcription systems used. The CTD appears not to be required for transcription from any promoter in a basal system comprising homogeneous or highly purified components. Dependence on the CTD can be reconstituted by introduction in this basal system of multiple additional factors, opening the way to biochemical dissection of CTD function and elucidation of its mechanism of action.

MATERIALS AND METHODS

Construction and Purification of CTD-Less RNA Polymerase II. For construction of the *RPB1-Xa* gene, *Kpn* I–*Bam*HI and *Bam*HI–*Hin*dIII fragments were prepared by PCR of the *RPB1* gene with the following primers: 5′-CCCCTGGTACCGGTGCATTTGATGTG-3′, 5′-CCCCGGATCCTCGACCTTCAATTGCTTCACCATAAGCAGCAAATGGAGACGTGGCTT-3′, 5′-CCCCGGATCCTTTGGAGTCTCCTCACCAGGCTT-3′, and 5′-CCCCAAGCTTAGAAGTTGGACGGACG-3′. The *Kpn* I–*Hin*dIII region of *RPB1* was replaced with the two PCR products to give *RPB1-Xa*, and an *Eco*RI–*Hin*dIII fragment containing the entire gene was inserted between the *Eco*RI and *Hin*dIII sites of YCp50 (29). The *URA3* marker was replaced by *LEU2*, by insertion of a *Xho* I (filled in)–*Sal* I fragment in the *Sal* I–*Sma* I region, to give YCpL5. Construction of YCpL14 was the same, except the second and third PCR primers listed above were replaced by 5′-CCCCCGGATCCTGCGTAATCCGGGACGTCGTAAGGATATGCTTCACCATAAGCAGCAAATGG-3′ and 5′-CCCCCGGATCCATTGAAGGTCGAATTGAAGGTCGATTTGGAGTCTCCTCACCAGGC-3′. YCpL5 and YCpL14 were transformed into yeast strain Z26 [a *ura3-52 leu2-3 leu2-112 his3-Δ300 rpb1-187*::*HIS3*/pRP112 (*URA3* CEN4 *RPB1*), (30)] by the lithium acetate procedure (31), followed by 5-fluoroorotic acid selection for loss of the plasmid-bearing wild-type *RPB1*. Wild-type and mutant RNA polymerase IIs were purified as described (32), except that no protease inhibitors were added to DEAE HPLC buffer C. Purified polymerases (230 μg) were treated with bovine factor Xa (FXa) (from S. Krishnaswarmy, Emory University) coupled to CNBr-activated Sepharose 4B (1 ml, Sigma; FXa activity equivalent to 180 μg of free enzyme, assayed with *N*-benzoyl-Ile-Glu-Gly-Arg *p*-nitroanilide, Sigma) in 50 mM Tris·Cl, pH 8.0/175 mM NaCl/3 mM dithiothreitol/1 mM $CaCl_2$ for 10 hr at 4°C. Uncleaved enzyme was removed by binding to 1 ml of 8WG16 monoclonal antibody-Sepharose (32) at 4°C for 1 hr. Cleaved enzymes were concentrated by adsorption to a Mono Q HR 5/5 HPLC column (Pharmacia) and elution with

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Abbreviations: FXa, factor Xa; CTD, C-terminal domain.

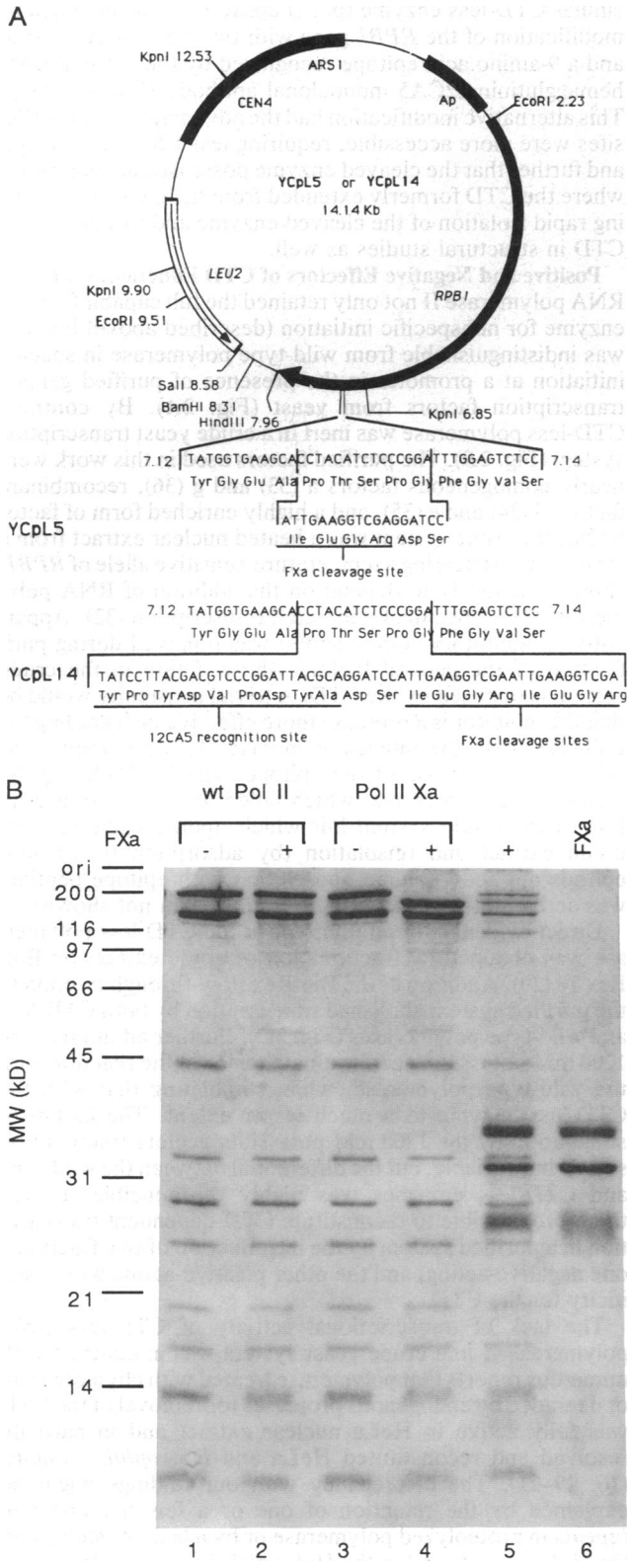

FIG. 1. Preparation of RNA polymerase II lacking the CTD. (*A*) Yeast expression plasmids YCpL5 and YCpL14 contained the *RPB1-Xa* and *RPB1-epi2FXa* genes, respectively, derived from the *RPB1* gene, which encodes the largest subunit of RNA polymerase II (1). In *RPB1-Xa*, 15 nt encoding three amino acids before the start of the CTD and two after (13) were replaced with 18 nt encoding a protease FXa cleavage site. In *RPB1-epi2FXa*, the replacement was with 57 nt encoding the influenza hemagglutinin epitope recognized by the 12CA5 monoclonal antibody (37), followed by two FXa cleavage sites. (*B*) Wild-type RNA polymerase II (wt Pol II) and mutated polymerase II (Pol II Xa), from cells expressing the *RPB1-Xa* gene, were treated with immobilized FXa (+, lanes 2 and 4, 12.5 μg of polymerase) or not (−, lanes 1 and 3, 12.5 μg of polymerase) and analyzed by electrophoresis in an SDS/12.5% polyacrylamide gel (38). A mixture of the two enzymes was digested with free FXa (6.6 μg) and run in the gel (lane 5, 7.5 μg of each) to show that the shift in mobility of the largest subunit upon digestion was due to cleavage and not an electrophoretic anomaly. FXa alone was analyzed as well (lane 6, 6.6 μg).

a linear gradient of 0.1–1.5 M potassium acetate in 50 mM Tris·Cl, pH 7.5/15% (vol/vol) glycerol/2 mM dithiothreitol/1 mM EDTA.

***In Vitro* Transcription.** Basal transcription factors were as follows: fraction VI factor a [3 ng, concentrated on a Mono Q HR 5/5 HPLC column, (33)], factor b [2 μl, (28)], recombinant yeast TATA-binding protein [100 ng, (34)], homogeneous factor e [50 ng, purified by chromatography on Bio-Rex 70, DE52, heparin-Sepharose, and hydroxylapatite; W. J. Feaver, personal communication, and ref. 35], and fraction VI factor g [50 ng, (36)]. Assays were done with a template containing the yeast *CYC1* promoter (pGAL4CG$^-$) or promoters as specified in 37 mM Hepes, pH 7.5/100 mM potassium acetate/0.7 mM ATP/0.7 mM CTP/5 μM UTP/[α-^{32}P]UTP at 0.4 mCi/ml/2.5 mM dithiothreitol/5 mM magnesium acetate/2.5 mM magnesium sulfate/3.7 mM EGTA, (1 Ci = 37 GBq) as described (28). Assays with nuclear extract were as above, except poly(dG·dC) at 33 mg/ml and 4 mM phosphoenolpyruvate were added.

RNA Polymerase II Nonspecific Initiation/Chain-Elongation Assay. The nonspecific assay used was as described in Edwards *et al.* (32). Briefly, the activity was determined by conversion of ^{32}P-labeled GTP to polymeric form with a poly(C) template. Reactions contained 5 μg of poly(C) template (Sigma), 70 mM Tris·Cl (pH 8.0), 3 mM magnesium chloride, 40 mM ammonium sulfate, 5 mM dithiothreitol, and 0.5 mM GTP. Reactions were at 24°C for 20 min and were stopped by spotting onto DE81 paper (Whatman), which was washed with 500 ml of 5% (wt/vol) anhydrous dibasic sodium phosphate, air-dried, and counted by scintillation for radioactivity.

The nonspecific RNA polymerase II activity presented in Fig. 3*B* was measured by a method similar to that of Thompson *et al.* (16). Reactions were performed under the conditions for promoter-specific transcription assays in Fig. 3*B*, except that 0.2 mM GTP was added, and sheared, denatured calf thymus DNA (500 ng) was the template. After 30 min at 24°C, reactions were stopped with 4 μl of 2.5% SDS/0.15 M sodium pyrophosphate and were spotted onto DE81 paper, which was treated as above, except that the washing buffer also contained 0.5% sodium pyrophosphate/0.1% SDS.

RESULTS

CTD-Less RNA Polymerase II. For the preparation of RNA polymerase II lacking a CTD, the gene for the largest subunit (*RPB1*) was modified by replacement of five amino acids from the junction between the CTD and the rest of the subunit (13) with a FXa protease cleavage site (Fig. 1*A*). The modified gene (*RPB1-Xa*) was introduced in yeast in place of the wild-type gene by a plasmid shuffle, yielding a strain dependent on the modified gene for growth. (The viability of the strain shows the CTD can be separated from the rest of the polymerase by a flexible linker.) Polymerase purified from this strain by a procedure devised for the wild-type enzyme (32) could be cleaved with FXa to truncate RPB1 without effect on the other subunits as described in the *Materials and Methods* (Fig. 1*B*). The resulting pol II ΔCTD was indistinguishable from wild-type enzyme in the conventional assay of nonspecific initiation/chain elongation (data not shown). A

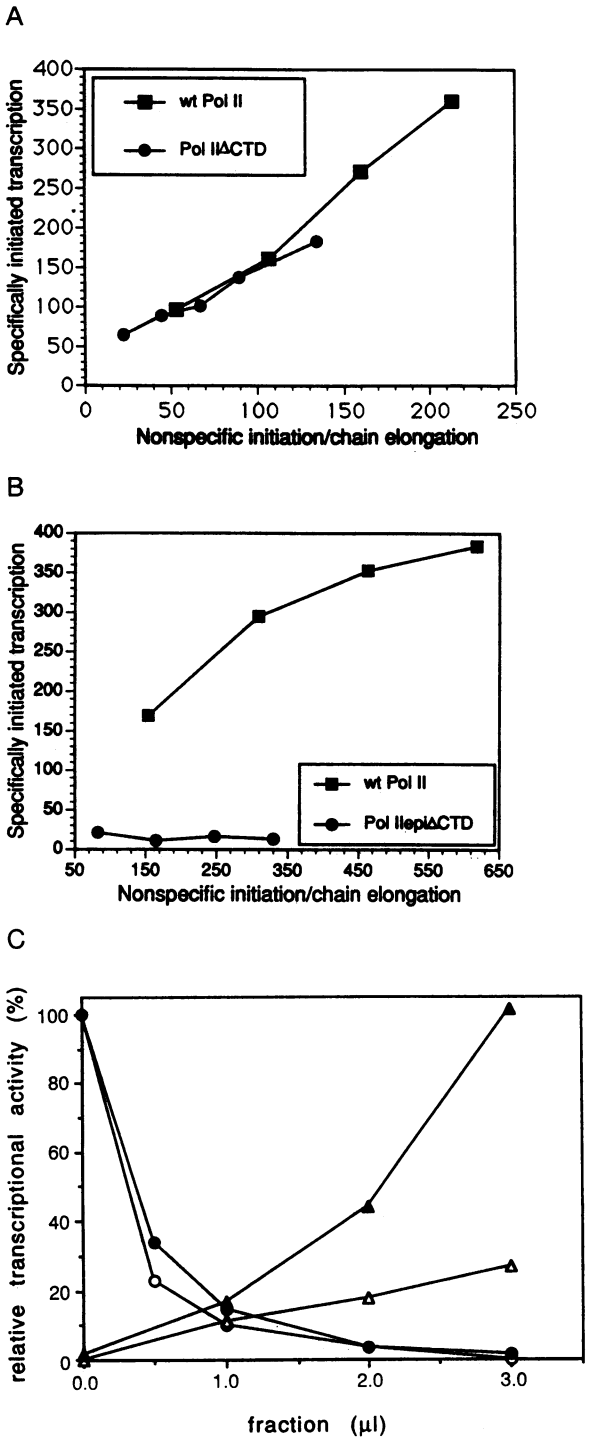

FIG. 2. Specifically initiated transcription by wild-type and CTD-less RNA polymerase IIs in crude and purified systems. (*A*) Transcription in purified system. Wild-type polymerase and CTD-less polymerase were assayed with basal factors a, b, e, and g, as described. Radioactivity in specifically initiated transcripts was quantitated and plotted as a function of the polymerase activity in nonspecific initiation/chain elongation. (*B*) Transcription in crude system. Polymerases as described in *A* and in Fig. 1, except with Pol II epiΔCTD rather than Pol II ΔCTD, were assayed with nuclear extract from yeast strain Y260-1 heated for 12 min at 42°C to inactivate the endogenous polymerase (32). Radioactivity in specifically initiated transcripts was quantitated and plotted as in *A*. (*C*) Effect of Bio-Rex 70 fractions on transcription in purified system. The Bio-Rex 70 column was run as described (28). Bio-Rex flow-through (BRFT) was prepared from the heat-treated nuclear extract described in *B*, and Bio-Rex 1200 mM potassium acetate eluate (BR1200) was prepared from whole-cell extract and precipitated with 72% ammonium sulfate. BR1200 from nuclear extract gave similar results. Transcription was performed as in *A*, except that factors b and g were DEAE-5-PW fractions (28). Symbols: filled, wild-type RNA polymerase II; open, pol IIepiΔCTD; circles, + BRFT; triangles, + BRFT + BR1200.

similar CTD-less enzyme (pol II epiΔCTD) was obtained by modification of the *RPB1* gene with two FXa cleavage sites and a 9-amino acid epitope recognized by the anti-influenza hemagglutinin 12CA5 monoclonal antibody [Fig. 1*A*, (37)]. This alternative modification had the advantages that the FXa sites were more accessible, requiring less FXa for cleavage, and further that the cleaved enzyme possessed an epitope tag where the CTD formerly extended from the molecule, allowing rapid isolation of the cleaved enzyme and location of the CTD in structural studies as well.

Positive and Negative Effectors of CTD Function. CTD-less RNA polymerase II not only retained the full capability of the enzyme for nonspecific initiation (described above) but also was indistinguishable from wild-type polymerase in specific initiation at a promoter in the presence of purified general transcription factors from yeast (Fig. 2*A*). By contrast, CTD-less polymerase was inert in a crude yeast transcription system (Fig. 2*B*). The purified factors used in this work were nearly homogeneous factors a (33) and g (36), recombinant factors d (34) and e (35), and a highly enriched form of factor b (28); the crude system was a heated nuclear extract from a strain of yeast bearing a temperature-sensitive allele of *RPB1*, shown previously to depend on the addition of RNA polymerase II for specifically initiated transcription (32). Apparently an inhibitory component(s) was removed during purification of the general factors whose action in the crude system is opposed by the CTD. A trivial possibility would be that the inhibitor is a protease more effective in degrading the CTD-less than the intact polymerase. This possibility was ruled out by the use of the epitope-tagged CTD-less polymerase described above, which failed to support transcription in the crude system but which upon incubation with crude extract and reisolation (by adsorption to an anti-epitope antibody column and elution with epitope peptide) was active in the reconstituted system (data not shown).

Direct evidence for an inhibitor of the CTD-less polymerase was obtained by fractionation of crude extract on Bio-Rex 70 (28). Addition of the Bio-Rex flow-through fraction to the purified system abolished transcription by both CTD-less and wild-type polymerases (Fig. 2*C*). Further addition of the 1200 mM potassium acetate eluate restored the reaction with the wild-type polymerase, while stimulating that with the CTD-less enzyme to a much lesser extent. The extent of stimulation by the 1200 mM potassium acetate fraction was somewhat variable, but the differential between the wild-type and CTD-less enzymes was highly reproducible. It was therefore possible to reconstitute CTD-dependent transcription in a purified system by the introduction of two fractions, one negative-acting, and the other positive-acting with specificity for the CTD.

The lack of transcriptional activity of CTD-less RNA polymerase II in a crude yeast system was in contrast with numerous reports that polymerase treated with chymotrypsin or degraded by endogenous proteases for removal of the CTD was fully active in HeLa nuclear extract and in partially resolved and reconstituted HeLa and *Drosophila* systems (16, 19–21). The discrepancy with our findings might be explained by the retention of one or a few heptapeptide repeats in proteolyzed polymerase or by a lack of inhibitor in the HeLa extract and in the HeLa and *Drosophila* transcription systems used by others. To test the generality of our results, we repeated the experiments with the adenoviral major late promoter, the *Schizosaccharomyces pombe ADH1* promoter, and the *Saccharomyces cerevisiae ADC1*,

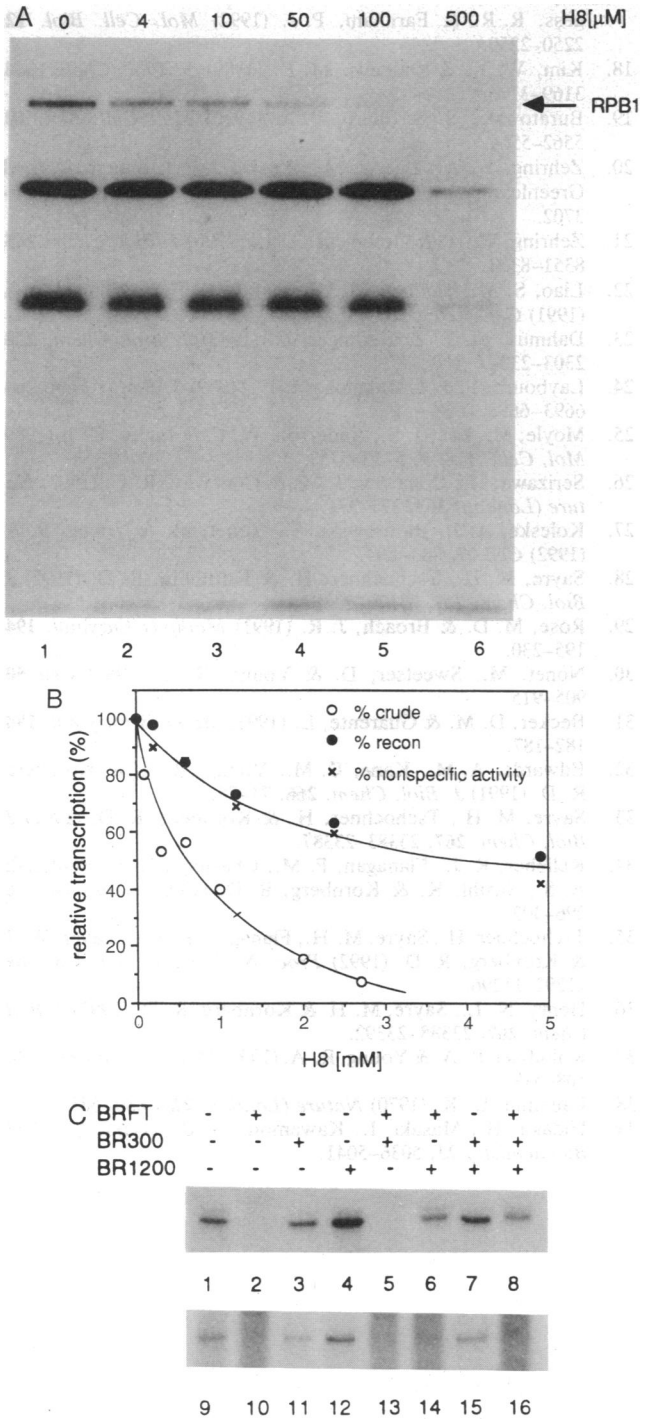

FIG. 3. Effects of the protein kinase inhibitor H8 on factor b-associated CTD kinase activity and RNA polymerase II transcription. (*A*) Inhibition by H8 (Seikagaku America, Inc.) of the phosphorylation of the largest subunit of RNA polymerase II (RPB1) by factor b. The kinase assay, done as described (5), entails reaction of [γ-^{32}P]ATP with purified RNA polymerase II in the presence of factor b, followed by SDS/PAGE and autoradiography. Two prominent bands besides that due to RPB1 arise from contaminants, unrelated to factor b and its substrates, for reasons discussed previously (5) and are distinguished here by lack of sensitivity to H8. recon, Reconstituted. (*B*) Inhibition by H8 of RNA polymerase II transcription in crude (○) and purified (●) systems and in nonspecific initiation/chain elongation (x). All reactions were done with wild-type RNA polymerase II under the conditions of Fig. 2, except that the ATP concentration was 30 μM. (*C*) Effects of Bio-Rex 70 fractions on transcription in the purified system in the presence of kinase inhibitor H8. Transcription proteins were as described in *B*, except that RNA polymerase II and factor g were supplied by a fraction from hydroxylapatite (28) containing both activities. Bio-Rex fractions were all derived from nuclear extract. Reactions 9–16 contained 5 mM H8. BRFT, Bio-Rex flow-through.

CYC1, *GAL1*, *HIS4*, and *PYK1* promoters. In all cases, the CTD-less polymerase was active in purified but not crude transcription systems (data not shown; *ADC1*, *GAL1*, and *HIS4* promoters were tested only in the purified system).

Requirement for CTD-Phosphorylation in a Crude System. These results were extended with the use of a protein kinase inhibitor termed H8, which binds competitively with ATP (39). H8 was shown (26) to inhibit the CTD kinase activity of factor δ, the rat homolog of yeast factor b. H8 also proved effective against factor b kinase, causing 50% inhibition at $\approx$10 μM (Fig. 3*A*). Others have observed H8 inhibition of RNA polymerase II transcription in crude or partially fractionated HeLa systems (M. Dahmus, personal communication), and H8 abolished transcription in the crude yeast system as well (Fig. 3*B*). H8 was much less inhibitory toward transcription with purified factors, and the effect in this system was attributable to inhibition of RNA polymerase rather than protein kinase, as shown by the effect of H8 on the polymerase alone (Fig. 3*B*). Sensitivity to H8 was restored in the purified system by addition of the Bio-Rex flow-through fraction found previously to be involved in CTD-dependent transcription (Fig. 3*C*). The Bio-Rex 1200 mM potassium acetate eluate, which reversed the effect of the flow-through fraction in the absence of H8, failed to do so in its presence. An intermediate fraction, eluting from Bio-Rex at 300 mM potassium acetate, was unable to replace either the flow-through or 1200 mM potassium acetate fractions. The capacity of the same Bio-Rex flow-through fraction to both inhibit CTD-less polymerase and confer H8-sensitivity upon the wild-type enzyme attests to its specificity as a negative effector of transcription. If H8 action in these systems was on factor b kinase, then CTD-phosphorylation must be involved in the reversal of inhibition by the positive effector in the Bio-Rex 1200 mM eluate. Finally, although factor b kinase activity and the CTD are dispensable in the purified system, factor b is nonetheless required for transcription with the CTD-less polymerase in this system (data not shown), so the factor must play an additional role(s) besides phosphorylation of the CTD.

DISCUSSION

Our results extend previous investigations of the CTD in several respects. (*i*) The lack of CTD-dependence in a transcription system reconstituted with highly purified proteins was found for all promoters tested and may be general, in contrast with the variation among promoters reported in less well-resolved systems in the past. (*ii*) The restoration of CTD function by the introduction of two additional chromatographic fractions in the purified system suggests that CTD action is not direct but rather occurs through a multifactorial mechanism. The two chromatographic fractions used were crude, and extensive purification will be required to identify the particular factors involved. (*iii*) CTD phosphorylation is evidently part of a positive mechanism for overcoming the effect of a negative-acting component(s). This finding may resolve a paradox arising from previous studies: on the one hand, the CTD is unique to RNA polymerase II and so is unlikely to be involved in a fundamental step of the transcription process; on the other hand, evidence has been presented for a role of the CTD in the transition from transcription initiation to elongation (14). Inhibition of transcription and its relief by a mechanism involving the CTD can accommodate both observations: the inhibitory and stimulatory factors are presumably specific for

RNA polymerase II transcription; and the block due to inhibition might occur at the transition to elongation. This interplay between positive and negative effectors may be a key control process in RNA polymerase II transcription.

Genetic studies have revealed several proteins, SIN1 (12) and SRBs (27) in particular, that interact with the CTD and that might be the negative and positive effectors of transcription identified here. Further fractionation of the reconstituted system will clarify their roles and identify other components involved in CTD function.

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1. Young, R. A. (1991) *Annu. Rev. Biochem.* **60**, 689–715.
2. Chao, D. M. & Young, R. A. (1991) *Gene Expression* **1**, 1–4.
3. Cadena, D. & Dahmus, M. (1987) *J. Biol. Chem.* **262**, 12468–12474.
4. Feaver, W. J., Gileadi, O. & Kornberg, R. D. (1991) *J. Biol. Chem.* **266**, 19000–19005.
5. Feaver, W. J., Gileadi, O., Li, Y. & Kornberg, R. D. (1991) *Cell* **67**, 1223–1230.
6. Gileadi, O., Feaver, W. J. & Kornberg, R. D. (1992) *Science* **257**, 1389–1392.
7. Conaway, J. W., Bradsher, J. N. & Conaway, R. C. (1992) *J. Biol. Chem.* **267**, 10142–10148.
8. Serizawa, H., Conaway, R. C. & Conaway, J. W. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 7476–7480.
9. Fischer, L., Gerard, M., Chalut, C., Lutz, Y., Humbert, S., Kanno, M., Chambon, P. & Egly, J.-M. (1992) *Science* **257**, 1392–1395.
10. Lu, H., Zawel, L., Fisher, L., Egly, J.-M. & Reinberg, D. (1992) *Nature (London)* **358**, 641–645.
11. Nonet, M. L. & Young, R. A. (1989) *Genetics* **123**, 715–724.
12. Peterson, C. L., Kruger, W. & Herskowitz, I. (1991) *Cell* **64**, 1135–1143.
13. Allison, L. A. & Ingles, C. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2794–2798.
14. Usheva, A., Maldonado, E., Goldring, A., Lu, H., Houbavi, C., Reinberg, D. & Aloni, Y. (1992) *Cell* **69**, 871–881.
15. Suzuki, M. (1990) *Nature (London)* **344**, 562–565.
16. Thompson, N. E., Steinberg, T. H., Aronson, D. B. & Burgess, R. R. (1989) *J. Biol. Chem.* **264**, 11511–11520.
17. Buermeyer, A. B., Thompson, N. E., Strasheim, L. A., Burgess, R. R. & Farnham, P. J. (1992) *Mol. Cell. Biol.* **12**, 2250–2259.
18. Kim, W. Y. & Dahmus, M. E. (1989) *J. Biol. Chem.* **264**, 3169–3176.
19. Buratowski, S. & Sharp, P. A. (1990) *Mol. Cell. Biol.* **10**, 5562–5564.
20. Zehring, W. A., Lee, J. M., Weeks, J. R., Jokerst, R. S. & Greenleaf, A. L. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3698–3702.
21. Zehring, W. A. & Greenleaf, A. L. (1990) *J. Biol. Chem.* **265**, 8351–8353.
22. Liao, S. M., Taylor, I. C., Kingston, R. E. & Young, R. A. (1991) *Genes Dev.* **5**, 2431–2440.
23. Dahmus, M. E. & Kedinger, C. (1983) *J. Biol. Chem.* **258**, 2303–2307.
24. Laybourn, P. J. & Dahmus, M. E. (1989) *J. Biol. Chem.* **264**, 6693–6698.
25. Moyle, M., Lee, J. S., Anderson, W. F. & Ingles, C. J. (1989) *Mol. Cell. Biol.* **9**, 5750–5753.
26. Serizawa, H., Conaway, J. W. & Conaway, R. C. (1993) *Nature (London)* **363**, 371–374.
27. Koleske, A. J., Buratowski, S., Nonet, M. & Young, R. A. (1992) *Cell* **69**, 883–894.
28. Sayre, M. H., Tschochner, H. & Kornberg, R. D. (1992) *J. Biol. Chem.* **267**, 23376–23382.
29. Rose, M. D. & Broach, J. R. (1991) *Methods Enzymol.* **194**, 195–230.
30. Nonet, M., Sweetser, D. & Young, R. A. (1987) *Cell* **50**, 905–915.
31. Becker, D. M. & Guarente, L. (1991) *Methods Enzymol.* **194**, 182–187.
32. Edwards, A. M., Kane, C. M., Young, R. A. & Kornberg, R. D. (1991) *J. Biol. Chem.* **266**, 71–75.
33. Sayre, M. H., Tschochner, H. & Kornberg, R. D. (1992) *J. Biol. Chem.* **267**, 23383–23387.
34. Kelleher, R. J., Flanagan, P. M., Chasman, D. I., Ponticelli, A. S., Struhl, K. & Kornberg, R. D. (1992) *Genes Dev.* **6**, 296–303.
35. Tschochner, H., Sayre, M. H., Flanagan, P. M., Feaver, W. J. & Kornberg, R. D. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 11292–11296.
36. Henry, N. L., Sayre, M. H. & Kornberg, R. D. (1992) *J. Biol. Chem.* **267**, 23388–23392.
37. Kolodziej, P. A. & Young, R. A. (1991) *Methods Enzymol.* **194**, 508–519.
38. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
39. Hidaka, H., Masaki, I., Kawamoto, S. & Sasaki, Y. (1984) *Biochemistry* **23**, 5036–5041.