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Viral transactivators E1A and VP16 interact with a large complex that is associated with CTD kinase activity and contains CDK8

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ABSTRACT

Previously, we showed that the viral transactivator proteins E1A and VP16 specifically interact with a cellular CTD kinase activity *in vitro*. We now report that E1A and VP16 complexes contain human CDK8, a newly identified member of the cyclin-dependent kinase family that has been shown to be a component of the RNA polymerase II (RNAP II) holoenzyme complex. The presence of CDK8 in the E1A- and VP16-containing complexes is specific for a functional activation domain of these viral transactivators, strongly suggesting that this association is relevant for the transactivation function of E1A and VP16. We show that CDK8 is associated with CTD kinase activity and that CDK8 co-fractionates with E1A- and VP16-associated CTD kinase activity over several chromatography columns. Therefore, CDK8 is likely responsible for the E1A- and VP16-associated CTD kinase activity. Gel filtration chromatography indicates that the E1A- and VP16-associated CTD kinase activity has a molecular size of ~1.5 MDa and contains cyclin C and the human homolog of SRB7 in addition to CDK8. This implies that E1A and VP16 associate with the RNAP II holoenyzme. We also looked at the transcriptional activity of CDK8 and found that CDK8 can function as a transcriptional activator when fused to the DNA binding domain of GAL4. Surprisingly, the ability of GAL4–CDK8 to activate transcription in this assay was not dependent on the kinase activity of CDK8, since a kinase-deficient mutant of CDK8 stimulated transcription nearly as well as wild-type GAL4–CDK8. This suggests that CDK8 may play a role in transcription that is distinct from its ability to function as a CTD kinase.

INTRODUCTION

Transcription of eukaryotic genes is a complex and highly regulated process that is comprised of several discrete steps, each of which is subject to regulation and requires the presence of multiple protein factors. Recent work has shown that transcription is mediated by a large multi-protein complex termed the RNA polymerase II (RNAP II) holoenzyme that was originally discovered in yeast (1–3). In addition to the multi-subunit RNA polymerase II (RNAP II) enzyme, the yeast holoenzyme contains general transcription factors (GTFs), which are necessary for RNAP II to initiate transcription at specific sites, and a mediator complex, which is required for proper response to transcriptional regulatory factors. The mediator complex contains proteins, known as SRB (suppressor of RNA polymerase B) proteins, that were originally identified as dominant extragenic suppressors of a mutant form of RNAP II in which the largest subunit contained partial deletions of the C-terminal domain (CTD) (4,5). In mammalian cells, a large multisubunit complex containing RNAP II and GTFs has been described (6). More recently, RNAP II holoenzyme complexes have been isolated from human cells that also contain homologs of the yeast SRB proteins (7,8).

The CTD of RNAP II contains an unusual, highly repetitive structure consisting of tandem repeats of the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser (9,10). Although its precise role in transcription remains unclear, the function of the CTD is thought to be mediated through protein–protein interactions with SRB proteins and other components of the transcription complex. These interactions are likely regulated by phosphorylation of the CTD. The CTD is a target for phosphorylation by a number of protein kinases, including a TFIIH-associated kinase (11), the cyclin-dependent kinases CDC2 and CDK2 (12,13) and several partially purified activities (13–15). Two of these kinases are associated with the RNAP II holoenzyme: the TFIIH-associated kinase and SRB10/11. The catalytic component of TFIIH is CDK7 (also known as MO15) (16–19). Addition of TFIIH containing wild-type CDK7 reconstitutes transcription in a TFIIH-dependent *in vitro* system, while a kinase-deficient form of TFIIH supports only abortive initiation, suggesting that the kinase activity of CDK7 is required for transcription at a stage after the formation of the first phosphodiester bond (20). The SRB10/11 proteins are a CDK/cyclin pair that are found in RNAP II holoenzymes isolated from *Saccharomyces cerevisiae* (21). Holoenzymes lacking SRB10/11 kinase activity are deficient in CTD phosphorylating

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activity and show defects in transcription *in vivo*, although they are not defective for transcription using a purified *in vitro* system (21). The SRB10 protein is identical to UME5 (22), SSN3 (23) and ARE1 (24), proteins identified in independent genetic screens. SRB10 is also structurally related to the human CDK8 protein (originally named K35) (25). CDK8, isolated in a search for human protein kinases that might play a role in cell cycle control (26), encodes a protein of 464 amino acids with a predicted molecular mass of 53 kDa (25). CDK8 associates with cyclin C *in vitro* and *in vivo*. Cyclin C is the closest known mammalian relative of SRB11. Like their yeast counterparts, both CDK8 and cyclin C are found associated with the human RNAP II holoenzyme (8).

It is likely that cellular CTD kinases are involved in mediating the action of at least some viral transactivator proteins as several especially potent viral transactivator proteins can specifically target cellular CTD kinases. For example, the herpes simplex virus VP16 protein is known to interact with TFIIH (27,28) and the human immunodeficiency virus Tat proteins specifically interact with the Tat-associated kinase (TAK) (13,29,30). More recently, we have identified a cellular CTD kinase activity that specifically interacts with the activation domains of VP16 and the adenovirus E1A proteins *in vitro* (31). The VP16- and E1A-associated kinase activities were shown to be related to each other but distinct from TFIIH and TAK (31).

In this report, we show that E1A and VP16 associate with a large complex, presumably the RNAP II holoenzyme, that contains CDK8, cyclin C and the human homolog of SRB7. We also show that CDK8 is associated with CTD kinase activity and that CDK8 co-fractionates with the E1A- and VP16-associated CTD kinase activity over several chromatography columns. This suggests that the E1A- and VP16-associated CTD kinase is most likely CDK8. We further demonstrate that CDK8 is capable of activating transcription when artificially targeted to the promoter. Surprisingly, the ability of CDK8 to stimulate transcription is not dependent on the kinase activity of CDK8. However, our results indicate that CDK8 possesses a weak activation domain at its C-terminus, a region not conserved among other CDKs. Possible roles for CDK8 in transcription are discussed.

MATERIALS AND METHODS

Plasmids

Bacterial expression plasmids used to express fusions of glutathione-*S*-transferase (GST) to the activation domains of the E1A and VP16 proteins (31) or the Tat proteins (32) have been described previously. The construct used for expression of GST–CTD was a gift from W. Dynan and colleagues (33). For construction of GAL4–CDK7, CDK7 sequences were PCR amplified from a CDK7-encoding plasmid (obtained from D.O. Morgan), inserted into a modified version of the plasmid pGEM3Z+ (Promega) to generate pGEM-CDK7 and the sequence was confirmed by di-deoxy sequencing. The fragment from pGEM-CDK7, encoding the CDK7 sequences was then cloned into pSG424 (34), which encodes the DNA binding domain (a.a. 1–147) of GAL4 directed by the SV40 early promoter. For construction of GAL4–CDK8, CDK8 sequences were excised from a CDK8/Bluescript expression vector (25) and inserted into pSG424. The D173A mutant was constructed using the Transformer Site Directed Mutagenesis kit (Clontech). To construct GAL4–D173A, the region of CDK8 containing the mutation was removed from the D173A/Bluescript expression vector and inserted into the corresponding region of GAL4–CDK8. The sequence of the D173A mutant was verified by di-deoxy sequencing. The GAL4–CDK8NX mutant contains the *Nco*I to *Xba*I fragment of CDK8 (amino acid residues 318–464) in the pSG424 vector. To generate the FLAG–CDK8 fusions, CDK8 sequences obtained from the GAL4–CDK8 plasmid were inserted into pFLAG-CMV-2 (Kodak), which codes for an eight amino acid tag 5′ of the multiple cloning site, yielding pFLAG-CDK8. The construct pFLAG-D173A was generated by digestion of GAL4–D173A to obtain a fragment of CDK8 containing the mutation which was then inserted into pFLAG-CDK8 such that the mutation replaced the wild-type sequence.

Transfections and CAT assays

All transfections were performed using a calcium phosphate transfection procedure (35). For analysis of transactivation by GAL4 fusions, HeLa cells in DMEM + 10% fetal bovine serum (Gibco) were seeded onto 6 cm dishes one day prior to transfection to yield 50% cell confluency. Each plate was transfected with 2 µg GAL4 parental or fusion plasmid + 2 µg pG6(–83)HIVLTRΔTAR-CAT (36) + 0.5 µg pSV-βgal (37) + 5 µg salmon sperm DNA. At ~48 h post-transfection, cells were harvested for CAT and β-galactosidase assays. Results of CAT assays were quantified on a Betascope 603 blot analyzer (Betagen). Reaction conditions were adjusted so that all results used for quantitation of CAT activity had <50% acetylation of the total chloramphenicol. Likewise, all measurements used for quantitation of β-galactosidase activity had OD_{420} values of <0.4.

Immunochemical analysis

Immunoprecipitations were performed as described (38). Immune complexes were washed three times in EBC buffer (50 mM Tris–HCl pH 8.0, 120 mM NaCl, 0.5% NP-40 and 5 mM DTT) and resuspended in Laemmli sample buffer. Immunoblot analysis was carried out as described previously (31) using enhanced chemoluminescence for detection of immunoreactive proteins. The rabbit anti-CDK8 (25) and anti-CDK7 (39) polyclonal antibodies were used at a dilution of 1:5000, the anti-cyclin C antibody (40) was used at a dilution of 1:2000, and the anti-hSRB7 antibody (7) was used at a dilution of 1:400. One-dimensional peptide analysis using V8 protease (Pierce) was performed as described (38).

Partial protein purification

Fractionation of HeLa cell nuclear extracts over phosphocellulose P11 and Resource Q columns was described previously (31). The peak of the E1A- and VP16-associated kinase activities eluted from the Resource Q column at 270–300 mM KCl. This peak was concentrated using a Centricon-30 column and applied to a Superose 6 FPLC column (Pharmacia) equilibrated with 50 mM Tris pH 8.0, 10% glycerol, 0.2 mM EDTA, 100 mM KCl, 0.5 mM DTT and 0.5 mM PMSF. Proteins were eluted with equilibration buffer at 0.2 ml/min and fractions (400 µl) were analyzed directly by immunoblot analysis or for CTD kinase activity. The molecular size of the complex was determined by extrapolation from molecular weight standards.

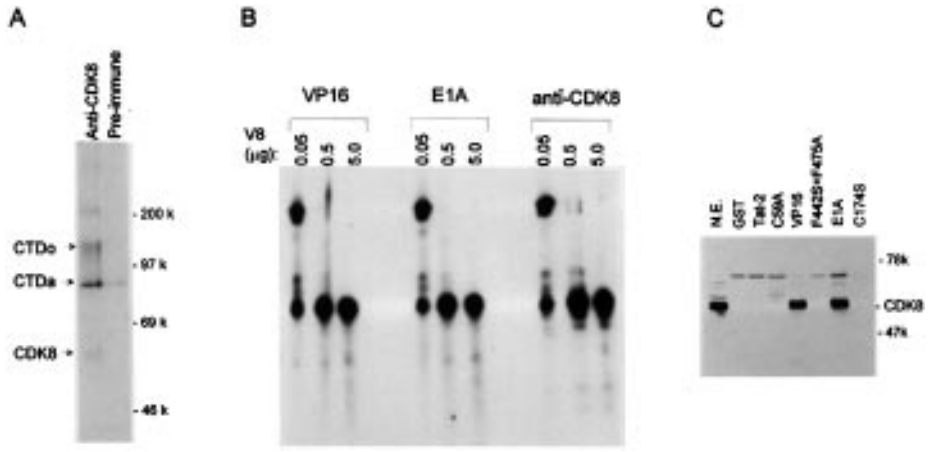

Figure 1. CDK8 is associated with CTD kinase activity and is present in E1A- and VP16-containing complexes. (**A**) Anti-CDK8 rabbit polyclonal antibody (1 μl) or the corresponding preimmune serum was incubated with a HeLa cell nuclear extract for 1 h on ice, the complexes were immunoprecipitated by the addition of Protein A Sepharose beads, washed and incubated with CTD kinase reaction mix (see Materials and Methods). The bands corresponding to the CTDo, CTDa and phosphorylated form of CDK8 are indicated. (**B**) Kinase reactions were performed as described in Materials and Methods using wild-type GST–VP16, wild-type GST–E1A, or the anti-CDK8 antibody. The 58–59 kDa phosphorylated products of these reactions were excised from the gel and subjected to one-dimensional peptide mapping using the indicated amounts of V8 protease as described previously (38). (**C**) The indicated GST-fusion proteins were bound to glutathione beads, incubated with a HeLa cell nuclear extract, and washed as described in Materials and Methods. The complexes were analyzed by immunoblot analysis using a rabbit polyclonal antibody raised against human CDK8 (25). The lane marked N.E. represents HeLa cell nuclear extract that was loaded onto the gel and used as a positive control.

CTD kinase assays

Kinase assays were performed as described previously (31). Briefly, fusions of viral transactivator proteins expressed in bacteria as GST-fusions were purified as described (13,29) and bound to HeLa cell nuclear extracts (31) for 60 min at 4°C with gentle rocking. Complexes were washed three times with EBC buffer containing 0.03% SDS, followed by one wash with Kinase Buffer (50 mM Tris pH 7.4, 10 mM $MgCl_2$ and 5 mM DTT). Kinase assays were performed by adding 25 μl of a mix containing Kinase Buffer, 2.5 mM $MnCl_2$, 200 ng GST-CTD, 5 μM ATP and 5 μCi [γ-^{32}P]ATP (NEN, 3000 Ci/mmol) to the bead complexes and incubating for 60 min at room temperature. Complexes were pelleted briefly, denatured in Laemmli sample buffer, and resolved by 9% SDS–PAGE.

RESULTS

Previously, we reported that a CTD kinase activity associates with the viral transactivator proteins E1A and VP16 *in vitro* (31). The interaction of a CTD kinase with E1A or VP16 was dependent on a functional activation domain of these proteins, suggesting that the interaction is relevant for the transactivation function of E1A and VP16. We also showed that the kinase activities that associate with E1A and VP16 are biochemically related to each other but distinct from two other known CTD kinase activities, TFIIH and TAK. Recently, the cloning of a novel member of the cyclin-dependent kinase family, CDK8, was reported (25). Given the sequence similarity of human CDK8 to the *S.cerevisiae* SRB10 protein, a kinase involved in CTD phosphorylation (21), we investigated whether human CDK8 is associated with CTD kinase activity and whether it interacts with E1A or VP16.

CDK8 is associated with CTD kinase activity and is present in E1A- and VP16-containing complexes

To test whether CDK8 is associated with CTD kinase activity, a CTD kinase assay was performed as described in Materials and Methods using GST–CTD as a substrate. Hyperphosphorylation of the GST–CTDa substrate leads to the generation of the more slowly migrating GST–CTDo form; the appearance of the CTDo form is indicative of CTD kinase activity (41). An anti-CDK8 antibody (25) was used to immunoprecipitate CDK8 from a HeLa cell nuclear extract. The immune complexes were washed and a CTD kinase assay was performed. As seen in Figure 1A, the CTDo form was generated in reactions using the anti-CDK8 antibody but not the preimmune serum. There was also an increase in the level of phosphorylation of the CTDa form. This experiment indicates that CDK8 is associated with CTD kinase activity. Results presented below using a kinase-deficient form of CDK8 (Fig. 4) further suggest that CDK8 possesses CTD kinase activity.

In addition to phosphorylation of the CTD, we noted that a protein of ~58 kDa became phosphorylated in the CDK8 immune complex kinase reactions (Fig. 1A). A band of similar size was also detected in wild-type E1A- and VP16-containing complexes, but not in complexes containing the transactivation-deficient mutants (31). To determine whether the proteins phosphorylated in the GST–E1A and GST–VP16 reactions are related to the protein phosphorylated in the CDK8 immune complexes, we analyzed this band by one-dimensional peptide mapping using V8 protease (Fig. 1B). Digestion of the protein phosphorylated in the GST–E1A or GST–VP16 reactions gave a similar pattern as that seen with the phosphorylated product of the CDK8 immune complex kinase reaction, suggesting that these phosphoproteins are highly related. We believe that this protein represents autophosphorylation of CDK8. Evidence to support this is: (i) the migration of this phosphoprotein is consistent with the migration of CDK8 (56–59 kDa) as detected by immunoblot analysis [Fig. 1C, (25)], (ii) the presence of this phosphoprotein in kinase reactions correlates with the peak levels of CDK8 as detected by immunoblot analysis over several chromatography columns (Fig. 2 and data not shown), (iii) this band is not detected when using a kinase-deficient mutant form of CDK8 (Fig. 4), and (iv) the migration of this band is retarded when using an epitope-tagged

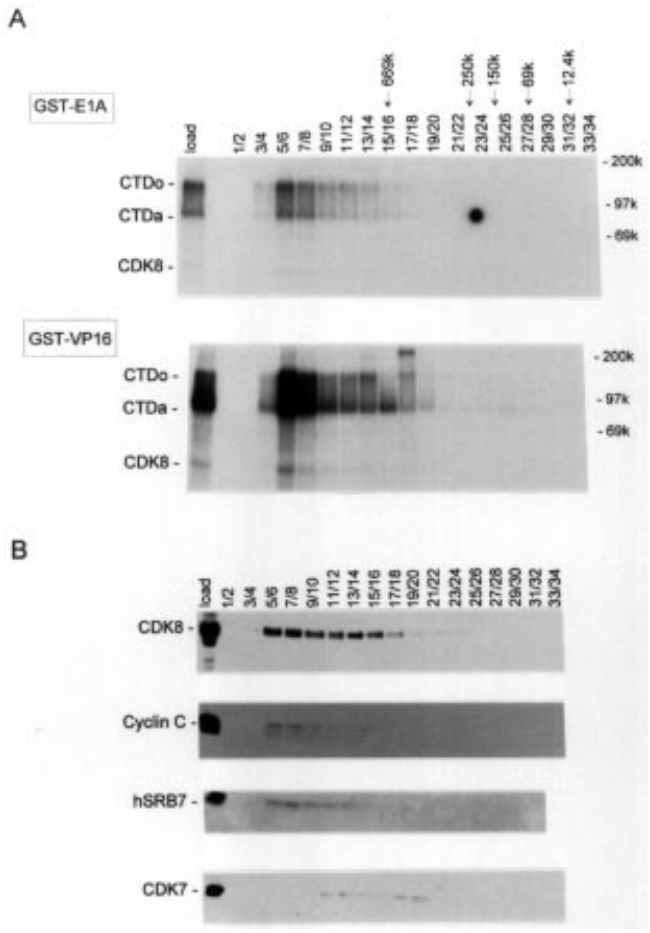

Figure 2. E1A- and VP16-associated kinase activity is present in a large complex that contains CDK8, cyclin C and hSRB7. HeLa cell nuclear extract was fractionated over a phosphocellulose, the 0.5 M KCl eluate was then applied to a Resource Q column, and the peak of E1A- and VP16-associated CTD kinase activity from the Resource Q column was then passed over a Superose 6 gel filtration column (see Materials and Methods). (**A**) Aliquots of fractions from the Superose 6 column were analyzed for E1A- and VP16-associated CTD kinase activity as described in Materials and Methods. (**B**) Aliquots of fractions were analyzed directly for the presence of CDK8, cyclin C, hSRB7 or CDK7 by immunoblotting as described in Materials and Methods.

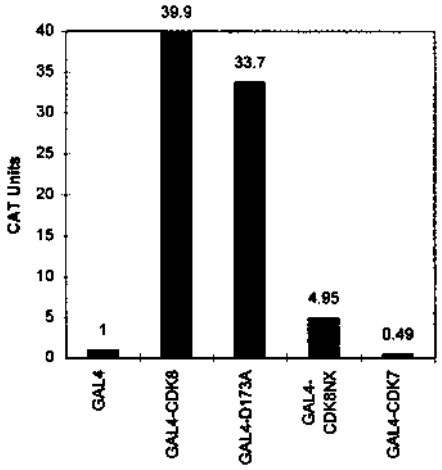

Figure 3. Transactivation by CDK8. HeLa cells in 6 cm dishes were transfected by the calcium phosphate method (35) with 2 µg of the indicated GAL4 expression plasmids (as described in the text), 2 µg of the CAT reporter plasmid G6(–83)HIVLTRΔTAR containing six GAL4 binding sites upstream of the HIV-1 promoter containing a deletion of the TAR element (36), and 0.5 µg of pSVβ-gal (37) used as an internal control. Cells were harvested at 48 h post-transfection and extracts were analyzed for CAT activity (47) and for β-galactosidase activity. A CAT unit is arbitrarily defined as the amount of activity obtained from the GAL4 vector alone after normalization to β-galactosidase activity. This experiment was performed in duplicate and is expressed as the average of the two duplicate plates with standard deviations <13%. The experiment shown was repeated several independent times with similar results.

form of CDK8 (Fig. 4). These results indicate that CDK8 or a highly related protein is present in the E1A and VP16 complexes.

To confirm that CDK8 associates with E1A and VP16 complexes, we looked for the presence of CDK8 in E1A and VP16 complexes by immunoblot analysis. A HeLa cell nuclear extract was incubated with GST–E1A and GST–VP16 fusion proteins as described in Material and Methods and an immunoblot was performed with the anti-CDK8 antibody (Fig. 1C). The results showed that CDK8 was present in complexes containing wild-type E1A or VP16, but not in complexes containing the transactivation-deficient mutants C174S (substitution of Ser for Cys at position 174 of E1A) and F442S+F475A (substitution of Ser for Phe at position 442 and substitution of Ala for Phe at position 475 of VP16). This experiment also showed that CDK8 does not interact detectably with the HIV Tat protein, consistent with the biochemical characterization of the Tat-, E1A- and VP16-associated kinases that indicated that the Tat-associated kinase is distinct from the E1A- or VP16-associated kinase (31).

These results indicate that CDK8 is found associated with wild-type but not transactivation-deficient mutant forms of E1A and VP16 and that CDK8 is likely to be responsible for the E1A- and VP16-associated CTD kinase activity.

CDK8 co-fractionates with E1A- and VP16-associated CTD kinase activity and is present in a large complex that also contains cyclin C and hSRB7

To further characterize the E1A- and VP16-associated CTD kinase activities, we partially purified the viral-associated kinase activities. HeLa cell nuclear extracts were fractionated over a phosphocellulose P11 column and the 0.5 M KCl eluate was then passed over a Resource Q ion exchange column (31). The peak of E1A- and VP16-associated CTD kinase activity coincided precisely with peak levels of CDK8 as determined by immunoblot analysis (not shown). These fractions also contained the highest levels of cyclin C and the human homolog of the yeast SRB7 protein (hSRB7), a component of the RNAP II holoenzyme mediator subcomplex. The majority of CDK7, another CTD kinase present in the holoenzyme, eluted from the Resource Q column at a lower salt concentration than the peak of E1A- and VP16-associated CTD kinase activity, although low levels were detected in the peak fractions (not shown).

Since CDK8 is a component of the RNAP II holoenzyme complex (8), we were interested to determine whether the E1A- and VP16-associated CTD kinase activity is associated with RNAP II holoenzyme complex or represents a distinct CDK8-containing complex. To determine the molecular size of the CTD kinase activity, the peak of kinase activity eluted from the Resource Q column was subjected to gel filtration chromatography over a

Superose 6 column. As seen in Figure 2, the peak of E1A- and VP16-associated kinase activity eluted as a large complex that was estimated to be ~1.5 MDa. This is consistent with the reported size of the holoenzyme complex (5,7,8). We next looked for the presence of known components of the mediator subcomplex for which reagents were available to us—CDK8, cyclin C and hSRB7. The immunoblot analysis shown in Figure 2, demonstrates that these proteins are present in fractions 3 through 18, with the highest levels seen in fractions 5 through 8. This is similar to the levels of E1A- and VP16-associated CTD kinase activity observed in these fractions. In contrast, CDK7 was detected predominantly in fractions 11 to 22. These results indicate that E1A and VP16 associate with a large complex that contains CDK8, cyclin C and hSRB7, presumably the RNAP II holoenzyme and suggest that E1A and VP16 may target the mediator subcomplex of the RNAP II holoenzyme.

CDK8 activates transcription when fused to a DNA binding domain

We have previously proposed a model in which some viral transactivators function by recruiting a CTD kinase to the promoter (13,31). If CDK8 is important in mediating the response to E1A and VP16, we reasoned that CDK8 might activate transcription in the absence of E1A or VP16 if it was artificially targeted to the promoter. For this purpose, the cDNA clone of CDK8 (25) was inserted into the pSG424 vector (34) which encodes the GAL4 DNA binding domain (a.a. 1–147) driven by the SV40 early promoter. The GAL4–CDK8 plasmid was transfected into HeLa cells along with a reporter plasmid, G6(–83)HIVLTRΔTAR (36), that contains six GAL4 binding sites upstream of a promoter based on the HIV-1 LTR that is fused to the chloramphenicol acetyltransferase (CAT) gene. As shown in Figure 3, the GAL4–CDK8 plasmid produced a 40-fold increase in expression from the reporter plasmid relative to a construct containing the GAL4 DNA binding domain only (after normalization to β-galactosidase activity to control for transfection efficiencies). A similar result was obtained using a different reporter plasmid, G5E1B-CAT-SP (42), which contains an Sp1 site upstream of the E1B TATA element (43). Transactivation was dependent on the presence of the GAL4 DNA binding sites as GAL4–CDK8 did not increase expression from a reporter construct that lacked the GAL4 DNA binding sites (43).

To determine whether the kinase activity of CDK8 is required for transactivation activity, we tested a mutant of CDK8, D173A, in which the aspartate at position 173 (in subdomain VII) is changed to alanine. To assay the transactivation activity of this mutant, D173A was cloned into the GAL4 vector and the transactivation activity was measured following transfection of HeLa cells. Surprisingly, this mutant displayed high levels of transactivation activity, although it reproducibly gave slightly lower levels of transactivation than the wild-type protein (Fig. 3). To confirm that this mutation renders CDK8 non-functional for kinase activity, FLAG-tagged wild-type CDK8 and the D173A mutant were overexpressed in HeLa cells, immunoprecipitated with an antibody directed against the FLAG epitope, and a CTD kinase reaction was performed (Fig. 4). With wild-type FLAG–CDK8, the appearance of the CTDo form and the phosphorylated form of FLAG–CDK8 were observed but, as expected, no autophosphorylation product or hyperphosphorylation of the CTD was observed with the D173A mutant. The wild-type and mutant form of FLAG–CDK8 were expressed at equivalent levels as

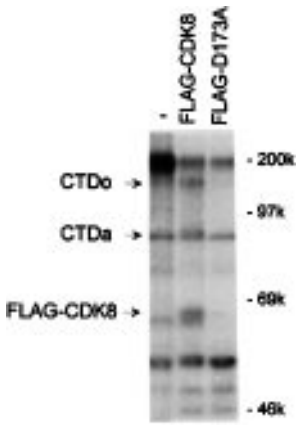

Figure 4. CDK8–D173A is defective for CTD kinase activity. Immunoprecipitations were performed with the M2 anti-FLAG monoclonal antibody (10 µl, Kodak) using lysates from untransfected (–) HeLa cells or cells transfected with wild-type FLAG–CDK8 or the FLAG–D173A mutant. Immune complexes were washed and CTD kinase assays were performed as described in Materials and Methods. The bands corresponding to the CTDo, CTDa, and phosphorylated form of FLAG–CDK8 are indicated.

detected by immunoprecipitation from [^{35}S]methionine-labeled transfected cells (data not shown). Together, these results indicate that CDK8 is capable of activating transcription independent of its kinase activity under the conditions of this assay.

Since the kinase activity of CDK8 appears not to be required for the transactivation function of CDK8, we postulated that CDK8 might contain an 'activation' domain that could interact with components of the RNAP II holoenzyme and serve to recruit the holoenzyme to the promoter. Because CDK8 contains a region at its C-terminus that is not conserved among other CDKs, we tested whether this region could function in the transactivation assay by fusing the C-terminal 147 amino acid residues (318–464) to the GAL4 DNA binding domain. As shown in Figure 3, this construct stimulated transcription ~5-fold. While this is clearly less than wild-type, it suggests that this region may be involved in interactions with other components of the RNAP II holoenzyme complex.

We also tested whether CDK7 could activate transcription when expressed as a fusion with the GAL4 DNA binding domain. As shown in Figure 3, GAL4–CDK7 was unable to activate expression from the reporter plasmid. We have confirmed that the GAL4–CDK7 protein is expressed in transfected HeLa cells by immunoprecipitation of GAL4–CDK7 following metabolic labeling of cells with [^{35}S]methionine (data not shown). Therefore, the ability of a CDK to function as a transactivator when expressed as a GAL4 fusion protein may be unique to CDK8.

DISCUSSION

The sequence similarity between human CDK8 and the yeast SRB10 protein, a component of the yeast mediator complex, suggested that CDK8 may play a role in CTD phosphorylation and transcriptional regulation. The work reported in this study is consistent with such a role for CDK8. First, we showed that CDK8 is tightly associated with CTD kinase activity. CTD kinase activity is present in CDK8-containing immune complexes (Fig. 1A) and CDK8 co-fractionates with CTD kinase activity over three chromatography columns (Fig. 2 and data not shown).

The result that the kinase-deficient FLAG–D173A mutant does not phosphorylate the CTD (Fig. 4) further supports the idea that CDK8 is a CTD kinase. Recent work from E. Lees and colleagues has also shown that a CDK8/cyclin C complex displays kinase activity for the CTD (40). *Drosophila* CDK8 has also been shown to be associated with CTD kinase activity (44). However, it is still formally possible that CDK8 associates with another kinase that phosphorylates the CTD.

Second, we showed that two viral transactivators, E1A and VP16, interact with a CDK8-containing complex (Figs 1B and C). We currently do not know whether the interaction between CDK8 and the viral activator proteins is a direct physical interaction. It is possible that E1A and VP16 directly contact another component of the holoenzyme complex, so that CDK8 is detected in association with E1A and VP16 as a result of direct binding to another holoenzyme protein. However, we have demonstrated that the interaction is specific for a functional activation domain of E1A and VP16. Transactivation-deficient mutants of E1A and VP16 fail to associate with CDK8, strongly suggesting that this interaction with the CDK8-containing complex is important for the transactivation function of these viral transactivators. CDK8 does not, however, interact with another strong viral transactivator protein, Tat. This is perhaps not too surprising, considering that Tat, unlike E1A and VP16, interacts with a downstream RNA element and therefore may contact different components of the transcription complex. Tat interacts with another cellular CTD kinase, TAK, which has been proposed to mediate Tat transactivation (13).

We have also shown that the CDK8-containing complex targeted by E1A and VP16 is a large complex (~1.5 MDa) that also contains cyclin C and hSRB7 (Fig. 2). CDK7 fractionates differently than the other proteins and is not detected in fractions that have the highest levels of E1A- and VP16-associated kinase activity. The yeast counterparts of CDK8, cyclin C and hSRB7 have been found in the mediator subcomplex of the holoenzyme, while CDK7 is not thought to reside in the mediator subcomplex (1). This suggests that E1A and VP16 may target the mediator subcomplex of the holoenyzme. This has previously been demonstrated for VP16 by Young and colleagues (3), but to our knowledge has not been shown for E1A. Therefore, targeting of the mediator complex may be a general mechanism used by activators in recruitment of the holoenzyme to the promoter. Our results indicate that this may not be universal mechanism, however, since the HIV Tat protein does not appear to interact with the CDK8-containing complex (Fig. 1C and data not shown).

Finally, we showed that CDK8 can activate transcription from a reporter plasmid when artificially targeted to the promoter (Fig. 3). Furthermore, the C-terminal region of CDK8 alone can act as a weak activator of transcription. The C-terminal 87 amino acids of CDK8 contains 14% glutamine, with a stretch of five continuous glutamines between amino acid residues 378 to 464. Interestingly, *Drosophila* CDK8 contains 65% glutamine in this region, with two continuous stretches of glutamines from positions 373 to 400 and 423 to 441. This is suggestive of a glutamine-rich type of activation domain at the C-terminus of CDK8. This region is not present in other CDKs.

Although the kinase activity of CDK8 appears to be dispensable for the ability of CDK8 to activate transcription in this assay, it is likely that the kinase activity of CDK8 is required *in vivo*. Liao *et al.* (21) have found that a kinase-deficient mutant of the yeast counterpart of CDK8, SRB10, results in a large decrease in galactose-induced transcription *in vivo*. In our artificial system, GAL4–CDK8 probably functions by recruiting the holoenzyme to the promoter but once present at the promoter, it is possible that other CTD kinases in the RNAP II holoenzyme complex, such as endogenous CDK8 or perhaps CDK7, could compensate for the loss of GAL4–CDK8 kinase activity. It also remains possible that the kinase activity of GAL4–CDK8 may be necessary under certain conditions not assayed here, such as in response to some activators or in response to growth regulatory signals.

Although CDK8 can clearly function as a transcriptional activator, it is not nearly as powerful as E1A. In experiments similar to that shown in Figure 3, GAL4–E1A produced at least a 20-fold higher level of activation of expression from the reporter construct than the GAL4–CDK8 construct (43). One interpretation of this result is that while recruitment of the CDK8 complex by E1A is important for the transactivation function of E1A, other protein–protein interactions, such as that with TFIID (45) are required for full transactivation by E1A.

How does CDK8 activate transcription? It is possible that CDK8 can function in recruitment of the RNAP II holoenzyme complex to the promoter. This likely occurs when CDK8 is artificially targeted to the promoter through a fusion with a DNA binding domain. This may also occur under more physiological conditions through an association with DNA-bound activators such as E1A and VP16. The finding that GAL4–CDK8 activates transcription is consistent with results in the yeast system, where components of the yeast mediator complex have been shown to activate transcription when fused to a DNA binding domain (23,46). Ptashne and colleagues have proposed that recruitment of the RNAP II holoenzyme complex to the promoter through a single activator-holoenzyme contact is sufficient for gene activation (46). Our results are consistent with this proposal. The finding that the C-terminus of CDK8 alone can activate transcription, albeit less efficiently than the full length protein, suggests that this region may contact other components of the holoenzyme complex. The failure of CDK7 to activate transcription as a GAL4 fusion suggests that not all interactions with holoenzyme components result in gene activation.

Finally, it is possible that CDK8 plays a dual role in transcription. CDK8 may function through protein–protein interactions to recruit the RNAP II holoenzyme complex to the promoter and it may also act catalytically by phosphorylating the CTD or possibly other components of the RNAP II holoenzyme complex.

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