Purification and Characterization of an RNA Polymerase II Phosphatase from Yeast*

Ross S. Chambers and Caroline M. Kane‡

From the Department of Molecular and Cell Biology, Division of Biochemistry and Molecular Biology, University of California, Berkeley, California 94720

RNA polymerase (RNAP) II is subject to extensive phosphorylation on the heptapeptide repeats of the C-terminal domain (CTD) of the largest subunit. An activity that is required for the dephosphorylation of yeast RNAP II in vitro has been purified from a yeast whole cell extract by >30,000-fold. The yeast CTD phosphatase activity copurified with two bands with apparent molecular masses of 100 and 103 kDa. The properties of the yeast CTD phosphatase are similar to those of a previously characterized CTD phosphatase from HeLa cells. These properties include stimulation by the general transcription factor IIF (TFIIF), competitive inhibition by RNAP II, magnesium dependence, and resistance to okadaic acid. Both the HeLa and yeast CTD phosphatases are highly specific for their cognate polymerases. Neither phosphatase functions upon the polymerase molecule from the other species, even though the heptapeptide repeats of the CTDs in yeast RNAP II and mammalian RNAP II are essentially identical. The activity of the highly purified CTD phosphatase is stimulated >300-fold by a partially purified fraction of TFIIF. Recombinant TFIIF did not substitute for the TFIIF fraction, indicating that an additional factor present in the TFIIF fraction is required for CTD phosphatase activity. These results show that yeast contains a CTD phosphatase activity similar to that of mammalian cells that is likely composed of at least two components, one of which is 100 and/or 103 kDa.

The largest subunit of RNAP II contains a highly conserved C-terminal domain composed of multiple heptapeptide repeats with the consensus sequence Tyr-*Ser*-Pro-*Thr*-Ser-*Pro*-Ser1 (1). The number of repeats varies in different organisms, with 26 in yeast and 52 in mammals. The exact function of the CTD in transcription is not clear. Genetic studies have shown the CTD to be essential in vivo; however, yeast can tolerate deletions leaving a minimum of eight repeats (2). In vitro, the CTD is not required in a reconstituted transcription system with the adenovirus major late promoter (3), but is required at promoters lacking a consensus TATA sequence (4, 5). The CTD interacts with a variety of transcription factors in vitro, including TATA-binding protein, TFIIF, TFIIE, and the SRB complex (6–8). The association of the SRB complex and other factors with RNAP II forms what has been termed the holoenzyme. This complex is thought to be recruited to promoters in vivo and to mediate activated transcription (9).

The CTD is subject to extensive phosphorylation in vivo, with a stoichiometry of approximately one phosphate per repeat (1). Phosphorylation predominantly occurs on serine residues, with small amounts on threonine and tyrosine residues. The unphosphorylated form of RNAP II is designated RNAP IIA, whereas the phosphorylated form is designated RNAP IIO. A variety of studies have shown that RNAP IIA and RNAP IIO have distinct functions during transcription and that each round of transcription is associated with the reversible phosphorylation of the CTD. RNAP IIA preferentially assembles into a preinitiation complex with transcription factors on promoter DNA (10), whereas elongation of the transcript is almost exclusively catalyzed by RNAP IIO (11). Phosphorylation of RNAP IIA occurs sometime during the initiation of transcription and is thought to trigger the release of RNAP II from the preinitiation complex (12). RNAP IIO is presumably dephosphorylated at or after termination of transcription to regenerate RNAP IIA for the next round of transcription. CTD phosphorylation appears to be an important mechanism in regulating RNAP II activity in vivo, and changes in the phosphorylation state of RNAP II are associated with major changes in the pattern of transcription. For example, serum stimulation of quiescent cells, heat shock, and viral infection all dramatically alter cellular transcription and also result in a change in the phosphorylation state of RNAP II (13–15).

Many different protein kinases have been characterized that can phosphorylate the CTD in vitro. Two yeast protein kinases, CTK1 and KIN28, have been shown to be important for phosphorylation of RNAP II in vivo. Disruption of the CTK1 gene results in a large decrease in RNAP II phosphorylation in vivo (16). The null mutant ctk1 cells, although viable, exhibit slow growth and other phenotypes, but it is not known what role CTK1 has in transcription. The KIN28 kinase is found associated with the general transcription factor IIIH, which assembles into the preinitiation complex (17). The KIN28 gene is essential, and loss of the KIN28 kinase activity results in a dramatic decrease in RNAP II phosphorylation and transcriptional activity (18).

Much less is known about CTD phosphatases. A CTD phosphatase activity has been purified from HeLa cells that is highly specific for RNAP II and dephosphorylates the CTD processively (19). Two proteins with apparent molecular masses of 205 and 150 kDa copurify with the HeLa CTD phosphatase activity, although it is not clear which contains the catalytic activity. In contrast to CTD kinases, HeLa CTD phosphatase does not use recombinant CTD as a substrate (20). Furthermore, CTD phosphatase is competitively inhibited by RNAP IIB, a form of RNAP II that lacks the CTD. These and

*This work was supported by Grant DMB-920-5583 from the National Science Foundation and Grant GM 34963 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 510-642-4118; Fax: 510-642-7846; E-mail: cmkane@mendel.berkeley.edu.

1 The abbreviations used are: RNAP, RNA polymerase; CTD, C-terminal domain; TF, transcription factor; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis.
other results suggested that a docking site exists on RNAPII that CTD phosphatase must first bind before it can gain access to the CTD. HeLa CTD phosphatase is also stimulated 5-fold by TFIIIF, and the stimulation can be inhibited by TFIIH. The minimal region of TFIIIF sufficient to stimulate CTD phosphatase is the C-terminal 160 amino acids of the RAP74 subunit (20).

Serine/threonine protein phosphatases have been classified into four families, PP1, PP2A, PP2B, and PP2C, based on biochemical properties and amino acid sequence (21). PP1, PP2A, and PP2B are all inhibited by okadaic acid, although with differing sensitivities. PP2B requires calcium ions for activity, and PP2C requires magnesium ions. HeLa CTD phosphatase has been classified as a type 2C phosphatase based on its requirement for magnesium ions and its resistance to okadaic acid. This report describes the purification and characterization of a type 2C phosphatase from yeast specific for the CTD of RNAPII.

EXPERIMENTAL PROCEDURES

Materials—Radiolabeled ribonucleotide [γ-32P]ATP (600 Ci/mmol) was obtained from DuPont NEN. Blue Sepharose CL-6B, Q-Sepharose Fast Flow, SP-Sepharose Fast Flow, phenyl-Sepharose HR 5/5, Sepharose 12 HR 10/30, and Mono Q HR 5/5 were obtained from Pharmacia Biotech Inc. Bio-Gel HTP (hydroxylapatite) and Bio-Rex 70 were obtained from Bio-Rad. DEAE-cellulose (DE52) and phosphocellulose P-11 were obtained from Whatman. Ni2+-nitrilotriacetic acid-agarose was obtained from Qiagen Inc. Centricron 30 microconcentrators were obtained from Amicon, Inc. The yeast strain YPH/TBF1.6HIS (ade2-101 ura3-52 lys2-801 trp1Δ63 his3Δ200 leu2Δ1 tfb1Δ LEU2 YCP50/TPP16HIS) as well as highly purified and recombinant yeast TFIIIF were kindly provided by the laboratory of Dr. R. Kornberg (Stanford University). Yeast RNAPII (wild-type, Δ1, and Δ2), human TFIIH, and human RAP74 A137–556 were kindly provided by Dr. A. Edwards (McMaster University), Dr. M. Dahmus (University of California, Davis, CA), and Dr. Z. Burton (Michigan State University), respectively.

Buffers—3× lysis buffer contained 450 mM Tris acetate, pH 7.8, 150 mM potassium acetate, 60% glycerol, 3 mM EDTA, 3 mM DTT, 3 mM phenylmethylsulfonyl fluoride, 6 µM pepstatin A, 6 µg/ml chymostatin, and 1.8 µM leupeptin. Buffer A contained 50 mM Tris acetate, pH 7.8, 20% glycerol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 mM DTT. Buffer B contained 20 mM HEPES/KOH, pH 7.6, 20% glycerol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 mM DTT. Buffer C contained 50 mM Tris acetate, pH 7.8, 20% glycerol, 0.1 mM EDTA, and 0.5 mM DTT. Buffer D was the same as buffer C except it contained 10 mM MgCl₂, 10 mM 2-mercaptoethanol, 0.5 µM okadaic acid, and 0.025% Tween 20.

Polyacrylamide Gel Electrophoresis—SDS-PAGE was carried out according to the method of Laemmli (22). Native polyacrylamide gel electrophoresis was carried out on 6% polyacrylamide gels with 50 mM Tris acetate, pH 7.8, 20% glycerol, 1 mM EDTA, and 10 mM potassium acetate. The running buffer was the same as the gel buffer except no glycerol was added. Native gels were prerun for 2 h at 8 V/cm and then run for 4.5 h at 20 V/cm at 4 °C. To recover proteins from the native polyacrylamide gel, the gel was sliced into 5-mm portions and washed, and the gel fragments were soaked overnight in 4 volumes of buffer F with 20 mM potassium acetate on a rotating platform at 4 °C. Polyacrylamide gels were stained with either Coomassie Blue or silver (23). Polyacrylamide gels were also prestained with Coomassie Blue before silver staining to increase sensitivity (24).

Preparation of [γ-32P]RNA Polymerase II—[γ-32P]RNAPII II was prepared by incubating either yeast or calf thymus RNAPII I with a partially purified TFIIH-associated CTD kinase and 2.5 µM [γ-32P]ATP (750 µCi) in buffer E for 1 h at 30 °C. The [γ-32P]-labeled RNAPII was purified on DEAE-52 as described previously (10). Calf thymus RNAPII and yeast RNAPII radiolabeled with [32P] to similar specific activities. The radio-labeled band corresponding to the largest subunit of RNAPII II was identified by the following criteria. (i) It comigrates on SDS-PAGE with the silver-stained largest subunit of RNAPII II; (ii) the presence of the band is dependent on the addition of RNAPII II to the kinase reaction; and (iii) the band undergoes a mobility shift on SDS-PAGE when extensively phosphorylated, characteristic of the largest subunit of RNAPII. Each mole of RNA polymerase IIA containing ~0.4 mole of RNAPII lacking subunits 4 and 7 (25) was used in preparing [γ-32P]RNAPII II. No differences were observed in using this enzyme as a substrate in CTD phosphatase assays compared with wild-type RNAPII II or RNAPII lacking subunit 9 (26), either in the presence or absence of the SP 0.48 fraction.

Purification of Proteins—TFIIH-associated CTD kinase was partially purified from a yeast whole cell extract using Bio-Rex 70, phosphocellulose, and Ni2+-nitrilotriacetic acid-agarose chromatography as described previously (27). CTD kinase activity was assayed according to Payne and Dahmus (28). The stimulatory fraction, containing TFIIIF and used in CTD phosphatase assays, was prepared from material that bound to SP-Sepharose during the CTD phosphatase purification (see below). The SP-Sepharose column was developed with a 0.05–1.2 M potassium acetate in buffer C, with the stimulatory activity eluting at 0.48 M potassium acetate (SP 0.48 fraction). HeLa CTD phosphatase was purified as described previously (19).

Protein Quantitation—Protein concentrations were determined using the protein dye assay (Bio-Rad) with bovine serum albumin as the standard.

Glycerol Gradient Sedimentation—Glycerol gradients were prepared in buffer C with 40 mM potassium acetate and glycerol concentrations from 15 to 35%. Thyroglobulin (669 kDa), catalase (240 kDa), and bovine serum albumin (66 kDa) were used as molecular mass markers. The gradients were centrifuged at 150,000 × g in an SW 41 rotor for 16 h at 4 °C.

Renaturation of Proteins—Proteins purified by SDS-PAGE were renatured according to the method of Conaway et al. (29).

CTD Phosphatase Assay—Samples of CTD phosphatase were dialyzed against buffer C containing 0.025% Tween 20 and 10 mM potassium acetate. The samples were incubated with [γ-32P]RNAPII II and 6 µg of the SP 0.48 fraction containing TFIIIF in buffer F in a final volume of 20 µl. The reactions were incubated at 30 °C for 30 min unless otherwise indicated. Reactions were stopped by the addition of 6 × Laemmli buffer (22), heated at 100 °C for 5 min, and electrophoresed on an SDS-5% polyacrylamide gel. The gel was dried, and the band corresponding to the largest subunit of RNAPII II was quantitated using a PhosphorImager (Molecular Dynamics, Inc.). The assay was linear until 40% of the label was removed. The amount of [γ-32P]RNAPII II in the assay was ~2 fmol (0.1 nm). One unit of CTD phosphatase activity releases 1 pmol of 32P/min. CTD phosphatase activity was also detected using an assay that specifically measures released 32P (30).

Purification of CTD Phosphatase—The yeast strain YPH/TBF1.6HIS was grown at 30 °C to mid-log phase in 200 liters of 1% Bacto-yeast extract, 2% Bacto-peptone, and 2% glucose; harvested by centrifugation (1-kg yield); washed in cold distilled water; and stored at 0 °C in 3 × 3 lysis buffer. All steps were carried out at 4 °C. A whole cell extract was prepared as described previously (31), with the following modifications. Polyethyleneimine was omitted since this resulted in loss of CTD phosphatase activity, and cells were disrupted in a bead beater (Biospec Products, Inc.) with 8 × 1 min bursts interrupted with 2 min of cooling on ice. The extract was dialyzed against buffer A containing 0.05 mM potassium acetate and stored as four equal aliquots at −80 °C. Each aliquot was diluted with buffer B to a protein concentration of 5 mg/ml, adjusted to 0.15 mM potassium acetate, and loaded onto a DE52 column (25 × 5 cm) equilibrated in buffer B with 0.15 mM potassium acetate. The DE52 column was washed with 1 column volume of buffer B with 0.15 mM potassium acetate and step-eluted with 2 column volumes of buffer B containing 0.25 mM potassium acetate followed by 2 column volumes of buffer B with 0.45 mM potassium acetate.

CTD phosphatase activity eluted in the 0.45 mM potassium acetate fraction and was dialyzed against buffer C containing 0.025 mM potassium acetate, adjusted to 0.05 mM potassium acetate, and loaded onto SP-Sepharose (15 × 2.5 cm) and Q-Sepharose (12 × 2.5 cm) columns connected in tandem and equilibrated in buffer C containing 0.05 mM potassium acetate. After the sample was loaded, the columns were washed with 2 column volumes of buffer C with 0.05 mM potassium acetate. The SP-Sepharose column was disconnected, and the Q-Sepharose column was developed with a 300-ml gradient of 0.05–1.2 mM potassium acetate in buffer C. CTD phosphatase activity eluted at 0.86 mM potassium acetate. The Q-Sepharose fractions containing CTD phosphatase activity were pooled; dialyzed against buffer C containing 10 mM potassium phosphate, pH 8.0, and 50 mM potassium acetate; and loaded onto blue Sepharose CL-6B (8 × 1.5 cm) and Bio-Gel HTP (8 × 2.5 cm) columns.

4 R. S. Chambers and C. M. Kane, unpublished observations.
Identification of a yeast CTD phosphatase activity—Yeast CTD phosphatase activity was measured by monitoring the removal of $^{32}$P radiolabel from the largest subunit of RNAP II. To prepare the phosphatase substrate, yeast RNAP IIA was phosphorylated using a partially purified yeast TFIH-associated CTD kinase in the presence of [$\gamma$-$^{32}$P]ATP. In addition to the largest subunit of RNAP II, a number of other proteins in the CTD kinase preparation were radiolabeled and served as controls for the specificity of phosphatase activity (Fig. 1). Previous studies showed that HeLa CTD phosphatase is okadaic acid-resistant and dependent on magnesium ions for activity, thereby classifying it as a type 2B phosphatase (19).

Phosphatase assays were therefore performed in the presence of 0.5 $\mu$M okadaic acid, sufficient to inhibit type 1 and 2A protein phosphatase activities, and with magnesium ions to activate type 2C phosphatases (32). 2-Glycerophosphate, a substrate of nonspecific acid and alkaline phosphatases, was included to inhibit these activities. In addition, samples of yeast CTD phosphatase were dialyzed against buffer with 0.1 mM EDTA, thereby inhibiting the calcium-dependent type 2B phosphatases. A yeast whole cell extract was fractionated on a DE52 column, and an aliquot of the DE 0.45 fraction was incubated with the $^{32}$P-labeled RNAP II for various times and analyzed by SDS-PAGE and autoradiography (Fig. 1A). The DE 0.45 fraction contained an activity that specifically removed $^{32}$P radiolabel from the largest subunit of RNAP II in a time-dependent manner. In contrast, a Bio-Rex 70 chromatography fraction derived from the same yeast whole cell extract contained phosphatase activities that removed radiolabel from all the $^{32}$P-labeled proteins except RNAP II, indicating that the largest subunit of RNAP II was relatively resistant to dephosphorylation by other type 2C phosphatases (Fig. 1B). The apparent phosphatase activity in the DE 0.45 fraction was not due to proteolysis of the CTD of RNAP II since no smaller peptides were generated even following a 2-h incubation with 50 times more enzyme. Furthermore, the release of $^{32}$P and not $^{32}$P-peptides was detected using a $^{32}$P release assay (data not shown) (30).

Purification of Yeast CTD Phosphatase—A summary of the purification of a CTD phosphatase activity is presented in Table I. The DEAE column step separated the CTD phosphatase activity from almost all of the nonspecific phosphatase activity, nucleic acids, and >90% of the protein. The SP-Sepharose column step removed the general transcription factors and a large proportion of the RNAP II. After this step, the CTD phosphatase activity was highly dependent on the addition of the SP 0.48 fraction that contained TFIIF. The CTD phosphatase activity recovered from the Q-Sepharose column was stimulated 10-fold by the SP 0.48 fraction. The level of stimulation increased after several subsequent column steps, resulting in fractions from the Mono Q column step being stimulated >300-fold by the SP 0.48 fraction. No phosphatase activity was detected in the SP 0.48 fraction. The Bio-Gel HTP column fractionated the remaining RNAP II from the CTD phosphatase activity. The CTD phosphatase enzyme has a high affinity for the anion exchange resins DEAE and Q-Sepharose, suggesting that it is a highly acidic protein. Two bands with apparent molecular masses of 100 and 103 kDa comigrated with CTD phosphatase activity on the Bio-Gel HTP, phenyl-Superose, Superose 12, and Mono Q columns in CTD phosphatase assays and on SDS-PAGE (data not shown) (Fig. 2). The 100- and 103-kDa proteins stained very poorly with silver; thus, polyacrylamide gels were prestained with Coomassie Blue to increase the sensitivity of detection with silver. CTD phosphatase activity eluted with an apparent molecular mass of 125
kDa on Superose 12, but sedimented with an apparent molecular mass of only 61 kDa in glycerol gradients, suggesting that it has a monomeric and likely an elongated structure (data not shown). The glycerol gradient fractions were also examined by SDS-PAGE and Coomassie Blue staining. Activity again comigrated with the 100- and 103-kDa bands. The Mono Q-purified CTD phosphatase was separated by SDS-PAGE as well as assayed for CTD phosphatase activity. CTD phosphatase activity only comigrated with the 100- and 103-kDa bands (Fig. 3). It remained possible that other poorly staining proteins might be present that could contain the CTD phosphatase activity. To test this, a sample of the Mono Q-purified CTD phosphatase was separated by SDS-PAGE. The 178-, 100/103-, and 66-kDa bands were excised, and the protein was extracted, renatured, and analyzed in a CTD phosphatase assay and again by SDS-PAGE. CTD phosphatase activity was detected only with the renatured 100/103-kDa proteins (Fig. 4). The Mono Q-purified CTD phosphatase preparation contained ~20 μg of the 100/103-kDa proteins from 1 kg of starting material.

Characterization of the CTD Phosphatase Activity—CTD phosphatase was inactive in the absence of divalent cations and had 50% maximal activity with 1 mM MgCl₂ (Fig. 5). CTD phosphatase activity was also supported with CaCl₂ or MnCl₂. In keeping with the PP2C categorization of the CTD phosphatase, the protein phosphatase inhibitors okadaic acid and vanadate had no effect on activity at concentrations up to 1 mM, respectively (data not shown). In contrast, other phosphatase activities present in the whole cell extract were only slightly inhibited with 400 mM potassium acetate; however, >90% of the activity was inhibited in reactions containing 250 mM magnesium acetate (data not shown). In contrast, other phosphatase activities present in the whole cell extract were only slightly inhibited with 400 mM potassium acetate. In keeping with the PP2C categorization of the CTD phosphatase, the protein phosphatase inhibitors okadaic acid and vanadate had no effect on activity at concentrations up to 10 μM and 1 mM, respectively (data not shown).

Yeast CTD phosphatase appears to be specific for dephosphorylating RNAP II (Fig. 1). To further investigate the specificity, yeast and HeLa CTD phosphatases were tested with yeast [³²P]RNAP II and mammalian [³²P]RNAP II. Yeast CTD phosphatase dephosphorylated yeast RNAP II, but not calf thymus RNAP II (Fig. 5A, lanes 1–4); conversely, HeLa CTD phosphatase dephosphorylated calf thymus RNAP II, but not yeast RNAP II (lanes 5–8). Furthermore, yeast TFIIH and not human TFIIH stimulated yeast CTD phosphatase (Fig. 6B). In addition, yeast CTD phosphatase did not dephosphorylate calf thymus RNAP II in the presence of human TFIIH (data not shown).

Previous studies showed that HeLa CTD phosphatase is competitively inhibited by RNAP II (20). The addition of a 200-fold excess of unlabeled yeast RNAP II over yeast [³²P]RNAP II to a CTD phosphatase assay resulted in inhibition of CTD phosphatase activity (Fig. 6C). A 100-fold excess of unlabeled yeast RNAP II over [³²P]RNAP II resulted in a 50% inhibition of CTD phosphatase activity (data not shown).
contrast, the addition of up to a 400-fold excess of calf thymus RNAP II over yeast \[^{32}\text{P}]\text{RNAP II} did not inhibit yeast CTD phosphatase activity (data not shown) (Fig. 6C). The addition of an equal number of moles of yeast TFIIF did not overcome the inhibition by yeast RNAP II (data not shown).

As mentioned above, after the SP-Sepharose column step, detection of CTD phosphatase activity was highly dependent on the addition of the SP 0.48 fraction that contained TFIIF. The activity of the CTD phosphatase recovered from the Mono Q column was stimulated \(>300\)-fold by the addition of the SP 0.48 fraction. Substituting recombinant yeast TFIIF (Tfg1 and Tfg2) or recombinant Tfg1 (33) for the SP 0.48 fraction gave no stimulation of activity (data not shown). Recombinant TFIIF did stimulate CTD phosphatase activity 3-fold when added to the whole cell extract or the DE 0.45 fraction and 4.5-fold when added to the Q-Sepharose fraction (data not shown) (Fig. 6B).

**DISCUSSION**

Yeast contains a protein phosphatase activity that is highly specific for dephosphorylating the largest subunit of yeast RNAP II. Many of the properties of the yeast CTD phosphatase are similar to those of the HeLa enzyme (19). These similarities include specificity for RNAP II as a substrate, stimulation of activity by the general transcription factor IIF, inhibition by excess RNAP II, resistance to the phosphatase inhibitor okadaic acid, requirement for magnesium ions for activity, and inhibition by high concentrations of salt (19). The magnesium requirement and resistance to okadaic acid would characterize the yeast CTD phosphatase, like the HeLa enzyme, as a type 2C phosphatase. However, the yeast CTD phosphatase activity is also supported by calcium ions, a property not seen before in the type 2C class of enzymes (21). The HeLa CTD phosphatase cannot use calcium ions to support activity, but can use zinc, which did not support activity with the yeast enzyme. Other magnesium-dependent phosphatases are unaffected by calcium, inhibited (34), or stimulated (35). Millimolar amounts of calcium are required for significant activity of the yeast CTD phosphatase, and calcium does not support a level of activity higher than with magnesium alone. Thus, the enzyme is unlikely to be regulated by calcium in vivo. However, this unusual property could be used to distinguish it from other phosphata-
Yeast CTD Phosphatase

The highly purified yeast CTD phosphatase activity is stimulated ~300-fold by an SP-Sepharose fraction from the CTD phosphatase purification that contains TFIIF. Highly purified HeLa CTD phosphatase is stimulated only 5-fold by recombinant human TFIIF (20). Surprisingly, recombinant yeast TFIIF did not stimulate highly purified yeast CTD phosphatase, but did stimulate partially purified fractions of CTD phosphatase. These results suggest that an additional essential factor for CTD phosphatase activity is fractionated from the 100/103-kDa component at the SP-Sepharose column step in the purification. If mammalian CTD phosphatase requires a similar factor, then it must remain stably associated since the HeLa enzyme showed no dramatic decline in activity after many purification steps and was not dependent on other fractions for activity (19). This difference in purified complexes might be analogous to the differential stability seen with TATA-binding protein-TATA-binding protein-associated factor complexes, which are stable in mammalian extracts, but dissociate during purification in yeast (38).

SDS-PAGE analysis of the highly purified yeast CTD phosphatase shows two bands with apparent molecular masses of 100 and 103 kDa. Glycerol gradient sedimentation and gel filtration analysis indicate a monomeric structure with an elongated shape. It is not clear if the two proteins represent two different forms of CTD phosphatase or if one of them is an unrelated protein. Microsequences from three peptides derived from the 100/103-kDa proteins match a single yeast gene of previously unknown function, predicting a highly acidic protein with a molecular mass of 83 kDa. The yeast genome sequence data base contains five genes with homology to type 2C phosphatases with predicted molecular masses ranging from 32 to 64 kDa. None of these has detectable similarity to the 83-kDa protein. Since serine/threonine protein phosphatases are a

3 R. S. Chambers and M. E. Dahmus, unpublished observations.

4 J. W. Conaway, personal communication.

5 J. Archambault, R. S. Chambers, G. Pan, M. Kobor, B. Andrews, C. M. Kane, and J. Greenblatt, manuscript in preparation.
highly conserved family of enzymes, the lack of detectable homology of the gene encoding the 83-kDa protein to other phosphatases presents two possibilities. The 83-kDa protein may represent a new specific class of protein phosphatases. Alternatively, this protein may have a regulatory role in CTD phosphatase function. The isolation and characterization of CTD phosphatase from yeast now allow a combined biochemical and genetic analysis to distinguish these possibilities. In addition, the function of this enzyme during transcription can now be examined in detail.

Acknowledgments—We gratefully acknowledge Rodney Weilbaecher for preparation of the calf thymus RNAP IIA. We also thank members of the laboratory for helpful comments during the course of this study and for review of this manuscript.

REFERENCES

1. Dahmus, M. E. (1994) Prog. Nucleic Acids Res. 48, 143–179
2. West, M. L., and Corden, J. L. (1995) Genetcs 140, 1163–1169
3. Kim, W.-Y., and Dahmus, M. E. (1989) J. Biol. Chem. 264, 3169–3176
4. Chambers, R. S., and Dahmus, M. E. (1994) J. Biol. Chem. 269, 26243–26248
5. Chambers, R. S., Wang, B. Q., Burton, Z. F., and Dahmus, M. E. (1995) J. Biol. Chem. 270, 14692–14699
6. Dahmus, M. E. (1994) Prog. Nucleic Acids Res. 48, 143–179
7. West, M. L., and Corden, J. L. (1995) Genetics 140, 1223–1233
8. Chambers, R. S., and Dahmus, M. E. (1994) J. Biol. Chem. 269, 26243–26248
9. Chambers, R. S., Wang, B. Q., Burton, Z. F., and Dahmus, M. E. (1995) J. Biol. Chem. 270, 14692–14699
10. Dahmus, M. E. (1994) Prog. Nucleic Acids Res. 48, 143–179