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Recombinant yeast TFIID, a general transcription factor, mediates activation by the gene-specific factor USF in a chromatin assembly assay

(transcriptional regulation/coactivators/Sp1)

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ABSTRACT **The TATA box-binding transcription factor TFIID from *Saccharomyces cerevisiae* was tested for its ability to mediate regulatory factor functions both in a cell-free system reconstituted with other general initiation factors (purified from HeLa cells) and in a combined nucleosome assembly–transcription system. In the latter assay recombinant yeast TFIID, expressed in and purified from bacteria, was sufficient to prevent nucleosome assembly-mediated repression and to mediate transcriptional enhancement of the adenovirus major late promoter by the gene-specific activator USF. In contrast, recombinant yeast TFIID was unable to mediate activation by USF in the system reconstituted only with purified general factors. Under the same conditions a partially purified natural yeast TFIID was able to mediate activation by both USF and Sp1 (assayed with the human immunodeficiency virus promoter), but to a lesser extent than observed with a partially purified natural human TFIID. The implications of these findings are discussed with respect to the structure of the yeast and human TATA factors and the possible involvement either of specific TFIID modifications or of coactivators.**

The changes in gene expression during cellular responses to growth, developmental, hormonal, and viral signals are regulated in large part at the transcriptional level. Variations in the transcription patterns are often reflected in altered chromatin structures, most likely caused by sequence-specific DNA-binding proteins that in turn regulate transcription initiation from specific genes (reviewed in refs. 1 and 2). *In vitro* studies have suggested a possible role of nucleosomal or higher-order chromatin structures as general repressors of both class III (reviewed in ref. 3) and class II genes (29, 30). Recent genetic studies in yeast also argue strongly for a general nucleosome-mediated repression that is reversed by gene-specific activators (4). Studies of general transcription factors from human cells have shown that the human TATA box-binding transcription factor (hTFIID), the key factor in the assembly of a functional preinitiation complex (5–7), is necessary and sufficient, when prebound to the DNA, to prevent nucleosome-mediated repression. The stable binding of TFIID by the nucleosome-containing template forms a committed complex that requires only the addition of the other general factors (TFIIA, -B, -E, and -F and RNA polymerase II) to initiate transcription (8). Moreover, in coupled transcription–nucleosome assembly systems in which rapid chromatin assembly otherwise precludes productive interactions of TFIID and the other general factors, the presence of viral (pseudorabies IE) and cellular (upstream stimulatory factor, USF) regulatory proteins are shown to markedly stimulate transcription by facilitating productive TFIID interactions (9–11). In the case of USF, the level of activation was greater than that observed in the absence of the chromatin assembly system.

To further investigate this problem we have studied initially the function of yeast TFIID in cell-free systems reconstituted with human general initiation factors in the presence and absence of a chromatin assembly system. It was known that yeast TFIID (yTFIID) consisted of a single polypeptide (12) that could functionally replace the human counterpart in basal-level transcription from a minimal promoter (reviewed in refs. 7 and 12) and the isolation of the corresponding cDNA (reviewed in refs. 12–15) provided the opportunity to work with a homogeneous, well-defined factor. Moreover, although the ability of mammalian factors to function in yeast cells was known (references in refs. 7 and 12), the ability of the purified yeast TFIID to mediate regulatory factor function in conjunction with the other general factors from human cells was unclear.

Here we show that recombinant yTFIID ($yTFIID^R$) (12–15), purified after expression in bacteria, can both prevent nucleosome-mediated repression and mediate USF induction in a combined chromatin assembly–transcription system. We also show that natural yTFIID ($yTFIID^N$), in contrast to $yTFIID^R$, can qualitatively substitute for a natural hTFIID ($hTFIID^N$) in mediating the action of both USF and Sp1 in a purified reconstituted system. These results suggest that the regulatory functions of yTFIID may involve either its modification or at least one cofactor.

MATERIALS AND METHODS

Preparation of Extracts and Transcription Factors. TFIIA, -B, and -D were purified from HeLa nuclear extracts (5) essentially as described (16) and consisted of the single-stranded DNA-agarose fraction of TFIIB (0.3 mg of protein per ml), the DEAE-cellulose (Whatman DE52) fraction of TFIID (0.5 mg/ml), and either the DEAE-cellulose fraction of TFIIA (4.8 mg/ml) or a derived fraction (0.1 M KCl flowthrough, 2.8 mg/ml) resulting from Mono S (Pharmacia) chromatography. TFIIE and -F were copurified by chromatography on phosphocellulose and DE52 as described (16) and by subsequent chromatography on a Mono Q column. The TFIIE/F fractions employed were eluted at 0.3 M KCl (0.4 mg of protein per ml) in a 0.1–0.5 M KCl gradient in buffer A containing 10% (vol/vol) glycerol and 0.03% Nonidet P-40 (5). RNA polymerase II (0.25 mg/ml) was purified from HeLa nuclear pellet extracts (M.M., A. Roy, and R.G.R., unpublished work).

Chromatin Assembly. Extracts were prepared from *Xenopus laevis* eggs essentially as described by Laskey *et al.* (17)

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Abbreviations: ML, major late; HIV-1, human immunodeficiency virus type 1; BSA, bovine serum albumin; hTFIID and yTFIID, human and yeast TFIID; superscript R, recombinant; superscript N, natural.

with modifications reported by Almouzni and Mechali (18). Assembly reaction mixtures contained 10 μl of the egg extract, 1 μl of DNA, about 0.7 μl of 25 mM $MgCl_2$ (final concentration, 1.2 mM), and 2 μl of TFIID (about 5 ng of yTFIID^R) and/or USF in buffer A (5). Buffer, salt, and carrier protein (bovine serum albumin, BSA) concentrations were adjusted if TFIID and/or USF were omitted. The circular DNA template (200 ng/10 μl of egg extract), pML(C_2AT) (19), was pretreated with topoisomerase I (Promega; 1.5 units/500 ng of template) for 45 min at 30°C. After assembly for 2 hr at 30°C, an equal volume of elongation buffer [6 mM ATP/1 mM UTP/0.2 mM 3′-*O*-methyl-GTP/40 μM CTP (ultrapure; Pharmacia)/20 mM $MgCl_2$/70 mM KCl/30% glycerol] was added and the reaction mixtures were incubated at 30°C for 1 hr. A small part (2.5 μl) of the reaction mixture was mixed with 400 μl of a "stop" solution (7 M urea/0.1 M LiCl/0.5% SDS/10 mM EDTA, pH 8.0/10 mM Tris·HCl, pH 7.9 at 4°C/0.3 M NaOAc, pH 4.8, with yeast tRNA at 50 μg/ml), extracted with phenol, precipitated with 2-propanol, and analyzed for supercoiling of the plasmid DNA by electrophoresis in a 1.5% agarose gel with Tris acetate/EDTA buffer, blotting on a nylon membrane, and hybridization with pML(C_2AT) probe labeled with ^{32}P by random priming.

Transcription Reactions. To carry out transcription reactions after assembly of nucleosomes, 10 μl of the elongation reaction mixture was mixed with 10 μl of HeLa nuclear extract and 13 μl of a buffer containing 20 units of RNasin (Promega), 20 units of RNase T1 (P-L Biochemicals), 10 μCi (0.37 MBq) of [α-^{32}P]CTP, 8 mM $MgCl_2$, 8 mM Tris·HCl (pH 8.1 at 4°C), 0.8 mM EDTA, and 7.5% glycerol, which brought the reaction to standard transcription conditions. When the general transcription factors were employed instead of a nuclear extract, the additions were 2 μl of TFIIB, 2 μl of TFIIE/F, 1 μl of RNA polymerase II (all in buffer A with 0.1 M KCl) and 4 μl of buffer A with 0.1 M KCl. Transcription was carried out for 1 hr at 30°C and the reaction mixtures were processed basically as described (8). Transcription mixtures with purified DNA contained 2 μl of TFIIA (DEAE or Mono S fraction), 1 μl of TFIIB, 2 μl of TFIIE/F, 1 μl of RNA polymerase II, 1.5 μl of hTFIID^N or 2 μl of yTFIID^N or about 1 ng of yTFIID^R (all in buffer A with 0.1 KCl) and about 2 ng of transcription factor Sp1. The final buffer concentrations were 25 mM Hepes·KOH (pH 8.2), 6 mM Tris·HCl (pH 7.7 at 4°C), 0.14 mM EDTA (pH 8.0), 5 mM 2-mercaptoethanol, 5 mM dithiothreitol, 3 mM $MgCl_2$, 0.5 mg of BSA per ml, 70–80 mM KCl, and 10–12% glycerol. The reactions contained DNA at 1–2.5 μg/ml [pML(C_2AT) and pΔ53] or 5 μg/ml (pMHIVWT). The constructs are described in the legend of Fig. 3.

RESULTS

Bacterially Expressed yTFIID^R Effects Reversal of Nucleosome-Mediated Expression. The coupled chromatin assembly–transcription system employed an egg extract (from *X. laevis*) that has been shown to allow chromatin assembly under physiological conditions (17), either a recombinant yeast protein (yTFIID^R) (19) or a partially purified human factor (hTFIID^N) as a source of TFIID, and either a crude nuclear extract or partially purified proteins as a source of the other general initiation factors. yTFIID^R was purified following expression of the corresponding cDNA in *Escherichia coli* and consisted of a single 27-kDa polypeptide of about 95% purity (13).

In the first step of a three-step protocol (Fig. 1), nucleosomes were assembled on a relaxed circular plasmid containing the adenovirus ML promoter by incubation with the egg extract for 2 hr in the presence of either yTFIID^R or hTFIID^N. In the second step ATP, Mg^{2+}, and nucleoside triphosphates were added to allow higher-order folding of

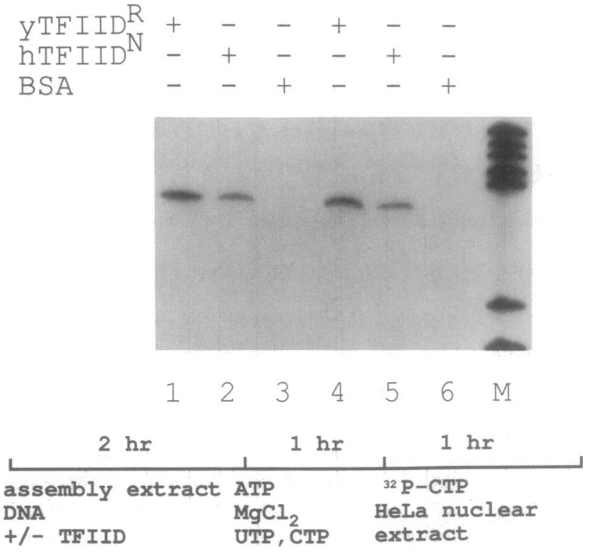

FIG. 1. Reversal of nucleosome-mediated repression by hTFIID^N and by yTFIID^R. Transcription from the adenovirus major late (ML) promoter was analyzed as a function of the presence of hTFIID^N or yTFIID^R during chromatin assembly as indicated. HeLa nuclear extract (lanes 1–3) or a heat-treated TFIID-deficient nuclear extract (20) (lanes 4–6) was employed as a source of transcription factors. The marker (M) is pBR322 digested with *Hae* III. Chromatin assembly and transcription were performed as explained in the text and shown in the graphic.

chromatin (21) and to suppress background transcription due to RNA polymerase III (8). Following assembly the plasmid was supercoiled and displayed the typical nucleosome protection pattern in an agarose gel after digestion with micrococcal nuclease (data not shown; ref. 11). In the third step radiolabeled CTP and either a fully competent HeLa nuclear extract or derived extract depleted of TFIID by heat treatment (5) was added and transcription was allowed to proceed for 1 hr. As shown in Fig. 1, and in agreement with earlier studies (8), the absence of exogenous TFIID in the nucleosome assembly phase resulted in a template refractory to transcription initiation upon later addition of either a complete (lane 3) or a TFIID-deficient (lane 6) nuclear extract. However, the presence of either yTFIID^R (lanes 1 and 4) or hTFIID^N (lanes 2 and 5) during assembly resulted in comparable levels of transcription in the same assay. This analysis clearly showed that yTFIID^R is capable of reversing nucleosome-mediated repression of transcription initiation.

yTFIID^R Mediates Induction by USF During Chromatin Assembly. We next employed the coupled nucleosome assembly–transcription assay to analyze the ability of yTFIID^R to mediate activation of the adenovirus ML promoter by USF, a cellular activator that binds to a site just upstream of the TATA element and that was partially purified as previously described (22). The protocol (Fig. 2, *Lower*) was the same as that described above, except that the source of general initiation factors in the last step was either a crude nuclear extract (Fig. 2*B*) or partially purified factors and RNA polymerase II (Fig. 2*A*). When exogenous TFIID and USF were added together with the assembly extract, induction by USF was 2- to 3-fold with yTFIID^R versus 4- to 8-fold with hTFIID^N when purified components were used as a source of general transcription factors (Fig. 2*A*). Potentiation of transcription by USF was higher for both yTFIID^R and hTFIID^N when a complete nuclear extract was used as a source of the other transcription factors (Fig. 2*B*). One explanation for this observation is that the nuclear extract contains additional components which potentiate upstream factor function and which are limiting in the reconstituted

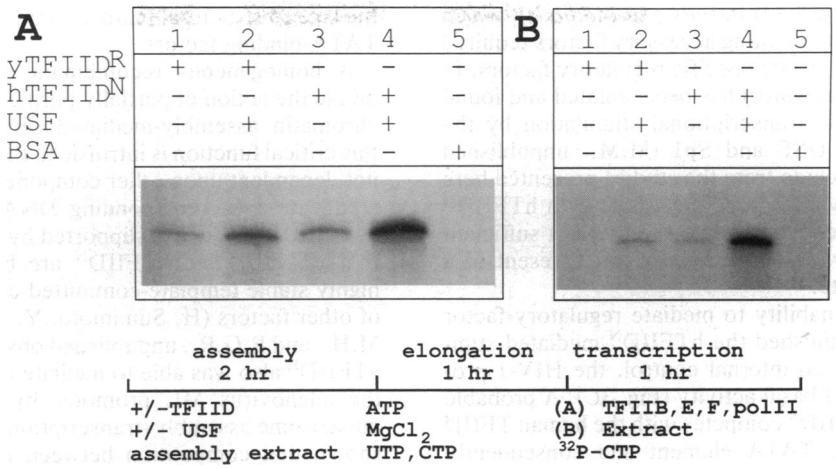

FIG. 2. Activation of transcription by USF via hTFIID^N and yTFIID^R in a chromatin assembly assay. Transcription from the ML promoter was analyzed in the absence or presence of USF and/or hTFIID^N and yTFIID^R during chromatin assembly as indicated. Transcription was carried out either with purified general initiation factors TFIIB, -E, -F and RNA polymerase II (pol II) (*A*) or with a HeLa nuclear extract (*B*). Chromatin assembly and transcription were performed as indicated in the graphic.

factors. Other experiments (data not shown) demonstrated that activation by USF via yTFIID^R was almost totally abolished when both TFIID and USF were incubated with the template prior to addition of the assembly extract. All these results are in agreement with previous studies showing that competition between nucleosome assembly and preinitiation complex assembly leads to an increased degree of stimulation by USF (11) and that prebinding of TFIID can diminish (by raising the basal activity) the effect of a regulatory factor (9).

In the present assay system, promoter activity was dependent upon the addition of TFIID during chromatin assembly, with virtually no detectable transcription when only USF was present (data not shown; see also ref. 11). This indicates that USF alone cannot efficiently maintain an open chromatin configuration for binding of the general factors, despite the proximity of the USF site to the TATA box (22).

yTFIID^N But Not yTFIID^R Mediates USF Activation in a System Reconstituted with Partially Purified General Transcription Factors. In the above analysis with yTFIID^R, modifications or accessory factors important for the function of upstream activators (USF) might have been contributed by the chromatin assembly extract. To investigate this possibility we compared the abilities of yTFIID^R, hTFIID^N, and a partially purified yTFIID^N to mediate activation by USF in a system reconstituted only with partially purified general transcription factors (TFIIA, -B, -E, and -F) and RNA polymerase II. In this system USF showed no transcriptional enhancement in conjunction with yTFIID^R, the level of transcription from wild-type and control (lacking the USF binding site) templates being the same (Fig. 3*A*). To determine whether this lack of a regulatory factor response was promoter- or activator-specific, we analyzed in parallel activation of the HIV-1 promoter by a nearly homogeneous preparation of Sp1 (23). Sp1 was previously shown to activate the HIV promoter both *in vivo* (24) and *in vitro* (25). In the present assay system yTFIID^R activated basal-level transcription from the HIV promoter to a level comparable to that observed for the adenovirus ML core promoter lacking the USF site (Fig. 3*A*, lanes 3 and 5). However, analogous to the situation with USF on the ML promoter, no further activation of the HIV promoter by Sp1 was observed in the presence of yTFIID^R (Fig. 3*A*, lanes 5 and 6). Control experiments showed that both factors, Sp1 and USF, activated the corresponding promoters about 3-fold when partially purified hTFIID^N was included in the reconstituted system, with no significant effect on the control adenovirus ML promoter lacking upstream sequence elements (Fig. 3*B*). The stimulatory effects of Sp1 and USF were lower than previously reported (22, 25). This may reflect the modified purification procedures used for the general transcription

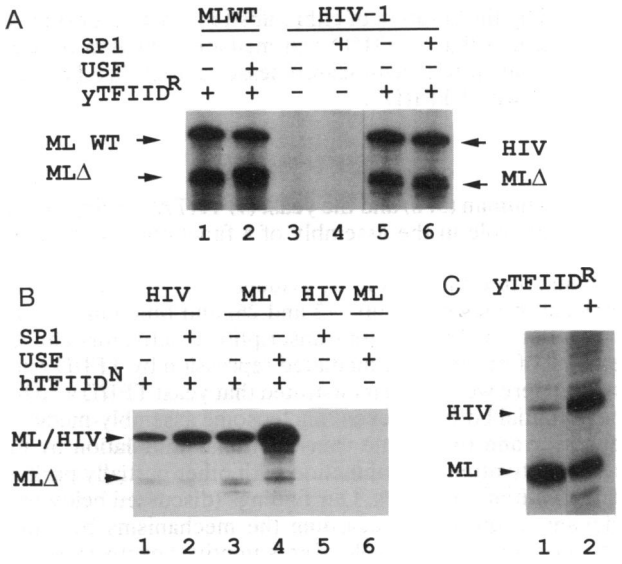

FIG. 3. Transcriptional activation by USF and Sp1 mediated by hTFIID^N and yTFIID^R in a reconstituted system of human general initiation factors. (*A*) Induction of the human immunodeficiency virus type 1 (HIV-1) promoter by Sp1 and the adenovirus ML promoter (wild type, WT) by USF via yTFIID^R. Lanes 3 and 4 show controls in which yTFIID^R was omitted to demonstrate that the system did not contain detectable amounts of hTFIID^N. (*B*) Activation by USF and Sp1 via hTFIID^N. Lanes 5 and 6 show the background in the absence of the hTFIID^N fraction and the presence of USF or Sp1 as indicated. *A* and *B* show transcription from ML nucleotides (−404 to +10) and HIV-1 (−109 to −8) promoters linked to a ML initiator sequence (−7 to +9) and to a G-free cassette (380 base pairs). As an internal control all reaction mixtures contained a ML core promoter (−53 to +10, pΔ53) linked to a shortened G-free cassette (about 300 base pairs). (*C*) Effect of addition of yTFIID^R (lane 2) to a transcription mixture including USF and hTFIID^N (lane 1). Analysis of transcripts from a HIV-1 wild-type promoter (−167 to +80) and a ML promoter (−261 to +33), both linked 5′ to the chloramphenicol acetyltransferase gene, was done by primer extension; vectors and protocols will be described elsewhere (H. Kato, M.H., and R.G.R.). Transcription was carried out with TFIIA (Mono S fraction), -B, -E, and -F and RNA polymerase II.

factors in the present work (*Materials and Methods*), which may have removed contaminating accessory factors required for maximal activation by site-specific regulatory factors. In fact, such a factor (coactivator) has been isolated and found to significantly increase transcriptional stimulation by the same preparations of USF and Sp1 (M.M., unpublished observations). We conclude from the studies presented here that the upstream activators were functional with $hTFIID^N$ and that $yTFIID^R$, by comparison, was either not sufficient to mediate these activation functions or was present in a regulatory-inactive state.

Consistent with its inability to mediate regulatory factor function, $yTFIID^R$ diminished the $hTFIID^N$-mediated stimulation by USF, while an internal control, the HIV-1 promoter, showed elevated basal activity (Fig. 3*C*). A probable explanation is that $yTFIID^R$ competes with the human TFIID for the binding to the TATA element and consequently suppresses induction by regulatory factors.

In a further analysis of the $yTFIID^R$ deficiency we analyzed the ability of $yTFIID^N$ to mediate transcriptional enhancement by the upstream activators. $yTFIID^N$ was isolated from a nuclear extract of *Saccharomyces cerevisiae* as described (12), and active fractions from either the first (heparin-Sepharose) or the second (Sephacryl S300) column were used. Fractions from each column effected a moderate induction (about 2-fold) by both USF and Sp1 in the purified-factor transcription assay (Fig. 4). Transcription from an internal control, the adenovirus ML core promoter, was not changed by the addition of either upstream activator (Fig. 4). We conclude that $yTFIID^N$ can mediate induction by USF and Sp1, albeit to a significantly lesser extent than has been observed with $hTFIID^N$.

DISCUSSION

Both the human (5, 6) and the yeast (7) TATA-binding factors play a key role in the assembly of a functional preinitiation complex. hTFIID also appears to play a role in the regulation of transcription initiation, as evidenced by studies showing physical (reviewed in refs. 12 and 26) and functional (9, 11) interactions of TFIID with transcriptional activators and the reversal of nucleosome-mediated repression by TFIID binding (8). Here we have demonstrated that yeast TFIID also has the potential both to prevent nucleosome assembly-mediated repressor and to mediate transcriptional stimulation by upstream activators in conjunction with other partially purified general initiation factors. Our findings (discussed below) are relevant to questions regarding the mechanisms by which activators function, the role of presumptive coactivators, and the role of structural features unique to human versus yeast TATA-binding factors.

A homogeneous recombinant $yTFIID^R$ was shown to mimic the action of partially purified $hTFIID^N$ in preventing chromatin assembly-mediated repression, suggesting that this critical function is intrinsic to the 27-kDa yeast factor and not dependent upon other components that might be associated with the corresponding DNA binding protein in the $hTFIID^N$. This idea is supported by recent studies indicating that $yTFIID^R$ and $hTFIID^N$ are both capable of forming highly stable template-committed complexes in the absence of other factors (H. Sumimoto, Y. Ohkuma, T. Yamamoto, M.H., and R.G.R., unpublished observations). Significantly, $yTFIID^R$ also was able to mediate a substantial induction of the adenovirus ML promoter by USF in the combined nucleosome assembly–transcription assay. It was previously shown that competition between nucleosome binding and initiation-complex formation results in a decrease in core promoter activity and a concomitant increase in the extent of stimulation by transcriptional activators (10, 11). Moreover, core promoter activity is generally high *in vitro* and low or undetectable *in vivo*, while the effects of activators are considerably greater *in vivo* than *in vitro*. Thus, superimposing the chromatin assembly system on the transcription assay may mimic more closely the *in vivo* situation and provide a more sensitive assay for regulatory factor functions and for TFIID structures necessary for these functions. Previous studies have documented that the presence of USF during assembly increases the number of functional promoters, implying a positive role of USF in TFIID binding (11). Results that failed to show activation by USF in a system reconstituted only with purified factors and $yTFIID^R$ are consistent with this idea but may also reflect modification of $yTFIID^R$ in the assembly system, either by the chromatin structure itself or by other factors in the assembly extract.

$yTFIID^R$ activated basal transcription from two different promoters, adenovirus ML and the HIV-1, in combination with partially purified general initiation factors (TFIIA, -B, -E, and -F) and RNA polymerase II, but failed to mediate induction by USF and Sp1 in the same assay system. By comparison, Pugh and Tjian (27), using partially purified general factors, did not detect induction of transcription by Sp1 with either expressed or natural yTFIID, whereas Berk and collaborators found the opposite result in less purified assay systems (reviewed in ref. 15). Our data support and extend the suggestion that $yTFIID^N$ carries a regulatory potential, by showing that both USF and Sp1 can activate via $yTFIID^N$ in a system reconstituted with purified general factors. However, the activation by USF and Sp1 was much

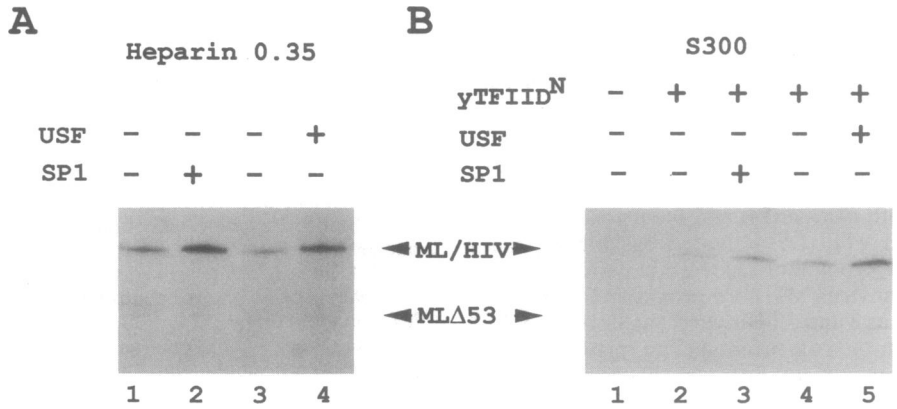

FIG. 4. Transcriptional activation by USF and Sp1 mediated by $yTFIID^N$ in a reconstituted system of human general transcription factors. Induction of the HIV-1 promoter by Sp1 and the adenovirus ML promoter by USF via partially purified $yTFIID^N$ from either a heparin-Sepharose column (0.35 M KCl) (*A*) or a Sephacryl S300 gel filtration column (*B*) is shown. The templates were the same as described in Fig. 3*A*. Transcription was carried out with TFIIA (DEAE fraction in *A* and Mono S fraction in *B*), -B, -E, and -F and RNA polymerase II.

more efficient with hTFIID^N than with yTFIID^N. Differences in induced transcription levels between the natural TFIIDs could indicate either distinct structural features in the proteins or differences in coactivators between the two species. The first possibility includes any type of structural (e.g., covalent) modification that could be species- and/or preparation-dependent, while the second includes a potentially novel class of accessory factors (coactivators) that may facilitate interactions between regulatory factors and any of the general transcription factors (e.g., TFIID; Fig. 5). The existence of a specific coactivator and putative adaptor has been predicted for the activator Sp1 (27). Based on a comparison of the properties of natural and recombinant TFIIDs, it was suggested that the coactivator was contained in the fractions of partially purified TFIID^N. Previous studies showed that hTFIID^N interactions on the ML promoter extended to sequences downstream of the transcription initiation site (5). By contrast, recombinant yeast and human TFIIDs show no interactions downstream of the transcription initiation site (A. Roy and R.G.R., unpublished observations). These observations indicate that putative coactivators might interact with TFIID and bind DNA downstream of the transcription initiation site (Fig. 5*A*) rather than bridge directly between TFIID and regulatory activators (reviewed in ref. 28).

We and others have reported recently the structure of a hTFIID (13–15). This protein and the TFIIDs from other organisms (reviewed in refs. 13–15) have about 80% amino acid sequence identity within a 180-residue carboxyl-terminal region that has been shown to be necessary and sufficient for DNA binding and basal transcription (20). In contrast, the amino termini of the TATA factors from human and yeast, as well as other species, are largely unrelated (reviewed in refs. 13–15). This has led to the suggestion that the amino-terminal domains might be involved in regulatory factor interactions and directly responsible for the different levels of activator-dependent transcription with yTFIID^R and hTFIID^N. However, that the yTFIID can mediate upstream factor activation in various assays suggests that the conserved carboxyl-terminal core can mediate at least a partial activator response. Direct comparisons of appropriately expressed TFIID carboxyl-terminal core domains with the corresponding full-length proteins should answer this question. Finally, it should be noted that even in a system containing hTFIID^N the stimulation by Sp1 and USF is not more than 3- to 10-fold. Thus we may need more potent activators and more sensitive assays, as well as further purification and characterization of TFIID^N and associated factors or coactivators, to more fully appreciate the structural requirements of TFIID for upstream factor activation.

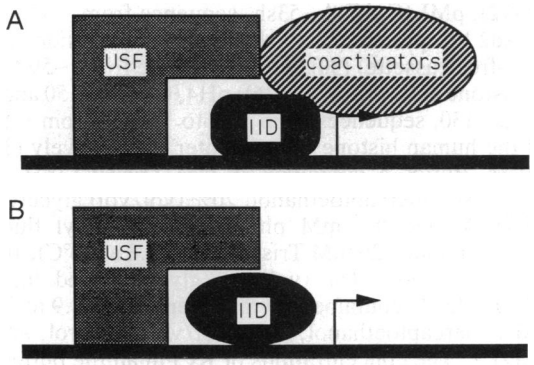

FIG. 5. Model for the involvement of putative coactivators in induced transcription. (*A*) Coactivators may associate downstream of the transcription initiation site and may interact with TFIID and regulatory factors. (*B*) Alternatively, or in addition, a physical or structural modification of TFIID might be necessary for a proper function in regulated transcription of class II genes. Explanations for these two possibilities are given in the text.

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