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Purification of a yeast TATA box-binding protein that exhibits human transcription factor IID activity

(transcription initiation factor/RNA polymerase II/*in vitro* transcription)

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ABSTRACT **By a series of conventional chromatographic procedures we have purified from whole-cell extracts of *Saccharomyces cerevisiae* yeast transcription factor IID (TFIID), which functionally substitutes for human TFIID in a complementation assay comprised of the adenovirus type 2 major late promoter and HeLa cell-derived RNA polymerase II, transcription factors IIA, IIB, and IIE. Similar to its human counterpart, yeast TFIID also exhibited specific binding to the adenovirus type 2 major late promoter TATA element, as shown by both DNase I footprinting and gel mobility shift assays. NaDodSO$_4$/PAGE analyses showed that a 27-kDa polypeptide coeluted with TFIID complementing activity through each chromatographic step. In agreement with this result and also suggesting that the native protein is a monomer, gel-filtration experiments indicated a molecular mass of 28 kDa for TFIID under nondenaturing conditions. That the 27-kDa polypeptide represented TFIID was further demonstrated by the ability of an HPLC-purified protein to bind specifically after renaturation to the adenovirus type 2 major late promoter TATA sequence.**

The activation and regulation of eukaryotic promoters is mediated through several types of DNA sequence elements and associated factors. Minimal or "core" promoter elements are necessary and sufficient for low-level initiation *in vitro* and consist primarily of a TATA element common to most class II genes (for review, see ref. 1). Activity of the core promoter is modulated, in turn, by promoter proximal and distal (enhancer) elements, which may contribute either to basal or to inducible or cell type-specific promoter activity (for review, see ref. 2). Proteins involved in the selective activation of specific genes through site-specific binding to these elements have been characterized extensively in recent years (for reviews, see refs. 3 and 4), in part because these factors can be readily identified and isolated by genetic and by affinity-purification and cloning techniques. Of special note has been the identification of distinct DNA-binding, activation, and regulatory domains, some of which are common to several proteins (for reviews, see refs. 3 and 4). At the same time relatively little is known about how the activating domains stimulate transcription by the general factors that interact with the core promoter element. The general factors include, in addition to RNA polymerase II, a set of ubiquitous transcription factors designated transcription factors IIA, IIB, IID, IIE, and IIF (TFIIA, TFIIB, TFIID, TFIIE, and TFIIF) (5–9). Although definitive studies have been hampered by the lack of homogeneous factors, studies with partially purified factors have indicated a number of distinct steps and corresponding preinitiation complexes in the overall activation pathway, as well as the steps at which individual factors act (1, 10–16).

In principle, any step in the overall activation pathway and, thus, any individual factor involved could be the target for a regulatory factor—whether the regulatory factor acts through specific proximal or distal elements or whether it acts via the TATA element itself. Considerable attention has been focused on the factor designated TFIID. This factor plays a primary role in promoter activation because it binds to the TATA element independently and at an early step (1) and because it plays a major role in template commitment (15). The possibility that TFIID is a direct target for various regulatory factors has been confirmed by our studies showing (*i*) that the cellular site-specific binding factors USF (17, 18), GAL4 (19), and ATF (20) interact cooperatively with TFIID at the promoter and, in the case of ATF, that these interactions lead to more efficient binding of TFIIB, TFIIE, and RNA polymerase II (20, 21), (*ii*) that a herpesvirus immediate-early protein stimulates transcription *in vitro* by facilitating TFIID interactions with the promoter (22, 23), and (*iii*) that prior promoter binding by TFIID precludes chromatin assembly-mediated repression (23, 24). Although a more detailed analysis of these (and other) promoter interactions would be facilitated by the availability of a purified TFIID, only a partial purification of the human factor has been possible (1).

Other studies have shown that yeast regulatory factors can activate transcription in mammalian cells (25, 26) and that mammalian factors can activate transcription in yeast (27–29). These results indicate that structure–function relationships in transcription factors are conserved and, more specifically, that regulatory factors can interact functionally with the general transcription machinery of heterologous cells. This hypothesis is further supported by the recent demonstration that a yeast TATA box-binding protein can substitute functionally for HeLa TFIID in a human *in vitro* transcription system (30, 31). It thus appears that a heterologous yeast/human cell-free system might prove useful for studying the molecular mechanisms of promoter activation. We therefore undertook the project of purifying yeast transcription factor TFIID. In this report we describe purification of a yeast TATA box-binding protein that mediates the reported TFIID activity.

MATERIALS AND METHODS

Purification of TFIID. Whole-cell yeast extracts from *Saccharomyces cerevisiae* were prepared (32) and dialyzed into buffer A [20 mM Hepes, pH 7.9/2 mM dithiothreitol/1 mM

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Abbreviations: Ad2, adenovirus type 2; MLP, major late promoter; TFIIA–F, transcription factors IIA–IIF; PMSF, phenylmethylsulfonyl fluoride.

phenylmethylsulfonyl fluoride (PMSF)/1 mM benzamidine/20 μM $ZnSO_4$/10% (vol/vol) glycerol/10 mM $MgCl_2$/0.2 mM EDTA] containing 0.1 M NaCl, and the dialyzed sample (2500 mg of protein) was loaded onto a heparin–Sepharose column (4 × 20 cm) equilibrated with buffer A plus 0.1 M NaCl. The column was washed with the same solution and eluted with a linear gradient of 0.1–1.0 M NaCl in buffer A. TFIID activity, which was measured by a complementation assay with human transcription factors (see below), eluted around 0.35 M NaCl. Active fractions were pooled and brought to 45% saturation with ammonium sulfate by dialysis against buffer B (0.2 M Tris·HCl, pH 7.9/1 mM EDTA/20 mM 2-mercaptoethanol/0.2 mM PMSF/0.2 mM benzamidine/10 mM $MgCl_2$) containing saturated ammonium sulfate. The protein precipitates were recovered by centrifugation, dissolved in buffer A, dialyzed against the same buffer containing 0.5 M NaCl, and subjected to chromatography on a Sephacryl S-300 gel filtration column (2.5 × 90 cm) equilibrated in the same solution. TFIID complementing activity eluted at a position of ≈28 kDa relative to molecular-mass markers run in parallel. Active fractions from the gel filtration column were dialyzed against buffer C [20 mM Tris·HCl, pH 7.9/0.2 mM EDTA/10 mM 2-mercaptoethanol/0.2 mM PMSF/10% (vol/vol) glycerol] containing 0.1 M NaCl and loaded onto a DEAE-Sepharose column (0.8 × 1.2 cm) equilibrated with the same solution. Flow-through fractions from this column were applied directly to a single-stranded DNA–agarose column (0.8 × 1.2 cm), washed with buffer C containing 0.1 M NaCl, and eluted with a linear gradient of 0.1–0.8 M NaCl in buffer C. Active fractions, which eluted around 0.3 M NaCl, were pooled, diluted with buffer C to 0.1 M NaCl, and loaded onto an SP-Sepharose column (0.8 × 1.0 cm) preequilibrated with buffer C containing 0.1 M NaCl. This column was subsequently washed with buffer C containing 0.1 M NaCl and eluted with a linear gradient of 0.1–0.8 M NaCl in buffer C. TFIID complementing activity eluted around 0.3 M NaCl.

Preparation of HeLa Cell Factors and Transcription Assays. HeLa cell nuclear extracts were prepared essentially as described (33). TFIIA (DE52 fraction) was partially purified through phosphocellulose and DEAE-cellulose. TFIIB (single-stranded DNA–agarose fraction), TFIIE (Bio-Gel A 1.5 fraction), and TFIID (DE52 fraction) were partially purified by described methods (6, 34). RNA polymerase II was purified from HeLa nuclear pellets by the method of Bitter (35). Definition of activity units for each factor has been described (21).

Standard transcription reaction mixtures (25 μl) contained 12 mM Tris·HCl (pH 7.9), 40 mM Hepes (pH 8.4), 60 mM KCl, 12% (vol/vol) glycerol, 8 mM $MgCl_2$, 100 μM ATP and UTP, 25 μM [α-^{32}P]CTP, 0.4 μg of pML(C_2AT) (6), and the following amounts of transcription factors: TFIIA (1.0 μg), TFIIB (0.85 unit), TFIIE (0.7 unit), TFIID (0.75 unit), and RNA polymerase II (0.7 unit). Because a guanine-free cassette (6) was used as template, 0.2 unit of RNase T1 and 0.1 mM 3′-*O*-methylguanosine triphosphate were also present in the reaction. After 45-min incubation at 30°C, TFIID complementation activity was measured by using either the DEAE-paper disk assay or autoradiography of polyacrylamide gel-fractionated transcription products (1).

Footprint Analyses. Labeled DNA fragments for footprint analyses were prepared as follows (1). Plasmid pSmaF was digested with *Hin*dIII, and the resulting fragments were end-labeled with [α-^{32}P]dATP by using the Klenow fragment of DNA polymerase I. The end-labeled DNA fragments were then digested with *Xho* I, and the fragment containing the adenovirus type 2 major late promoter (Ad2 MLP) [456 base pairs (bp)] was purified by PAGE; specific activity of 20,000 cpm/ng was usually obtained. About 0.5 ng of ^{32}P-labeled DNA fragment was used in each assay.

Footprint analyses were done under buffer conditions identical to the *in vitro* transcription reactions, except that nucleotides were omitted and the promoter was present at a 10-fold lower molar concentration. General footprinting protocol was conducted as described (1, 19, 20).

Gel Mobility Shift Assay. The labeled DNA fragment used for the gel shift assay was prepared from an *Ava* II/*Rsa* I digest of plasmid pMLH1 (12). The 184-bp DNA fragment containing the MLP (position −138 to +46) was purified by PAGE and end-labeled with [α-^{32}P]dATP by using the Klenow fragment of DNA polymerase I as described above.

Gel shift assays were done under transcription conditions, except that nucleotides were omitted, promoter concentration was 10-fold lower, and $MgCl_2$ concentration was reduced to 5 mM. One femtomole (50,000 cpm/ng) of end-labeled Ad2 MLP fragment was incubated with protein fractions at 30°C for 40 min, and protein–DNA complexes were resolved from free DNA by electrophoresis on 4% polyacrylamide gel with running buffer containing 25 mM Tris·HCl, pH 8.3, 190 mM glycine, 5 mM $MgCl_2$, and 1 mM EDTA. The gel was cast in running buffer supplemented with 5 mM $MgCl_2$, 10% (vol/vol) glycerol, and 0.5 mM dithiothreitol.

HPLC Fractionation of SP-Sepharose-Purified TFIID. Reverse-phase HPLC was performed on a Vydac 214TP54 C_4 column (0.46 × 25 cm). The elution system consisted of a linear gradient of acetonitrile (5–80% solvent B over 30 min) with trifluoroacetic acid. Solvent A was 0.1% (vol/vol) trifluoroacetic acid in water, and solvent B was 0.1% (vol/vol) trifluoroacetic acid in acetonitrile/water (9:1) (vol/vol). Elution was done at room temperature at a flow rate of 1.5 ml/min, and 0.5-ml fractions were collected.

RESULTS

Yeast TFIID was purified from whole-cell extracts by the chromatographic steps that have been detailed. A HeLa-derived complementation assay (consisting of human RNA polymerase II and factors TFIIA, TFIIB, and TFIIE) templated with the Ad2 MLP was used to follow activity. As reported by others (30, 31) and observed by us yeast whole-cell extracts contain large amounts of nonspecific inhibitors that completely block specific transcription of the Ad2 MLP when these extracts are added to an active HeLa nuclear extract-derived transcription system (data not shown). These inhibitors were partially separated from yeast TFIID by chromatography of the yeast extracts on heparin-Sepharose (refs. 30, 31 and data not shown), and care was taken to collect appropriate active fractions. Further chromatography of the crude yeast TFIID on a gel-filtration column resulted in a single peak of activity that exhibited a molecular mass of 28 kDa relative to molecular mass standards (Fig. 1 *A* and *B*). Table 1 shows that significant purification of TFIID was effected by just these two columns. To see whether any distinct polypeptides tracked with TFIID activity, proteins present in the Sephacryl S-300 column fractions were separated by $NaDodSO_4$/PAGE and detected by silver staining. The only protein that correlated with TFIID transcription activity was the 27-kDa polypeptide indicated by an arrow in Fig. 1*C*. Careful inspection of Fig. 1 *B* and *C* indicates that concordance was not perfect between TFIID activity and the presence of the 27-kDa polypeptide. Explanation is provided by the fact that an inhibitor of transcription elutes just after TFIID on this column. This inhibitor can be efficiently removed by chromatography on SP-Sepharose (data not shown). The data in Fig. 1*B* also indicate that yeast TFIID was as efficient as human TFIID in facilitating transcription of the Ad2 MLP promoter in the HeLa-derived complementation system.

TFIID activity and the 27-kDa polypeptide continued to coelute through the final three chromatographic steps of the

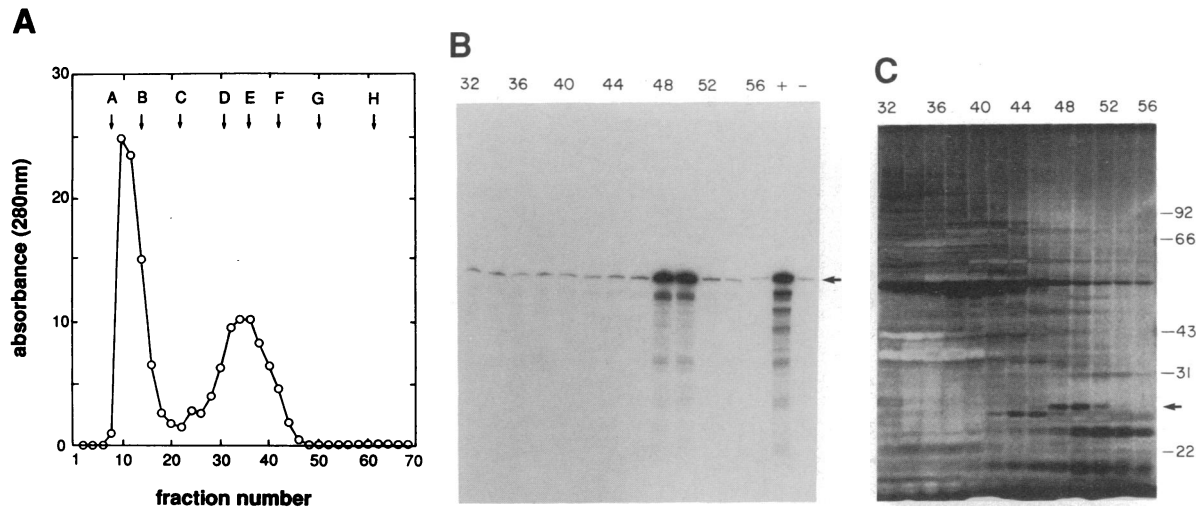

FIG. 1. Gel filtration of heparin-Sepharose-purified yeast TFIID on Sephacryl S-300 column. Transcriptionally active fractions from the heparin-Sepharose column were analyzed as described. (*A*) A_{280} profile of Sephacryl S-300 column fractions. Lettered arrows indicate the elution positions of molecular mass markers run in parallel under identical conditions. Standards used included blue dextran (A); ferritin, 400 kDa (B); catalase, 230 kDa (C); aldolase, 160 kDa (D); bovine serum albumin, 66 kDa (E); ovalbumin, 43 kDa (F); chymotrypsinogen, 25 kDa (G); and lysozyme, 14 kDa (H). (*B*) TFIID complementation assays of column fractions (2-μl aliquots); numbers indicate the Sephacryl column fractions assayed. The + and − lanes indicate control assays that served, respectively, as positive (plus HeLa TFIID) and negative (HeLa complementation factors only) controls. These assays indicated that elution of TFIID activity was in fractions 48–50. (*C*) Sephacryl S-300 fraction polypeptide patterns monitored by silver staining of $NaDodSO_4$/polyacrylamide gels. Column fractions assayed (5-μl aliquots) and migration of molecular-size standards are indicated on horizontal and vertical axes, respectively. Arrow, migration of 27-kDa polypeptide, which coelutes with transcription complementation activity.

purification procedure: DEAE–Sepharose (data not shown), single-stranded DNA–agarose (data not shown), and SP–Sepharose (Fig. 2 *A* and *B*). On the final SP-Sepharose column relative levels of TFIID activity correlated nearly perfectly with relative abundance of the 27-kDa polypeptide (compare lanes 36–40 in Fig. 2 *A* and *B*). In addition, assessment of site-specific binding activity of SP–Sepharose-purified TFIID by DNase I footprinting with probe containing the Ad2 MLP revealed coincidence with TFIID activity (Fig. 2*C*). The pattern of protection, extending from −35 to −20 and centered on the TATA element, represented a subset of the sequences protected by human TFIID (−47 to +35; + lane in Fig. 2*C* and ref. 1). This pattern resembled that seen by others (30) for binding of partially purified yeast TFIID to the Ad2 MLP. Additionally, much fainter protection on the −70 region, which has a TATAAA sequence on the opposite strand, was detected in fraction 38. Although this protection was not seen with the partially purified human TFIID (1), its TATA-sequence binding specificity was demonstrated by competition analyses with oligonucleotides containing either wild-type or mutated TATA sequences (data not shown).

A very significant overall increase in specific activity of TFIID was achieved by this multi-column purification scheme (see Table 1 for details). The magnitude of this purification is underscored by $NaDodSO_4$/polyacrylamide gel analysis of proteins in the SP-Sepharose column fractions (Fig. 2*A*). The 27-kDa TFIID species was estimated to represent ≈95% of protein present in the peak fraction from SP-Sepharose.

To demonstrate unambiguously that the 27-kDa polypeptide species actually represented TFIID, SP-Sepharose-purified TFIID was subjected to high-resolution HPLC on a C_4 hydrophobic column (for the particular SP–Sepharose fraction used, the single-stranded DNA–agarose chromatography step was omitted). The 27-kDa polypeptide and DNA-binding activity on the MLP were localized in the HPLC column fractions by $NaDodSO_4$/PAGE (Fig. 3*A*) and mobility shift assays (Fig. 3*B*). HPLC column fraction 75 contained only the 27-kDa polypeptide and most of the site-specific binding activity. To verify further the specificity of the DNA–protein complex seen in the gel shift assay, specific DNA-binding assays were done with HPLC fraction 75 with and without oligonucleotide competitors containing Ad2 MLP wild-type and mutant TATA sequences (core sequences indicated at top of Fig. 3*C*). The wild-type sequence effectively competed for TFIID binding at the lowest concentration tested, whereas the mutant sequence showed significant competition only at much higher levels (Fig. 3*C*, compare TATAAAA lanes with TAGAGAA lanes). This result shows that the 27-kDa polypeptide binds specifically to the Ad2 MLP TATA element and supports the view that TFIID transcriptional activity resides in the same protein. These results appear to conflict with a recent report indicating that TFIIA is essential for detection of TFIID interactions with the TATA element by gel shift assays (16); this discrepancy may reflect differences in purity of the yeast TFIID used.

Table 1. Purification of TATA box-binding protein (TFIID) from yeast

Fraction	Protein, mg	Volume, ml	Activity, units	Specific activity, units/mg	Relative specific activity	Recovery, %
Whole-cell extract	2500	100	—	—	—	—
Heparin-Sepharose	86	60	2000	23	1	100
Sephacryl S-300	0.32	20	1000	3,100	130	50
ss DNA–agarose	0.092	2.3	680	7,400	320	26
SP-Sepharose	0.018	0.44	310	17,000	740	16

This table summarizes the purification of TFIID from 100 g of yeast. The activity of TFIID in whole-cell extracts was not determined because of the presence of inhibitory components. ss, Single-stranded.

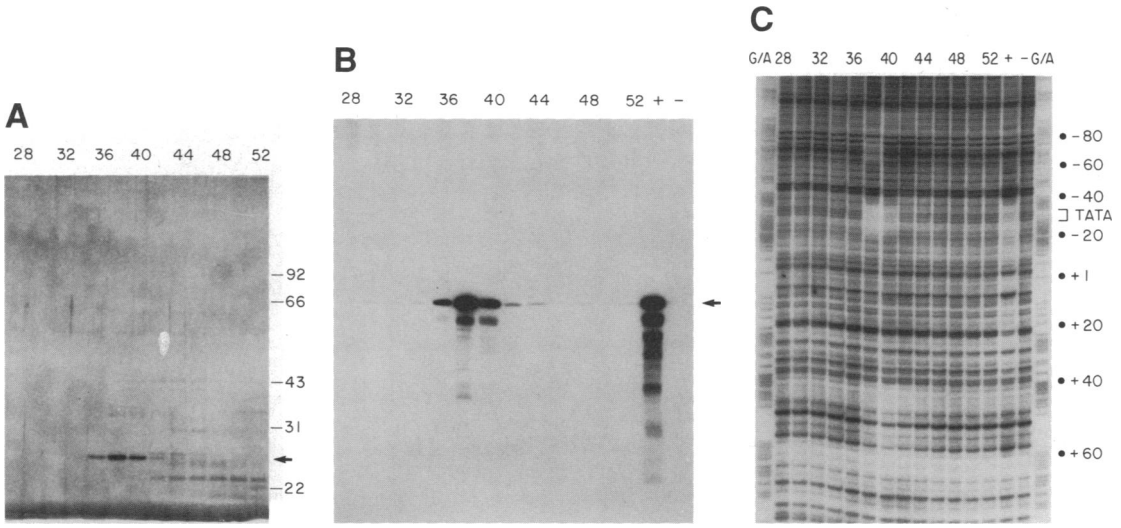

FIG. 2. SP-Sepharose column chromatography of single-stranded DNA–agarose-purified TFIID. Single-stranded DNA–agarose fractions that contained TFIID complementing activity were obtained and chromatographed on an SP-Sepharose column as described. (*A*) Protein distribution in column fractions (2.5-μl aliquots) monitored by $NaDodSO_4$/PAGE and detected by silver staining. (*B*) Assay of TFIID transcription complementation activity in column fractions (1-μl aliquots assayed). *A* and *B* are labeled as in Fig. 1 legend. (*C*) Site-specific binding of TFIID in SP-Sepharose column fractions assessed by DNase I footprinting. Aliquots (1 μl) of the fractions indicated at gel top were analyzed for binding to the Ad2 MLP as indicated. Numbers at right indicate nucleotide positions relative to transcription initiation site. Lanes indicated with + and − show footprint reactions containing, respectively, HeLa TFIID or no added factor. End lanes show G+A sequencing reactions.

DISCUSSION

The TATA factor (TFIID) plays a central role in eukaryotic promoter activation because it both facilitates productive promoter interactions by the general factors (including RNA polymerase II) and serves as target for the action of various regulatory factors (see Introduction). As a major step toward more detailed studies of structure, function, and regulation of eukaryotic TATA factors, we purified to apparent homogeneity a TATA box-binding protein from *S. cerevisiae*. Our conclusion that the purified 27-kDa polypeptide mediates all or part of the TFIID site-specific binding and functional activities is based on the following: (*i*) copurification of the 27-kDa polypeptide and transcriptional activity, measured in a HeLa cell-derived TFIID-dependent complementation assay, through several chromatographic steps, (*ii*) coelution of TFIID transcriptional activity and site-specific binding activity from the final SP-Sepharose column, (*iii*) an observed molecular mass of 28 kDa for native TFIID (detected by a functional assay) on gel filtration, and (*iv*) renaturation of site-specific binding activity from reverse-phase HPLC fractions containing only the 27-kDa polypeptide. Thus, we report here the complete purification of a eukaryotic TATA box-binding protein associated with TFIID activity. Assuming no TFIID activity was lost in the initial chromatographic step, the overall degree of purification was ≈20,000-fold from crude extracts.

The apparent native molecular mass of yeast TFIID is quite small compared with the estimated molecular mass of 120 kDa of the corresponding HeLa factor (13). This difference could reflect proteolysis during purification, as seen for other yeast transcription factors (36–38), even though protease-deficient yeast strains were used. However, preliminary sequence analysis of a yeast gene that encodes the HPLC-purified 27-kDa polypeptide indicates that little or no proteolysis occurs (unpublished observations). A more intriguing possibility is that the partially purified mammalian TFIID is a multimeric structure, consisting of a TATA box-binding component and one or more associated components. If the latter were essential for mammalian promoter activation, then these associated components would have to be present in stoichiometric excess in the HeLa fractions and function in association with the yeast factor; alternatively, a basal level of promoter activation might be mediated (by the TATA box-binding factor) without any additional components, especially if the latter were involved primarily in regulatory factor interactions (see below). Perhaps relevant to these possibilities is the recent demonstration of a virus-encoded heterodimeric initiation factor that binds to TATA-like elements in vaccinia promoters (39).

Consistent with the apparent smaller size of the yeast TFIID and in agreement with earlier studies of a partially purified yeast factor (30), DNase I footprinting demonstrated interactions of the purified factor only with the TATA region (−35 to −20) of Ad2 MLP. In contrast, studies with human TFIID showed interactions on the MLP extending from −47 to +35 (1). These differences in binding properties of yeast and mammalian factors could reflect basic differences in TFIID structure or mechanism of TFIID action in these organisms. However, our current lack of a comparably purified human TFIID precludes firm conclusions on this point. Moreover, it is also true that human TFIID interactions on some promoters are restricted to a small region encompassing the TATA element (1, 19, 20, 40), although recent studies have indicated that other (upstream) site-specific binding factors may facilitate such interactions as part of their function (19, 20).

In concurrence with results of previous studies with less-purified factors (30, 31), we have shown that a purified yeast protein can substitute for human TFIID to mediate formation of functional preinitiation complexes with heterologous general (core promoter) factors. However, and related to the above question of possible differences between mammalian and yeast TATA factors, whether yeast or mammalian regulatory factors (e.g., acting at proximal or distal elements) can stimulate promoter function under these conditions (with yeast TFIID and human TFIIA, TFIIB, TFIIE, and RNA polymerase II) remains to be seen. The ability of regulatory factors to function in heterologous cell types and the known conservation of some functional domains in regulatory proteins suggest that this stimulation may, indeed, occur. However, there might be less flexibility and evolutionary conservation of structural (and functional) relationships among the total complement of core promoter factors—as already sug-

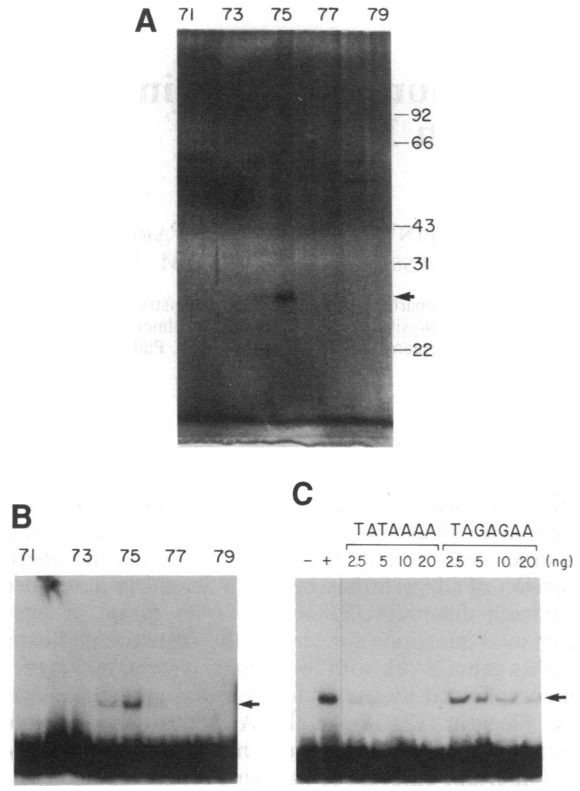

FIG. 3. Analysis of TFIID by reverse-phase HPLC. SP-Sepharose fractions containing TFIID complementing activity were analyzed by reverse-phase HPLC. Column fractions were evaporated to dryness in a Speed-Vac concentrator before analysis for specific DNA binding. (*A*) $NaDodSO_4$/PAGE analysis of 100-μl aliquots of HPLC column fractions; this gel was stained with silver. Fraction numbers, molecular mass markers, and position of 27-kDa polypeptide are indicated as in Fig. 1 legend. (*B*) Site-specific DNA-binding activity of renatured fractions as monitored by the gel shift assay. The dried HPLC column fractions were dissolved in 1 μl of 6 M guanidine·HCl and kept at room temperature for 4 hr. Subsequently, 50 μl of buffer D [20 mM Tris·HCl, pH 7.9/0.2 mM EDTA/10 mM β-mercaptoethanol/20% (vol/vol) glycerol/0.1 M KCl/0.2 mM PMSF] was added, and renaturation proceeded for 4 hr at room temperature. A 1-μl aliquot of each renatured fraction was incubated with ^{32}P-labeled DNA probe containing the Ad2 MLP TATA element, and protein–DNA complexes were resolved as described. Numbers indicate HPLC column fractions, and an arrow indicates the TFIID–TATA element complex. (*C*) Binding specificity of the observed protein–DNA complex. Gel-shift analyses were done as for *B* except that 1 μl of fraction 75 was incubated with an unlabeled competitor oligonucleotide containing Ad2 MLP wild-type or mutant TATA-box sequences along with the labeled Ad2 MLP TATA-element probe. Lanes labeled − and + indicate reactions without protein (−) or with renatured TFIID (+) in the absence of competing oligonucleotides. TATAAAA and TAGAGAA indicate, respectively, the wild-type and mutated sequences near the center (position −31 to −25) of the 31-bp duplex DNA competitors that extend from position −45 to −15 (1). Nanogram amounts of competitor DNA in each incubation and position of protein–DNA complex are indicated by numbers and arrow, respectively.

gested by reasonable differences between the subunit structures of yeast and mammalian RNA polymerases (36) and by the apparent inability of yeast RNA polymerase II to function in a HeLa-derived complementation system (unpublished results). Despite these latter concerns, the availability of pure yeast TFIID clearly provides us with a valuable reagent for examining transcriptional regulatory mechanisms for class II genes in varied eukaryotes.

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1. Nakajima, N., Horikoshi, M. & Roeder, R. G. (1988) *Mol. Cell. Biol.* **8,** 4028–4040.
2. Maniatis, T., Goodbourn, S. & Fischer, J. A. (1987) *Science* **236,** 1237–1245.
3. Ptashne, M. (1988) *Nature (London)* **335,** 683–689.
4. Johnson, P. F. & McKnight, S. L. (1989) *Annu. Rev. Biochem.* **58,** 799–839.
5. Matsui, T., Segall, J., Weil, P. A. & Roeder, R. G. (1980) *J. Biol. Chem.* **255,** 11992–11996.
6. Sawadogo, M. & Roeder, R. G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4394–4398.
7. Reinberg, D. & Roeder, R. G. (1987) *J. Biol. Chem.* **262,** 3310–3321.
8. Zheng, X.-M., Moncollin, V., Egly, J.-M. & Chambon, P. (1987) *Cell* **50,** 361–368.
9. Flores, O., Maldonado, E., Burton, Z., Greenblatt, J. & Reinberg, D. (1988) *J. Biol. Chem.* **263,** 10812–10816.
10. Davison, B. L., Egly, J.-M., Mulvihill, E. R. & Chambon, P. (1983) *Nature (London)* **301,** 680–686.
11. Fire, A., Samuels, M. & Sharp, P. A. (1984) *J. Biol. Chem.* **259,** 2509–2516.
12. Hawley, D. K. & Roeder, R. G. (1985) *J. Biol. Chem.* **260,** 8163–8172.
13. Reinberg, D., Horikoshi, M. & Roeder, R. G. (1987) *J. Biol. Chem.* **262,** 3322–3330.
14. Van Dyke, M. W., Roeder, R. G. & Sawadogo, M. (1988) *Science* **241,** 1335–1338.
15. Van Dyke, M. W., Sawadogo, M. & Roeder, R. G. (1989) *Mol. Cell. Biol.* **9,** 342–344.
16. Buratowski, S., Hahn, S., Guarente, L. & Sharp, P. A. (1989) *Cell* **56,** 549–561.
17. Sawadogo, M. & Roeder, R. G. (1985) *Cell* **43,** 165–175.
18. Sawadogo, M. (1988) *J. Biol. Chem.* **263,** 11994–12001.
19. Horikoshi, M., Carey, M. F., Kakidani, H. & Roeder, R. G. (1988) *Cell* **54,** 665–669.
20. Horikoshi, M., Hai, T., Lin, Y.-S., Green, M. R. & Roeder, R. G. (1988) *Cell* **54,** 1033–1042.
21. Hai, T., Horikoshi, M., Roeder, R. G. & Green, M. R. (1988) *Cell* **54,** 1043–1051.
22. Abmayr, S. M., Workman, J. L. & Roeder, R. G. (1988) *Genes Dev.* **2,** 542–553.
23. Workman, J. L., Abmayr, S. M., Cromlish, W. A. & Roeder, R. G. (1988) *Cell* **55,** 211–219.
24. Workman, J. L. & Roeder, R. G. (1987) *Cell* **51,** 613–622.
25. Kakidani, H. & Ptashne, M. (1988) *Cell* **52,** 161–167.
26. Webster, N., Jin, J. R., Green, S., Hollis, M. & Chambon, P. (1988) *Cell* **52,** 169–178.
27. Lech, K., Anderson, K. & Brent, R. (1988) *Cell* **52,** 179–184.
28. Schena, M. & Yamamoto, K. R. (1988) *Science* **241,** 965–967.
29. Metzger, D., White, J. H. & Chambon, P. (1988) *Nature (London)* **334,** 31–36.
30. Buratowski, S., Hahn, S., Sharp, P. A. & Guarente, L. (1988) *Nature (London)* **334,** 37–42.
31. Cavallini, B., Huet, J., Plassat, J.-L., Sentenac, A., Egly, J.-M. & Chambon, P. (1988) *Nature (London)* **334,** 77–80.
32. Wang, C. K. & Weil, P. A. (1989) *J. Biol. Chem.* **264,** 1092–1099.
33. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
34. Digman, J. D., Martin, P. L., Shastry, B. S. & Roeder, R. G. (1983) *Methods Enzymol.* **101,** 582–598.
35. Bitter, G. A. (1983) *Anal. Biochem.* **128,** 294–301.
36. Sentenac, A. (1985) *CRC Crit. Rev. Biochem.* **18,** 31–91.
37. Wiederrecht, G., Shuey, D. J., Kibbe, W. A. & Parker, C. S. (1987) *Cell* **48,** 507–515.
38. Wu, C., Wilson, S., Walker, B., Dawid, I., Paisley, T., Zimarino, V. & Ueda, H. (1987) *Science* **238,** 1247–1252.
39. Broyles, S. S., Yuen, L., Shuman, S. & Moss, B. (1988) *J. Biol. Chem.* **263,** 10754–10760.
40. Huang, D.-H., Horikoshi, M. & Roeder, R. G. (1988) *J. Biol. Chem.* **263,** 12596–12601.