Multiple Functions of the Nonconserved N-Terminal Domain of Yeast TATA-Binding Protein

Mark Lee[1] and Kevin Struhl

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

Manuscript received December 15, 2000
Accepted for publication January 26, 2001

ABSTRACT

The TATA-binding protein (TBP) is composed of a highly conserved core domain sufficient for TATA-element binding and preinitiation complex formation as well as a highly divergent N-terminal region that is dispensable for yeast cell viability. *In vitro*, removal of the N-terminal region domain enhances TBP-TATA association and TBP dimerization. Here, we examine the effects of truncation of the N-terminal region in the context of yeast TBP mutants with specific defects in DNA binding and in interactions with various proteins. For a subset of mutations that disrupt DNA binding and the response to transcriptional activators, removal of the N-terminal domain rescues their transcriptional defects. By contrast, deletion of the N-terminal region is lethal in combination with mutations on a limited surface of TBP. Although this surface is important for interactions with TFIIA and Brf1, TBP interactions with these two factors do not appear to be responsible for this dependence on the N-terminal region. Our results suggest that the N-terminal region of TBP has at least two distinct functions *in vivo*. It inhibits the interaction of TBP with TATA elements, and it acts positively in combination with a specific region of the TBP core domain that presumably interacts with another protein(s).

THE TATA-binding protein (TBP) plays a central role in eukaryotic transcription, being required for accurate transcriptional initiation by all three nuclear RNA polymerases (Hernandez 1993; Struhl 1994; Burley and Roeder 1996). TBPs from a wide variety of eukaryotes and archea have a highly conserved 180-residue C-terminal core domain (80% identical between yeast and human) that is sufficient for TATA-element binding and preinitiation complex formation. TBPs also contain an N-terminal region that is very divergent across species with respect to both length and sequence and whose function is poorly understood. Human TBP does not support yeast cell growth, but species-specific difference maps to the C-terminal core domain, not the N-terminal region (Cormack *et al.* 1991; Gill and Tjian 1991). Indeed, a single amino acid change within the core domain enables human TBP to substitute for all of the essential activities of yeast TBP *in vivo* (Cormack *et al.* 1994).

The TBP core domain and full-length TBP behave similarly in many biochemical assays, but there are several differences that provide insight into the function of the N-terminal domain. First, deletion of the N-terminal region results in enhanced binding and bending of DNA relative to full-length TBP (Horikoshi *et al.* 1990; Lieberman *et al.* 1991), primarily by increasing the rate of TBP-TATA association (Kuddus and Schmidt 1993). As a consequence, DNA binding by TBP mutants defective for the TBP-TATA interaction can be restored by removal of the N-terminal domain (Lee *et al.* 1992). Second, the yeast TBP core domain readily forms transcriptionally inert dimers and higher order oligomers *in vitro* (Kato *et al.* 1994; Coleman *et al.* 1995). Although the physiological relevance of TBP dimerization is controversial (Jackson-Fisher *et al.* 1999; Geisberg and Struhl 2000), the N-terminal domain inhibits oligomerization of full-length TBP, potentially enhancing the probability that TBP is monomeric under physiologic conditions (Campbell *et al.* 2000). Third, a peptide or a monoclonal antibody corresponding to a portion of the N-terminal region selectively blocks TATA-dependent transcription by RNA polymerases II and III while sparing RNA polymerase I and TATA-less promoter transcription (Lescure *et al.* 1994). Fourth, yeast and human NC2 are most effective in repressing transcription in reactions reconstituted with TBP from the same species, and maximal repression requires the N-terminal region (Goppelt and Meisterernst 1996). Fifth, removal of the N-terminal region of yeast TBP increases interactions with human TBP-associated factors (TAFs) and the formation of a hybrid TFIID complex (Zhou and Berk 1995). Sixth, the N-terminal region of human TBP mediates cooperative binding with the small nuclear RNA activating protein (SNAP) complex to the U6 promoter and enhances U6 transcription (Mittal

Corresponding author: Kevin Struhl, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115. E-mail: kevin@hms.harvard.edu

[1] *Present address:* Department of Medicine, Rm. S-101, Stanford University Medical Center, Stanford, CA 94305.

and Hernandez 1997). Taken together, these results suggest a regulatory role for the N-terminal domain in governing the interactions of TBP with the TATA element, with other transcription factors, and with TBP itself. However, the physiological relevance of these biochemical properties of the TBP N-terminal region remains to be established.

In yeast cells, expression of the TBP core domain at physiological levels has minimal phenotypic consequence, indicating that the 63-residue N-terminal region of TBP is dispensable for viability and the response to transcriptional activators (Cormack *et al.* 1991; Reddy and Hahn 1991). However, overexpression of the TBP core domain inhibits cell growth, particularly on nonfermentable carbon sources (Gill and Tjian 1991; Zhou *et al.* 1991), and this toxicity is suppressed by concurrent overexpression of Std1(Msn3) (Ganster *et al.* 1993). Std1 directly associates with TBP, and deletion of the N-terminal region qualitatively alters this interaction (Tillman *et al.* 1995). Apart from these studies, however, relatively little is known about the function of the TBP N-terminal region *in vivo*.

To examine the physiological role of the N-terminal region of yeast TBP, we deleted this region in the context of a large number of yeast TBP mutants. Our results suggest that the N-terminal region of TBP has at least two distinct functions *in vivo*. It inhibits the interaction of TBP with TATA elements, and it functions in concert with a specific surface of TBP that presumably interacts with another transcriptional regulatory protein.

MATERIALS AND METHODS

DNA molecules: TBP mutants used in this study have been described previously (Cormack and Struhl 1993; Lee and Struhl 1995, 1997). The N-terminally deleted derivatives in Table 1 derive from pBC83, a *TRP1*-marked centromeric plasmid with the 2.4-kb *Eco*RI-*Bam*HI fragment containing the yeast *TBP* gene with a deletion of residues 4–63 (Cormack *et al.* 1991). The N-terminally deleted derivatives in Figure 2 derive from pML3095, a derivative of pBC83 in which a FLAG epitope was engineered at the N terminus of the TBP core domain by subcloning of annealed oligonucleotides. Serial deletions of the N-terminal region were generated by PCR using the wild-type *TBP* gene as template.

Phenotypic analyses: TBP derivatives were assayed for their ability to support cell growth by spotting 10^4 and 10^5 cells on glucose medium supplemented with casamino acids and 5-fluoroorotic acid, using the plasmid shuffle assay and strain BYΔ2 (Cormack *et al.* 1991); growth was monitored after 2–3 days incubation at 30°. S1 nuclease protection assays for measuring RNA levels and β-galactosidase assays for determining Gal4-dependent activation were performed as described elsewhere (Cormack and Struhl 1992; Lee and Struhl 1995; Iyer and Struhl 1996). Intracellular levels of N-terminally deleted TBPs were determined by Western blotting using monoclonal antibody mAb58C9 (Santa Cruz Biotechnologies) against the FLAG epitope. To avoid the confounding effects of homeostatic mechanisms on TBP levels, mutant TBP levels were measured in a strain containing hemagglutinin-tagged TBP (Lee and Struhl 1997).

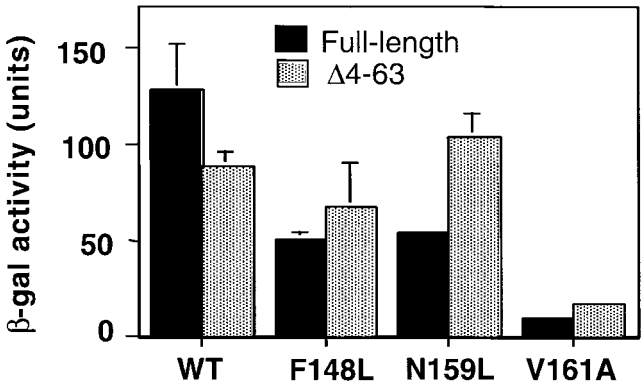

Figure 1.—N-terminal truncation can rescue the transcriptional phenotypes of a subset of activation-deficient DNA-binding mutants of yeast TBP. β-Galactosidase activity of a Gal4-dependent *LacZ* reporter in wild-type and mutant TBP strains grown with galactose as the sole carbon source (inducing conditions) is shown. Standard errors are given as error bars. Solid bars, full-length TBP derivatives; stippled bars, N-terminally truncated derivatives (Δ4-63).

RESULTS

Deletion of the N-terminal region rescues the activation-deficient phenotype of a subset of DNA-binding mutants: Certain TBP mutants deficient in the response to transcriptional activators contain mutations on the DNA-binding surface that dramatically reduce TATA-element binding *in vitro* (Kim *et al.* 1994; Arndt *et al.* 1995; Lee and Struhl 1995). As removal of the N-terminal region can restore TBP-TATA association *in vitro* for some TBP mutants with DNA-binding defects (Lee *et al.* 1992), we examined the genetic properties of N-terminally deleted versions of three TBP mutants deficient for DNA binding and transcriptional activation (Lee and Struhl 1995). All three N-terminally deleted derivatives support yeast cell growth at levels comparable to their full-length counterparts (Table 1). As previously reported (Lee and Struhl 1995), the F148L, N159L, and V161A derivatives are compromised for Gal4-dependent activation when compared to wild-type TBP (39, 42, and 8% of wild-type activity, respectively; Figure 1). Removal of the N-terminal region from wild-type TBP results in a 40% decrease in Gal4-dependent activation, in accord with previous results (Cormack *et al.* 1991; Reddy and Hahn 1991). By contrast, N-terminal deletion confers increased transcriptional activation by the N159L, V161A, and perhaps the F148L derivatives.

Deletion of the N-terminal region of TBP does not affect *HIS3* TATA-element utilization: The results above and previous biochemical experiments indicate that the N-terminal region can act as an inhibitor of TBP function. For this reason, we examined whether the N-terminal region of TBP affects TATA-element utilization at the *HIS3* promoter in a manner similar to that of other general inhibitors of TBP function such as Mot1 (Collart 1996), NC2 (Lemaire *et al.* 2000), and TAF130

TABLE 1

Viability of defined TBP mutants with and without the N-terminal domain

TBP derivative	Biochemical defect	Viability as	
		Full length TBP	ΔN terminus
Wild type (TBP^+)	None	+++	+++
L114K	DNA binding	+	+
S118L	DNA binding	+++	++
N159L	DNA binding	++	++
V161A	DNA binding	+++	+++
F148L	DNA binding	+++	+++
D130A	TFIIA interaction	+++	++
R137A	TFIIA interaction	+++	−
D130A, R137A	TFIIA interaction	++	−
Y139A	TFIIA interaction	+++	+++
E186A	TFIIB interaction	−	−
E188A	TFIIB interaction	+++	+++
L189K	TFIIB interaction	−	−
L134A, E222A	TFIIF, Pol II interaction	+++	++
E108A, L134A, L189A, E222A	TFIIB, TFIIF, Pol II interaction	++	++
R137W	TDS4 interaction	++	−
A140R	TDS4 interaction	++	−
F152E	TDS4 interaction	+++	−
F155S	TDS4 interaction	+++	−
I160H	TDS4 interaction	+	−
G162Y	TDS4 interaction	+++	+
R220A	TDS4 interaction	++	++
Y224K	TDS4 interaction	+	+
Y231V	TDS4 interaction	++	++
R238D	TDS4 interaction	++	−
A135T	Decreased Pol I, Pol II	++	−
K138W	TFIIA?	++	−

(Moqtaderi *et al.* 1996). The *HIS3* promoter contains a noncanonical TATA-like element (T_C) that is responsible for initiation from the +1 site and a consensus TATA element (T_R) that is responsible for initiation from the +13 site (Iyer and Struhl 1995). Loss of Mot1, NC2, or TAF130 results in a dramatic decrease in T_C-dependent transcription from the +1 site, but does not affect T_R-dependent transcription from the +13 site. In contrast, deletion of the N-terminal region of TBP does not affect the pattern of transcriptional initiation, and hence TATA-element utilization, at the *HIS3* promoter region (Figure 2). In addition, the N-terminally deleted version of TBP has minimal effects on transcription by RNA polymerases I and III. Thus, by the criterion of TATA-element utilization at the *HIS3* promoter, the N-terminal region of TBP behaves differently from other proteins that can function as inhibitors of TBP function.

Mutations within the core domain of TBP render the N-terminal domain essential for viability: In addition to its negative effect on DNA binding, the N-terminal region of TBP has been postulated to participate in protein-protein interactions with other components of the transcription apparatus (Lescure *et al.* 1994; Zhou and Berk 1995; Goppelt and Meisterernst 1996). Deletion of the N-terminal region has minimal effects on growth and on transcription by all three nuclear RNA polymerases. Thus, if the N-terminal region mediates physiologically important functions, such functions must be also performed by another region of TBP or by another protein(s).

To search for such potential redundant functions, we constructed N-terminally deleted versions of a panel of TBP mutants with specific defects for interactions with TFIIA (Stargell and Struhl 1995; Lee and Struhl 1997), TFIIB (Kim *et al.* 1994; Lee and Struhl 1997), TFIIF, RNA polymerase II (Lee and Struhl 1997), and the RNA polymerase III factor Brf1 (Cormack and Struhl 1993). Deletion of the N-terminal region from TBP mutants deficient for interaction with DNA, TFIIB, TFIIF, and RNA polymerase II produces only modest changes in growth (Table 1). In contrast, N-terminal deletion of a subset of mutations involving the TFIIA-

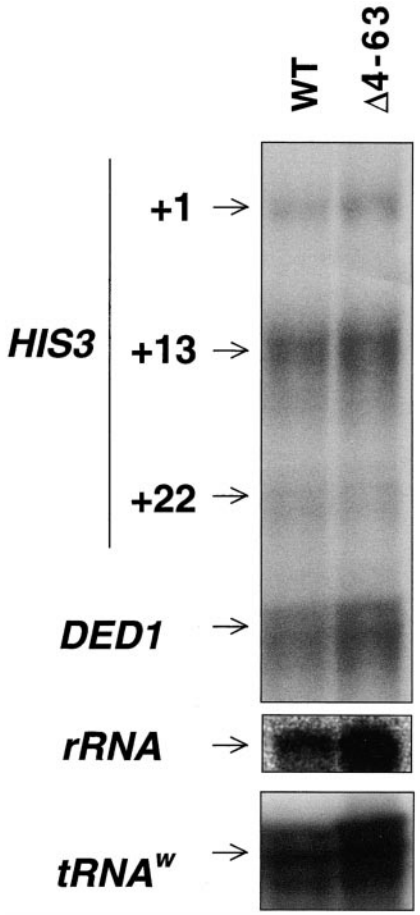

FIGURE 2.—Deletion of the N terminus of TBP does not significantly alter Pol I, II, or III transcription *in vivo*. S1 nuclease protection assays for determination of *HIS3*, *DED1*, rRNA, and $tRNA^w$ transcription levels in yeast strains supported by either full-length or N-terminally deleted (Δ4-63) TBP are shown. All bands in the right lane are <50% higher in intensity than in the left lane and likely represent a difference in total RNA used for each assay.

and Brf1-interaction surfaces results in lethality. Although removal of the N-terminal domain does not destabilize wild-type TBP (CORMACK *et al.* 1991; REDDY and HAHN 1991; GANSTER *et al.* 1993), it might cause protein instability of TBP derivatives with mutations within the core domain. To address this possibility, FLAG-tagged versions of these inviable N-terminally deleted TBP mutants were generated to facilitate detection of their protein levels *in vivo*. Unexpectedly, three of these mutants (A135T, K138W, and R238D) are viable in the context of this N-terminal FLAG epitope tag (Table 2). The ability of an unrelated peptide (*i.e.*, FLAG) to functionally substitute for the N terminus in these three mutants suggests that these mutants, in the absence of the N terminus, are unstable or misfolded. The remainder of the inviable N-terminally truncated TBP mutants, however, are inviable in the context of the FLAG epitope, and their *in vivo* protein levels (with the possible exception of F152E) are comparable to the level achieved by N-terminally deleted wild-type TBP (Figure 3).

TABLE 2

Viability of N-terminally truncated TBP derivatives with and without an N-terminal FLAG epitope

TBP derivative	Viability as Full-length TBP	ΔN terminus	FLAG-ΔN terminus
Wild type (TBP^+)	+++	+++	+++
A135T	++	−	+
K138W	++	−	++
R238D	++	−	+
R137A	+++	−	−
A140R	++	−	−
F152E	+++	−	−
F155S	++	−	−
I160H	+	−	−

N-terminal-dependent mutations map to a limited surface of TBP and define a new TBP function: The mutations that confer dependence on the N-terminal domain lie in close proximity within the three-dimensional structure of the TBP core domain (Figure 4). Specifically, the five positions define a limited surface on the convex face of TBP that lines the inner portion of the groove formed by helix H2, helix H2′, and the segments preceding strand S1 and connecting helix H2 and strand S1′. Amino acid substitutions at neighboring positions where the side chains project away from this putative interaction surface do not yield this phenotype. For example, N159, I160, and V161 are adjacent residues; but, unlike I160, the side chains of N159 and V161 project, away from the convex surface of TBP, and mutations at these positions do not confer this phenotype.

Despite the overlap of this surface with the regions previously implicated in interactions with TFIIA and Brf1, disruption of interaction with neither of these two factors appears to be responsible for this N-terminal dependence. First, only a subset of the mutations that disrupt either TFIIA or Brf1 interaction yield this pheno-

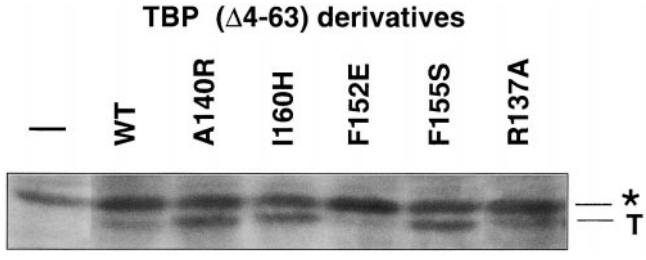

FIGURE 3.—Western blot analysis of inviable N-terminally truncated TBP mutants. Whole-cell extracts from yeast strains supported by wild-type TBP and coexpressing the indicated TBP derivatives were analyzed with monoclonal antibody mAb58C9. *, cross-reacting band.

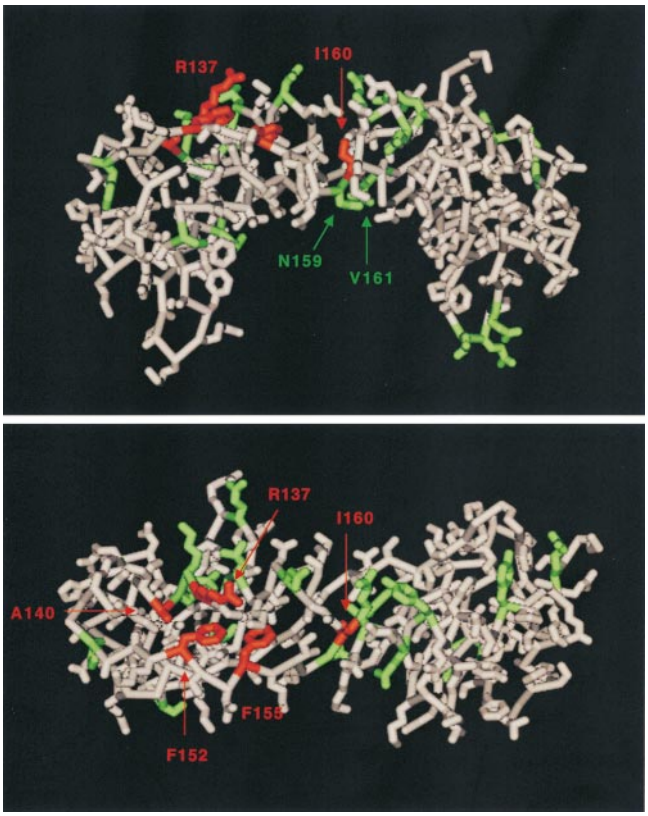

FIGURE 4.—TBP mutants with N-terminal dependence map to a discrete surface of TBP. Two views of an X-ray crystallographic structure of yeast TBP (based on coordinates from NCBI Entrez MMDB file 1TBP; CHASMAN *et al.* 1993) are shown. Structure renditions were generated by Cn3D version 3.0 (NCBI). Residues given in green represent positions tested in this study in which full-length and N-terminally truncated derivatives exhibited identical phenotypes. Residues given in red indicate positions at which mutations conferred N-terminal dependence. Top, view of TBP molecular saddle perpendicular to the intramolecular dyad axis. Bottom, view of the convex surface of TBP molecular saddle along the intramolecular dyad axis, looking into the groove formed by helix H2, helix H2′, and the segments preceding strand S1 and connecting helix H2 and strand S1′.

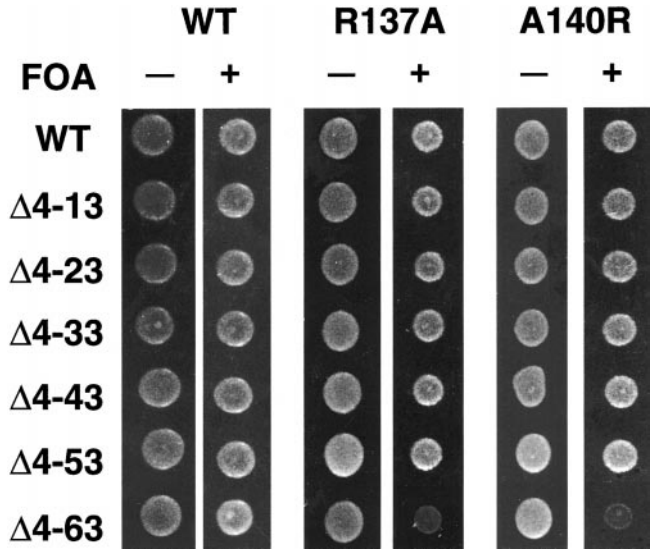

FIGURE 5.—Sequential truncation of the N terminus of TBP. Viability of TBP derivatives with successive deletions of the N-terminal domain was assessed by growth on medium containing 5-fluoroorotic acid (FOA) in a plasmid shuffle assay. R137A and A140R respectively affect the TFIIA- and Brf1-interaction surfaces.

type (Table 1). Second, Y139A, which is 20–30 times more deficient for TFIIA interaction than D130A, R137A (LEE and STRUHL 1997), is unaffected by removal of the N terminus. Third, overexpression of Brf1 fails to suppress the requirement for the N-terminal domain in these mutants (data not shown), even though it does suppress the Pol III defect (CORMACK and STRUHL, 1993). Std1(Msn3) interacts directly with yeast TBP *in vitro* and *in vivo*, and this interaction is modulated by the N-terminal domain of TBP (TILLMAN *et al.* 1995). However, neither overexpression nor deletion of *STD1* relieves the N-terminal dependence of these TBP mutants (data not shown).

A 10-amino-acid segment of the N-terminal region is sufficient to restore viability to TBP mutants dependent on the N terminus: The above results suggest that, in the absence of a particular interaction(s) normally mediated by the core domain of TBP, at least one function of the N-terminal region becomes essential for yeast cell growth. To localize the region within the N terminus that performs this function, we analyzed a series of successive deletions within the N-terminal region in the context of two TBP mutants that require the N-terminal domain: R137A (TFIIA-interaction mutant) and A140R (Brf1-interaction mutant). Remarkably, all but the final 10 amino acids of the N-terminal domain could be removed without affecting the viability of these two TBP mutants (Figure 5). This 10-amino-acid segment likely encodes a specific function, as an unrelated sequence (FLAG) could not be functionally substituted (Table 2).

DISCUSSION

A role for the N-terminal domain in DNA binding by TBP *in vivo*: The association of TBP with the TATA element is a crucial regulatory step in the formation of productive transcription complexes *in vivo*. Direct recruitment and stabilization of TBP at the promoter through fusions with heterologous DNA-binding domains activates transcription (CHATTERJEE and STRUHL 1995; KLAGES and STRUBIN 1995; XIAO *et al.* 1995), and natural transcriptional activators enhance TATA occupancy by TBP at chromosomal promoters (KLEIN and STRUHL 1994; KURAS and STRUHL 1999; LI *et al.* 1999). A subset of TBP mutations that selectively impair activated transcription maps to the TBP-TATA interface (KIM *et al.* 1994; ARNDT *et al.* 1995; LEE and STRUHL 1995). In this report, we demonstrate that removal of

the N-terminal region from these DNA-binding mutants of TBP can partially restore their activation competency. This result provides evidence that the inhibitory effect of the TBP N-terminal domain on DNA binding, long recognized *in vitro* (Horikoshi *et al.* 1990; Lieberman *et al.* 1991; Lee *et al.* 1992; Kuddus and Schmidt 1993), has physiological relevance.

More generally, our results indicate that the N-terminal region of TBP has an autoinhibitory role *in vivo*. In otherwise wild-type cells, loss of this autoinhibitory function does not significantly affect cell growth or general transcriptional functions of TBP. In part, the minimal phenotype caused by loss of the TBP N-terminal region might be due to functional redundancy with other negative regulators of TBP function such as NC2 (Gadbois *et al.* 1997; Kim *et al.* 1997, 2000; Prelich 1997; Lemaire *et al.* 2000; Xie *et al.* 2000), Mot1 (Auble *et al.* 1994, 1997; Collart 1996; Madison and Winston 1997), the N terminus of TAF145 (Kokubo *et al.* 1998), and the NOT complex (Collart and Struhl 1994; Collart 1996). Nevertheless, we suspect that loss of this autoregulatory function might result in the generation of a hyperfunctional TBP, thereby explaining why overexpression of the TBP core domain strongly inhibits cell growth even in the presence of wild-type TBP (Gill and Tjian 1991; Zhou *et al.* 1991).

Functional redundancy between the N terminus and a surface of the TBP core domain: We examined the effect of N-terminal deletion in the context of TBP mutations representing 25 unique positions with the core domain. Mutations at only 5 of those positions yield a lethal phenotype when combined with the N-terminal deletion (excluding the mutants that are rescued by the presence of an N-terminal FLAG epitope). Strikingly, these 5 positions define a limited surface of TBP that lines the inner portion of the groove formed by helix H2, helix H2′, and the segments preceding strand S1 and connecting helix H2 and strand S1′. This synthetic lethality between TBP mutations lacking the N-terminal region and those affecting a specific surface in the core domain provides genetic evidence for functional redundancy between these two regions of TBP.

These observations provide evidence both for a positive function for the N-terminal region of TBP and for a previously unidentified surface of the TBP core domain that interacts with a transcriptional regulatory protein(s). The factor(s) that interacts with this surface of the TBP core domain is unknown, although TFIIA, TFIIB, and Brf1 do not appear to be strong candidates. A role for the TBP N terminus in interactions with other transcription factors is consistent with observations *in vitro* in which the presence of the N-terminal domain affects the association of TBP with NC2 (Goppelt and Meisterernst 1996), TAFs (Zhou and Berk 1995), Std1 (Tillman *et al.* 1995), and the SNAP complex (Mittal and Hernandez 1997).

We consider two models by which the N-terminal region of TBP performs a positive function. In one model, the N-terminal domain stabilizes the interaction between TBP and the factor that interacts with the surface of the core domain identified by virtue of the synthetic lethal effects described above. The role of the N-terminal region of human TBP in mediating cooperative binding with the SNAP complex at the U6 promoter (Mittal and Hernandez 1997) is a precedent for such a model, although the SNAP complex and its target site (PSE) do not exist in yeast cells. In a second model, the apparent positive role of the N-terminal region might actually be a negative function that is redundant with a distinct negative regulatory function that is mediated by the limited surface of the TBP core domain. In this view, synthetic lethality results from the simultaneous elimination of multiple negative regulators of TBP function. In accord with this model, the region immediately adjacent to yeast TBP core domain is required both for the synthetic lethal interactions observed here (Figure 5) and for the toxicity observed upon overexpression of the core domain (Zhou *et al.* 1991). By either model, our results and previous observations (Zhou and Berk 1995; Goppelt and Meisterernst 1996; Mittal and Hernandez 1997) suggest that the evolutionarily divergent N-terminal region coevolved with other transcriptional regulators to mediate certain species-specific functions of TBP.

We thank Martin Schmidt for providing *STD* constructs, Laurie Stargell for helpful discussions at the initial stages of this work, and Lisete Fernandes and Ada Garcia for assistance in the preparation of this manuscript. We also thank Susanna Chou, Sukalyan Chatterjee, and Irene Wu for their discussions and critical reading of the manuscript. This work was supported by a predoctoral fellowship to M.L. from the Howard Hughes Medical Institute and by research grants to K.S. from the National Institutes of Health (GM 30186 and GM 53720).

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Communicating editor: M. Hampsey