

Yeast and human TFIIDs are interchangeable for the response to acidic transcriptional activators in vitro

Raymond J. Kelleher III,¹ Peter M. Flanagan,¹ Daniel I. Chasman,¹ Alfred S. Ponticelli,² Kevin Struhl,² and Roger D. Kornberg^{1,3}

¹Department of Cell Biology, Stanford University School of Medicine, Stanford, California 94305 USA; ²Departments of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115 USA

Previous work showed that human TFIID fails to support yeast cell growth, although it is nearly identical to yeast TFIID in a carboxy-terminal region of the molecule that suffices for basal, TATA-element-dependent transcription in vitro. These and other findings raised the possibility that TFIID participates in species-specific interactions, possibly with mediator factors, required for activated transcription. Here, we report that human TFIID and amino-terminally truncated derivatives of yeast TFIID are fully functional in support of both basal transcription and the response to acidic activator proteins in a yeast in vitro transcription system. Conversely, and in contrast to previously published results, yeast TFIID supports both basal and activated transcription in reactions reconstituted with human components. This functional interchangeability of yeast and human TFIIDs argues strongly against species specificity with regard to TFIID function in basal transcription and the response to acidic activator proteins. In addition, our results suggest that any intermediary factors between acidic activators and TFIID are conserved from yeast to man.

[Key Words: Mediator; TFIID; transcriptional activation; acidic activators; in vitro transcription]

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Transcription factor IID (TFIID), which binds the TATA element of RNA polymerase II promoters, was first defined as a fraction from HeLa cells required for basal transcription (i.e., transcription in the absence of activator proteins). Activity was subsequently shown to reside in a 37-kD polypeptide from human cells (hTFIID), a 27-kD polypeptide from the yeast *Saccharomyces cerevisiae* (yTFIID), and molecules of comparable size from other organisms (Cavallini et al. 1989; Hahn et al. 1989; Horikoshi et al. 1989; Schmidt et al. 1989; Hoffmann et al. 1990; Kao et al. 1990; Peterson et al. 1990). Much as yTFIID will replace the human protein in basal transcription in the HeLa transcription system, so the HeLa TFIID fraction can be substituted in a yeast system (Buratowski et al. 1988; Flanagan et al. 1990). In keeping with this functional conservation, the sequence of the carboxy-terminal 180 amino acids of TFIID is 81% identical between yeast and man, and this carboxy-terminal domain, produced either by expression of a deleted gene or by partial proteolysis, is active in basal transcription in the HeLa transcription system (Horikoshi et al. 1990; Peterson et al. 1990; Lieberman et al. 1991).

In contrast with the evolutionary conservation of the carboxy-terminal domain of TFIID, the amino-terminal

region varies widely in size and sequence, even among closely related organisms. A number of observations have led to the notion that the amino-terminal region is required for transcriptional activation. Deletion of this region from hTFIID eliminates the response to the activator Sp1 (Peterson et al. 1990). Similarly, yTFIID, with its different amino-terminal region, has been reported to substitute only in basal but not in GAL4-VP16-activated transcription in the HeLa system (Peterson et al. 1990). It was suggested that the amino-terminal region interacts with factors known as mediators or coactivators that are required for activated transcription. Recently, however, the amino-terminal region of yTFIID was shown to be dispensable for viability of yeast, so the amino-terminal region cannot be solely responsible for regulated transcription in this organism (Cormack et al. 1991; Gill and Tjian 1991; Reddy and Hahn 1991; Zhou et al. 1991).

Despite the capacity of the carboxy-terminal domain of yTFIID to perform all essential functions in yeast, hTFIID with a nearly identical carboxy-terminal sequence was inadequate for cell growth (Cormack et al. 1991; Gill and Tjian 1991). This deficiency of hTFIID might be attributed to a failure to support effects of activators in yeast, in keeping with the previous observation that yTFIID is deficient in activated transcription in the HeLa system. Here, we report on studies leading to the opposite conclusion—that human and yeast TFIIDs

³Corresponding author.

and carboxy-terminal fragments are all functionally equivalent in basal and activated transcription in vitro.

Results

A TFIID-dependent yeast transcription system responsive to acidic activator proteins

Yeast extracts have been resolved into five fractions, designated a, b, c, d, and e, which are sufficient along with RNA polymerase II for basal transcription in the absence of activator proteins (Flanagan et al. 1990). Fraction d could be replaced by recombinant yTFIID isolated from *Escherichia coli*. Upon purification of fraction a, the response of the transcription system to acidic activator proteins was lost, but this response could be restored by the addition of a mediator distinct from the basal transcription factors (Flanagan et al. 1991). As fraction e was further purified, fraction c became dispensable for basal transcription. In this study, a scheme was employed that results in the copurification of mediator and all factors required for basal transcription, with the exception of TFIID, through two chromatographic steps (P.M. Flanagan, R.J. Kelleher, H. Tschochner, M.H. Sayre, and R.D. Kornberg, in prep.). The resulting protein fraction is en-

riched ~200-fold with respect to mediator, RNA polymerase II, and yeast initiation factors a, b, and e. Addition of TFIID to this fraction was necessary and sufficient for basal transcription (Fig. 1B). The further addition of fraction c had no stimulatory effect on basal transcription but was important for activation. The complete transcription system supported high levels of activation by GAL4-VP16 and GCN4 with full-length yTFIID (26- and 32-fold, respectively; see Fig. 2), and permitted an analysis of the activities of yTFIID derivatives and hTFIID in basal and activated transcription.

Carboxy-terminal domain of yTFIID is sufficient for basal and activated transcription in a yeast system in vitro

To assess the importance of the amino-terminal region of yTFIID in basal and activated transcription in vitro, derivatives lacking part or all of this region were expressed in *E. coli* and purified (Fig. 1A). Derivative yTFIID207C consists of an initiator methionine fused to the 207 carboxy-terminal amino acids, resulting in the deletion of residues 2–33. Derivative yTFIID179C consists of an initiator methionine fused to the 179-amino-acid, conserved carboxy-terminal domain, deleting resi-

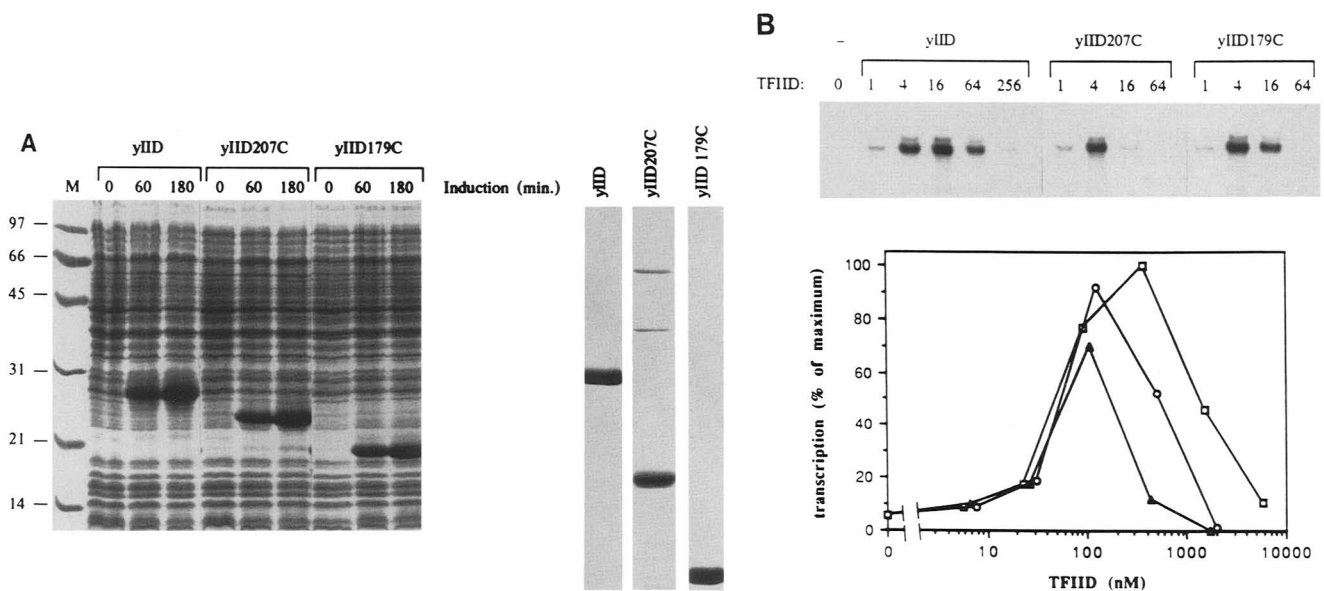


Figure 1. Bacterial expression and activity in basal transcription of yTFIID and amino-terminally truncated derivatives. (A) Full-length yTFIID (yIID) and derivatives consisting of the 207 carboxy-terminal residues (yIID207C) and the 179 carboxy-terminal residues (yIID179C) fused to an initiator methionine, were expressed in *E. coli*, and purified. (Left) SDS-PAGE analysis of total bacterial protein immediately prior to induction and 1 and 3 hr following induction. (Right) SDS-PAGE analysis of the purified fractions used in transcription assays. (B) Full-length yTFIID and the truncated derivatives were tested for the ability to support basal transcription in a TFIID-dependent reconstituted yeast transcription system. Relative amounts of TFIID assayed are given in multiples of 18.75 ng. Reactions were performed with pGAL4CG- (100 ng), which contains a single GAL4-binding site upstream of the yeast *CYC1* promoter fused to a G-less cassette (Lue et al. 1989), in the presence of fraction c, mediator, yeast initiation factors a, b, e, and RNA polymerase II, as described in Results. Radioactivity incorporated into specific transcripts, shown in the autoradiograph, is expressed as a percentage of the maximum (obtained with 300 ng of yTFIID) and plotted as a function of TFIID concentration. (□) yTFIID; (▲) yTFIID207C; (○) yTFIID179C.

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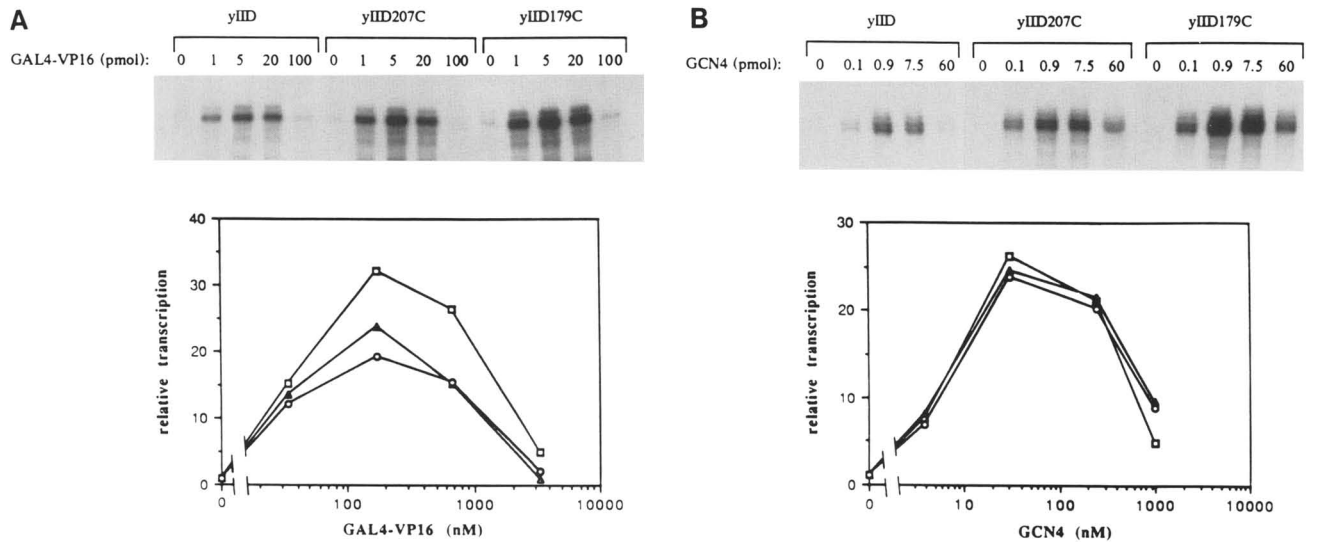


Figure 2. Mediator-dependent response of full-length yTFIID and amino-terminally truncated derivatives to GAL4-VP16 and GCN4. (A) The indicated amounts of GAL4-VP16 were added to transcription reactions, as in Fig. 1B, with the indicated form of TFIID (75 ng) and pGAL4CG⁻ (25 ng). Radioactivity incorporated into specific transcripts, shown in the autoradiograph, was normalized to that obtained in the absence of activator for each form of TFIID and plotted as a function of the GAL4-VP16 concentration (For description of symbols, see Fig. 1B). (□) yTFIID; (▲) yTFIID207C; (○) yTFIID179C. (B) The indicated amounts of GCN4 were added to transcription reactions, as in Fig. 1B, with the indicated form of TFIID (75 ng) and pSP{GCN4}²CG⁻ (25 ng), which contains two copies of a GCN4-binding site upstream of the yeast *CYC1* promoter fused to a G-less cassette (Flanagan et al. 1990). Radioactivity incorporated into specific transcripts, shown in the autoradiograph, was normalized to that obtained in the absence of activator for each form of TFIID and plotted as a function of GCN4 concentration (see A for description of symbols). (C) Fully reconstituted transcription reactions, containing 100 ng of pGAL4CG⁻ (left) or pSP{GCN4}²CG⁻ (right) and the indicated form of TFIID, were performed in the absence (-) or presence (+) of activator protein (1 pmole) and/or highly enriched mediator fraction. All reactions also contained fraction c, RNA polymerase II, and initiation factors a, b, and e (as described in the Materials and methods). As reported previously (Flanagan et al. 1991), a slight activation of the reconstituted system may be observed in the absence of added mediator, presumably as the result of contamination of fractions supplying yeast general initiation factors with mediator activity. Maximal activation is nonetheless dependent on added mediator.

dues 2–61. Both derivatives exhibited higher DNA-binding activity than full-length yTFIID in gel mobility-shift assays (R.J. Kelleher and W.J. Feaver, unpubl.), as noted previously (Horikoshi et al. 1990).

The activities of the truncated derivatives in support of basal transcription were similar to that of full-length yTFIID in several respects (Fig. 1B). First, the dependence of transcription on low levels of the TFIID molecules was nonlinear, perhaps reflecting the equilibrium binding of the molecules to the TATA element. Second, there was a well-defined optimum, at $\sim 10^{-7}$ M for the derivatives, and $\sim 4 \times 10^{-7}$ M for the full-length molecule. The slightly lower optimal concentration for the derivatives might be the result of their somewhat higher affinity for DNA (see above). Finally, all forms of TFIID were very inhibitory at the highest levels tested. In view of the purity of the TFIID preparations and the relatively low concentration at which inhibition was observed (10^{-6} –

10^{-7} M), this effect may be specific and not attributable to a contaminant.

The two truncated derivatives of TFIID were indistinguishable from the full-length molecule in support of activation by GCN4 and only slightly less effective than the full-length molecule in activation by GAL4-VP16 (Fig. 2A,B). The small difference in behavior might be explained by the presence of two activator-binding sites in the GCN4-responsive template, as opposed to one site in the GAL4 template, or the amino terminus might make some minor contribution to the response to GAL4-VP16. The overall levels of transcription were higher with the truncated TFIID derivatives (see autoradiographs, Fig. 2A,B, top), probably reflecting the increased DNA-binding activities of the derivatives. No effect of the activators was seen in the absence of added TFIID (data not shown), demonstrating that activation was entirely the result of the added form of TFIID and

not any of the full-length molecule contaminating the fractions used to reconstitute transcription.

Although the carboxy-terminal domain of yTFIID supported activation by GAL4-VP16 and GCN4, ruling out a requirement for the amino-terminal region, the possibility remained that the amino-terminal region functions as a negative element, as indicated in other circumstances (Horikoshi et al. 1990, and see above). According to this hypothesis, mediator would be required to relieve the inhibitory effect of the amino-terminal region, whereas activators would function through direct contact with the carboxy-terminal domain of TFIID or another general initiation factor. If this were the case, mediator would only be required for activation with full-length TFIID but not with truncated derivatives. However, we found that mediator was essential for activation by GAL4-VP16 and GCN4 with both full-length and amino-terminally truncated TFIID in a fully reconstituted yeast transcription system (Fig. 2C). Taken together, our results indicate that the amino-terminal region of yTFIID does not play a significant role in the response of the general transcription machinery to these acidic activators and that if yTFIID does participate in regulatory interactions with acidic activators or the mediator, these interactions are restricted to the carboxy-terminal domain.

hTFIID and yTFIIDs are functionally interchangeable in transcription in vitro

The capacity of the carboxy-terminal domain of yTFIID to support both basal and activated transcription in the yeast system and the sequence conservation of this domain between yeast and man lead one to expect that hTFIID would be fully functional in yeast as well. And yet, as mentioned above, hTFIID fails to support yeast cell growth. To determine whether this failure of hTFIID reflects a general transcriptional defect, we investigated the activity of hTFIID in the yeast system *in vitro*.

Recombinant hTFIID supported basal transcription from both the yeast *CYC1* promoter and the adenoviral major late promoter (Fig. 3A). As described above for yTFIID and amino-terminally deleted derivatives, the concentration dependence of transcription at low levels of hTFIID was nonlinear. Transcription was maximal at $\sim 10^{-7}$ M, and higher concentrations were inhibitory. The maximal level of basal transcription obtained with hTFIID from the *CYC1* and adenoviral major late promoters was $\sim 45\%$ and 70% , respectively, of that obtained with full-length yTFIID from the same promoters. Recombinant hTFIID also supported activation by both GAL4-VP16 and GCN4 over the same range of activator concentration as observed with yTFIID and derivatives (Fig. 3B,C). We can therefore rule out a general defect of hTFIID in basal transcription or in the response to acidic activators of either mammalian or yeast origin in the yeast system.

Because hTFIID supports the effect of acidic activators in the yeast transcription system, yTFIID ought to do the same in the HeLa transcription system. To investigate

this point, TFIID activity in a HeLa nuclear extract was inactivated by heat treatment (Nakajima et al. 1988). Addition of yTFIID to the heated extract restored both basal and activated transcription to levels similar to those obtained with unheated extract (Fig. 4). The amino-terminally truncated yTFIID derivatives yTFIID179C and yTFIID207C also fully restored the response of the heated extract to GAL4-VP16 (data not shown), further emphasizing the sufficiency of the conserved carboxy-terminal domain for this response. Thus, human and yeast TFIIDs are interchangeable between human and yeast systems with regard to both basal transcription and effects of acidic activators.

Discussion

The findings reported here resolve two paradoxes regarding the structure and function of TFIID. First, it was noted previously that the carboxy-terminal domain of TFIID, $>80\%$ conserved between yeast and man, is sufficient for basal transcription in the HeLa system, yet hTFIID is inadequate for yeast cell growth (Horikoshi et al. 1990; Peterson et al. 1990; Cormack et al. 1991; Gill and Tjian 1991). The present results demonstrate that hTFIID is fully functional in a yeast transcription system *in vitro*, so the insufficiency *in vivo* cannot be due to a general defect in support of yeast RNA polymerase II transcription. A number of alternatives may be considered: hTFIID may fail to support RNA polymerase II transcription of one or a small number of essential genes; it may not respond to other types of activation domains (i.e., nonacidic); it may fail to act as required at RNA polymerase III promoters (Margottin et al. 1991; Simmen et al. 1991); or it may fail to fulfill an essential role unrelated to transcription.

A second paradox arose from the previous proposal (Peterson et al. 1990) that the amino-terminal region of TFIID, although highly divergent across species, is involved in the response to a "universal" activator, GAL4-VP16, which stimulates transcription of responsive genes in both yeast and HeLa transcription systems (Sadowski et al. 1988; Chasman et al. 1989; Kelleher et al. 1990). This proposal was based on the failure of yTFIID to support activation by GAL4-VP16 in a HeLa transcription system (Peterson et al. 1990). We have now shown that on the contrary, yTFIID does support a response to GAL4-VP16 in the HeLa system comparable to that observed with the endogenous hTFIID; and although the reason for the discrepancy is unclear, our positive result seems more likely to be correct than the previous negative one. Moreover, our findings are in accord with the recent demonstrations that yTFIID supports a GAL4-VP16 effect in the HeLa system in the context of a chromatin template (Workman et al. 1991) and that yTFIID can partially restore the GAL4-VP16 response of a HeLa nuclear extract subjected to an appropriate heat treatment (White et al. 1991). Our findings do not bear on a possible role of the amino-terminal region of hTFIID in the response to other classes of activator, such as Sp1 (Peterson et al. 1990).

The growth of yeast cells bearing only the carboxy-terminal domain of yTFIID has been variously reported to be indistinguishable from that of wild-type yeast (Cormack et al. 1991; Reddy and Hahn 1991) or to be much impaired (Gill and Tjian 1991; Zhou et al. 1991). In the latter case, the poor growth phenotype was attributed to the deletion of a small amino-terminal region of yTFIID between residues 48 and 57 (Zhou et al. 1991). In the present study, yTFIID derivatives both containing (yTFIID207C) and lacking (yTFIID179C) these residues exhibited comparable activities to the full-length molecule both in basal transcription and in the response to acidic activators, indicating that the deletion of this region does not impose any general transcriptional defect. These findings from transcription *in vitro* are in agreement with a report that inducible transcription from the *GAL1* and *CYC1* promoters *in vivo* is unaffected by deletion of the amino-terminal region of yTFIID (Reddy and Hahn 1991). In addition, yeast strains bearing only the carboxy-terminal domain of yTFIID are viable under selective conditions requiring activation by GCN4,

ACE1, and GAL4 proteins (Cormack et al. 1991). Although we did observe minor differences in optimal concentrations for basal transcription among yTFIID and the amino-terminally truncated derivatives, these differences are unlikely to account for the importance of the amino-terminal region *in vivo* in some studies, because the growth defect was observed over a wide range of expression levels of the carboxy-terminal domain (Zhou et al. 1991). Rather, deletion of the amino-terminal region may affect the expression of one or a small number of essential genes in a manner analogous to that noted with reporter genes bearing upstream elements of the *CUP1* or *HSP* promoters (Zhou et al. 1991).

The inhibitory effect of TFIID at high concentrations found here resembles that observed previously *in vitro* (Kelleher et al. 1990) and *in vivo* (Gill and Ptashne 1988; Triezenberg et al. 1988; Meyer et al. 1989; Kelleher et al. 1990; Martin et al. 1990) at high concentrations of activator proteins, and it may have a similar explanation: TFIID may sequester another general initiation factor with which it interacts in the transcription process. Ex-

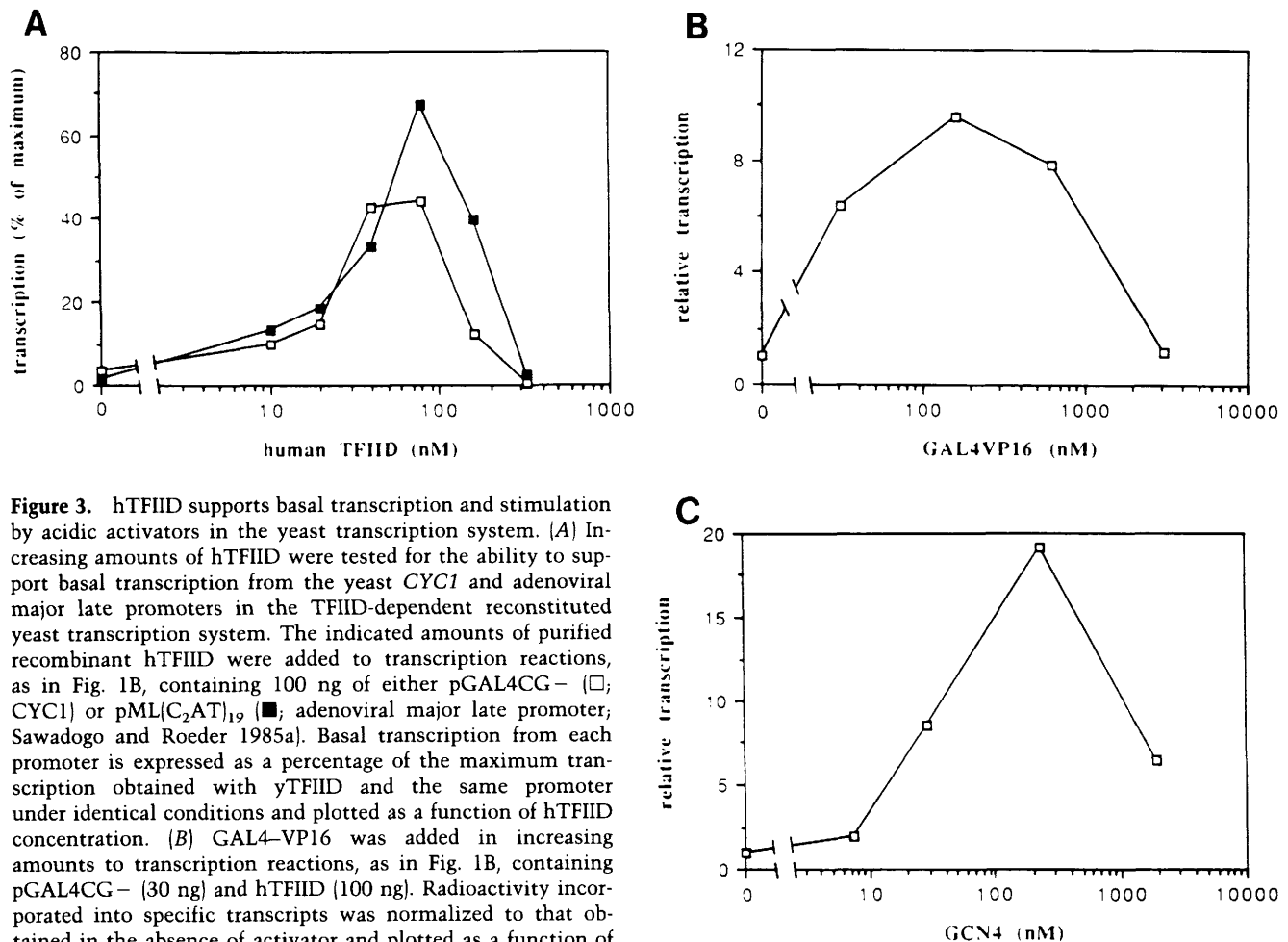


Figure 3. hTFIID supports basal transcription and stimulation by acidic activators in the yeast transcription system. (A) Increasing amounts of hTFIID were tested for the ability to support basal transcription from the yeast *CYC1* and adenoviral major late promoters in the TFIID-dependent reconstituted yeast transcription system. The indicated amounts of purified recombinant hTFIID were added to transcription reactions, as in Fig. 1B, containing 100 ng of either pGAL4CG- (□; *CYC1*) or pML(C₂AT)₁₉ (■; adenoviral major late promoter; Sawadogo and Roeder 1985a). Basal transcription from each promoter is expressed as a percentage of the maximum transcription obtained with yTFIID and the same promoter under identical conditions and plotted as a function of hTFIID concentration. (B) GAL4-VP16 was added in increasing amounts to transcription reactions, as in Fig. 1B, containing pGAL4CG- (30 ng) and hTFIID (100 ng). Radioactivity incorporated into specific transcripts was normalized to that obtained in the absence of activator and plotted as a function of GAL4-VP16 concentration. (C) GCN4 was added in increasing amounts to transcription reactions, as in Fig. 1B, containing pSP(GCN4)₂CG- (30 ng) and hTFIID (100 ng). Radioactivity incorporated into specific transcripts was normalized to that obtained in the absence of activator and plotted as a function of GCN4 concentration.

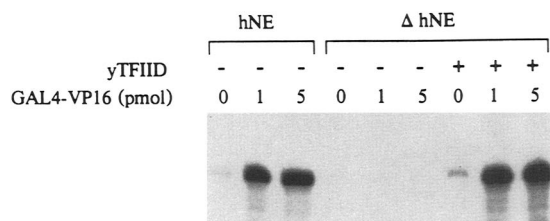


Figure 4. yTFIID supports a GAL4–VP16 response in a HeLa transcription system. The indicated amounts of GAL4–VP16 were added to transcription reactions containing pGAL4MLG – (100 ng), with a single GAL4-binding site upstream of the adenovirus major late promoter fused to a G-less cassette (Lue et al. 1989), and untreated HeLa nuclear extract (hNE), heat-treated HeLa nuclear extract, or heat-treated HeLa nuclear extract (Δ hNE) supplemented with recombinant yTFIID.

pression of TFIID at high levels in vivo impairs cell growth (Reddy and Hahn 1991), suggesting that the effect we find in vitro may indeed be functionally significant. The possibility remains, however, that the inhibitory effect has a trivial explanation. While it was probably not caused by a contaminant, for the reasons mentioned above, it could have been the result of nonspecific interaction of TFIID with the template DNA or self-aggregation of the protein.

Stimulation of yeast RNA polymerase II transcription by the acidic activators GAL4–VP16 and GCN4 is dependent on a mediator distinct from the general initiation factors (Kelleher et al. 1990; Flanagan et al. 1991). The action of such acidic activator proteins and mediator may involve contact(s) with general initiation factors, and TFIID has been implicated as a target of such interactions (Sawadogo and Roeder 1985b; Horikoshi et al. 1988; Peterson et al. 1990; Stringer et al. 1990; Ingles et al. 1991). The ability of the carboxy-terminal domain of yTFIID to support activation by GAL4–VP16 and GCN4 in a mediator-dependent fashion eliminates the amino-terminal region as a potential target of interactions with either acidic activation domains or the mediator. Likewise, the capacity of yTFIID to support effects of GAL4–VP16 in the HeLa transcription system shows that the amino-terminal region of the human protein is dispensable for activation and further suggests that any interactions of acidic activators or a putative HeLa mediator with TFIID are not species-specific. The lack of a requirement for the divergent amino-terminal region and the interchangeability of the conserved carboxy-terminal domain of TFIID between yeast and human systems described here could account for the evolutionary conservation of the response to acidic activators. If the conserved carboxy-terminal domain of TFIID is a target of mediator, our results suggest further that the mediator itself is conserved between yeast and man.

Materials and methods

Preparation of TFIID

The full-length yeast TFIID gene was modified to create an *NdeI*

site at the ATG by replacement of sequences between the start site and the *DraI* site at amino acid 6 with a synthetic oligonucleotide. Amino-terminally truncated derivatives were generated by the polymerase chain reaction with upstream primers containing *NdeI* sites followed by internal TFIID-coding sequences. The complete coding sequence and the two PCR-derived deletions were then cloned as *NdeI*–*BamHI* fragments into the T7 polymerase expression vector pET11a and expressed in *E. coli* strain BL21(DE3) as described (Studier and Moffat 1986). The amino acid sequence of the yTFIID179C derivative was confirmed by determination of the nucleotide sequence. The expressed yTFIID and yTFIID179C polypeptides were purified to homogeneity by a chromatographic scheme to be described elsewhere (D.I. Chasman et al., in prep.). The yTFIID207C derivative was purified to near homogeneity by chromatography over DE52 and heparin–Sepharose essentially as described (Hoey et al. 1990). hTFIID was expressed in BL21(DE3) from the T7 polymerase expression vector pET3a containing a full-length HeLa TFIID cDNA (generous gift of A. Hoffmann and R. Roeder). The bacterial lysate in buffer A (300 mM HEPES–KOH (pH 7.6), 10% glycerol, 1 mM EDTA, 0.5 mM DTT, 1 mM PMSF; concentration of KCl in mM in parentheses) containing 0.2 M KCl was applied to a DE52 column equilibrated in the same buffer. The flowthrough was diluted to 100 mM KCl and applied to a heparin–Sepharose column equilibrated in buffer A (100). The column was successively eluted with buffer A containing 0.2 M, 0.6 M, and 1 M KCl. The 0.6 M eluate was dialyzed against buffer A (90) and applied to a Q–Sepharose column equilibrated in buffer A (90). The flow through from Q–Sepharose was then applied to a SP–5PW column equilibrated in buffer A (90). The column was washed with buffer A (200) and developed with a linear gradient from 0.2–1 M KCl. The peak of hTFIID eluted at \sim 0.38 M KCl and was estimated to be 50% pure by SDS-PAGE.

In vitro transcription

GAL4–VP16 protein (Chasman et al. 1989) and GCN4 protein (generous gifts of C. Ampe and T. Steitz) were expressed in *E. coli* and purified. Fraction c was prepared from yeast nuclear extracts as described previously (Flanagan et al. 1990). For all transcription reactions except those in Figure 2C, yeast initiation factors a, b, e, and RNA polymerase II, in addition to mediator, were supplied by a single fraction enriched \sim 200-fold by chromatography of yeast whole-cell extracts on Bio-Rex70 (Bio-Rad) and DE52 (Whatman) by methods described elsewhere (P.M. Flanagan, H. Tschochner, R.J. Kelleher, M.H. Sayre, and R.D. Kornberg, in prep.). Basal and activated transcription reactions were reconstituted with 8–10 μ g of this fraction, 3 μ g of fraction c, the indicated amount of TFIID, and activator protein, where appropriate. For the transcription reactions in Figure 2C, yeast initiation factors a, b, and RNA polymerase II were prepared as described (Edwards et al. 1990; Feaver et al. 1991; Flanagan et al. 1991). Mediator, prepared as described (Flanagan et al. 1991), was subjected further to high-performance liquid chromatography (HPLC) on a Bio-Gel heparin–5PW column. Yeast initiation factor e was obtained from further chromatography of the single enriched fraction, described above, on a SP–5PW column (H. Tschochner, unpubl.). HeLa nuclear extract was heated for 15 min at 47°C to inactivate endogenous TFIID activity (Nakajima et al. 1988). Recombinant yTFIID employed in the HeLa transcription assays was prepared as described (Flanagan et al. 1990). Yeast and HeLa transcription reactions were performed and analyzed as described previously for yeast nuclear extracts (Lue et al. 1989; Kelleher et al. 1990).

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