Requirement for acidic amino acid residues immediately N-terminal to the conserved domain of *Saccharomyces cerevisiae* TFIID

Qiang Zhou, Martin C.Schmidt¹ and Arnold J.Berk

Department of Microbiology and Molecular Genetics, Molecular Biology Institute, University of California at Los Angeles, Los Angeles, CA 90024-1570, USA

¹Present address: Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA

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TFIID binds to TATA boxes and initiates the assembly of general transcription factors and pol II on promoters. TFIID proteins from various species consist of a highly conserved carboxy terminal domain and very divergent amino terminal domains. We investigated the function of the non-conserved amino terminal domain (residues 1–60) of *Saccharomyces cerevisiae* TFIID (YIID, 240 residues) by testing the ability of a series of YIID amino terminal deletion mutants to complement a YIID deficient yeast strain. Mutants with deletions up to amino acid 48 restored the YIID deficient yeast strain to an apparently wild type phenotype. However, deletion up to position 57 or 60 produced yeast strains which formed extremely small colonies. Moreover, overexpression of YIIDΔ2-57 or YIIDΔ3-60 protein in the presence of wild type YIID resulted in a dominant-negative inhibition of growth. No difference between the basal transcriptional activity of wild type YIID and these amino terminal deletion mutants was observed *in vitro*. However, transcriptional activation *in vivo* of promoter–*lacZ* fusions showed that the YIIDΔ2-57 deletion affects the ability of certain promoters (*CUP1* and an HSP *UAS*–*CYCI* promoter hybrid promoter) to respond to upstream factor stimulation. At least one inducible promoter, *PHO5*, was not affected by this deletion. The defect produced by YIIDΔ2-57 was due to the deletion of several acidic residues present between residues 48 and 57. The results show that the conserved carboxy terminal domain of YIID is sufficient for cell viability. However, an acidic region just amino terminal to the conserved domain is required for normal growth and transcription control in most yeast strains.

Key words: gene regulation/TFIID/transcription/transcription factor/transcriptional activation

Introduction

Gene expression is controlled to a large extent through the regulation of transcription initiation (Maniatis *et al.*, 1987). TFIID is one of the general transcription factors required for the expression of most, if not all, genes transcribed by RNA polymerase II (Davison *et al.*, 1983; Fire *et al.*, 1984; Nakajima *et al.*, 1988). The binding of TFIID to the TATA

box promoter element initiates an ordered assembly of the components of the general transcriptional machinery, including TFIIA, IIB, IIE, IIF and RNA polymerase II (Buratowski *et al.*, 1989). This multiprotein complex is required for basal level transcription initiation from a minimal promoter containing only a TATA box and initiation site. However, gene-specific regulation of transcription and the rate of transcription initiation are largely determined by specific transcriptional activators that interact with enhancers and upstream promoter elements. These transactivators are generally bipartite in structure, containing a DNA-binding domain and a transcription activation domain (Mitchell and Tjian, 1989). At present, it is not clear how these activation domains stimulate transcription by the general transcriptional machinery.

The gene encoding TFIID has recently been cloned from a variety of organisms, making it feasible to address some of the questions concerning the mechanism of transcriptional stimulation by upstream activators. A comparison of the TFIID sequences from *Saccharomyces cerevisiae* (Cavallini *et al.*, 1989; Hahn *et al.*, 1989; Horikoshi *et al.*, 1989; Schmidt *et al.*, 1989b), *Schizosaccharomyces pombe* (Fikes *et al.*, 1990; Hoffmann *et al.*, 1990b), *Drosophila* (Hoey *et al.*, 1990), *Arabidopsis* (Gasch *et al.*, 1990) and human (Hoffmann *et al.*, 1990a; Kao *et al.*, 1990; Peterson *et al.*, 1990) indicates that the protein is composed of a highly divergent amino terminus of varying length, and a very conserved carboxy terminus of ~180 amino acid residues. For *S.cerevisiae* TFIID (YIID), the conserved domain begins at residue 61. Mutational analysis of YIID protein demonstrated that the conserved 180 amino acid carboxy terminus is sufficient for DNA binding and basal levels of transcription *in vitro* (Horikoshi *et al.*, 1990). Partial proteolysis of YIID showed that the conserved carboxy terminus forms a folded domain separate from the protease sensitive amino terminal domain (Lieberman *et al.*, 1991). The non-conserved amino termini of the human and *Drosophila* TFIID proteins have been implicated in the process of transcriptional activation by upstream activators (Pugh and Tjian, 1990). *In vitro* experiments with Sp1 and CTF suggested that partially purified TFIID fractions from human and *Drosophila* cells contain co-activators that are probably dispensable for basal transcription but are essential for transcriptional activation by upstream activators. It was demonstrated with cloned TFIID proteins that these co-activators may function through the amino terminal portion of TFIID and that co-activator–TFIID interactions are species specific (Peterson *et al.*, 1990).

To study the function of the non-conserved amino terminus of YIID protein *in vivo*, we constructed a series of amino terminal deletion mutants. We found that while the first 49 amino acids of YIID's amino terminus were dispensable for its ability to complement a YIID deficient yeast strain, further deletion up to amino acids 57 and 60 produced a defective transcription factor. Our experiments indicate that in many

yeast strains, the defective YIID protein responds poorly to signals from upstream activating sequences in a subset of yeast promoters.

Results

Amino terminal deletions of YIID to residues 57 and 60 produced yeast strains which formed extremely small colonies

To characterize the function of the non-conserved amino terminal domain of YIID, we constructed a series of amino terminal deletion mutants and tested their ability to allow cell growth in the absence of wild type YIID. The structures of wild type YIID protein and its amino terminal deletion mutants YIID Δ 2-41, YIID Δ 2-48 and YIID Δ 2-57 are shown in Figure 1A. Open reading frames encoding wild type YIID and these three amino terminal deleted forms were placed in the yeast expression vector pBM258 (Johnston and Davis, 1984), under the control of the yeast *GAL1* promoter. The plasmid pBM258 contains the *URA3* gene for yeast transformation and *CEN4* DNA for efficient segregation. The resulting constructs pBMYIID, pBMYIID Δ 2-41, pBMYIID Δ 2-48 and pBMYIID Δ 2-57 were transformed into a diploid yeast strain, MSY101, in which one chromosomal copy of the *SPT15* gene encoding YIID was substituted with the *LEU2* gene. Ura⁺ transformants were sporulated and tetrads were isolated and dissected. Since *SPT15* is an essential gene (Eisenmann *et al.*, 1989; Cavallini *et al.*, 1989), spores which grew on galactose-containing medium (YEPG) but not on glucose-containing medium (YEPD) were selected and the *LEU2* substitution of the endogenous *SPT15* gene and presence of the YIID expression plasmids were subsequently verified by Southern blot analysis (data not shown).

The growth of the selected strains which contained either wild type YIID or amino terminal deleted YIID expressed from the plasmids as the only source of TFIID activity was compared on a rich medium plate containing galactose (YEPG) (Figure 1B). As expected, wild type YIID produced from the plasmid pBMYIID could complement the YIID deficient strain, producing colonies (MSY103) of similar size to those of cells (BJ1991) with the endogenous YIID-encoding gene (*SPT15*). Amino terminal deletions up to amino acid 41 (YB120) or 48 (YB121) produced colonies of similar size, indicating that the first 48 amino acids of YIID are not critical for normal cell growth. However, the amino terminal deletion to position 57 of YIID produced a yeast strain (MSY122, Figure 1B) which formed much smaller colonies on media than did wild type or the other amino terminal deletion mutants. Growth curves in liquid YEPG medium showed that MSY122 initially grew with about twice the generation time of BJ1991 and reached saturation at about one-twentieth the concentration of BJ1991 (Figure 1C). BJ1991, MSY103 and MSY122 exhibited similar viability with >80% of log phase cells forming colonies on YEPG plates. The amino terminal deletion Δ 3-60 of YIID resulted in a phenotype similar to that of MSY122 (data not shown).

Overexpression of YIID Δ 2-57 protein in the presence of the wild type *SPT15* gene resulted in a dominant-negative initiation of growth

Poor growth of MSY122 cells could have been due to a defect in the function of the Δ 2-57 deletion mutant or

Fig. 1. Amino terminal deletion to residue 57 of YIID produced a yeast strain which formed extremely small colonies. (A) The structures of wild type YIID and the amino terminal deletion mutants YIID Δ 2-41, YIID Δ 2-48 and YIID Δ 2-57. The 60 amino acid amino terminal non-conserved domain and 180 amino acid carboxy terminal conserved domain of the wild type YIID are represented by a closed bar and an open bar, respectively. (B) The ability of wild type YIID, YIID Δ 2-41, YIID Δ 2-48 and YIID Δ 2-57 to complement a null mutation in the *S.cerevisiae* *SPT15* gene. Cells containing a null mutation (*spt15::LEU2*) in the YIID-encoding gene *SPT15* on chromosome V and various expression plasmids are grown on galactose-containing rich medium to induce the expression of wild type YIID (MSY103), YIID Δ 2-41 (YB120), YIID Δ 2-48 (YB121) and YIID Δ 2-57 (MSY122). (C) Growth curves of MSY103, MSY122 and BJ1991. Log phase cultures were diluted into fresh YEPG media and grown at 30°C with vigorous shaking. Optical density at 600 nm (OD₆₀₀) was measured for each strain at various time points as indicated.

Fig. 2. Dominant-negative inhibition of growth by amino terminal mutants of YIID. Haploid yeast strain (BJ1991) containing wild type *SPT15* gene on chromosome V was transformed with various plasmids expressing wild type YIID (YB150), YIIDΔ2-41 (YB151), YIIDΔ2-48 (YB152), YIIDΔ2-57 (YB153), YIIDΔ3-60 (YB154), YIIDΔ2-48EED-VVA (YB156) or YIIDΔ2-48K-L (YB155) under the control of the *GAL1* promoter. The growth of the resulting strains on medium containing 2% raffinose (A) or 2% galactose (B) is shown.

expression of reduced levels of the mutant protein due to protein instability. We were not able to measure YIIDΔ2-57 levels directly since we have not been able to raise specific antiserum against it, probably because it is virtually equivalent to the conserved TFIID domain shared by mammalian TFIID. To determine if YIIDΔ2-57 is expressed as a stable protein, we asked whether its expression from plasmid pBMYIIDΔ2-57 would inhibit the growth of cells with a wild type endogenous *SPT15* gene. Low level expression of mutant YIID would not be expected to produce a dominant-negative phenotype, whereas high level expression of a defective protein might, especially since the mutant protein would be expected to bind to TATA boxes (Horikoshi *et al.*, 1990). Haploid cell BJ1991 containing a wild type YIID-encoding gene (*SPT15*) on chromosome V was transformed with either pBMYIIDΔ2-57 (resulting in strain YB153) for expression of truncated YIIDΔ2-57 protein, or, as a control, pBMYIID (resulting in strain YB150) for expression of wild type YIID. The *GAL1* promoter controlling YIID expression in these vectors is relatively strong when fully induced in media with 2% galactose. By performing immunoblots on cell extracts using an anti-YIID rabbit serum, we found that the YIID concentration was increased ~5- to 10-fold in cells transformed with pBMYIID in 2% galactose media compared with cells without a YIID expression vector (data not shown). (This antiserum was specific for the amino terminus of YIID and did not detect YIIDΔ2-57.) YB153 (expressing YIIDΔ2-57 from the *GAL1* promoter) had a normal colony size on medium containing 2% raffinose (a non-fermentable sugar that does not induce or repress the *GAL1* promoter, Figure 2A) but formed only extremely small colonies on medium containing 2% galactose (which induces the *GAL1* promoter, St John and Davis, 1981, Figure 2B). In contrast, YB150, containing the plasmid pBMYIID, grew well on both media. Expression of YIIDΔ3-60 from the *GAL1* promoter (in YB154 cells) produced a similar phenotype to YB153. In contrast, cells expressing YIIDΔ2-41 (YB151) or YIIDΔ2-48 (YB152) mutant proteins in the presence of wild

Fig. 3. A comparison of the amino acid sequences at the junction between the amino terminal divergent domain and the carboxy terminal conserved domain of TFIID proteins from *S.cerevisiae*, *Pichia heedii*, *Kluyveromyces lactis* and *S.pombe*. Alignment of the various TFIID sequences is based on the strong homology present in the carboxy terminal conserved domain of all TFIID proteins. The acidic amino acids are in bold-face type. The basic residues are underlined. Numbers 51 and 61 indicate amino acid residues 51 and 61 of the *S.cerevisiae* TFIID protein (YIID).

type YIID did not display any significant change in their growth behavior (Figure 2). Therefore, when expressed from the induced *GAL1* promoter, the mutant yeast TATA factors with amino terminal deletions to residues 57 and 60 had a dominant-negative effect on cell growth. This observation indicates that the small colony phenotype associated with strain MSY122 was not caused by insufficient amounts of YIIDΔ2-57 protein present in the cell, since low levels of YIIDΔ2-57 would not be expected to produce a dominant-negative phenotype. The dominant-negative effect of the YIIDΔ2-57 deletion mutant expressed from the *GAL1* promoter was also observed in six other yeast strains with various genotypes (MSY101, LJY153, LJY155, YM214, YM259 and BJ5409; Materials and methods), indicating that this phenotype is not restricted to strain BJ1991.

Acidic residues in the amino terminal domain are required for normal YIID function

We noticed that there were one basic and three acidic residues between amino acids 48 and 57 in YIID protein. A similar number of acidic and basic residues was also present just amino terminal to the conserved domain of

TFIID from *S.pombe* and two other species of budding yeast (C.C.Kao and A.J.Berk, unpublished results; Figure 3). To examine if these charged amino acids are required for YIID to function properly *in vivo*, two more mutants were constructed in the YIID Δ 2-48 background. In one construct (pBMYIID Δ 2-48EED-VVA), the three negatively charged residues present in this region (Glu52, Glu54 and Asp56) in YIID Δ 2-48 were replaced with neutral amino acids (Val52, Val54 and Ala56). In the second construct (pBMYIID Δ 2-48K-L), the positively charged lysine residue at position 55 was changed to leucine. The two constructs were transformed into BJ1991 and the effect on cell growth of expressing these two YIID mutants was studied. Like cells expressing YIID Δ 2-48 (YB152), cells producing the YIID Δ 2-48K-L point mutant (YB156) grew normally in both galactose and raffinose containing media (Figure 2). However, YIID Δ 2-48EED-VVA had a dominant-negative effect on cell growth (YB155, Figure 2) similar to YIID Δ 2-57. To determine if the acidity or the particular sequence of this region is critical for YIID function, a third YIID mutant (YIID Δ 2-48EED-DDE) was constructed with substitutions of aspartate for glutamate at positions 52 and 54, and glutamate for aspartate at position 56. Like YB152 (YIID Δ 2-48) and YB156 (YIID Δ 2-48K-L), cells producing the YIID Δ 2-48EED-DDE protein (YB157) grew normally in both galactose and raffinose containing media (Figure 4). These results demonstrate a requirement for acidic residues but not for the basic residue in a region just amino terminal to the conserved domain of YIID for the proper function of this protein in *S.cerevisiae*.

Amino terminal deletion Δ 2-57 of YIID produces a defective transcription factor

Plasmid pBMYIID Δ 2-57 is the only source of TFIID activity in strain MSY122. The *GAL*I promoter used in this expression vector is a relatively strong promoter when fully induced. We wanted to determine if YIID Δ 2-57 causes the poor growth phenotype in MSY122 only when it is expressed from the *GAL*I promoter at a high level. To test this, we plated the cells (Figure 5) on a series of minimal medium

plates with decreasing amounts of galactose, which leads to a gradual decrease in *GAL*I promoter activity (L.Durrin and M.Grunstein, personal communication). The amount of total sugar in the medium was kept constant for each plate by the addition of raffinose. MSY103 (Figure 5, lower left corner), which expressed YIID exclusively from pBMYIID, grew normally until the galactose concentration was <0.12% in the medium. It finally lost viability on medium containing 0.01% galactose, indicating insufficient YIID to support growth. When YIID Δ 2-57 was expressed from pBMYIID Δ 2-57 in the presence of endogenous wild type YIID (YB153, upper right corner), the dominant-negative poor growth phenotype of YB153 disappeared at galactose concentrations <0.5%. At this galactose concentration, YB153 was indistinguishable from YB150 (upper left corner), which expressed wild type YIID from both the plasmid pBMYIID and the endogenous *SPT15* gene. However, MSY122, which expressed YIID Δ 2-57 from pBMYIID Δ 2-57 as its only source of TFIID activity, formed only extremely small colonies through the entire range of galactose concentrations (lower right corner). Similar results were observed on plates of rich media with varying galactose concentration (data not shown). Therefore, the poor growth phenotype of MSY122 persisted through a range of expression levels of YIID Δ 2-57, even when the level was not high enough to produce the dominant-negative effect in a strain with a wild type endogenous *SPT15* gene. These results indicate that the phenotype observed in strain MSY122 is not simply caused by overproduction of YIID Δ 2-57 protein from the *GAL*I promoter. Rather, the poor growth phenotype reflects a functional deficiency of the YIID Δ 2-57 transcription factor.

Wild type YIID and YIID Δ 2-57 have similar basal transcriptional activity *in vitro*

Mutational analysis of YIID protein demonstrated that the 180 amino acid conserved carboxy terminus is sufficient for DNA binding and basal level transcription *in vitro* (Horikoshi *et al.*, 1990). To determine if the phenotype observed with the YIID Δ 2-57 deletion could be caused by a decrease in

Fig. 4. Acidic residues in a region just amino terminal to the conserved domain of YIID are critical for YIID function. Strain BJ1991 was transformed with various plasmids expressing YIID Δ 2-48 (YB152), YIID Δ 2-48EED-VVA (YB156), YIID Δ 2-48K-L (YB155) or YIID Δ 2-48EED-DDE (YB157) under the control of the *GAL*I promoter. Colonies of the resulting strains are shown on medium containing 2% raffinose (A) or 2% galactose (B).

Fig. 5. The effect of decreasing the level of wild type YIID or YIIDΔ2-57 on cell growth. The four yeast haploid strains analyzed are diagrammed at the left. YB150 and YB153 both have the wild type chromosomal YIID-encoding gene (*SPT15*). MSY103 and MSY122 are *SPT15* null mutants (*spt15::LEU2*). In galactose-containing medium, YB150 and MSY103 both express full-length YIID from the plasmid pBMYIID, while YB153 and MSY122 express YIIDΔ2-57 from plasmid pBMYIIDΔ2-57. The effect of decreasing the level of YIID or YIIDΔ2-57 on the growth of these four strains is shown on the right. Numbers indicated above or below each plate represent the media galactose and raffinose concentrations of each corresponding plate.

Fig. 6. Basal level transcriptional activity of *in vitro* translated wild type and amino terminal deleted YIID proteins. **(A)** SDS-polyacrylamide gel analysis of wild type YIID (lane 1) and the amino terminal deleted YIID mutants YIIDΔ2-41 (lane 2), YIIDΔ2-48 (lane 3) and YIIDΔ2-57 (lane 4). Proteins were expressed in a rabbit reticulocyte lysate *in vitro* translation system in the presence of [³⁵S]methionine. **(B)** Primer extension analysis of basal level transcriptional activity. Transcription from the adenovirus early 1B promoter was measured in reactions containing no exogenously added YIID (lane 1), control reticulocyte lysate (lane 2), *in vitro* translated full-length YIID (lanes 3–5), YIIDΔ2-41 (lanes 6–8), YIIDΔ2-48 (lanes 9–11) or YIIDΔ2-57 (lanes 12–14). Based on the autoradiogram of the SDS-polyacrylamide gel shown in (A), equal numbers of *in vitro* translated YIID and the amino terminal deleted mutant protein molecules were assayed in these reactions. The expected extension products are indicated.

the basal level transcriptional activity of the YIID Δ 2-57 protein due to the slight difference between our construct and the constructs used by Horikoshi *et al.*, we carried out *in vitro* transcription studies. Wild type and amino terminal truncated YIID proteins were expressed in a rabbit reticulocyte lysate *in vitro* translation system in the presence of [³⁵S]methionine (Figure 6A, lanes 1–4). Based on the autoradiogram of the SDS protein gel, equal numbers of *in vitro* translated YIID and its amino terminal deletion mutant protein molecules were assayed for their ability to reconstitute transcription from the adenovirus E1B promoter in *in vitro* transcription reactions (Schmidt *et al.*, 1989a). Results shown in Figure 6B demonstrated that each of YIID's amino terminal deletion mutants was not only active, but had a similar specific activity to that of wild type YIID.

Amino terminal deletion Δ 2-57 of YIID affects the ability of certain promoters to respond to upstream factor stimulation *in vivo*

To further characterize the defect of the YIID Δ 2-57 mutation, we investigated the ability of cells expressing the mutant protein to induce three regulated promoters *in vivo*. Transcription from the yeast *CUP1* promoter (Karin *et al.*, 1984), *PHO5* promoter (Nakao *et al.*, 1986) and an HSP *UAS*–*CYC1* hybrid promoter (Kobayashi and McEntee, 1990) was quantified by assaying β -galactosidase activity expressed from the *Escherichia coli lacZ* gene placed under the control of each of these promoters on *CEN4*-containing plasmids. When the mutant TATA factor YIID Δ 2-57 was the only source of TFIID activity present in the cell (MSY122), cells did not survive the DNA transformation procedure. We therefore introduced the reporter plasmids into a strain (YB153) containing the wild type *SPT15* gene on chromosome V and the plasmid pBMYIID Δ 2-57, which expresses YIID Δ 2-57 protein upon galactose induction. Cells were grown in raffinose-containing medium to prevent expression of YIID Δ 2-57, and then diluted into either fresh

raffinose-containing medium or galactose-containing medium to induce the expression of YIID Δ 2-57. After approximately three cell doublings, cells were subjected to the type of inductions appropriate to their reporter plasmid. The *CUP1* gene is induced rapidly by CuSO₄ (Karin *et al.*, 1984). The *PHO5* gene is induced by lowering the concentration of inorganic phosphate (P_i) in the medium (Han *et al.*, 1988b). The HSP *UAS*–*CYC1* hybrid promoter can be induced by a brief heat shock at 37°C (Kobayashi and McEntee, 1990). There was only a minimal effect of YIID Δ 2-57 induction on growth rate after expression for only three generations. In control experiments, transcription was also measured from these reporter plasmids present in BJ1991 cells together with the wild type YIID expressing plasmid pBMYIID or the vector pBM258 without an insert.

Assay of β -galactosidase (Table I and Figure 7) following these induction protocols indicated that the three promoters tested differ in their sensitivity to the amino terminal deletion Δ 2-57 in the YIID protein. In cells expressing YIID Δ 2-57 mutant protein, *CUP1* promoter activity following CuSO₄ induction was only increased ~1.6-fold relative to its activity before induction, whereas a 7.6-fold increase was observed in control cells expressing wild type YIID from the expression vector and a 7.8-fold increase in cells containing the expression vector alone (Figure 7A). The YIID Δ 2-57 mutation, however, did not have a significant effect on the basal level activity of the *CUP1* promoter observed in the absence of added Cu²⁺. Moreover, while there was only 1.4-fold induction of the activity of HSP *UAS*–*CYC1* hybrid promoter in cells expressing YIID Δ 2-57 after heat shock, a 3.2- or 4.6-fold increase was found in control cells expressing wild type YIID from the expression vector or containing the vector alone (Figure 7B). However, following removal of inorganic phosphate from the medium, cells producing the YIID Δ 2-57 mutant protein and carrying the *PHO5*–*lacZ* reporter plasmid yielded approximately the same fold induction of β -galactosidase activity as did control

Table I. YIID Δ 2-57 affects the ability of certain promoters to respond to upstream factor stimulation *in vivo*

Reporter plasmid	Strain	Medium	β -Galactosidase activity		
			Uninduced (U)	Induced (U)	Fold induction
<i>CUP1</i> – <i>lacZ</i>	pBMYIID	raf	12.5	103.1	8.2
		gal	10.9	82.6	7.6
	pBM258	raf	9.1	92.1	10.1
		gal	8.6	66.8	7.8
	pBMYIID Δ 2-57	raf	14.0	123.4	8.8
		gal	10.8	17.6	1.6
<i>HSE</i> – <i>CYC1</i> – <i>lacZ</i>	pBMYIID	raf	3.7	14.0	3.8
		gal	2.4	7.9	3.3
	pBM258	raf	4.1	13.7	3.3
		gal	2.6	8.9	3.4
	pBMYIID Δ 2-57	raf	3.1	12.4	4.0
		gal	1.9	2.7	1.4
<i>PHO5</i> – <i>lacZ</i>	pBMYIID	raf	81.3	263.0	3.2
		gal	40.9	181.5	4.4
	pBM258	raf	93.5	306.7	3.3
		gal	44.2	191.0	4.3
	pBMYIID Δ 2-57	raf	88.7	320.5	3.6
		gal	31.9	134.1	4.2

See legend to Figure 7. Units (U) of uninduced and induced β -galactosidase are in nmol *O*-nitrophenyl- β -D-galactoside cleaved per min per mg protein.

Fig. 7. YIIDΔ2-57 affects the ability of certain promoters to respond to upstream factor stimulation *in vivo*. Transcription from the *CUP1* promoter (A), HSP *UAS* (*HSE*)–*CYC1* hybrid promoter (B) and *PHO5* promoter (C) was quantified by assaying β -galactosidase activity expressed from the *lacZ* gene placed under the control of these promoters in reporter plasmids. The reporter plasmids were introduced into yeast strains containing the plasmid pBMYIID, the vector pBM258 or the plasmid pBMYIIDΔ2-57. pBMYIID, pBM258 or pBMYIIDΔ2-57 direct expression of wild type YIID, no protein or YIIDΔ2-57 respectively in galactose-containing medium (gal, closed bar), but not in raffinose-containing medium (raf, open bar). The graph represents the fold induction of the β -galactosidase activities expressed from the *CUP1* promoter (A), *HSE*–*CYC1* hybrid promoter (B) or *PHO5* promoter (C) in various yeast strains upon inductions appropriate to each promoter.

cells (Table I and Figure 7C). Therefore, induction from the *PHO5* promoter was not affected by the expression of YIIDΔ2-57 under these conditions. The observation that the *PHO5* promoter responded normally indicates that the decreased induction observed with both the *CUP1* and HSP *UAS*–*CYC1* hybrid promoters was not due to a general toxic effect of YIIDΔ2-57 expression for three generations. In summary, these experiments indicate that in strain BJ1991 the amino terminal deletion Δ2-57 of YIID interferes with the ability of some but not all yeast promoters to respond to upstream factor stimulation *in vivo*.

Discussion

We have identified a small region just amino terminal to the conserved domain of the yeast TFIID factor (YIID) which is required for normal transcriptional activation by upstream activators in some yeast promoters *in vivo*. Deletion of YIID residues 2–41 or 2–48 had no observable effect on yeast cell growth. However, deletion of residues 2–57 or 3–60 (the entire non-conserved amino terminal domain) resulted in cells which formed only extremely small colonies. Furthermore, whereas expression of wild type YIID, YIIDΔ2-41 and YIIDΔ2-48 from the induced *GAL1* promoter had no effect on the growth of cells with a wild type YIID gene, expression of YIIDΔ2-57 and YIIDΔ3-60 from the *GAL1* promoter resulted in a dominant inhibition of cell growth. This dominant-negative phenotype indicates that the defect in cell growth produced by the YIIDΔ2-57 and YIIDΔ3-60 deletions was not due to poor expression or low stability of the mutant proteins. Low levels of the

Table II. Yeast strains used in this study

Strain	Genotype	Source
BJ1991	MAT α <i>prb1 pep4 gal2 leu2 trp1 ura3</i>	E.Jones
SEY2101	MAT α <i>ura3 leu2 suc2 gal2 ade2</i>	S.Emr
MSY100	MAT α /MAT α BJ1991 $\times$ SEY2101	This study
MSY101	MAT α /MAT α <i>SPT15/spt15::LEU2</i> the rest is the same as MSY100	This study
YB120	MAT α <i>spt15::LEU2 gal2 leu2 ura3/pBMYIIDΔ2-41</i>	This study
YB121	MAT α <i>spt15::LEU2 gal2 leu2 ade2 ura3/pBMYIIDΔ2-48</i>	This study
MSY103	MAT α <i>spt15::LEU2 gal2 leu2 ade2 trp1 ura3/pBMYIID</i>	This study
MSY122	MAT α <i>spt15::LEU2 gal2 leu2 ura3/pBMYIIDΔ2-57</i>	This study
LJY153	MAT α <i>trp1 lys2 ade2 leu2 ura3 his3 hsf1::HIS3</i>	M.Grunstein
LJY155	MAT α <i>trp1 lys2 ade2 leu2 ura3 his3 hsf1::HIS3 sir3::LEU2</i>	M.Grunstein
YM214	MAT α <i>his3 lys2 ade2 ura3</i>	M.Grunstein
YM259	MAT α <i>his3 lys2 ade2 ura3</i>	M.Grunstein
BJ5409	MAT α <i>ura3 trp1 leu2 his3</i>	E.Jones
YB150	= BJ1991 containing pBMYIID	This study
YB151	= BJ1991 containing pBMYIIDΔ2-41	This study
YB152	= BJ1991 containing pBMYIIDΔ2-48	This study
YB153	= BJ1991 containing pBMYIIDΔ2-57	This study
YB154	= BJ1991 containing pBMYIIDΔ3-60	This study
YB155	= BJ1991 containing pBMYIIDΔ2-48EED-VVA	This study
UB156	= BJ1991 containing pBMYIIDΔ2-48K-L	This study
YB157	= BJ1991 containing pBMYIIDΔ2-48EED-DDE	This study
YB158	= BJ1991 containing pBMYIID and pLD3(TRP)	This study
YB159	= BJ1991 containing pBMYIIDΔ2-57 and pLD3(TRP)	This study
YB160	= BJ1991 containing pBM258 and pLD3(TRP)	This study
YB161	= BJ1991 containing pBMYIID and pHS(TRP)	This study
YB162	= BJ1991 containing pBMYIIDΔ2-57 and pHS(TRP)	This study
YB163	= BJ1991 containing pBM258 and pHS(TRP)	This study
YB164	= BJ1991 containing pBMYIID and pMH313(TRP)	This study
YB165	= BJ1991 containing pBMYIIDΔ2-57 and pMH313(TRP)	This study
YB166	= BJ1991 containing pBM258 and pMH313(TRP)	This study

mutant protein would not be expected to produce a dominant-negative phenotype in the presence of wild type YIID. YIIDΔ2-57 had equivalent basal transcriptional activity *in vitro* to wild type YIID and can, therefore, bind to TATA boxes. This is expected from the analysis of the effects of YIID deletions on *in vitro* transcriptional and DNA binding activity by Horikoshi *et al.* (1990). Therefore, the dominant-negative effect of YIIDΔ2-57 expressed from the induced *GAL1* promoter *in vivo* is probably due to a functional deficiency of the mutant protein when it replaces wild type YIID on TATA boxes.

Although not sufficient for normal cell growth, the conserved domain of YIID was able to support yeast cell viability. This indicates that the expression of essential yeast genes is not vastly perturbed by deletion of the non-conserved amino terminal domain. However, the small colony size produced by cells expressing the YIID conserved domain alone indicates that the expression of essential genes is not optimal.

The nine residue region between amino acids 48 and 57 is rich in charged amino acids, containing three acidic and one basic residue. A comparison of the TFIID sequences from *S.cerevisiae* (Cavallini *et al.*, 1989; Hahn *et al.*, 1989; Horikoshi *et al.*, 1989; Schmidt *et al.*, 1989b), budding yeast species *Pichia heedii* and *Kluyveromyces lactis* (C.C.Kao and A.J.Berk, unpublished data) and fission yeast *S.pombe* (Fikes *et al.*, 1990; Hoffmann *et al.*, 1990) show that they all contain several negatively charged residues within this small region (Figure 3). When the three negatively charged residues present in this region in YIID (Glu52, Glu54 and Asp56) were replaced with neutral amino acids (Val52, Val54 and Ala56), the resulting YIID mutant behaved similarly to the YIIDΔ2-57 deletion mutant. However, when substitutions of aspartate for glutamate at positions 52 and 54, and glutamate for aspartate at position 56 were made within this small region, the resulting YIID mutant (YIIDΔ2-48EED-DDE) did not interfere with cell growth. Mutation of lysine at position 55 to leucine in YIIDΔ2-48 had no effect on cell growth either. These results demonstrate a requirement for acidic residues in the region just amino terminal to the conserved domain of *S.cerevisiae* TFIID. *S.pombe* TFIID has been shown to fully substitute *in vivo* for *S.cerevisiae* TFIID, even though an alignment of the amino acid sequences of both TFIID proteins reveals little homology amino terminal to the conserved domain (Fikes *et al.*, 1990). The *S.pombe* protein may be able to substitute for the *S.cerevisiae* protein because it also contains several acidic amino acids just amino terminal to the conserved domain.

Why does the deletion of residues 48–57 of YIID result in poor cell growth? We tested the obvious possibility that deletion of this region affects transcription by assaying expression from three regulated promoters after expression of YIIDΔ2-57 from the *GAL1* promoter for three generations in cells containing the wild type YIID gene. We found that uninduced expression levels from the *CUP1* and an HSP *UAS*–*CYC1* promoter hybrid control regions were unaffected by expression of YIIDΔ2-57 for three cell doublings. However, induced expression from these regulatory regions was reduced compared with that of control cells expressing the wild type YIID from the *GAL1* promoter or cells containing the *GAL1* expression vector without an inserted YIID gene. On the other hand, uninduced and induced expression from the *PHO5* regulatory region was not

significantly affected by expression of YIIDΔ2-57 under these conditions. This observation indicates that the inability to induce the *CUP1* and HSP *UAS*–*CYC1* promoter hybrid control regions normally was not due to a generalized toxic effect of the expression of YIIDΔ2-57. The results demonstrate that the region of YIID between residues 48 and 57 is required for normal transcriptional activation by the ACE1 transcription factor for the *CUP1* promoter (Fürst *et al.*, 1988) and the heat-shock transcription factor for the HSP *UAS*–*CYC1* promoter (Sorger and Pelham, 1988; Wiederrecht *et al.*, 1988). This poor response to upstream activating sequences is probably responsible for the poor growth phenotype observed for YIIDΔ2-57 and YIIDΔ3-60 *in vivo*. Peterson *et al.* (1990) demonstrated that the non-conserved amino terminus of human TFIID is required for transcriptional activation by transcription factors SP1 and CTF *in vitro*. The results reported here show that transcriptional stimulation *in vivo* in yeast by transcription factors ACE1 and HSF requires the non-conserved amino terminal sequence of YIID between residues 48 and 57.

While this manuscript was in preparation, we learned that Steven Hahn (Fred Hutchinson Cancer Research Center, Seattle) had constructed similar amino terminal deletion mutants of YIID, but did not observe the growth impaired phenotype with his mutant yeast strains (S.Hahn, unpublished results). We obtained his strains which express wild type YIID or amino terminal deleted YIID (YIIDΔ1-60), and transformed them with our construct pBMYIIDΔ2-57. We found that expression of YIIDΔ2-57 in these two strains did not produce the poor growth phenotype which was observed in the six strains tested in our experiments. Consequently, there is at least one yeast strain in which the amino terminal deletion of YIID does not interfere with cell growth. It may be informative in future work to determine what function(s) in the Hahn strain suppresses the phenotype of the YIIDΔ2-57 mutation.

Materials and methods

Recombinant plasmids and oligonucleotides

To create deletions and amino acid substitutions in the amino terminal non-conserved domain of YIID, the polymerase chain reaction (PCR) was employed using pAB24 (Schmidt *et al.*, 1989b) as the initial template. M13 universal sequencing primer 5'-GTTTTCCAGTCACGACGTT-3', which hybridizes to pAB24 at a position downstream of the YIID open reading frame, was used in all PCR reactions. The other oligonucleotides used in PCR reactions to introduce deletions or amino acid substitutions at the amino terminus of YIID and to prime the extension of the coding strands were: Δ41, 5'-GACGGATCCATTATGGAGAGTGAAGAGGACATAAAA-3' for creating YIIDΔ2-41 deletion; Δ48, 5'-GACGGATCCATTATGGCT-ACCCCAGAATCTGAAAAA-3' for creating YIIDΔ2-48 deletion; Δ57, 5'-GACGGATCCATTATGGCCTCCGCCACATCAGGTATTG-3' for creating YIIDΔ2-57 deletion; Δ60, 5'-GACGGATCCATTATGGCATCA-GGTATTGTTCCAACAC-3' for creating YIIDΔ3-60 deletion; Δ48K-L, 5'-GACGGATCCATTATGGCTACCCCAGAATCTGAATTAGACAC-CTCCGCCACA-3' for the replacement of Lys55 with Leu55 in YIIDΔ2-48; Δ48EED-VVA, 5'-GACGGATCCATTATGGCTACCCCAGTTTCT-GTTAAAGCCACCTCCGCCACATC-3' for the replacement of Glu52, Glu54 and Asp56 with Val52, Val54 and Ala56 in YIIDΔ2-48; Δ48EED-DDE, 5'-GACGGATCCATTATGGCTACCCCAGACTCTGACAAA-GAAACCTCCGCCACATC-3' for the replacement of Glu52, Glu54 and Asp56 with Asp52, Asp54 and Glu56 in YIIDΔ2-48.

PCR reactions, which amplified DNA fragments encoding YIID proteins with various deletions or amino acid substitutions at its amino terminus, were carried out essentially as described (Kao *et al.*, 1990). The PCR-amplified DNA fragments were cleaved with *Bam*HI restriction enzyme and inserted in the correct orientation into the *Bam*HI site of the yeast expression vector pBM258 (Johnston and Davis, 1984), under the control of the *GAL1* promoter. The resulting plasmids were designated

pBMYIIDΔ2-41, pBMYIIDΔ2-48, pBMYIIDΔ2-57, pBMYIIDΔ3-60, pBMYIIDΔ2-48K-L, pBMYIIDΔ2-48EED-VVA and pBMYIIDΔ2-48EED-DDE, respectively. To be certain that the phenotype produced by YIIDΔ2-57 was due to the deletion of residues 2–57 and not to other mutations introduced during construction, several independent constructions of pBMYIIDΔ2-57 were prepared. After transformation into BJ1991, all produced the dominant-negative inhibition of cell growth in media with 2% galactose. Two independent constructs were used to transform MSY101. Sporulation of the transformants yielded haploid cells with identical growth phenotypes on galactose plates. No alterations in pBMYIIDΔ2-57 plasmids were detected when they were recovered from yeast cells by transformation of *E. coli* and digested with several four-base specific restriction enzymes.

To construct plasmid pBMYIID, which expresses full-length YIID in yeast, a *Bam*HI site was engineered at the *Spe*I site in front of the YIID open reading frame in pAB24. A 1.2 kb *Bam*HI fragment containing the entire YIID gene and its 3' transcription termination signal was subsequently isolated from the modified plasmid and inserted into the *Bam*HI site of pBM258 behind the *GAL*I promoter.

To construct plasmids producing amino terminal deleted YIID proteins in an *in vitro* translation system, the *Bam*HI digested PCR fragments encoding YIIDΔ2-41, YIIDΔ2-48 and YIIDΔ2-57 were cloned into the *Bam*HI site in the polylinker region of pBluescript II SK (Stratagene), under the control of the bacteriophage T3 promoter. The plasmids thus obtained were designated pBSYIIDΔ2-41, pBSYIIDΔ2-48 and pBSYIIDΔ2-57. To generate pBSYIID expressing wild type YIID *in vitro*, a *Bam*HI site was first introduced at the –10 position of the YIID open reading frame in pAB24. A *Bam*HI fragment containing the YIID gene was cloned into the *Bam*HI site of pSP64 (Promega) behind the SP6 promoter, creating plasmid pSP64-YIID. Subsequently, an *Eco*RI–*Sal*I DNA fragment containing the entire YIID open reading frame was isolated from pSP64-YIID and inserted into pBluescript II KS between the *Eco*RI and *Sal*I sites, resulting in plasmid pBSYIID.

The *CUP*I promoter–*lacZ* gene fusion pLD3(TRP) and the *PHO*5 promoter–*lacZ* gene fusion pMH313(TRP) were kindly provided by L. Durrin and M. Grunstein. pMH313(TRP) was essentially the same as pMH313 (Han and Grunstein, 1988), except that the *URA*3 marker in the vector (pSEYC102, Emr *et al.*, 1986) was replaced with the *TRP*I marker. The *CUP*I promoter in pLD3(TRP) contains sequences between –241 and +57 of the *CUP*I gene (Karin *et al.*, 1984) subcloned into the modified pSEYC102 vector. To prepare the HSP *UAS*–*CYC*1 hybrid promoter–*lacZ* gene fusion, pHS(TRP), an *Eco*RI–*Cl*aI fragment containing two copies of the heat-shock element (HSE), *CYC*1 TATA box, and a piece of DNA encoding the amino terminal portion of the *lacZ* protein was isolated from pCTHSE (Kobayashi and McEntee, 1990) and cloned into the modified pSEYC102 vector between the *Eco*RI and *Cl*aI sites.

Yeast strains

Yeast strains used in the study are listed in Table II. MSY101 was constructed by replacing one of the *SPT*15 alleles of MSY100 with the *LEU*2 gene. The 2.2 kb *Xho*I–*Sal*I fragment containing the entire *LEU*2 gene (Andreadis *et al.*, 1982) was engineered with *Xba*I linkers and inserted into the *Spe*I and *Xba*I sites (–105 to +512 relative to the initiating AUG codon of the TFIID open reading frame) of pAB24 to create pAB24LEU. Genomic DNA from *Leu*⁺ transformants was analyzed by Southern blot to confirm that the *LEU*2 gene had inserted at the *SPT*15 locus (data not shown).

In vitro transcription and translation

The *in vitro* transcription and translation reactions used to express the wild type YIID and its amino terminal deletion mutant proteins YIIDΔ2-41, YIIDΔ2-48 and YIIDΔ2-57 were performed according to the manufacturer (Promega) with T3 RNA polymerase (Stratagene). pBSYIID, pBSYIIDΔ2-41, pBSYIIDΔ2-48 and pBSYIIDΔ2-57 (3 μg each), linearized at an *Xho*I site downstream of each open reading frame, were transcribed with 50 units of T3 RNA polymerase. One-fifth of each RNA sample was used in the *in vitro* translation reaction containing rabbit reticulocyte lysate and [³⁵S]methionine (20 μCi, 1200 Ci/mmol). For quantitative analysis of translated proteins, aliquots (3 μl each) were analyzed by SDS–PAGE. Since YIID and its three amino terminal deletion mutants all have the same numbers of methionine residues, they are labeled to similar specific activity. The amount of full-length YIID or truncated YIID proteins used in transcription assays was standardized by densitometric scanning of the protein bands shown in the autoradiogram of Figure 3A.

Transcription assays

Transcriptional activities of the wild type YIID and its amino terminal deletion mutants produced *in vitro* were assayed as described (Schmidt *et al.*, 1989a). The plasmid template used in the assay was pAD1B which contains the

adenovirus type 2 early 1B promoter region. Phosphocellulose B and C fractions of a HeLa cell nuclear extract containing RNA polymerase II and general transcription factors but not TFIID activity were used to reconstitute transcription from the E1B promoter. Oligonucleotide B₁₇₅₀ was used for primer extension assays.

Media, gene induction methods and β-galactosidase assays

YEPR, YEPG and YEPD represent rich media containing 2% raffinose, galactose and glucose respectively. SR-ura-trp and SG-ura-trp stand for raffinose- or galactose-containing minimal media lacking uracil and tryptophan respectively. High and low phosphate media were prepared as described (Han *et al.*, 1988). Cells containing the *CUP*I–*lacZ* reporter plasmids (YB158–160) were grown in SR-ura-trp media till confluent, and then washed and aliquoted into either SR-ura-trp or SG-ura-trp medium. After 13.5 h incubation at 30°C, these cells were induced for 30 min in the same medium with 1 mM CuSO₄ (Han *et al.*, 1988). Strains containing the HSP *UAS*–*CYC*1–*lacZ* reporter plasmids (YB161–163) were grown in SR-ura-trp media till confluent, diluted into either SR-ura-trp or SG-ura-trp medium, incubated at 25°C for 15 h, and finally induced with a 60 min heat shock at 37°C (Kobayashi and McEntee, 1990). Cells containing the *PHO*5–*lacZ* plasmids (YB164–166) were grown in SG-ura-trp media till confluent, and aliquoted into SR-ura-trp or SG-ura-trp medium. After 8 h incubation at 30°C, these cells were washed and induced in either low phosphate YEPR or low phosphate YEPG medium for 6 h. β-Galactosidase assays were performed as described (Guarente, 1983).

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