Incorporation of *D o;ophila* TAF110 into the yeast TFIID complex does not permit the Sp1 glutamine-rich activation domain to function *in i o*

Marie Keaveney and Kevin Struhl*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

Abstract

***Background*: Acidic activation domains function across eukaryotic species, and hence stimulate transcription by a conserved molecular mechanism. In contrast, glutamine-rich activation domains function in flies, mammals, and fission yeasts but not in the budding yeast *Saccharomyces cerevisiae*. The glutamine-rich activation domain of Sp1 interacts with TAF110, and it has been suggested that this interaction is important for transcriptional activation. *S. cerevisiae* does not contain a homologue of TAF110, suggesting a potential mechanism to account for the failure of glutamine-rich activation domains to stimulate transcription.**

***Results*: Here, we have artificially recruited *Drosophila* TAF110 into the yeast TFIID complex by fusing it to yeast TBP. The resulting TFIID complex supports normal cell growth, but it is unable to mediate Sp1-dependent activation.**

***Conclusions*: Thus, the interaction of glutamine-rich activation domains with TAF110 is insufficient for transcriptional activation *in vivo*, indicating that other targets within the *Pol*II machinery are necessary.**

Introduction

Transcriptional activator proteins bind to enhancer elements and stimulate gene expression via activation domains. It is generally believed that the activation domains function by interacting with components of the RNA polymerase II (*Pol*II) transcription machinery and thereby recruiting this *Pol*II machinery to the TATA and initiator elements of promoters (Struhl 1996; Ptashne & Gann 1997; Keaveney & Struhl 1998). There are many potential activator targets in both the TFIID and the *Pol*II holoenzyme complexes, but it is unclear which of these are physiologically significant.

Activation domains are operationally defined by their ability to stimulate transcription when tethered to DNA-binding domains, and they have been classified into different types (acidic, glutamine-rich, proline-rich, isoleucine-rich) largely based on the predominant residues in the primary amino acid sequence. Acidic activation domains are functionally interchangeable across widely divergent eukaryotic organisms, suggesting that they operate by a conserved mechanism involving conserved targets in the *Pol*II machinery. In contrast, glutamine-rich activation domains are inactive in *Saccharomyces cerevisiae* (Kunzler *et al.* 1994; Ponticelli *et al.* 1995), although they function in mammals, flies, and the fission yeast *Schizosaccharomyces pombe* (Remacle *et al.* 1997). The functional distinction between the two yeast species is not particularly surprising, because *S. pombe* is evolutionary distant from *S. cerevisiae*, and in several respects, more closely related to mammalian cells (Baldauf & Palmer 1993; Wainright *et al.* 1993; Glick 1996). Many *S. cerevisiae* transcriptional regulatory proteins (e.g. TAF61, Gal11, Snf5, Cyc8, Hap1, Hap2, Mcm1, Pho2) contain long stretches of glutamine residues, but in all cases tested, there is no evidence that such regions are functionally important. In apparent contrast to these results, a recent report suggested that the fusion of Sp1 or two other glutamine-rich regions to the Gal4 DNA-binding domain (residues 1–147) can stimulate transcription in *S. cerevisiae* (Xiao & Jeang 1998). However, such activation was extremely weak (1–2% of the maximal

Communicated by: Akira Ishihama
* *Correspondence:* E-mail: kevin@hms.harvard.edu

level) and observed only on multicopy reporters, and it is confounded by the cryptic activation domain that is present in the Gal4(1–147) moiety (Lin *et al.* 1988).

The failure of glutamine-rich activators to stimulate transcription in budding yeasts strongly suggests that the target(s) of this class of activators is absent or functionally divergent in *S. cerevisiae*. Although it was initially suggested that yeast and human TBP differed in their ability to support the response to the glutamine-rich activator Sp1 *in vitro* (Pugh & Tjian 1990), subsequent experiments have indicated that functional distinctions between these TBPs do not account for the failure of glutamine-rich activators to function in *S. cerevisiae* (Ponticelli *et al.* 1995). More recently, it has been suggested that TAF110, a component of the *Drosophila* TFIID complex, is a target for mediating the response to glutamine-rich activators. TAF110 interacts *in vitro* with the glutamine-rich activation domains of Sp1 and other proteins (Hoey *et al.* 1993), and the strength of this biochemical interaction is correlated with the degree of transcriptional activation *in vivo* (Gill *et al.* 1994). TFIID reconstitution experiments indicate that TAF110 is required for the response to Sp1; a combination of TBP, TAF250 and TAF110 confers a modest activation by Sp1, and the addition of TAF150 confers robust Sp1-dependent activation (Chen *et al.* 1994). TAF110 also interacts with a variety of other activators (Schulman *et al.* 1995; DeFalco & Childs 1996; Lala *et al.* 1996; Coustry *et al.* 1998). Interestingly, *S. cerevisiae* does not contain a homologue of TAF110, although it contains homologues for all other TAFs found in flies and mammals (Moqtaderi *et al.* 1996). Taken together, these observations prompt the hypothesis that glutamine-rich activators might fail to function in *S. cerevisiae* due to a lack of TAF110.

Here, we have tested this hypothesis by generating an artificial *S. cerevisiae* TFIID complex containing *Drosophila* TAF110. The resulting TFIID complex supports normal cell growth, but is unable to mediate Sp1-dependent activation. Thus, the presence of TAF110 in the TFIID complex is not sufficient to reconstitute Sp1-mediated transcriptional enhancement in *S. cerevisiae*, indicating that other components of the *Pol*II machinery are necessary for species-specific activation by glutamine-rich activation domains.

Results

Experimental design

To determine if the lack of a TAF110 homologue is the sole limiting factor in *S. cerevisiae*, we wished to incorporate *Drosophila* TAF110 into the yeast TFIID complex. Because it seemed unlikely that TAF110 would stably associate with yeast TFIID, we artificially generated a stable association by fusing TAF110 to yeast TBP (Fig. 1A). In addition, we fused TBP to the N-terminal 308 residues of TAF110, because this region is necessary and sufficient for *in vitro* and two-hybrid interactions with the glutamine-rich activation domains of Sp1 (Hoey *et al.* 1993; Gill *et al.* 1994). The TAF110 derivatives were fused directly to the

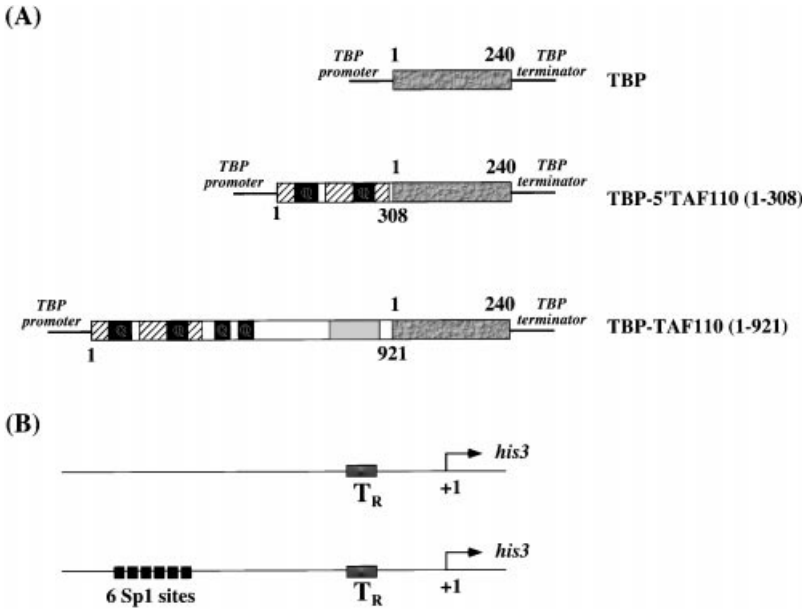

Figure 1 TBP derivatives and promoters used in this study. (A) Full-length yeast TBP (grey bar; residues 1–240) was fused to the indicated portions of TAF110 (open bar with serine/threonine-rich, glutamine-rich and highly charged regions shown, respectively, as hatched, black and light grey bars. All TBP derivatives are expressed under the control of the natural TBP promoter and termination sequence. (B) Diagram of the minimal *his3* promoter (T_R TATA element) and the *his3* promoter containing six upstream Sp1 binding sites.

N-terminus of TBP because DNA-binding domains (Chatterjee & Struhl 1995; Klages & Strubin 1995; Xiao *et al.* 1995), acidic activation domains (Keaveney & Struhl 1998), and TFIIA subunits (Stargell & Struhl 1995) have been fused to TBP at this location without affecting TBP function. The fusion of TAF110 to yeast TBP ensures that TAF110 is actually incorporated into the yeast TFIID complex.

Plasmids expressing the TAF110-TBP fusions were introduced into yeast strains that have previously been used to analyse transcriptional activation by Sp1 (Ponticelli *et al.* 1995). These strains contain chromosomally located *his3* derivatives with zero or six Sp1 binding sites (GC boxes) upstream of the *his3* TATA region, as well as multicopy plasmids that do or do not express full-length Sp1 or an Sp1/Gcn4 fusion (Fig. 1B). Transcriptional activation by Sp1 is determined by the ability of the relevant strains to grow on media containing aminotriazole (AT), a competitive inhibitor of the *his3* gene product.

TAF110-TBP fusions support yeast cell viability, but do not permit Sp1 to activate transcription

Expression of the TBP-TAF110 fusions (or TBP itself) in the above strains does not confer increased growth in the presence of AT (Fig. 2, compare panel D to panels A–C). In fact, there is reduced growth in the strain that over-expresses Sp1 and contains the six Sp1 binding sites in the *his3* promoter (panel D). This repression phenomenon has been observed before (Ponticelli *et al.* 1995), and it most likely reflects Sp1 binding at its target

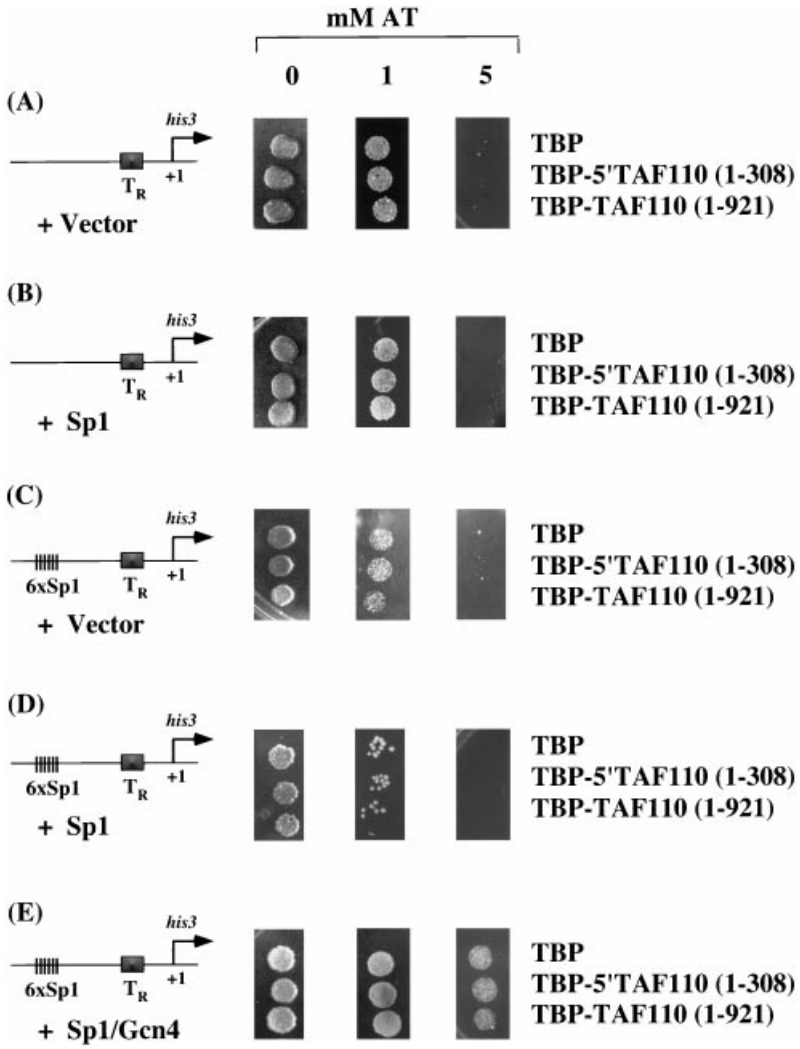

Figure 2 Sp1 fails to mediate transcriptional activation, even in the presence of TBP-TAF110 hybrid proteins. Strains containing the indicated TBP derivative (right side) and *his3* promoter and Sp1 derivative (left side) were assayed for *his3* expression by growth on synthetic complete (SC) medium containing the indicated concentrations of aminotriazole (AT).

sites and reducing the access of the *Pol*II machinery to the *his3* promoter. Further evidence for Sp1 binding was obtained by analysing a hybrid protein containing the acidic activation domain of Gcn4 fused to the DNA-binding domain of Sp1. In the strain containing six Sp1 binding sites, this Sp1-Gcn4 fusion protein stimulated high levels of *his3* expression, as evidenced by its ability to confer strong growth in the presence of high AT concentrations (panel E). Thus, Sp1 binds avidly to its target sites upstream of the *his3* promoter *in vivo*, but it fails to activate transcription, even in the presence of the TAF110-TBP fusion proteins.

The failure of the TAF110-TBP fusions to mediate Sp1-dependent activation could be explained by a failure to form functional TFIID complexes due to competition with wild-type TBP molecules expressed from the normal chromosomal locus. To eliminate this possibility, we performed similar experiments in strains deleted for the chromosomal TBP locus. Specifically, we generated derivatives of the four above strains in which the sole copy of TBP was present on a *URA3*-marked centromeric plasmid. These strains were transformed by plasmids expressing the TBP-TAF110 fusions, and the *URA3*-marked plasmid containing wild-type TBP was selectively removed by growth in a medium containing 5-fluoro orotic acid (FOA).

The resulting strains, which contain the TAF110-TBP fusions as the sole source of TBP, grow indistinguishably from the wild-type strains in the presence of FOA (Fig. 3A) and in other conditions, indicating that the fusion proteins do not detectably affect cell physiology. Thus, fusion of the full length or amino terminal portion of TAF110 does not significantly affect the biological function of TBP. Most importantly, as yeast TAFs are essential for cell viability (Reese *et al*. 1994; Poon *et al*. 1995; Moqtaderi *et al*. 1996), these results indicate that TAF110 is integrated into a functional yeast TFIID complex. The fusion proteins can be detected by Western blot analyses, and there is no evidence for a fortuitous cleavage event that releases TBP (Fig. 3B).

In strains containing TAF110-TBP fusions as the sole source of TBP, we observe no evidence for Sp1-mediated activation through its binding sites upstream of the *his3* promoter (Fig. 4). Instead, the strain containing Sp1 and the *his3* promoter with Sp1 binding sites (panel C) shows a decreased growth in the presence of AT, again providing evidence for DNA binding, but not transcriptional activation *in vivo*. Thus, the presence of TAF110 in a functional yeast TFIID complex is not sufficient to reconstitute Sp1-mediated transcriptional activation in *S. cerevisiae* cells.

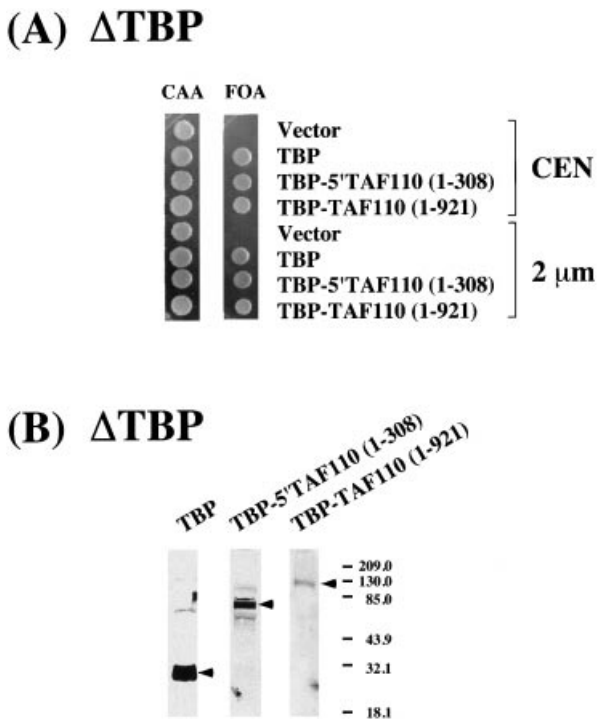

Figure 3 TBP-TAF110 derivatives support normal cell growth. (A) Complementation assays. Centromeric or multicopy (2μ) plasmids expressing the indicated TBP derivative were introduced into BYΔ2 cells, and the resulting strains were tested on glucose minimal medium containing casamino acids (CAA) or 5-fluoro orotic acid (FOA). Growth in FOA-containing medium shuffles out the plasmid expressing wild-type TBP, and hence depends on the introduced TBP derivative. (B) Western blot analysis. Electrophoretically separated proteins (50 μg) from yeast strains which are deleted for the chromosomal copy of TBP but contain the indicated TBP derivative on a multicopy plasmid were probed with an anti-TBP antibody, and visualized using the alkaline phosphatase detection system. Arrows indicate the position of full-length TBP derivatives as determined by comparison with molecular weight markers at right.

Discussion

The experiments described here indicate that the incorporation of TAF110 into the yeast TFIID complex does not permit the glutamine-rich activation domain of Sp1 to stimulate transcription *in vivo*. It is extremely unlikely that the failure to activate represents a negative result due to some peculiarity of the experimental design. Specifically, we have shown that the Sp1 DNA-binding domain can functionally interact with its target sequences *in vivo*, and that the TBP-TAF110 hybrid protein can support normal cell growth, and hence is transcriptionally competent for thousands of natural yeast genes. Moreover, the TBP-TAF110 hybrid

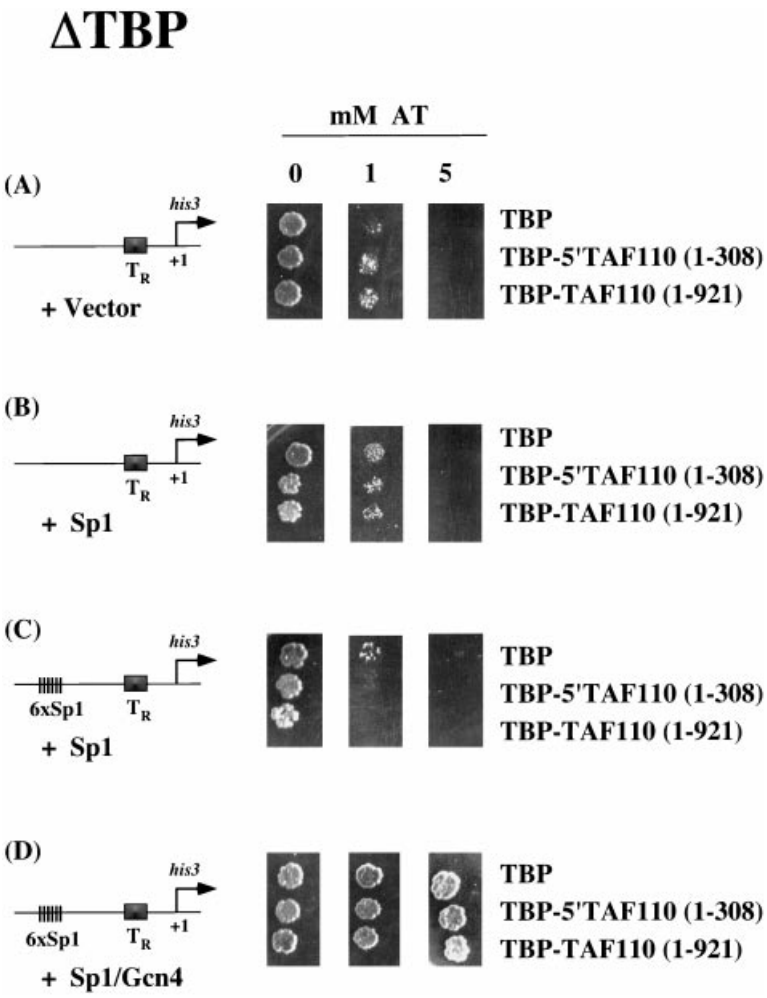

Figure 4 TBP-TAF110 derivatives do not support Sp1-dependent activation even in the absence of chromosomally encoded TBP. The experiment was performed as described in Fig. 2, except that the indicated hybrid proteins are the only source of TBP.

protein appears to be full-length, and there is no evidence for proteolysis leading to free TBP. Although we can not directly assess the structure or function of the TAF110 moiety in the context of the TBP-TAF110 hybrid protein, related TAF110 hybrid proteins are capable of two–hybrid interactions with the Sp1 glutamine-rich activation domain in a manner consistent with biochemical interactions (Gill *et al.* 1994). Thus, our results indicate that the inability of the Sp1 glutamine-rich activation domain to function in *S. cerevisiae* is not due simply to the absence of a TAF110 homologue.

There are several explanations to account for why the glutamine-rich activation domain of Sp1 cannot stimulate transcription in yeast cells, even when TAF110 is incorporated into a functional TFIID complex. One possibility is that the biochemical (Hoey *et al.* 1993) and two-hybrid (Gill *et al.* 1994) interactions of the Sp1 glutamine-rich activation domain with TAF110 might not occur in the context of a transcription complex assembled at the promoter under physiological conditions. In this view, the Sp1 glutamine-rich activation domain would not stimulate transcription via TAF110, but would rather utilize another target(s) that is absent or functionally diverged in *S. cerevisiae*. In accordance with this possibility, *S. pombe* supports the response to glutamine-rich activation domains *in vivo* (Remacle *et al.* 1997), even though it does not appear to have a homologue of TAF110 (approximately 70% of the genome has been sequenced). Thus, even though it is possible *in vitro* to generate conditions for a TAF110-dependent response to the Sp1 glutamine-rich activation domain (Chen *et al.* 1994), TAF110 might not be required (or even important) for this response *in vivo*.

Alternatively, the interaction between the Sp1 glutamine-rich activation domain and TAF110 might occur under physiological conditions, but be insufficient by itself to stimulate transcription *in vivo*. In this regard, *Drosophila* TAF250 and TAF150 were

important for Sp1 to activate transcription in a TAF110-dependent manner *in vitro* (Chen *et al.* 1994); perhaps the yeast homologues of TAFs 250 and 150 (TAF130 and Tsm1, respectively) are incapable of supporting Sp1 mediated activation. Finally, the failure of the Sp1 glutamine-rich activation domain to stimulate transcription in *S. cerevisiae* might not reflect differences in targets *per se*, but rather basic differences in promoter organization. For example, glutamine-rich activation domains function in a variety of organisms (fission yeasts, flies and mammals) whose promoters contain TATA elements located approximately 30 nucleotides upstream from the initiation site. In contrast, the distance between TATA elements and mRNA initiation sites in *S. cerevisiae* promoters (40–100 bp) is larger and more variable.

Glutamine-rich regions are found in a variety of mammalian and yeast transcriptional regulatory proteins. In general, glutamine-rich regions in yeast proteins do not support transcriptional activation, although a recent report indicated that certain glutamine-rich regions can activate transcription to a very limited extent (Xiao & Jeang 1998). This observation suggests that a high proportion of glutamine residues might not be relevant for transcriptional activation, but rather for some other biological function. In this regard, a detailed analysis of the Sp1 activation domain indicates that bulky hydrophobic residues are much more important than glutamine residues for transcriptional activation (Gill *et al.* 1994). Furthermore, some glutamine-rich domains fail to interact with TAF110 (Hoey *et al.* 1993). For these reasons, not all transcriptional activators with a preponderance towards glutamine-rich regions need to function by the same molecular mechanism or to interact with the same coactivators.

Despite the above complexities involving glutamine-rich activation domains, it is clear that Sp1 represents a transcriptional activator protein whose function is species-specific. Our results strongly argue against the idea that species-specificity is determined by the presence or absence of TAF110. In addition, the results suggest that transcriptional activation by Sp1 cannot be explained simply by an interaction with TAF110 that recruits TFIID to promoters, and that other targets within the *Pol*II machinery are necessary.

Experimental procedures

DNAs

The yeast TBP-*Drosophila* TAF110 fusion DNAs were constructed by amplifying the relevant *Drosophila* TAF110 fragments from the plasmid pTb-TAF110. The 5′TAF110 (950 bp) and full-length TAF110 fragments (2780 bp) were digested with *Mfe*I and *Eco*RI and cloned into the *Eco*RI site immediately upstream of the yeast TBP open reading frame in the YCplac22-RI/TBP vector (Lee & Struhl 1997). These fusions cassettes are all under the control of the TBP promoter and are followed by the TBP termination signal. High copy *TRP1*- and *URA3*-marked versions of these DNAs were constructed in the yeast vectors YEplac112 and YEplac195, respectively (Gietz & Sugino 1988).

Yeast strains

The starting strains used for Sp1-dependent activation assays have previously been described (Ponticelli *et al.* 1995). For the experiment in Fig. 2, plasmids expressing the TAF110-TBP fusions were introduced into strains containing a wild-type chromosomal TBP locus. For the experiments in Fig. 4, the starting strains were all deleted for the chromosomal TBP locus, with TBP function being provided by a human TBP mutant with a single amino acid change at position 231 (R231K), the lysine substitution being essential for cell viability in *S. cerevisiae* (Cormack *et al.* 1994). Plasmids expressing the TAF110-TBP hybrid proteins were introduced into these strains, and the TBP-R231K plasmid was shuffled out.

Expression of TBP derivatives in yeast

Yeast strains were grown in casamino acid media lacking tryptophan to mid-log phase. The cells were subsequently harvested and lysed in a buffer containing 25 mM Tris-phosphate (pH 6.7) and 2 mM PMSF by vortexing with glass beads. For Western blot detection, 30–50 μg of protein was electrophoresed on 10% SDS–polyacrylamide gels and electroblotted to a nitrocellulose membrane. Primary antibody hybridization using a polyclonal anti-TBP (dilution 1:5000) was followed by a rabbit secondary antibody (1:10 000 dilution) incubation. Subsequent washing of the membrane was performed by standard techniques, and the proteins were visualized using the alkaline phosphatase detection system.

Phenotypic analysis

Complementation assays for the TBP-TAF110 fusions were performed in the yeast strain BYΔ2 using a plasmid shuffling technique (Cormack *et al.* 1991). To assay for activated *his3* transcription, strains were purified on synthetic complete media lacking tryptophan and leucine. Serial dilutions (10^5–10^2) from overnight cultures were spotted on synthetic complete media lacking the amino acids histidine, tryptophan and leucine and containing increasing concentrations (0–50 mM) of aminotriazole (AT), a competitive inhibitor of the *his3* gene product. The plates were incubated at 30 °C for 3–4 days.

Acknowledgements

We thank Fred Ponticelli, Grace Gill and Laurie Stargell for the yeast strains, plasmid DNAs and anti-TBP antibody. This work

was supported by a National Institutes of Health Postdoctoral Fellowship (GM17903) to M.K. and by grants from the National Institutes of Health (GM30186) and the Lucille Markey Trust to K.S.

References

Baldauf, S.L. & Palmer, J.D. (1993) Animals and fungi are each other's closest relatives: congruent evidence from multiple proteins. *Proc. Natl. Acad. Sci. USA* **50**, 11558–11562.

Chatterjee, S. & Struhl, K. (1995) Connecting a promoter-bound protein to the TATA-binding protein overrides the need for a transcriptional activation region. *Nature* **374**, 820–822.

Chen, J.-L., Attardi, L.D., Verrijzer, C.P., Yokomori, K. & Tjian, R. (1994) Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. *Cell* **79**, 93–105.

Cormack, B.P., Strubin, M., Ponticelli, A.S. & Struhl, K. (1991) Functional differences between yeast and human TFIID are localized to the highly conserved region. *Cell* **65**, 341–348.

Cormack, B.P., Strubin, M., Stargell, L.A. & Struhl, K. (1994) Conserved and nonconserved functions of yeast and human TATA-binding proteins. *Genes Dev.* **8**, 1335–1343.

Coustry, F., Sinha, S., Maity, S.N. & DeCrombrugghe, B. (1998) The two activation domains of the CCAAT-binding factor CBF interact with the dTAF$_{II}$110 component of the *Drosophila* TFIID complex. *Biochem. J.* **331**, 291–297.

DeFalco, J. & Childs, G. (1996) The embryonic transcription factor stage specific activator protein contains a potent bipartite activation domain that interacts with several RNA polymerase II basal transcription factors. *Proc. Natl. Acad. Sci. USA* **93**, 5802–5807.

Gietz, R.D. & Sugino, A. (1988) New yeast-*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. *Gene* **74**, 527–534.

Gill, G., Pascal, E., Tseng, Z.H. & Tjian, R. (1994) A glutamine-rich hydrophobic patch in transcription factor Sp1 contacts the dTAF$_{II}$110 component of the *Drosophila* TFIID complex and mediates transcriptional activation. *Proc. Natl. Acad. Sci. USA* **91**, 192–196.

Glick, B.S. (1996) Cell biology: alternatives to baker's yeast. *Curr. Biol.* **6**, 1570–1572.

Hoey, T., Weinzierl, R.O.J., Gill, G., Chen, J.-L., Dynlacht, B.D. & Tjian, R. (1993) Molecular cloning and functional analysis of *Drosophila* TAF110 reveal properties expected of coactivators. *Cell* **72**, 247–260.

Keaveney, M. & Struhl, K. (1998) Activator-mediated recruitment of the RNA polymerase II machinery is the predominant mechanism for transcriptional activation in yeast. *Mol. Cell* **1**, 917–924.

Klages, N. & Strubin, M. (1995) Stimulation of RNA polymerase II transcription initiation by recruitment of TBP *in vivo*. *Nature* **374**, 822–823.

Kunzler, M., Braus, G.H., Georgiev, O., Seipel, K. & Schaffner, W. (1994) Functional differences between mammalian transcription activation domains at the yeast *GAL1* promoter. *EMBO J.* **13**, 641–645.

Lala, D.S., Mukherjee, R., Schulman, I.G., *et al.* (1996) Activation of specific RXR heterodimers by an antagonist of RXR homodimers. *Nature* **383**, 450–453.

Lee, M. & Struhl, K. (1997) A severely defective TATA-binding protein–TFIIB interaction does not preclude transcriptional activation *in vivo*. *Mol. Cell. Biol.* **17**, 1336–1345.

Lin, Y.-S., Carey, M.F., Ptashne, M. & Green, M.R. (1988) GAL4 derivatives function alone and synergistically with mammalian activators *in vitro*. *Cell* **54**, 659–664.

Moqtaderi, Z., Yale, J.D., Struhl, K. & Buratowski, S. (1996) Yeast homologues of higher eukaryotic TFIID subunits. *Proc. Natl. Acad. Sci. USA* **93**, 14654–14658.

Ponticelli, A.S., Pardee, T.S. & Struhl, K. (1995) The glutamine-rich activation domains of human Sp1 do not stimulate transcription in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **15**, 983–988.

Poon, D., Bai, Y., Campbell, A.M., *et al.* (1995) Identification and characterization of a TFIID-like multiprotein complex from *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **92**, 8224–8228.

Ptashne, M. & Gann, A. (1997) Transcriptional activation by recruitment. *Nature* **386**, 569–577.

Pugh, B.F. & Tjian, R. (1990) Mechanism of transcriptional activation by Sp1: evidence for coactivators. *Cell* **61**, 1187–1197.

Reese, J.C., Apone, L., Walker, S.S., Griffin, L.A. & Green, M.R. (1994) Yeast TAF$_{II}$s in a multisubunit complex required for activated transcription. *Nature* **371**, 523–527.

Remacle, J.E., Albrecht, G., Brys, R., Braus, G.H. & Huylebroeck, D. (1997) Three classes of mammalian transcription activation domain stimulate transcription in *Schizosaccharomyces pombe*. *EMBO J.* **16**, 5722–5729.

Schulman, I.G., Chakravati, D., Juguilon, H., Romo, A. & Evans, R.M. (1995) Interactions between the retinoid X receptor and a conserved region of the TATA-binding protein mediate hormone-dependent transactivation. *Proc. Natl. Acad. Sci. USA* **92**, 8288–8292.

Stargell, L.A. & Struhl, K. (1995) The TBP–TFIIA interaction in the response to acidic activators *in vivo*. *Science* **269**, 75–78.

Struhl, K. (1996) Chromatin structure and RNA polymerase II connection: Implications for transcription. *Cell* **84**, 179–182.

Wainright, P.O., Hinkle, G., Sogin, M.L. & Stickel, S.K. (1993) Monophyletic origins of the metazoa: an evolutionary link with fungi. *Science* **260**, 340–342.

Xiao, H., Friesen, J.D. & Lis, J.T. (1995) Recruiting TATA-binding protein to a promoter: transcriptional activation without an upstream activator. *Mol. Cell. Biol.* **15**, 5757–5761.

Xiao, H. & Jeang, K.-T. (1998) Glutamine-rich domains activate transcription in yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* **273**, 22873–22876.

Received: 13 January 1999
Accepted: 2 March 1999