

# The TBP-TFIIA Interaction in the Response to Acidic Activators in Vivo

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A yeast TBP mutant (N2-1) is described here that is defective specifically in responding to acidic activators in vivo. N2-1 does not support activation by Gal4, Ace1, and Gcn4, but appears unaffected for constitutive transcription, repression by the Cyc8-Tup1 and Not complexes, and transcription by polymerase I (Pol) and Pol III. In vitro, N2-1 fails to interact with TFIIA, but it associates normally with a TATA element, an acidic activation domain, and TFIIIB. Fusion of the small subunit of TFIIA to N2-1 restores activation function in vivo. Thus, an efficient interaction between TBP and TFIIA is required for transcriptional activation in vivo.

RNA Pol II transcription is regulated by gene-specific activator proteins that interact with enhancer elements located distal to the site of transcription initiation. Activators generally contain a DNA-binding domain and a separable activation domain (or domains) that stimulate the transcription machinery. Many activation domains are characterized by a large proportion of acidic amino acids. Such acidic activation domains function in a wide variety of eukaryotic species, which is indicative of a highly conserved mechanism of transcriptional activation.

Most models for activation invoke protein-protein interactions between activators bound at enhancer elements and the general transcription factors assembled at the TATA and initiation elements (1). These general factors include the TATA-box binding protein (TBP), TBP-associated factors (TAFs) that are components of the Pol II-specific TFIID complex, as well as TFIIA, -B, -E, -F, -H, -J, and Pol II (2). In vitro, activation domains can directly interact with TBP (3), TAF110 and TAF40 (4), TFIIA (5), TFIIIB (6), and TFIID (7). Biochemical studies suggest that some of these interactions are important for transcriptional activation in vitro (4, 8–10). In addition, several TBP mutants that support basal but not activated transcription in vitro have been described; one of these is specifically defective for interaction with TFIIIB, whereas the others show combinations of defects in interactions with an acidic activation domain (VP16), TFIIA, TFIIIB, or DNA (11). The significance of these interactions to the mechanism of activation in vivo is unknown.

Recruitment of TBP to the promoter in vivo can be a rate-limiting step for transcription that is enhanced by acidic activator proteins (12). Transient transcription ex-

periments in mammalian cells indicate that TBP plays a role in transcriptional activation (13, 14). Thus, interactions that facilitate the association of TBP with the TATA element are potential targets for acidic activators. However, little is known about which interactions are critical for the response to acidic activators in vivo. Here, we report the isolation and characterization of a TBP mutant protein (N2-1) whose properties indicate that the TBP-TFIIA interaction is essential for transcriptional activation by acidic activator proteins.

TBP is highly conserved throughout eukaryotic evolution (15) and is essential for transcription by all three nuclear RNA polymerases in vivo (16). The N2-1 derivative was isolated in a genetic screen for TBP derivatives that are competent for Pol III transcription but have another functional defect. Specifically, a set of complex and compact libraries of yeast TBP mutant proteins (17) was screened for derivatives that complemented the temperature-sensitive phenotype of TBP-F155S (where Phe<sup>155</sup> was changed to Ser), a Pol III-specific TBP mutant (17), and also conferred growth when present as the sole source of TBP (Fig. 1A). In addition, the strain with N2-1 as the only TBP grew slowly even at 30°C (Fig. 1B), and the cells exhibited a large and elongated morphology. This phenotype was not the result of reduced expression or instability of the mutant TBP, because immunoblot analysis of the mutant strain indicated amounts of TBP equal to those found in the wild type (Fig. 1C). Moreover, the structure of the N2-1 protein was not substantially compromised, because N2-1 was normal for Pol III transcription at the restrictive temperature (Fig. 1D).

To characterize the transcriptional properties of the N2-1 protein, we determined RNA levels for various genes in a strain with this derivative as the only TBP. Wild-type levels of Pol II transcription from the *DED1*, *HIS3*, *RPS4*, *TBP*, and *TRP3* genes occurred in cells grown at the permissive or restrictive temperature (Fig. 2, A and B). In

2. B. Schobert and J. K. Lanyi, *J. Biol. Chem.* **257**, 10306 (1982); J. K. Lanyi, *Physiol. Rev.* **70**, 319 (1990).
3. D. Oesterhelt and J. Tittor, *Trends Biochem. Sci.* **14**, 57 (1989); —, E. Bamberg, *J. Bioenerg. Biomembr.* **24**, 181 (1992).
4. R. Henderson et al., *J. Mol. Biol.* **213**, 899 (1990).
5. M. H. J. Koch et al., *EMBO J.* **10**, 521 (1991); M. Nakasako, M. Kataoka, Y. Amemiya, F. Tokunaga, *FEBS Lett.* **292**, 73 (1991); S. Subramaniam, M. Gerstein, D. Oesterhelt, R. Henderson, *EMBO J.* **12**, 1 (1993).
6. M. Kataoka et al., *J. Mol. Biol.* **243**, 621 (1994).
7. A. Blanck and D. Oesterhelt, *EMBO J.* **6**, 265 (1987); J. K. Lanyi, A. Duschl, G. W. Hatfield, K. M. May, D. Oesterhelt, *J. Biol. Chem.* **265**, 1253 (1990); J. Otomo, H. Tomioka, H. Sasabe, *Biochim. Biophys. Acta* **1112**, 7 (1992); J. Soppa, J. Duschl, D. Oesterhelt, *J. Bacteriol.* **175**, 2720 (1993).
8. T. Mogi, L. J. Stern, T. Marti, B. H. Chao, H. G. Khorana, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4148 (1988); S. Subramaniam, T. Marti, H. G. Khorana, *ibid.* **87**, 1013 (1990).
9. Abbreviations are as follows: D, aspartate; T, threonine; and N, asparagine. Mutants are denoted with the wild-type residue, the residue number, and the new residue (for example, D85T).
10. J. Tittor, U. Schweiger, D. Oesterhelt, E. Bamberg, *Biophys. J.* **67**, 1682 (1994).
11. J. L. Spudich, *Cell* **79**, 747 (1994).
12. A. Maeda, T. Ogurusu, T. Yoshizawa, T. Kitagawa, *Biochemistry* **24**, 2517 (1985); B. Schobert and J. K. Lanyi, *ibid.* **25**, 4163 (1986); C. Pande, J. K. Lanyi, R. H. Callender, *Biophys. J.* **55**, 425 (1989).
13. A. Dér, R. Tóth-Boconádi, L. Keszthelyi, *FEBS Lett.* **259**, 24 (1989); L. Keszthelyi, S. Száraz, A. Dér, W. Stoekenius, *Biochim. Biophys. Acta* **1018**, 260 (1990); A. Dér et al., *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4751 (1991).
14. T. Marti, S. J. Rösselet, H. Otto, M. P. Heyn, H. G. Khorana, *J. Biol. Chem.* **266**, 18674 (1991); T. Marti, H. Otto, S. J. Rösselet, M. P. Heyn, H. G. Khorana, *ibid.* **267**, 16922 (1992).
15. L. S. Brown and J. K. Lanyi, unpublished results.
16. J. K. Lanyi and D. Oesterhelt, *J. Biol. Chem.* **257**, 2674 (1982).
17. The D85T gene was expressed in *H. salinarum* Pho81 cells with a plasmid vector that contains a gene for novobiocin resistance. Envelope vesicles were prepared as described [J. K. Lanyi and R. E. MacDonald, *Methods Enzymol.* **56**, 398 (1979)].
18. S. A. Sundberg et al., *J. Bacteriol.* **172**, 2328 (1990).
19. A. Duschl, J. K. Lanyi, L. Zimányi, *J. Biol. Chem.* **265**, 1261 (1990).
20. A. Matsuno-Yagi and Y. Mukohata, *Biochem. Biophys. Res. Commun.* **78**, 237 (1977).
21. B. Scharf and M. Engelhard, *Biochemistry* **33**, 6387 (1994).
22. T. Ogurusu, A. Maeda, T. Yoshizawa, *J. Biochem.* **95**, 1073 (1984); B. Schobert, J. K. Lanyi, D. Oesterhelt, *J. Biol. Chem.* **261**, 2690 (1986).
23. G. Váró et al., in preparation.
24. J. Sasaki, unpublished results.
25. J. Tittor, D. Oesterhelt, R. Maurer, H. Desel, R. Uhl, *Biophys. J.* **52**, 999 (1987); J. K. Lanyi and V. Vodyanoy, *Biochemistry* **25**, 1465 (1986).
26. G. Váró et al., *Biophys. J.* **68**, 2062 (1995).
27. T. Kouyama, A. Nasuda-Kouyama, A. Ikegami, M. K. Mathew, W. Stoekenius, *Biochemistry* **27**, 5855 (1988); H. Otto et al., *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9228 (1989); L. Zimányi, Y. Cao, R. Needleman, M. Ottolenghi, J. K. Lanyi, *Biochemistry* **32**, 7669 (1993).
28. L. Zimányi, L. Keszthelyi, J. K. Lanyi, *Biochemistry* **28**, 5165 (1989).
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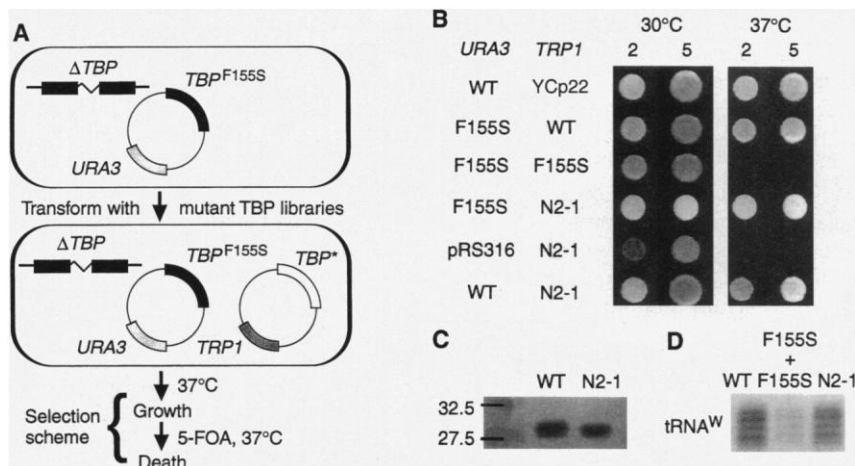
addition, amounts for Pol I-transcribed ribosomal RNA (rRNA) were similar to those found in the wild type and were unchanged by a temperature shift. Thus, N2-1 is fully functional for transcription by Pol I and Pol III, as well as for constitutive transcription by Pol II at both the permissive and restrictive temperatures.

In contrast, the N2-1 strain was severely

defective in the response to three acidic activator proteins, even when grown at 30°C. First, unlike wild-type cells, which exhibited an increase in *HIS3* transcription in the presence of a plasmid that constitutively expressed Gcn4, cells containing N2-1 showed amounts comparable to those produced in the absence of Gcn4 (Fig. 2B). Second, copper induction of the *CUP1*

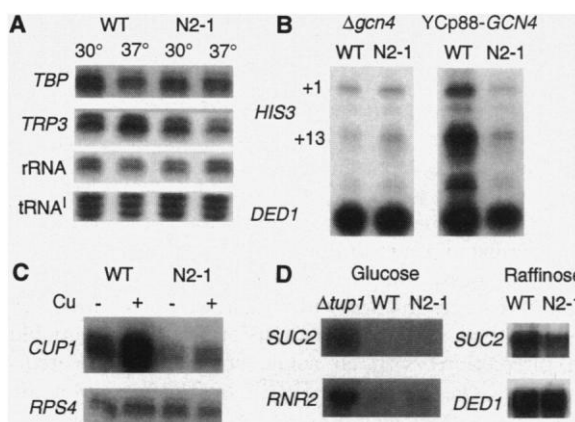
gene, which requires the Ace1 transcriptional activator (18), was also defective in the mutant strain (Fig. 2C). In the presence of copper, the wild-type strain showed a 10- to 12-fold increase in *CUP1* transcription, whereas the mutant strain showed less than a twofold increase. In the absence of exogenously added copper, *CUP1* RNA levels in the N2-1 strain were lower than in the wild-type strain, which is due primarily to the defect in Ace1-dependent activation (19). Third, with the use of the *lacZ* reporter YCp86-Sc3801 (20), we examined the response to the activator Gal4, which stimulates transcription in the presence of galactose. The addition of galactose caused a 400-fold increase in  $\beta$ -galactosidase activity in the wild-type strain, but only a twofold increase in the mutant strain (Table 1). Thus, the N2-1 strain is not competent for response to three acidic activators that induce the expression of physiologically unrelated sets of genes.

To address whether the activation-deficient TBP was able to respond to negative regulators, we examined repression by the Cyc8-Tup1 co-repressor complex (21) and by the Not complex (22). The Cyc8-Tup1 complex is required for the repression of at least five independently regulated sets of genes, including those regulated by glucose (*SUC2*) and DNA damage (*RNR2*). Under repressing conditions (glucose medium without DNA damage), *SUC2* and *RNR2* RNA levels in the N2-1 strain were indistinguishable from those in the wild-type strain (Fig. 2D), which indicates that repression by Cyc8-Tup1 was intact. If Not-dependent repression was compromised in N2-1, then the strain should have exhibited a preferential increase in *HIS3* transcription from the +1 initiation site (22). However, the ratio of +1 to +13 transcripts in the mutant strain was nearly 1:1 and comparable to wild-type levels (Fig. 2B). Although our results do not address whether TBP is a direct target of the Cyc8-Tup1 or Not com-



**Fig. 1.** Isolation and initial characterization of strains containing the N2-1 derivative of TBP. (A) The genetic selection. The starting strain, containing a chromosomal deletion of TBP and a centromeric *URA3* plasmid containing a ts Pol III-specific derivative of TBP (F155S), was transformed with the mutant TBP libraries (denoted by the asterisk) on a centromeric *TRP1* plasmid (17). Colonies were selected for growth at 37°C and then screened for the inability to grow on 5-fluoroorotic acid (5-FOA) at 37°C. (B) Growth of strains containing the indicated TBP derivatives (or pRS316 or YCp22 vectors) at 30°C and 37°C for 2 or 5 days. Approximately  $10^5$  cells were spotted to plates deficient for uracil and tryptophan. (C) Immunoblots with yeast TBP antibody specific to the NH<sub>2</sub>-terminus and 25  $\mu$ g of whole cell protein extract from the indicated strain grown at 30°C. Molecular weight standards (in kilodaltons) are shown. (D) S1 analyses of tryptophan transfer RNA (*tRNA*<sup>W</sup>) transcription with 10  $\mu$ g of total RNA from strains containing the indicated TBP derivatives after shifting to 37°C for 1 hour.

**Fig. 2.** Transcriptional analyses of the N2-1 strain. Total RNAs from wild-type (WT) and N2-1 strains (or derivatives) grown at 30°C (unless otherwise indicated) were hybridized to completion with an excess of the indicated oligonucleotide probes and treated with S1 nuclease (16). (A) Strains were grown at the permissive (30°C) or restrictive (37°C) temperatures for 1 hour and analyzed for *TBP* and *TRP3* mRNAs generated by Pol II, rRNA transcribed by Pol I, and isoleucine tRNA (*tRNA*<sup>I</sup>) synthesized by Pol III. (B) Activation by Gcn4. Strains with *gcn4* deleted that lack or contain YCp88-GCN4, a plasmid constitutively expressing Gcn4 (32), were analyzed for *HIS3* (+1 and +13 sites indicated) and *DED1* RNAs. Constitutive *HIS3* transcription is initiated equally from the +1 and +13 sites, whereas Gcn4-activated transcription is initiated preferentially from the +13 site; *DED1* is not affected by Gcn4 and serves as an internal control (33). (C) Copper induction of *CUP1* transcription. Strains were grown overnight in synthetic complete medium in the presence (+) or absence (–) of 100  $\mu$ M copper sulfate. Parallel reactions were performed with a probe for the ribosomal protein gene *RPS4* (34). (D) Repression by the Cyc8-Tup1 complex. Total RNA (10  $\mu$ g) from wild-type, N2-1, and *tup1* deletion strains grown in repressing conditions (glucose medium without DNA-damaging agents) was blotted to nylon filters and hybridized with *RNR2* or *SUC2* probes (35). For assessment of *SUC2* derepression, wild-type and N2-1 strains were grown in raffinose medium, and 10  $\mu$ g of total RNA was hybridized to *SUC2* and *DED1* (control) probes.



**Table 1.** Activation by Gal4.  $\beta$ -Galactosidase activities were from the reporter plasmid YCp86-3801, which contains the 365-bp *GAL1*, *GAL10* promoter fragment with four Gal4-binding sites, upstream of the *HIS3* TATA box, fused to the *E. coli lacZ* gene. Assays were performed with  $10^7$  cells harboring the indicated TBP derivative, cultured in media containing 2% of either glucose or galactose.

Strain	$\beta$ -Galactosidase activities (Miller units $\pm$ SD) with	
	Glucose	Galactose
Wild type	<1	430 $\pm$ 30
N2-1	<1	2 $\pm$ 1
K138T	<1	420 $\pm$ 20
Y139A	<1	47 $\pm$ 4

plexes, they do indicate that this activation-defective TBP is fully functional for at least two forms of global repression. This suggests that if TBP is a direct target of these negative regulators, then the surfaces for interaction with repressors are likely to be distinct from those of acidic activators.

The N2-1 protein contains two consecutive amino acid substitutions, Lys<sup>138</sup> to Thr and Tyr<sup>139</sup> to Ala (K138T; Y139A). Both singly substituted derivatives grew normally at 30°C and nearly as well as wild-type TBP at 37°C. When assayed for Gal4-dependent activation, the K138T derivative was indistinguishable from wild-type TBP, whereas the Y139A protein had only 11% of the activity of the wild type (Table 1). Thus, although Y139A had a more significant effect, both substitutions contribute to the severe activation defect and slow-growth phenotype of the N2-1 strain. Residues 138 and 139 lie within  $\alpha$  helix 2 on the upper surface of TBP (23) in a region of conserved basic amino acids that has been implicated as important for interaction with TFIIA in vitro (24).

To investigate the molecular basis of the activation defect, we produced the N2-1 protein in *Escherichia coli*, and its biochemical properties were compared with those of wild-type TBP. As assayed by coprecipitation with glutathione-S-transferase (GST)–VP16, the N2-1 protein was indistinguishable from wild-type TBP in its ability to interact with the VP16 acidic activation domain (Fig. 3A). In addition, mobility-shift (Fig. 3B) and deoxyribonuclease I footprinting assays demonstrated that the N2-1 protein binds with wild-type affinity to a

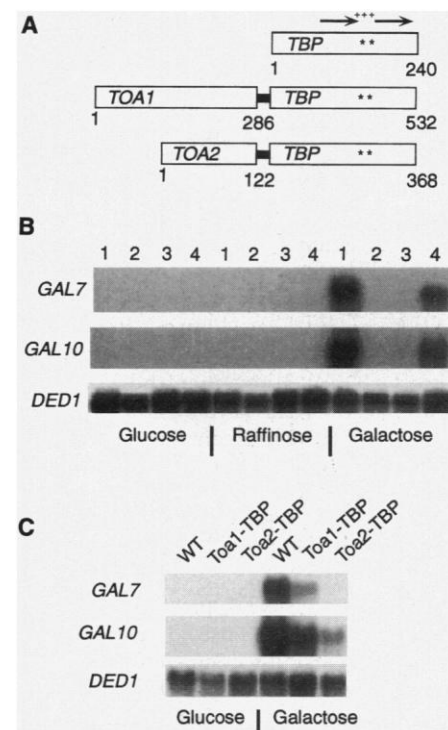
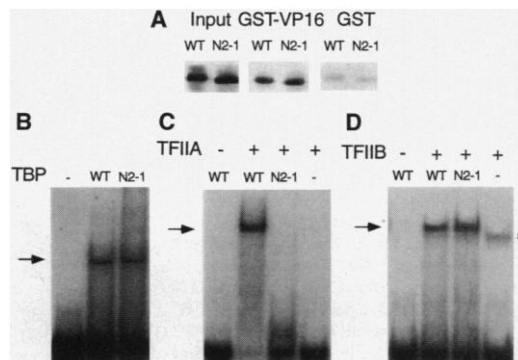
consensus TATA element, which indicates that the protein is correctly folded in vitro. However, N2-1 did not interact with TFIIA in a mobility-shift assay (Fig. 3C). TBP–TFIIA–DNA complexes were not visualized even when five times more N2-1 or TFIIA or both was used. In contrast, N2-1 interacted with TFIIIB in the same assay (Fig. 3D). Thus, these biochemical experiments indicate that the N2-1 protein is specifically defective for interaction with TFIIA.

We tested the hypothesis that loss of TFIIA interaction causes the activation deficiency in vivo by attempting to overcome the defect by increasing the concentration of TFIIA. Yeast TFIIA consists of two subunits, Toa1 and Toa2, and most likely exists as an ( $\alpha\beta$ )<sub>2</sub> tetramer (25). Because simultaneous overexpression of Toa1 and Toa2 failed to alleviate the activation-defective phenotype of N2-1, we increased the effective concentration of TFIIA by fusing the individual subunits to the N2-1 derivative of TBP (Fig. 4A). When Toa2 was fused to N2-1, activation by Gal4 was largely recovered (Fig. 4B). In galactose medium, the strain with the Toa2–N2-1 derivative showed nearly 30% of the GAL7 and GAL10 RNA levels in the wild type. GAL7 and GAL10 transcription was not observed in glucose or raffinose medium, indicating that activation by Toa2–N2-1 depends on Gal4; that is, the Toa2–N2-1 fusion did not bypass the need for a DNA-binding activator. Furthermore, the Toa2–N2-1 fusion rescued the temperature-sensitive, slow-growth, and cell morphology phenotypes, which indicates that it was having a general and positive effect and was not specific to

Gal4 activation. In contrast, fusion of Toa1 to wild-type TBP caused temperature-sensitive growth and markedly reduced Gal4 activation (Fig. 4C). This inhibition may reflect an artificially strong TBP–TFIIA interaction that results either in sequestering activators or coactivators away from their target promoters or blocking subsequent steps in the activation pathway. Taken together, these results indicate that the tran-

**Fig. 3.** Biochemical properties of the N2-1 derivative.

(A) Approximately 20  $\mu$ l of glutathione-agarose beads containing GST–VP16 or GST (6) were incubated with equal amounts of His-tagged (36) wild-type or mutant TBPs for 1 hour in 100  $\mu$ l of buffer containing 100 mM KCl, 20 mM Hepes (pH 7.9), 5 mM MgCl<sub>2</sub>, bovine serum albumin (20  $\mu$ g/ml), 0.2 mM EDTA, 1 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 10% glycerol, and 0.03% NP-40. The beads were washed five times with 200  $\mu$ l of buffer, bound proteins were eluted by boiling in 40  $\mu$ l of SDS–polyacrylamide gel electrophoresis (SDS–PAGE) loading buffer, separated by 10% SDS–PAGE, and blotted to nitrocellulose. Immunoblots were probed with TBP antibody specific to the NH<sub>2</sub>-terminus. (B) TATA element binding. A 45-bp fragment (0.5 ng) containing the adenovirus early 1B TATA box (37) was incubated with 20 ng of the indicated TBP derivative in the presence of 200 ng of poly(dGdC), 100 mM KCl, 40 mM Hepes (pH 7.9), 20 mM Tris (pH 7.5), 5 mM MgCl<sub>2</sub>, 10% glycerol, 1 mM DTT, and 0.5 mM PMSF. TBP–DNA complexes (arrows) were separated on a 5% acrylamide gel containing 0.5 $\times$  TBE and 2 mM MgCl<sub>2</sub> in both the gel and running buffer. (C) Interaction with TFIIA. Recombinant yeast TFIIA (25) was incubated with wild-type or mutant TBP and the TATA element probe and treated as described above except that MgCl<sub>2</sub> was omitted from the gel and running buffer. Under these conditions, only TBP–TFIIA–DNA complexes are stable. (D) Interaction with TFIIIB. His-tagged human TFIIIB was purified from *E. coli* and incubated with wild-type or N2-1 TBP and analyzed under the same conditions as in (C). The arrow indicates the position of the TBP–TFIIIB–DNA complex. The asterisk denotes a nonspecific binding activity that copurifies with recombinant TFIIIB from bacteria.



**Fig. 4.** TFIIA–TBP fusions. (A) TOA1 and TOA2 coding sequences (obtained by polymerase chain reaction) were cloned into an Eco RI site introduced upstream of wild-type TBP or N2-1 coding sequences to generate protein fusions between the TFIIA subunits and TBP separated by the linker peptide RIPFLI. The approximate location of the double mutation in N2-1 is denoted by asterisks, the conserved repeats of TBP by arrows, and the basic region by pluses. Amino acid numbers are indicated. (B) Total RNA (10  $\mu$ g) from strains containing the indicated TBP derivative, grown overnight in the presence of 2% galactose, raffinose, or glucose, were blotted to nylon filters and hybridized with GAL7, GAL10, or DED1 probes. Lanes 1, WT; lanes 2, N2-1; lanes 3, Toa1–N2-1; lanes 4, Toa2–N2-1. Analysis by phosphorimager revealed that GAL7 and GAL10 levels in the strain containing Toa2–N2-1 were nearly 30% of that observed in the wild-type strain. (C) Total RNA (10  $\mu$ g) from strains containing wild-type TBP that was or was not fused to the indicated TFIIA subunits was grown in the presence of 2% glucose or galactose and hybridized with GAL7, GAL10, or DED1 probes. In contrast to the Toa2–N2-1 fusion protein that restored galactose-induced Gal4 activation, the Toa1–TBP fusion protein inhibited Gal4 activation. The Toa1–TBP fusion protein showed a modest decrease in Gal4 activation.

scriptional activation defect of N2-1 is caused by its inability to interact efficiently with TFIIA.

Although there is considerable information about transcriptional activation in vitro, very little is known about the mechanisms that occur under physiological conditions. Because acidic activation domains can enhance recruitment of TBP to promoters in vivo (12), any interaction that facilitates the association of TBP with the TATA element is a potential target for acidic activators. The properties of the N2-1 derivative of TBP indicate that the TBP-TFIIA interaction is essential for the response to acidic activators in vivo. In vitro, TFIIA stabilizes the TBP-TATA element interaction, alters the conformation of TBP, and extends the deoxyribonuclease I footprint upstream of the TATA element (26, 27). Kinetic studies suggest that the activator-dependent assembly of a complex requiring TFIID and TFIIA can be a rate-limiting step in the production of preinitiation complexes in vitro (10). Taken together, these observations suggest that TFIIA plays an active role in TBP recruitment and in the response to activators in vivo. However, TFIIA may also contribute by counteracting repressor proteins that interact with TBP and block its interaction with the TATA element (28).

Whereas the N2-1 protein is severely defective in its response to acidic activators, it appears fully competent to support transcription from many Pol II promoters. This suggests that the TBP-TFIIA interaction plays a less important (and perhaps no) role in constitutive Pol II transcription in vivo. In apparent contrast, TFIIA strongly stimulates basal transcription in vitro (5, 29), and loss of TFIIA function causes a general reduction in Pol II transcription (30). However, it may be that N2-1 interacts weakly with TFIIA and that its amount is sufficient to support basal, but not activated, transcription in vivo. Alternatively, TFIIA may also carry out a biochemical function distinct from the TBP interaction (for example, displacement of negative regulators or interaction with TAFs) that is essential for constitutive Pol II transcription but not the response to acidic activators.

Although our results demonstrate the importance of the TBP-TFIIA interaction in the response to acidic activators in vivo, they do not address which component is the target of acidic activation domains. The simplest hypothesis is that the activation domain contacts TBP or TFIIA or both, thereby stabilizing the TBP-TFIIA-TATA element complex in the context of nuclear chromatin. However, it is possible that the activation domain interacts with proteins

that associate with TFIIA or TBP (for example, TAFs or TFIIB). The properties of the N2-1 protein are consistent with the observation that activation domains can stimulate recruitment of TBP to promoters in vivo (12), and they are in good accord with certain biochemical experiments indicating that activators function at an early step involving TBP and TFIIA (10). However, our results are not inherently inconsistent with other biochemical experiments that suggest that acidic activators can act at steps that occur after TBP binding (9).

In addition to elucidating the mechanism of transcriptional enhancement by acidic activators, the N2-1 and Toa2-N2-1 derivatives represent powerful tools for investigating other aspects of transcription in vivo. For example, the importance of the TBP-TFIIA interaction can be assessed at any native (or mutated) yeast promoter under any desired environmental or genetic condition. Moreover, by generating artificial promoters whose function depends on specific activators or on other proteins involved in transcription, these TBP derivatives can be used to classify activator proteins on a mechanistic basis. Finally, it is noteworthy that the N2-1 derivative supports cell growth despite the severe defect in its response to acidic activators. Although acidic activators perform biologically important roles in eukaryotic organisms, our results suggest that they may not be required for transcription of most yeast genes or for viability of the organism.

## REFERENCES AND NOTES

1. R. Tjian and T. Maniatis, *Cell* **77**, 5 (1994).
2. R. C. Conaway and J. W. Conaway, *Annu. Rev. Biochem.* **62**, 161 (1993); L. Zawel and D. Reinberg, *Prog. Nucleic Acid Res. Mol. Biol.* **44**, 67 (1993); S. Buratowski, *Cell* **77**, 1 (1994).
3. K. F. Stringer, C. J. Ingles, J. Greenblatt, *Nature* **345**, 783 (1990).
4. T. Hoey *et al.*, *Cell* **72**, 247 (1993); J. A. Goodrich, T. Hoey, C. J. Thut, A. Admon, R. Tjian, *ibid.* **75**, 519 (1993).
5. J. Ozer *et al.*, *Genes Dev.* **8**, 2324 (1994).
6. Y.-S. Lin, I. Ha, E. Maldonado, D. Reinberg, M. R. Green, *Nature* **353**, 569 (1991).
7. H. Xiao *et al.*, *Mol. Cell. Biol.* **14**, 7013 (1994).
8. C. J. Ingles, M. Shales, W. D. Cress, S. J. Triezenberg, J. Greenblatt, *Nature* **351**, 588 (1991).
9. Y.-S. Lin and M. R. Green, *Cell* **64**, 971 (1991); B. Choy and M. R. Green, *Nature* **366**, 531 (1993).
10. W. Wang, J. D. Gralla, M. Carey, *Genes Dev.* **6**, 1716 (1992); P. M. Lieberman and A. J. Berk, *ibid.* **8**, 995 (1994).
11. T. K. Kim *et al.*, *Nature* **369**, 252 (1994).
12. C. Klein and K. Struhl, *Science* **266**, 280 (1994); S. Chatterjee and K. Struhl, *Nature* **374**, 820 (1995); N. Klages and M. Strubin, *ibid.*, p. 822.
13. J. Colgan and J. L. Manley, *Genes Dev.* **6**, 304 (1992).
14. W. P. Tansey, S. Ruppert, R. Tjian, W. Herr, *ibid.* **8**, 2756 (1994).
15. L. A. Stargell and M. A. Gorovsky, *Mol. Cell. Biol.* **14**, 723 (1994).
16. B. P. Cormack and K. Struhl, *Cell* **69**, 685 (1992).
17. ———, *Science* **262**, 244 (1993).
18. P. Furst, S. Hu, R. Hackett, D. Hamer, *Cell* **55**, 705 (1988).
19. The difference in uninduced *CUP1* RNA levels between the two strains is partially explained by the fact that wild-type strains with *ACE1* deleted have one-fourth the amount of *CUP1* RNA in the absence of added copper (L. A. Stargell and K. Struhl, data not shown). Thus, in the uninduced state, most of *CUP1* transcription represents Ace1-dependent activation that is probably due to micromolar amounts of copper present in the media. Furthermore, the *CUP1* promoter is also regulated by the transcriptional activators Ace2 and heat shock factor [G. Butler and D. J. Thiele, *Mol. Cell. Biol.* **11**, 476 (1991); K. T. Tamai, X. Liu, P. Silar, T. Sosinowski, D. J. Thiele, *ibid.* **14**, 8155 (1994)]. Thus, the mutant TBP strain has smaller amounts of *CUP1* transcription in the absence of additional copper because it is not competent in responding to any of these activators. The *CUP1* oligonucleotide probe was CCCAGAGCAGCATGACTTCTTGGTTCCTTCAGACTTGTACCGCAGGGGATTTGTGCTGCTGCTGAATGTG.
20. V. L. Singer, C. R. Wobbe, K. Struhl, *Genes Dev.* **4**, 636 (1990).
21. C. A. Keleher, M. J. Redd, J. Schultz, M. Carlson, A. D. Johnson, *Cell* **68**, 709 (1992); D. Tzamarias and K. Struhl, *Nature* **369**, 758 (1994).
22. M. Collart and K. Struhl, *Genes Dev.* **8**, 525 (1994).
23. D. B. Nikolov *et al.*, *Nature* **360**, 40 (1992).
24. Although a yeast TBP derivative containing the K138L (where L is Leu) substitution fails to interact with human TFIIA in vitro (27), this observation is not relevant to the analysis here because the K138L protein interacts normally with yeast TFIIA (31). The double substitution K133L; K138L is slightly less efficient at interacting with yeast TFIIA in vitro and is ts at 37°C in vivo (31). High-copy suppression of the K133L; K138L derivative yielded *TDS4*, a gene encoding a component of the Pol III transcription machinery [S. Buratowski and H. Zhou, *Cell* **71**, 221 (1992)], and K138L appears to be compromised for Pol III transcription in vitro [T. K. Kim and R. G. Roeder, *J. Biol. Chem.* **269**, 4891 (1994)]. Although the K133L; K138L derivative has not been examined for transcriptional activation in yeast cells, it does respond to acidic activators in mammalian cells (14) and in yeast extracts (11); hence, it is unlikely that the slight defect in TFIIA interaction has a phenotypic consequence in vivo. In contrast, the K138T; Y139A derivative (N2-1) described here does not interact with yeast TFIIA in vitro and is fully functional for Pol III in vivo.
25. J. A. Ranish, W. S. Lane, S. Hahn, *Science* **255**, 1127 (1992).
26. S. Buratowski, S. Hahn, L. Guarente, P. A. Sharp, *Cell* **56**, 549 (1989); S. Hahn, S. Buratowski, P. A. Sharp, L. Guarente, *EMBO J.* **8**, 3379 (1989).
27. D. K. Lee, J. Dejong, S. Hashimoto, M. Horikoshi, R. G. Roeder, *Mol. Cell. Biol.* **12**, 5189 (1992).
28. D. T. Auble *et al.*, *Genes Dev.* **8**, 1920 (1994); J. A. Inostroza, F. H. Mermelstein, I. Ha, W. S. Lane, D. Reinberg, *Cell* **70**, 477 (1992).
29. K. Yokomori *et al.*, *Genes Dev.* **8**, 2313 (1994); X. Sun, D. Ma, M. Sheldon, K. Yeung, D. Reinberg, *ibid.*, p. 2336.
30. J. J. Kang, D. T. Auble, J. A. Ranish, S. Hahn, *Mol. Cell. Biol.* **15**, 1234 (1995).
31. S. Buratowski and H. Zhou, *Science* **255**, 1130 (1992).
32. I. A. Hope and K. Struhl, *Cell* **46**, 885 (1986).
33. K. Struhl, *Mol. Cell. Biol.* **6**, 3847 (1986).
34. C. Klein and K. Struhl, *ibid.* **14**, 1920 (1994).
35. D. Tzamarias and K. Struhl, *Genes Dev.* **9**, 821 (1995).
36. A. Hoffman and R. G. Roeder, *Nucleic Acids Res.* **19**, 6337 (1991).
37. C. C. Kao *et al.*, *Science* **248**, 1646 (1990).
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