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A protein kinase that phosphorylates the C-terminal repeat domain of the largest subunit of RNA polymerase II

(*Saccharomyces cerevisiae*/subunit II_0/heptapeptide repeat/fusion proteins/*Drosophila*)

JAE MOON LEE AND ARNO L. GREENLEAF

Department of Biochemistry, Duke University Medical Center, Durham, NC 27710

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ABSTRACT **The unique C-terminal repeat domain (CTD) of the largest subunit (II_a) of eukaryotic RNA polymerase II consists of multiple repeats of the heptapeptide consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. The number of repeats ranges from 26 in yeast to 42 in *Drosophila* to 52 in mouse. The CTD is essential *in vivo*, but its structure and function are not yet understood. The CTD can be phosphorylated at multiple serine and threonine residues, generating a form of the largest subunit (II_0) with markedly reduced mobility in $NaDodSO_4$/polyacrylamide gels. To investigate this extensive phosphorylation, which presumably modulates functional properties of RNA polymerase II, we began efforts to purify a specific CTD kinase. Using CTD-containing fusion proteins as substrates, we have purified a CTD kinase from the yeast *Saccharomyces cerevisiae*. The enzyme extensively phosphorylates the CTD portion of both the fusion proteins and intact subunit II_a, producing products with reduced electrophoretic mobilities. The properties of the CTD kinase suggest that it is distinct from previously described protein kinases. Analogous activities were also detected in *Drosophila* and HeLa cell extracts.**

Eukaryotic RNA polymerase II is a complex enzyme consisting of 8–12 subunits (for review, see ref. 1). The largest subunit can be resolved into three major forms in $NaDodSO_4$/polyacrylamide gels: II_0 II_a, II_b. Subunit II_a (≈215 kDa) appears to be the primary translation product. Subunit II_b (≈175 kDa) is a proteolyzed form of subunit II_a lacking the C-terminal domain (CTD), which is composed of multiple repeats of the consensus heptapeptide motif Tyr-Ser-Pro-Thr-Ser-Pro-Ser (2–4). Subunit II_0 is a form of the largest subunit with a highly phosphorylated CTD that can be generated *in vitro* or *in vivo*; it migrates markedly slower than subunit II_a in $NaDodSO_4$/polyacrylamide gels (5–7). RNA polymerase II0 (containing subunit II_0) is the predominant form of the enzyme in HeLa cells (8), but its relative abundance appears to be less in other mammalian cells (1, 8); it has not been well studied in most organisms.

Several possible functions for the CTD have been proposed (2, 3, 9): (*i*) interacting with certain transcription factors during initiation; (*ii*) anchoring RNA polymerase II to a structure within the nucleus; (*iii*) destabilizing of histone–DNA interactions during elongation (phosphorylated form II_0). The CTD is essential for cell viability (4, 10–12), but its precise function has not been elucidated. RNA polymerase II completely lacking the CTD is capable of initiating transcription at promoter sites in at least one factor-dependent *in vitro* system (4). On the other hand, antibody inhibition (13, 14) and photoaffinity labeling (15, 16) experiments support the idea that the CTD, probably in its phosphorylated form, plays an important role in specific transcription.

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As a step toward understanding the roles of CTD phosphorylation *in vivo*, we began efforts to purify protein kinases that phosphorylate this domain. We purified CTD-containing fusion proteins from *Escherichia coli* and used them as substrates to detect an activity that extensively phosphorylates the CTD. Here we report the purification and preliminary characterization of this CTD kinase from the yeast *Saccharomyces cerevisiae*.

METHODS

Construction and Purification of CTD Fusion Proteins. For the yeast CTD fusion protein construction, a 1.05-kilobase (kb) *Bgl* II–*Hin*dIII fragment from *RPO21* (2) that encodes the entire CTD and 48 upstream amino acids was isolated from plasmid pJH107 (from C. J. Ingles, University of Toronto) and inserted into the *Bam*HI–*Hin*dIII site in pUR290 (17). For the *Drosophila* fusion protein the 3.6-kb *Eco*RI fragment from *RpII215* encoding the CTD (18) was subcloned into pBR325, and the resulting 2.3-kb *Sal* I–*Hin*dIII fragment, containing 34 of the total 42 repeats (19), was inserted into the *Sal* I–*Hin*dIII site in pUR290. These recombinant plasmids were transformed into *E. coli* BMH 71-18 (17). The cells were grown in LB broth to OD_{590} = 0.7 at 37°C and induced with 1 mM isopropyl β-D-thiogalactoside for 2 hr at 30°C. The cells were harvested, resuspended at 1/20th their original volume in buffer A [25 mM Tris·HCl, pH 7.6/25 mM 2-mercaptoethanol/2 mM EDTA/1 mM phenylmethylsulfonyl fluoride (PMSF; Calbiochem)] plus 0.2 M NaCl, and lysed and centrifuged essentially as described (20). The β-galactosidase fusion proteins were completely soluble. Polyethylenimine [10% (wt/vol), pH 7.9] was added dropwise to a final concentration of 0.1%, and the extract was centrifuged at 10,000 × *g* for 30 min at 4°C. Solid ammonium sulfate was added to the supernatant to 33% saturation, and the resulting precipitate was collected at 10,000 × *g* for 30 min. The pellet was resuspended in buffer A and dialyzed to 0.1 M NaCl. The dialysate was passed through a phosphocellulose (P11, Whatman) column equilibrated with the same buffer and loaded onto a DEAE-cellulose (DE52, Whatman) column. The column was washed with 0.15 M NaCl in buffer A and fusion protein was eluted with 0.4 M NaCl in buffer A. The proteins were further purified by gel filtration on a Sephacryl S-300 column (Pharmacia). Typical yields were about 20 mg of protein per liter of culture. The yeast CTD fusion protein (Y-FP) was ≈90% proteolyzed, whereas the *Drosophila* CTD fusion protein (D-FP) was >50% intact (see Fig. 1).

Kinase Assay. Standard reaction mixtures (20 μl) contained yeast CTD fusion protein (final concentration of 0.15 mg/ml, for intact protein), Tris·HCl (25 mM, pH 7.8), $MgCl_2$ (10 mM), NaF (5 mM), PMSF (1 mM), and [γ-^{32}P]ATP (300 μM, 1–3 μCi; 1 μCi = 37 kBq). After incubation for 15 min at room temperature, reactions were terminated by the addition of

Abbreviations: CTD, C-terminal domain; PMSF, phenylmethylsulfonyl fluoride.

$NaDodSO_4$ sample buffer. Phosphorylated products were analyzed by $NaDodSO_4$/6% PAGE followed by autoradiography. For quantitative assays the labeled regions were then excised from unstained and dried gels with the autoradiogram as template, and radioactivity was measured by liquid scintillation counting. Phosphorylation of casein (Sigma C4765) was assayed both under standard conditions and by the method of Padmanabha and Glover (21), and products were analyzed by $NaDodSO_4$/15% PAGE. Assays on phosvitin (Sigma P1253) and histone (Sigma H5505) were done similarly.

Purification of Yeast CTD Kinase. Yeast cells were broken by using a Bead-Beater (Biospec Products, Bartlesville, OK). Typically 120 g of wet cells [CB001, a *pep4* strain from A. Sugino (National Institute of Environmental Health Sciences, Research Triangle Park, NC) grown in YEPD broth to OD_{600} = 10] was resuspended in buffer B (25 mM Tris·HCl, pH 7.6/25 mM KCl/1 mM EDTA/1 mM dithiothreitol) plus protease inhibitors [PMSF (1 mM) leupeptin (0.5 μg/ml; Boehringer Mannheim), pepstatin (0.5 μg/ml), antipain (1 μg/ml), and bestatin (0.35 μg/ml)] and disrupted with 200 ml of glass beads (0.5-mm diameter) in a full 375-ml container. For commercial yeast (Fleischmann), 40 g of dry yeast was used per batch. Cells were disrupted on ice 10 times for 30 sec, with 5-min intervals for cooling. After the supernatant was removed, the glass beads were washed with buffer B to make 400 ml total. After centrifugation at 15,000 × *g* for 30 min at 4°C the supernatant, typically 320 ml, was retained ("crude extract"). All subsequent steps were carried out at 1–4°C.

To the crude extract, 10% polyethylenimine (pH 7.9) was added dropwise to a final concentration of 0.3%. After centrifugation at 10,000 × *g* for 15 min, the pellet was resuspended in 80 ml of buffer B by Dounce homogenization and recentrifuged. The enzyme was eluted from the pellet with 80 ml of 0.4 M KCl in buffer B by homogenization and centrifugation ("polyethylenimine eluate"). Solid ammonium sulfate was added to 45% saturation, and the precipitate obtained by centrifugation at 15,000 × *g* for 30 min was resuspended in buffer H [25 mM Hepes, pH 7.6/0.1 mM EDTA/15% (vol/vol) glycerol/1 mM dithiothreitol/1 mM PMSF].

Eight such samples (each representing 120 g of wet cells and stored at −70°C if necessary) were then pooled, dialyzed to 0.2 M KCl in buffer H, and loaded onto a P11 column (150-ml bed volume) previously equilibrated with the same buffer. The column was washed extensively with the same buffer and the protein was eluted with a 0.2–0.8 M KCl gradient (800 ml). Each fraction was assayed and active fractions were pooled. The peak of the enzyme activity was eluted at 0.32 M KCl. The sample was dialyzed to 0.12 M KCl and applied to a DE52 column (20-ml bed volume) equilibrated with the same buffer. The enzyme was recovered in the flowthrough, directly applied to a 1-ml Mono S (FPLC, Pharmacia) column, and eluted with a 0.15–0.45 M KCl gradient. Activity was eluted at 0.35 M KCl. For sedimentation analysis, a 100-μl aliquot of one Mono S fraction was layered onto a 5-ml glycerol gradient (15–35%) in buffer H at 0.5 M KCl and centrifuged for 24 hr at 45,000 rpm in an AH262 rotor (Sorvall) at 2°C. Fractions (100 μl) were collected from the bottom of the tube and assayed.

Other Methods. Phospho amino acid analyses were carried out as described by Cooper *et al.* (22). *Drosophila* K_c cell nuclear extract and RNA polymerase were prepared as described (23). Yeast RNA polymerase II was a generous gift from J. Jaehning (Indiana University). Nuclear extract from HeLa cells was a gift from R. Roeder (Rockefeller University). $NaDodSO_4$/PAGE was done according to Laemmli (24), and silver staining was done by the method of Morrissey (25). Protein concentration was determined by the Coomassie G-250 binding assay with reagents from Bio-Rad.

RESULTS

CTD Phosphorylation in Yeast Extracts. Using β-galactosidase–CTD fusion proteins as substrates (see *Methods*), we assayed kinase activity in crude yeast extracts. When small amounts of extract were used, we could observe labeling of the fusion proteins above a background due to phosphorylation of endogeneous substrates (Fig. 1*B*). The intact fusion proteins were phosphorylated to produce two types of labeled product; one migrated in $NaDodSO_4$/polyacrylamide gels with unchanged mobility, while the other migrated with markedly reduced mobility (arrowheads, lanes 3 and 4), reminiscent of the subunit II_a/II_0 difference. Additionally, in the case of the yeast fusion protein another labeled band was observed with the mobility of the prominent proteolysis products slightly larger than β-galactosidase. We do not know whether this was due to phosphorylation of upstream sequences or residual CTD. Note that β-galactosidase was not phosphorylated.

The experiment of Fig. 1 indicated that two modes of CTD phosphorylation were occurring in the yeast extracts: one resulted in a marked mobility shift of the phosphorylated product and one did not. This report describes the CTD kinase activity responsible for generating the product with reduced mobility.

Purification of the CTD Kinase. The CTD kinase activity generating the fusion protein product with reduced mobility was purified from a defined yeast strain as summarized in Table 1 (see *Methods*). Similar results were obtained from commercial yeast. After the Mono S column chromatography

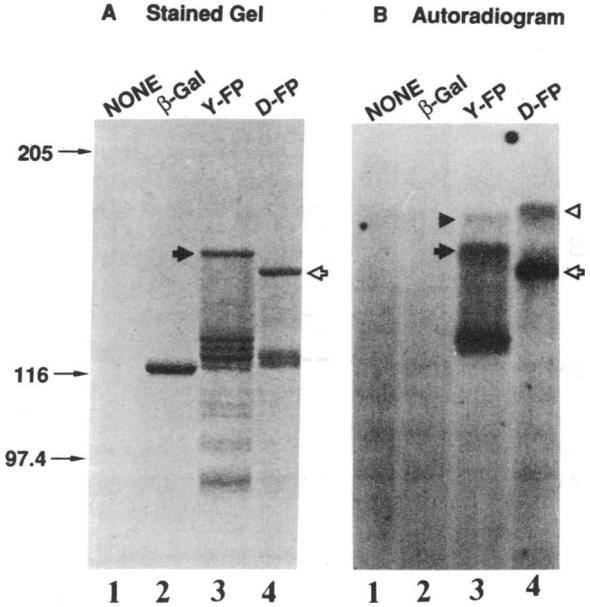

FIG. 1. Assay of CTD kinase activity in yeast crude extract. Yeast and *Drosophila* CTD fusion proteins (Y-FP and D-FP, respectively) as substrates and β-galactosidase as a control were incubated with yeast crude extract (0.2 μl, 2.2 μg of protein) prepared from cultured cells. After the reaction products were separated by $NaDodSO_4$/6% PAGE, the gel was stained with Coomassie blue R-250 (*A*), dried, and autoradiographed (*B*). Solid and open arrows indicate the bands of the intact fusion proteins for yeast (146 kDa) and *Drosophila* (142 kDa) respectively. Solid and open arrowheads in *B* indicate the labeled and reduced-mobility bands for Y-FP ("156" kDa) and D-FP ("164" kDa) respectively. Sizes (kDa) of marker proteins are indicated at left. Lane 1, extract alone; lane 2, extract plus β-galactosidase; lane 3, plus Y-FP; lane 4, plus D-FP.

Table 1. Purification of CTD kinase from yeast (960 g, wet weight)

Enzyme fraction	Volume, ml	Total protein, mg	Total activity, units	Specific activity, units/mg
Crude extract	2560	28,572	41,500	1.4
PEI eluate	640	5,376	80,230	14.9
Ammonium sulfate	52	3,172	63,700	20.1
Phosphocellulose	42	32	39,120	1,223
DEAE-cellulose	45	11	28,560	2,596
Mono S	1.5	0.28	18,210	65,036

One unit of enzyme activity incorporates 1 pmol of phosphate into substrate per minute under standard conditions (see *Methods*). The activity in the crude extract was not linear with respect to the amount added. Normally the total activity in the 400 mM KCl eluate of the polyethylenimine (PEI) precipitate was about twice that in the crude extract. This may be due to the removal of ATPases, protein phosphatases, or kinase inhibitors present in the crude extract. Note also that this CTD kinase bound to other cation-exchange and affinity columns tested (e.g., Bio-Rex 70, Affi-Gel Blue, ATP-agarose, *Drosophila* CTD fusion protein), at 0.1 M KCl.

step, the kinase was about 50% pure. These data indicate that the kinase is not an abundant enzyme.

Mono S enzyme was purified further by sedimentation through a glycerol gradient (Fig. 2), where it migrated with an *s* value consistent with a native molecular mass of ≈120 kDa.

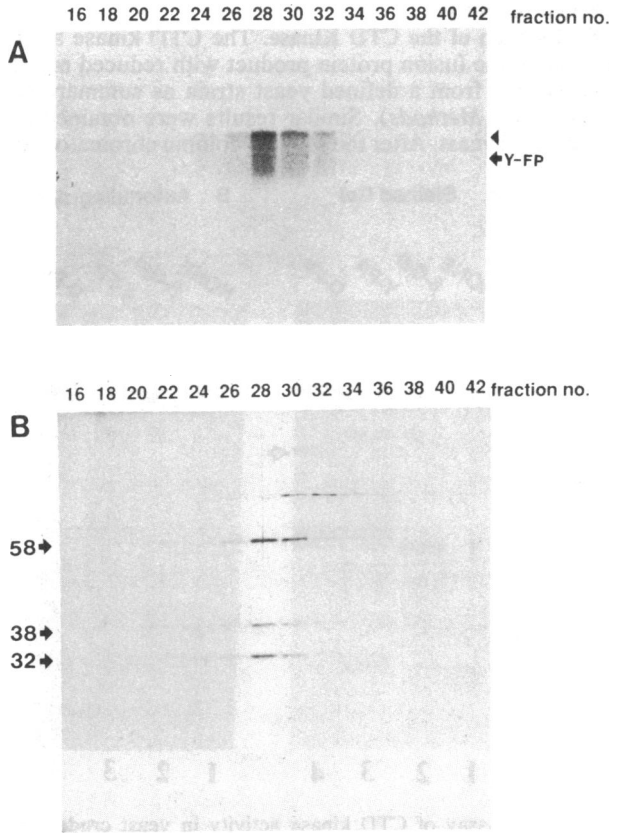

FIG. 2. Glycerol gradient sedimentation of yeast CTD kinase. Mono S-purified kinase was sedimented in a 15–35% glycerol gradient and 100-μl fractions were collected from the bottom of the tube. (*A*) Samples (0.2 μl) of alternate fractions were assayed with the yeast CTD fusion protein as a substrate under standard conditions and an autoradiogram was taken from the dried gel. Arrow, position of the intact yeast CTD fusion protein (Y-FP, 146 kDa); arrowhead, position of the phosphorylated, reduced-mobility product ("156" kDa). (*B*) Aliquots (10 μl) of the same fractions were analyzed in a $NaDodSO_4$/12% polyacrylamide gel and silver-stained. Apparent sizes (kDa) of kinase subunits are indicated at left.

Comparison of the activity profile and the stained protein gel of fractions across the activity peak showed that three polypeptides, of 58, 38, and 32 kDa, comigrated with the kinase activity. These three polypeptides also copurified from several other columns tested (data not shown). The intensities of the three protein bands in the stained gel suggest that they are present in equimolar amounts, and the native molecular mass of the kinase suggests that each polypeptide is present once. Thus the CTD kinase appears to be composed of one each of three different subunits (tentatively $\alpha\beta\gamma$).

Mobility Shift of Hyperphosphorylated Fusion Proteins. The CTD kinase always produced a labeled product with a lower electrophoretic mobility than the intact fusion protein substrate. To confirm that this was actually a phosphorylated form of the fusion protein and to test whether it was possible to convert quantitatively the fusion protein into the slower-migrating form, we tested higher ratios of enzyme to fusion protein. As shown in Fig. 3*A* (lanes 1–4), 24 units of kinase shifted quantitatively 1.5 μg of intact yeast fusion protein into the slower-migrating form, and this was the only labeled product detectable (Fig. 3*B*, lanes 1–4). The *Drosophila* fusion protein was similarly phosphorylated and shifted in mobility (Fig. 3, lanes 5 and 6). That the yeast CTD fusion protein contains 26 repeats of the heptapeptide motif and the *Drosophila* fusion protein contains 34 repeats may account for the difference in magnitude of the mobility shift between the two fusion proteins. Note also that only the intact forms of the fusion proteins were shifted in mobility (or detectably labeled).

Analysis of the phosphorylated amino acids in the labeled protein showed that phosphorylation occurred predominantly on serines and to a lesser extent on threonines; no labeled tyrosine was detected (data not shown). As estimated by measuring the radioactivity in excised gel bands, about 15–25 phosphates were incorporated into each shifted fusion

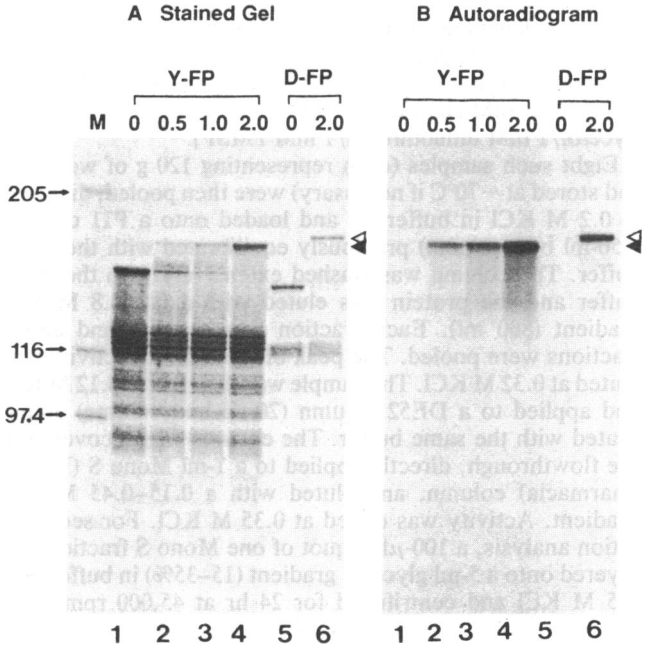

FIG. 3. Mobility shift of CTD fusion proteins by purified CTD kinase. Yeast and *Drosophila* CTD fusion proteins (Y-FP and D-FP) were incubated with various amounts (0–2.0 μl, as indicated) of CTD kinase (Mono S-purified, 12 units/μl) under standard conditions. Reaction products were analyzed in a $NaDodSO_4$/6% polyacrylamide gel, which was stained with Coomassie blue R-250 (*A*), dried, and autoradiographed (*B*). Solid and open arrowheads indicate the mobility-shifted forms of Y-FP and D-FP, respectively. Lane M: marker proteins, sizes (kDa) indicated on left.

protein molecule, for both yeast and *Drosophila*. Similar results were obtained by a trichloroacetic acid precipitation assay. Thus, the CTD kinase phosphorylates many but not all serine and threonine residues in the repeat domain. The distribution of the phosphates among the repeats was not investigated.

Phosphorylation of RNA Polymerase II by the CTD Kinase. We tested the ability of the CTD kinase to hyperphosphorylate subunit II_a of native yeast RNA polymerase II. Indeed, the kinase phosphorylated the largest subunit, producing a II_0-like form migrating conspicuously slower than subunit II_a in $NaDodSO_4$/polyacrylamide gels (Fig. 4, lanes 2 and 3). The CTD kinase also phosphorylated subunit II_a of *Drosophila* RNA polymerase II, dramatically shifting its electrophoretic mobility (lanes 4 and 5).

Characteristics of the Yeast CTD Kinase. Initial characterizations were performed with the yeast CTD fusion protein as substrate. The enzyme required Mg^{2+} (>1 mM) and ATP (K_m = 27 μM). The activity was not affected by Ca^{2+}, Ca^{2+}/calmodulin, cAMP, or cGMP over a wide range of concentrations. NH_4Cl up to 20 mM and Triton X-100 up to 0.5% had little effect on activity. The K_m for the fusion protein was 0.22 μM. The enzyme was tested on various substrates such as casein, phosvitin, and histone; none of these was phosphorylated by the enzyme.

CTD Kinase Activity in Nuclear Extracts of *Drosophila* and HeLa Cells. To test whether metazoans possess CTD kinase activities similar to those in yeast, we incubated the CTD fusion proteins with nuclear extracts from *Drosophila* K_c and human HeLa cells (Fig. 5). Both extracts phosphorylated the CTD fusion proteins and generated lower-mobility forms as described above. These data suggest that a protein kinase with properties similar to those of the yeast enzyme is also present in higher eukaryotes.

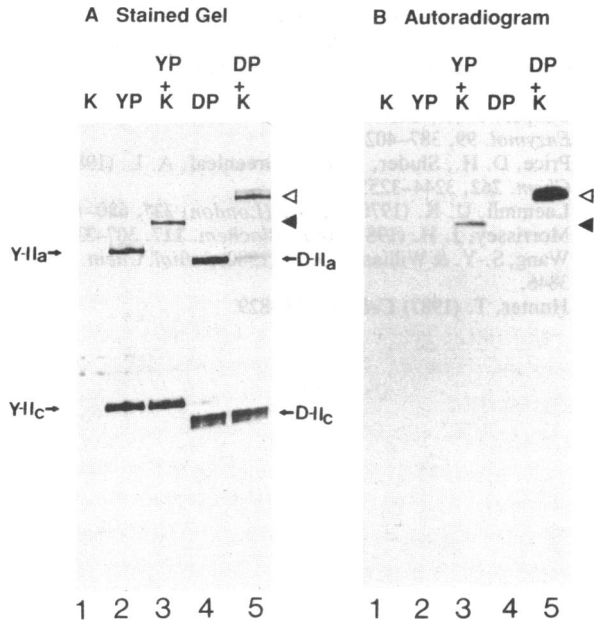

FIG. 4. Phosphorylation of RNA polymerase II by CTD kinase. Purified RNA polymerases II (2 μg) from yeast and *Drosophila* were incubated with 2 μl of Mono S-purified kinase under standard conditions. Reaction products were run in a $NaDodSO_4$/6% polyacrylamide gel, which was stained with silver (*A*), dried, and autoradiographed (*B*). Y-II_x and D-II_x indicate subunits II_x of yeast and *Drosophila* RNA polymerase II, respectively. Solid and open arrowheads indicate reduced-mobility bands of subunit Y-II_a and D-II_a, respectively. Lane 1, kinase alone; lane 2, yeast RNA polymerase II alone; lane 3, kinase plus yeast polymerase; lane 4, *Drosophila* RNA polymerase II alone; lane 5, kinase plus *Drosophila* polymerase. Note: molecular mass of II_c subunits is ≈140 kDa; proteins of less than about 100 kDa ran off this gel.

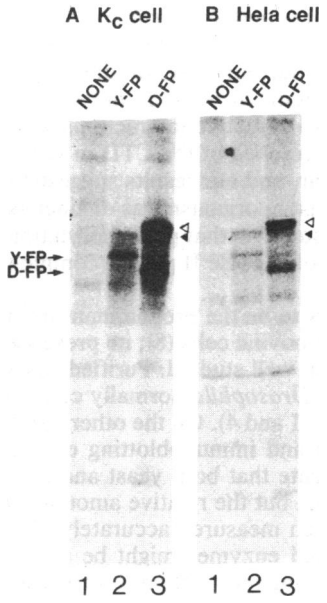

FIG. 5. CTD kinase activity in nuclear extracts of K_c and HeLa cells. Yeast and *Drosophila* CTD fusion proteins (Y-FP and D-FP) were incubated with nuclear extracts of *Drosophila* K_c cells (0.05 μl, 0.7 μg of protein) (*A*) or HeLa cells (0.2 μl, 1.7 μg of protein) (*B*) under standard conditions, and the reaction products were analyzed as described in the legend of Fig. 1. Lanes 1, extract alone; lanes 2, extract plus Y-FP; lanes 3, extract plus D-FP. The positions of intact Y-FP (146 kDa) and D-FP (142 kDa) are indicated at left. Solid and open arrowheads denote the reduced-mobility bands of Y-FP and D-FP, respectively.

DISCUSSION

A CTD consisting of the consensus sequence (Tyr-Ser-Pro-Thr-Ser-Pro-Ser)$_n$ has been described for the largest subunit of RNA polymerase II in yeast (2), mammals (3, 10), and *Drosophila* (4, 10). While the structure and function of the CTD are not known, it is likely that phosphorylation affects both. As a step toward investigating the physiological role of the CTD and its phosphorylation, we purified a CTD kinase from yeast.

The pronounced shift in electrophoretic mobility displayed by a CTD fusion protein upon hyperphosphorylation provided a convenient assay, which we used to purify the CTD kinase to near homogeneity. The enzyme, which is present in relatively low amounts in yeast extracts, appears to consist of one each of three subunits with molecular mass 58, 38, and 32 kDa. Its subunit structure, its purification behavior, its protein substrate specificity, and its other tested enzymatic properties suggest that the CTD kinase is distinct from previously purified protein kinases. Dahmus (5) showed that a calf thymus casein kinase I preparation phosphorylates subunit II_a of mammalian RNA polymerase II *in vitro* to generate a highly phosphorylated, slower-migrating form. Whether casein kinase I phosphorylates RNA polymerase II *in vivo* is not known. The CTD kinase we purified using our CTD fusion-protein mobility-shift assay appears to differ from casein kinase I in several respects.

The relationship between the extent of CTD phosphorylation and the mobility shift has yet to be investigated thoroughly, but it exhibits some interesting properties. For example, Figs. 3 and 4 show that virtually the only labeled product detectable is the shifted (hyperphosphorylated) form, even at intermediate stages of phosphorylation [the smears observed with the yeast fusion protein may be due to phosphorylation of proteolyzed forms (compare the *Drosophila* fusion protein)]. Thus the reaction appears to display a cooperativity or processivity that needs to be studied further. We have also not investigated the physical basis for the mobility shift itself, but such a consequence of hyperphosphorylation is not unique to RNA polymerase subunit II_0 (for example, see ref. 26).

Since the *Drosophila* CTD contains many more nonconsensus amino acids than that of either yeast or mammals (4, 10, 19) and, unlike the mammalian CTD, will not substitute

for the yeast CTD *in vivo* (10), it is perhaps surprising that it is a good substrate for CTD kinase activities from both yeast and mammals. On the other hand, our observations might indicate that its failure to function in yeast is not due to improper phosphorylation but to defective interactions with other yeast components. The presence of the CTD in virtually all eukaryotes studied so far, and our results suggesting that analogous CTD kinases exist in organisms as distinct as yeast, fruit flies, and humans, suggests that phosphorylation of the largest subunit of RNA polymerase II plays a common role in most eukaryotic cells.

RNA polymerase II0 appears to be the predominant form of the enzyme in HeLa but not bovine cells (8); its presence in most other cells has not been well studied. Purified RNA polymerases II from yeast and *Drosophila* normally contain no detectable II_0 (e.g., see refs. 1 and 4). On the other hand, *in vivo* phosphorylation (6, 7) and immunoblotting experiments (unpublished data) indicate that both yeast and *Drosophila* cells contain subunit II_0, but the relative amounts of II_a and II_0 *in vivo* have not been measured accurately. The absence of II_0 from the purified enzymes might be due to instability (8), selective loss during purification, or presence of only low amounts *in vivo*. Studies of the CTD kinase may help clarify this situation. With the purified kinase RNA polymerase II0 can also be generated *in vitro* (Fig. 4), and its properties studied.

When genes for the kinase subunits are cloned, *S. cerevisiae* genetics will afford straightforward approaches to test whether the enzyme is essential for viability, and in addition should allow experiments to test whether it actually phosphorylates the CTD *in vivo*. If it does, elucidating its properties should yield insights into the function of the CTD and the physiological significance of its phosphorylation. It will be informative to determine, for example, whether the kinase phosphorylates the CTD at a particular point in each transcription cycle, in each cell cycle, or noncyclically in response to regulatory signals. It will also be interesting to see if the CTD kinase is encoded by previously identified genes, such as cell division cycle (*CDC*) genes, several of which have been shown to encode protein kinases (for review, see ref. 27).

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1. Sentenac, A. (1985) *CRC Crit. Rev. Biochem.* **18,** 31–90.
2. Allison, L. A., Moyle, M., Shales, M. & Ingles, C. J. (1985) *Cell* **42,** 599–610.
3. Corden, J. L., Cadena, D. L., Ahearn, J. M. & Dahmus, M. E. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7934–7938.
4. Zehring, W. A., Lee, J. M., Weeks, J. R., Jokerst, R. S. & Greenleaf, A. L. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3698–3702.
5. Dahmus, M. E. (1981) *J. Biol. Chem.* **256,** 3332–3339.
6. Buhler, J. M., Iborra, F., Sentenac, A. & Fromageot, P. (1976) *FEBS Lett.* **71,** 37–41.
7. Bell, G. I., Valenzuela, P. & Rutter, W. J. (1977) *J. Biol. Chem.* **252,** 3082–3091.
8. Kim, W.-Y. & Dahmus, M. E. (1986) *J. Biol. Chem.* **261,** 14219–14225.
9. Sigler, P. B. (1988) *Nature (London)* **333,** 210–212.
10. Allison, L. A., Wong, J. K.-C., Fitzpatrick, V. D., Moyle, M. & Ingles, C. J. (1988) *Mol. Cell. Biol.* **8,** 321–329.
11. Nonet, M., Sweetser, D. & Young, R. A. (1987) *Cell* **50,** 909–915.
12. Bartolomei, M. S., Halden, N. F., Cullen, C. R. & Corden, J. L. (1988) *Mol. Cell. Biol.* **8,** 330–339.
13. Christmann, J. L. & Dahmus, M. E. (1981) *J. Biol. Chem.* **256,** 11798–11803.
14. Dahmus, M. E. & Kedinger, C. (1983) *J. Biol. Chem.* **258,** 2303–2307.
15. Bartholomew, B., Dahmus, M. E. & Meares, C. F. (1986) *J. Biol. Chem.* **261,** 14226–14231.
16. Cadena, D. L. & Dahmus, M. E. (1987) *J. Biol. Chem.* **262,** 12468–12474.
17. Rütter, U. & Müller-Hill, B. (1983) *EMBO J.* **2,** 1791–1794.
18. Biggs, J., Searles, L. L. & Greenleaf, A. L. (1985) *Cell* **42,** 611–621.
19. Jokerst, R. S., Weeks, J. R., Zehring, W. A. & Greenleaf, A. L. (1989) *Mol. Gen. Genet.* **215,** 266–275.
20. Rio, D. C., Laski, F. A. & Rubin, G. M. (1986) *Cell* **44,** 21–32.
21. Padmanabha, R. & Glover, C. V. C. (1987) *J. Biol. Chem.* **262,** 1829–1835.
22. Cooper, J. A., Sefton, B. M. & Hunter, T. (1983) *Methods Enzymol.* **99,** 387–402.
23. Price, D. H., Sluder, A. E. & Greenleaf, A. L. (1987) *J. Biol. Chem.* **262,** 3244–3255.
24. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
25. Morrissey, J. H. (1981) *Anal. Biochem.* **117,** 307–310.
26. Wang, S.-Y. & Williams, D. L. (1982) *J. Biol. Chem.* **257,** 3837–3846.
27. Hunter, T. (1987) *Cell* **50,** 823–829.