Engineering Dimer-stabilizing Mutations in the TATA-binding Protein*

Haiping Kou and B. Franklin Pugh‡

From the Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802

The TATA-binding protein (TBP) plays a central role in assembling eukaryotic transcription complexes and is subjected to extensive regulation including auto-inhibition of its DNA binding activity through dimerization. Previously, we have shown that mutations that disrupt TBP dimers in vitro have three detectable phenotypes in vivo, including decreased steady-state levels of the mutants, transcriptional derepression, and toxicity toward cell growth. In an effort to more precisely define the multimeric structure of TBP in vivo, the crystallographic dimer structure was used to design mutations that might enhance dimer stability. These mutations were found to enhance dimer stability in vitro and significantly suppress in vivo phenotypes arising from a dimer-destabilizing mutation. Although it is conceivable that phenotypes associated with dimer-destabilizing mutants could arise through defective interactions with other cellular factors, intragenic suppression of these phenotypes by mutations designed to stabilize dimers provides compelling evidence for a crystallographic dimer configuration in vivo.

Gene expression levels are derived from a net output of a dynamic interplay of positive and negative regulatory factors. The main steps leading toward gene activation include chromatin modification and remodeling, TBP delivery, and assembly of the RNA polymerase II holoenzyme (1–3). Each of these steps is the target of multiple regulatory factors. TBP delivery to promoters is directed by the positive action of transcriptional activators, acetylated histone tails, SAGA and TFIID delivery complexes, basal factors such as TFIIF and TFIID, and the TATA box. Counteracting this delivery are Mot1, NC2, a portion of TAF1 (termed TAND), the amino-terminal domain of TBP, and TBP auto-inhibition, which occurs through dimerization and occlusion of its DNA binding surface.

TBP auto-inhibition through dimerization is an evolutionary conserved process, occurring from yeast to mammals (4–15). The structure of TBP dimers has been defined crystallographically and through biochemical analysis. Dimer instability caused by mutations along the crystallographic dimer interface correlate with transcriptional derepression in yeast cells (8, 15), and this derepression occurs genome-wide at about 7% of all genes (16). Together, these findings indicated that TBP dimerization represents a physiologically important mechanism for auto-inhibiting its DNA binding activity. Nonetheless, the notion of an auto-inhibited TBP dimer has been sufficiently controversial (17) that we pursued the possibility of using the x-ray crystal structure of TBP dimers to design stabilizing mutations that might intragenically suppress a dimerization mutant.

We focused on two residues, Arg-98 and Arg-171, which lie on opposite sides of a TBP monomer. In the dimer configuration, Arg-98 of one monomer lies immediately across Arg-171 of the opposing monomer (11). We reasoned that changing one or the other to an acidic residue might generate a positive electrostatic interaction whose effect can be measured in vitro using dimerization and DNA binding assays. Previous in vivo data suggested that TBP normally resides as dimers only when not bound to DNA (8, 15). Under such circumstances, enhanced dimer stability might have little impact on its already auto-repressed state. Therefore, we utilized a previously characterized unstable dimer mutant N69R (8) and created additional mutations at Arg-98 and Arg-171 to assess whether such mutations might suppress phenotypes associated with the N69R mutation. Phenotypes include lowered steady-state protein levels of the mutants, transcriptional derepression of a basal promoter, and inhibition of cell growth (toxicity). We find that R98E and R171E partially suppress these phenotypes associated with N69R, which lends further support for a TBP dimer configuration in vivo that mimics the crystallographic dimer structure.

EXPERIMENTAL PROCEDURES

Plasmids and Strains—Mutant TBPs were created in pET16b-γTBP (xxx) by oligonucleotide-directed mutagenesis and sequence-verified, as described previously (15). pCalf/TxxT/PGK is a low copy plasmid harboring HA-tagged TBP mutants under the control of the PGK1 promoter, as described previously (15). “xxx” denotes the mutation. pADH-LacZ was described (18). pKH80 was constructed as described elsewhere (19). pPK315-spt/E240K was constructed by transferring an EcoRI-XhoI fragment containing the Spt3-401 allele from p3-401 (gift of Fred Winston). BY4705 (MATa ade2-1::his3 his3D200 leu2-3,112 lyt2Δ3 met15Δ3 trp1Δ63 ura3Δ0) and BY4742 (MATa his3Δ1 leu2Δ3 lys2Δ3 ura3Δ0) were used for all in vitro studies. Strain KH3 is an spt3Δ derivative of BY4705, created by deleting spt3 via homologous recombination using kanMX. YTW22 (MATa ura3-52 trplΔ1 his3Δ200 leu2Δ3 lys2Δ3::HIS3 ade2-101::hisG pro4Δ15::TRP1[pCW16-TBP-WT]) is a TBP plasmid shuffle strain (20). An spt3Δ version of this strain was created by deleting SPT3 via homologous recombination using kanMX.

GST Pull-down Assay—Purification of yeast TBP mutants and the GST pull-down assay were performed as described previously (15). GST concentrations were determined by comparison with known GST standards, within its linear range. The concentration of GST-181C and His-tagged TBP derivatives in each reaction was 20 and 45 nM, respectively. Although it was not possible to determine the monomer/dimer concentration of GST-181C on the resin and TBP off the resin, titration...
experiments suggest that GST-181C monomers were in excess over TBP monomers. Data were quantitated by densitometry of autoradiographs and plotted as described previously (9). In short, band intensities corresponding to the amount of TBP retained on the resin were normalized to GST-181C recovery and then fit to the equation \( y = \Delta S(1 - e^{-kt}) \). The parameters \( k \) and \( t \) represent the apparent net rate constant and time (min), respectively. Each curve was normalized to \( \Delta S \) so that the reaction coordinate (y) ranged between 0 and 1. Experiments were performed at least three times, and representative data are shown. Kinetic time courses were limited to about 3 h since TBP is intrinsically unstable when not bound to DNA (6). Irreversible inactivation and multiple dimer equilibria in these immobilized binding reactions preclude a meaningful interpretation of apparent rate constants.

**Other Assays**—Detailed procedures for the TBP immunoblots, \( \beta \)-galactosidase assays, electrophoretic mobility shift assays, and toxicity assays are described elsewhere (15).

**RESULTS**

**Arg-98 and Arg-171 Abut Each Other in the Crystallographic TBP Dimer**—The crystallographic structure of the conserved core of TBP is shown in Fig. 1A. We examined the dimer interface for amino acids that did not appear to be contributing positively to dimerization and that, if mutated, might generate a stabilizing interaction across the interface. As shown, Arg-98 of one monomer abuts directly against Arg-171 of the other monomer. Being of the same charge, these two residues might be generally repulsive and thus somewhat destabilizing to the dimer. Alternatively, hydrophobic interactions between the two side chains could contribute to dimer stability. In a monomer configuration, these two residues are far apart and thus are not expected to have any direct interactions. Both are also far from Asn-69 and thus are not expected to influence phenotypes associated with the dimer destabilizing N69R mutation through localized intramolecular interactions.

In an effort to create a more stable dimer, R98E and R171E mutations in His-tagged TBP were created individually and in combination. The mutant proteins were purified after overexpression in bacteria (Fig. 1B), and their dimerization and DNA binding properties were examined.

**R98E and R171E Stabilize TBP Dimers**—To measure relative TBP dimer stability, a previously described GST pull-down assay was employed (9, 15) in which the kinetics of TBP binding to resin-bound GST-181C are measured (Fig. 2A). GST-181C contains the carboxyl-terminal conserved DNA binding/dimerization core of TBP (illustrated in Fig. 1A) fused to glutathione S-transferase. Previously, we had demonstrated that, with both human and yeast TBP, slow binding of TBP to the GST-181C resin reflects slow dimer dissociation followed by rapid capture by resin-bound GST-181C (6, 9).

Kinetic assays were performed on wild type TBP, single mutants R98E and R171E, and the double mutant R98E-R171E. The data are presented in Fig. 2B, and their quantitations are presented Fig. 2C. Fitting each data set to a single exponential decay toward equilibrium (defined as 1.0 along the reaction coordinate) provides a measure of relative dimer stability. The presence of multiple dimerization equilibria (homo- and heterodimers of the mutants and GST-181C) renders quantitative estimates of individual rate constants impractical to assess. Nonetheless, R98E and R171E each displayed a slower approach to equilibrium than wild type TBP, which is consistent with the notion that these mutations form dimer-stabilizing interactions and thus are captured by the resin more slowly. The R98E-R171E double mutant displayed generally faster kinetics than the individual mutants, which is consistent with the removal of dimer-stabilizing interactions. These observations are consistent with behavior expected of a TBP dimer envisioned in the crystal structure.

**TBP(R171E) Has Slower DNA Binding Kinetics**—Previously, we had found that under conditions of limiting monomer concentrations (in which dimers are required to dissociate to detect DNA binding), TBP displays slow binding kinetics to TATA DNA (9). The slow binding was attributed to a slow rate-limiting dissociation of TBP dimers. If the R98E or R171E mutations stabilize TBP dimers, then these mutants might display slower DNA binding kinetics. In Fig. 3A, the binding of the various TBP mutants to TATA DNA was examined under optimal binding conditions in which the monomer concentration was not limiting. Under these conditions, TBP(R171E) bound DNA nearly as well as wild type. However, mutants containing the R98E mutation bound poorly. The weaker binding of the R98E mutants is expected since Arg-98 makes a stabilizing contact with DNA in the TBP/TATA crystal structure (21, 22). Because of the weak binding of R98E, we chose to analyze the DNA binding kinetics of only TBP(R171E).

Typical DNA binding kinetics using the electrophoretic mobility shift assay utilize a vast excess of protein when compared with DNA, resulting in only a small portion of the protein binding to DNA. In the case of TBP, which undergoes active monomer/inactive dimer transitions, it was necessary to keep the DNA concentrations near the total TBP protein concentration so that binding of the majority of the TBP population can be monitored. Otherwise, only the binding kinetics of the active monomer pool are captured. Monomer levels were made partially limiting by incubating 120 nM TBP (containing predomin-
Fig. 2. Dimer dissociation kinetics of TBP mutants. A, a schematic of the pull-down assay used to measure TBP dimer dissociation kinetics. B, a TBP immunoblot showing the amount of TBP mutants bound to GST-181C resin as a function of time of incubation. Time points are indicated above each lane. Note: some time courses have different time frames, 7% of the input was loaded, as indicated. Signals were quantified from two film exposures, using the exposure that was in the linear response range. C, quantitation of data from panel B. Data were fit to a single exponential approach toward the calculated equilibrium (designated at 1.0 along the reaction coordinate).
nM unlabeled TATA oligonucleotide (N, except that the radiolabeled TATA oligonucleotide was mixed with 50 nM magnesium).

and R171E in the purified TBP mutants (30 nM) were incubated with radiolabeled TATA plus an additional 50 nM unlabeled probe. As shown in Fig. 3, ard trace amounts of radiolabeled TATA DNA probe (N) in the absence of DNA, and then probe was added for 1 h. Lane 2

Figure 3. DNA binding kinetics of TBP(R171E). A, the indicated purified TBP mutants (30 nM) were incubated with radiolabeled TATA probe (N) at 23 °C for 40 min. Free and bound (TD) DNA complexes were separated using an electrophoretic mobility shift assay (Tris-magnesium). B, reactions containing 120 nM TBP (WT in the upper subpanel and R171E in the lower subpanel) were performed as in panel A, except that the radiolabeled TATA oligonucleotide was mixed with 50 nM unlabeled TATA oligonucleotide (lanes 4–9). After the indicated time, samples were analyzed. In lane 1, TBP was incubated for 4 h in the absence of DNA, and then probe was added for 1 h. Lane 2 lacked any added protein. Lane 3, unlabeled TATA oligonucleotide was omitted.

R98E and R171E increase the steady-state levels of TBP(N69R)—A general property of mutations that destabilize TBP dimers in vitro is that they are present at lower steady-state levels in vivo (8, 15). For example, TBP(N69R) is present in yeast cells at levels that are only about 10% of wild type TBP. If the R98E and R171E mutations stabilize TBP dimers in vivo, then addition of these mutations to TBP(N69R) might lead to increased steady-state levels of this protein. Since TBP(N69R) does not support cell viability, these experiments are necessarily performed in an otherwise wild type TBP background. Consequently, both homo- and heterodimers could form. However, if dimerization controls the steady-state levels of TBP, then the most stable dimer configuration is likely to dictate the steady-state levels of the TBP mutants. Since heterodimers of the N69R mutants with wild type TBP (having a single dimerization defect) are likely to be more stable than homodimers having two dimerization defects, we consider only the potential stabilizing interaction between the N69R mutants and the endogenous wild type TBP. As illustrated in Fig. 4A, TBP(N69R,R98E) and TBP(N69R,R171E) mutants are expected to create an additional bridging interaction across the dimer interface with endogenous wild type TBP, when compared with TBP(N69R). TBP(N69R,R98E,R171E) is expected to create two additional interactions.

To distinguish the TBP mutants from endogenous wild type TBP, the mutants were HA-tagged. All mutants were expressed from the PGK1 promoter. As shown in Fig. 4B, TBP(N69R) was present at a low steady-state level when compared with wild type TBP, as shown previously (8). Introduction of either R98E or R171E or both into TBP(N69R) resulted in an increase in the steady-state levels of these mutants. This outcome is consistent with the crystallographic prediction that these mutations enhance dimer stability through heterodimerization with the resident wild type TBP.

The fact that TBP interacts with other proteins potentially complicates interpretations of the mutant data. In particular, Arg-171 of TBP has genetic interactions with Glu-240 of Spt3 (23), a subunit of the chromatin-modifying and TBP delivery complex SAGA (24). To address whether SPT3 plays any role in altering the steady-state levels of TBP, the experiments were repeated in an spt3A strain (Fig. 4C), as well as in a strain harboring both a wild type SPT3 allele and an spt3–401 allele (Fig. 4D). The spt3–401 allele harbors an E240K mutation, which is suppressed by mutations at Arg-171 (23). In both strains, the intragenic presence of R98E and/or R171E conferred greater steady-state levels to the N69R mutants, indicating that altered Spt3-TBP interactions cannot account for the increased steady-state level afforded by R171E.

R98E and R171E Partially Suppress a Transcriptional Derepression Phenotype Associated with N69R—A second phenotype associated with TBP dimer mutants is transcriptional derepression. At a basal lacZ reporter gene containing a truncated ADH1 promoter, the presence of TBP(N69R) results in as much as a 200-fold increase in β-galactosidase activity (8). This transcriptional derepression occurs despite TBP(N69R) being present at very low levels. Our previous study suggested that decreased homo- and heterodimer stability (the latter with endogenous wild type), resulting in higher monomer levels, could account for the transcriptional derepression. Here, we test whether the intragenic presence of R98E and/or R171E with N69R could counteract this transcriptional derepression, resulting in lowered β-galactosidase output. Possible heterodimer configurations of the N69R derivatives with endogenous wild type TBP are depicted in Fig. 4A.

When R98E or R171E is introduced on the same molecule as N69R, β-galactosidase activity decreased when compared with N69R alone (Fig. 5A). This is a predicted outcome of enhanced dimer stability. The decrease in β-galactosidase activity occurred despite an overall increase in the protein level of the TBP mutants (Fig. 4B). Previously, we had shown that β-galactosidase activity is proportional to TBP(N69R) levels (8), and so the suppressive effects of R98E and R171E are significantly greater if normalized to TBP levels (not shown). The N69R,R98E,R171E triple mutant displayed an even lower level of β-galactosidase activity, which is expected if this mutant is making additional stabilizing interactions with the endogenous wild type TBP, as depicted in Fig. 4A.

It is possible that the decrease in β-galactosidase activity caused by the R98E and R171E mutations is due to a general disruption of the structure and thus function of TBP. However, as shown further below, TBP(R98E) and TBP(R171E) each support cell viability in the absence of wild type TBP, indicating that the essential functions of TBP remain intact.

It is also possible that the suppression by R171E is caused by a loss of functional interactions with Spt3. To test this possibility, the experiments were repeated in spt3A and SPT3/spt3–401 strains. In both cases, R98E and R171E partially suppressed the elevated β-galactosidase activity caused by N69R.
**Fig. 4. Immunoblot measuring in vivo levels of TBP mutants.** A, schematic of the types of relevant TBP encounters expected in the indicated strains presented in panels B–D. The charges at amino acids 98 and 171 are indicated, and the presence of the N69R mutations is indicated by a line having a T-junction. The wild type and mutant amino acid configurations are designated by black and white circles, respectively. In viewing this schematic, it is important to recognize that dimer stability is driven by a broad, largely hydrophobic interface, which is not illustrated here. 

B, strain BY4742 containing pCALF-T(XXX)(PGK) was grown in CSM-Leu plus 2% glucose to an A600 near 1, after which 0.5 ml of culture was subjected to gel electrophoresis and TBP immunoblot analysis. Bands were quantified by densitometry scanning of autorads, and the level of HA-TBP mutants was normalized to the endogenous (Endog.) TBP levels. In this particular immunoblot, the endogenous TBP bands were more diffuse, giving the erroneous appearance of being less abundant when compared with other panels.

C, strain KH8 (spt3/H9004 derivative of BY4705) was used.

D, strain BY4705 (SPT3) containing the plasmid pHK313-spt3(E240K) harboring the spt3–401 allele was used. All signals were quantified from two film exposures, using the exposure that was in the linear response range.
TBP(R98E) was shuffled in. used in which wild type TBP (pTFIID-Ura) was shuffled out and panel E
His. D harboring the spt3 A except that strain BY4705 (Ura plus 2% glucose to an A
galactopyranoside as a substrate are plotted in each bar graph. panel A as
strain and caused transcriptional derepression (Fig. 5E). Impor-
tantly, however, the level of derepression was significantly less with TBP(N69R,R98E) and TBP(N69R,R98E,R171E) when com-
pared with TBP(N69R,R171E). This pattern in the TBP(R98E)
background contrasts with the pattern observed in the wild type
TBP background (Fig. 5A). The higher level of derepression ob-
erved with TBP(N69R,R171E) is consistent with an absence of
potential 98–171-stabilizing interactions, which are present in
the other mutants (Fig. 5D). Taken together, the sets of in vivo
data presented in Figs. 4 and 5 are remarkably consistent with
behavior predicted form the crystallographic TBP dimer.

R98E and R171E Suppress the Toxicity Caused by N69R—
Expression of TBP(N69R) causes a dominant inhibition of cel-
ular growth, which is associated with dimer instability (15).
We examined whether R98E and R171E, either individually or
in combination, could suppress this toxicity. As shown in Fig. 6,
these mutants behaved in a manner consistent with their sup-
pression of the other phenotypes, causing a suppression of
toxicity in an SPT3/spt3–401 strain, either in liquid or in plate
assays (Fig. 6, A and B, respectively). Similar results were
obtained in wild type and spt3A strains (not shown). The sup-
pression of the toxicity caused by N69R is a predicted outcome
of R98E- and/or R171E-stabilizing TBP dimers.

R98E and R171E Are Synthetically Lethal—As part of a
fuller in vivo characterization of TBP(R98E) and TBP(R171E),
we examined whether they could support cell viability in the
absence of wild type TBP. As shown in Fig. 7A, both mutants
grew when wild type TBP, residing on a Ura-marked plasmid,
was shuffled out via 5-FOA selection. Both mutants grew
slowly, with TBP(R171E) growing the slowest. Interestingly,
the TBP(R98E,R171E) double mutant did not grow, whereas
when the two mutations are introduced into separate TBP
molecules, growth was obtained. As shown in Fig. 7B, these
mutants are capable of being expressed at normal levels prior
to the FOA shuffle, indicating that altered expression levels are
unlikely to account for the synthetic lethality.

The ability of the single mutants to grow but not the double
suggests a number of interpretations. First, it is possible that
Arg-98 and Arg-171 each make functionally redundant con-
tacts that are required to support cell growth. In this case,
growth is still achieved when one or the other is eliminated but
not when both are eliminated. Besides their interactions in the
crystallographic dimer, Arg-98 has crystal contacts with DNA,
and Arg-171 has genetic interactions with Spt3. No other in-
teractions of Arg-98 and Arg-171 are known. TBP-DNA and
TBP-Spt3 contacts could in principle be functionally redund-
ant, if both serve to enhance the binding of TBP to promoters.
To experimentally address this possibility, we examined whether other mutations that impair TBP-DNA binding are synthetically lethal in combination with a loss of SPT3. TBP mutations R196E, K201E, V203E, and L205R lie along the DNA binding surface of TBP and impair DNA binding (15). These mutants, however, support cell growth equally well in both wild type SPT3 and spt3/H9004 strains (Fig. 7C). Therefore, the intragenic synthetic lethality associated with R98E and R171E is unlikely to be attributed to a loss of functionally redundant TBP-DNA and TBP-Spt3 interactions.

Since Arg-98 and Arg-171 abut each other across the crystallographic dimer interface (Fig. 1A), a conceivable second interpretation is that altered interactions between R98E and R171E result in the inability to support cell growth. Nonetheless, any effects of altered interactions between R98E and R171E could be further exacerbated by impaired DNA binding (via R98E) and/or impaired Spt3 interactions (via R171E).

DISCUSSION

True to its central role in gene expression, TBP interacts with many positive and negative regulatory factors. Several TBP interactions have been defined at the structural level, allowing detailed structure/function studies to be conducted. Previously, we mutated 24 amino acids that lie at the crystallographic dimer interface and found a strong correlation between decreased dimer stability and three in vivo phenotypes: decreased steady-state TBP levels, increased transcriptional derepression, and growth impairment (15). This correlation suggested that TBP dimerization has an important function in vivo.

The crystallographic structure of TBP dimers provides a unique opportunity to not only design dimer-disrupting mutations but also to undertake the more challenging task of rationally designing mutations that potentially increase dimer stability. Inasmuch as the rigid dimer crystal structure might only approximate a more dynamic and flexible structure in solution and in vivo, it serves primarily as a guide in mutation design.
Arg-98 and Arg-171 lie on opposite sides of the TBP monomer. However, in the dimer structure, Arg-98 of one monomer abuts Arg-171 of the other monomer, possibly causing some steric repulsion due to their similar charge. This repulsive interaction might afford greater dynamics to the monomer-dimer equilibrium, which might limit TBP binding at some promoters. A prediction of the dimer structure is that a conversion of either Arg-98 or Arg-171 to an oppositely charged glutamate might increase dimer stability via generation of a salt bridge, whereas converting both to glutamate should not. This prediction was borne out when homodimer stability was measured in GST pull-down and DNA binding assays.

The ultimate goal of these experiments is to assess whether rationally designed dimer-stabilizing mutations could suppress \textit{in vivo} phenotypes associated with a dimer-defective TBP mutant. Based upon the dimer crystal structure, we wondered whether R98E or R171E could counteract N69R, thereby suppressing the three dimer-associated phenotypes. Indeed, significant, although not total, suppression was observed. Asn-69, Arg-98, and Arg-171 lie far apart in the monomer, ensuring that altered local interactions are unlikely to contribute to the suppression. In addition, most mutations along the concave surface of TBP elicit phenotypes like N69R, so there was no \textit{a priori} reason to think that mutations at Arg-98 or Arg-171 would have the opposite effect unless in a crystallographic dimer configuration.

Nevertheless, we consider two alternative possibilities. First, phenotypic suppression might be caused by general inactivation of TBP. This seems unlikely in that the single mutants individually support cell viability as the sole source of TBP and thus should be structurally intact. A second possibility is that Arg-98 and Arg-171, which normally interact with DNA and Sp3t, respectively, both contribute to TBP stabilization at promoters. Suppression of the N69R phenotype might occur if a loss of these interactions destabilizes promoter binding of the TBP mutants, which could result in less transcriptional derepression, less toxicity, and lower steady-state levels of the mutant proteins. Several experiments were conducted to address this possibility, including performing the suppression studies in an \textit{sp3t}a strain and a strain harboring both a wild type SPT3 allele and the \textit{sp3t}3–401 allele, which suppresses mutations at Arg-171. In all cases, similar suppression trends were observed, thereby ruling out a loss of Sp3t interactions as the sole basis for suppression by R171E. The loss of DNA contacts from the R98E mutations seems unlikely to be a major contributor to suppression since other mutations along the DNA binding (and dimerization) surface of TBP have more severe defects in DNA binding, and these mutants cause very high levels of transcriptional derepression. Although suppression of the N69R phenotypes cannot be readily explained by unstable promoter binding, we cannot exclude the possibility that potential loss of these interactions contributes to the phenotype.

A striking example of the specificity of suppression is demonstrated in Fig. 5, in which potential \textit{in vivo} heterodimer interactions of wild type TBP with TBP(N69R,R98E) and TBP(N69R,R171E) resulted in similar levels of suppression, whereas suppression with TBP(N69R,R98E,R171E), which is predicted to make an additional interfacial contact with wild type TBP, was greater. Moreover, when wild type TBP was mutated to R98E, and the same series of experiments was repeated, TBP(N69R,R98E) and TBP(N69R,R98E,R171E) behaved similarly, but TBP(N69R,R171E), which is predicted to have one less contact, displayed significantly higher levels of transcription. These series of outcomes are entirely consistent with a crystallographic dimer structure driving the phenotypes.

Why might TBP engage in an auto-inhibitory interaction? The prevailing evidence suggests that TBP has the ability to bind DNA promiscuously, and in that state, it can assemble a functional RNA polymerase II transcription complex (25). Since this unregulated transcription is likely to be detrimental to the cell, several mechanisms might have evolved to block unidirectional binding of TBP to chromosomal DNA. These include Mot1, which uses the energy of ATP hydrolysis to dissociate TBP-DNA complexes. The amino-terminal TAND domain of TAF1 engages in molecular mimicry by taking on the shape of a partially unwound and distorted TATA box and engaging the DNA binding surface of TBP (26). The nonconserved amino-terminal domain of TBP is also inhibitory to DNA binding (27). TBP dimerization adds another level of regulation by also preventing DNA binding through occlusion of the DNA binding surface of TBP. The TAF1 TAND domain and TBP dimerization appear to be partially redundant at a subset of lowly expressed genes in the yeast genome (16). Mot1 and another TBP inhibitor NC2 might work in concert to disassemble TBP from an otherwise active transcription complex (28) and thus are typically associated with active genes (16).

Acknowledgments—We thank Rieko Yakijima for technical assistance and Lata Chitikila, Melissa Durant, Kathryn Huisenga, Jordan Irvin, and Sara Zanton for numerous helpful suggestions throughout this study.

REFERENCES

1. Struhl, K. (1999) \textit{Cell} 98, 1–4
2. Pugh, B. F. (2000) \textit{Genes (Amst.)} 255, 1–14
3. Orphanides, G., Lagrange, T., and Reinberg, D. (1996) \textit{Genes Dev.} 10, 2657–2683
4. Taggart, A. K., and Pugh, B. F. (1996) \textit{Science} 272, 1331–1333
5. Perez-Howard, G. M., Weil, P. A., and Beecham, J. M. (1995) \textit{Biochemistry} 34, 8005–8017
6. Jackson-Fisher, A. J., Burton, S., Portnoy, M., Schneeweis, L., Coleman, R. A., Mitra, M., Chitikila, C., and Pugh, B. F. (1999) \textit{Biochemistry} 38, 11340–11349
7. Coleman, R. A., Taggart, A. K. P., Burton, S., Chica, II, J. J., and Pugh, B. F. (1999) \textit{Cell} 4, 451–457
8. Jackson-Fisher, A. J., Chitikila, C., Mitra, M., and Pugh, B. F. (1999) \textit{Mol. Cell} 3, 717–727
9. Coleman, R. A., and Pugh, B. F. (1997) \textit{Proc. Natl. Acad. Sci. U. S. A.} 94, 7221–7226
10. Coleman, R. A., Taggart, A. K., Benjamin, L. R., and Pugh, B. F. (1995) \textit{J. Biol. Chem.} 270, 13842–13849
11. Chasman, D. I., Flaherty, K. M., Sharp, P. A., and Kornberg, R. D. (1993) \textit{Proc. Natl. Acad. Sci. U. S. A.} 90, 8174–8178
12. Nikolov, D. B., Hu, S. H., Lin, J., Gasch, A., Hoffmann, A., Horikoshi, M., Chua, N. H., Roeder, R. G., and Burley, S. K. (1992) \textit{Nature} 360, 40–46
13. Katz, K., Makino, Y., Kishimoto, T., Yamauchi, J., Katz, S., Muramatsu, T., and Tamura, T. (1994) \textit{Nucleic Acids Res.} 22, 1179–1185
14. Icard-Liepkalns, C. (1993) \textit{Biochem. Biophys. Res. Commun.} 193, 453–459
15. Keu, H., Irvin, J. D., Huisenga, K. L., Mitra, M., and Pugh, B. F. (2003) \textit{Mol. Cell} 22, 3186–3201
16. Chitikila, C., Huisenga, K. L., Irvin, J. D., Mitra, M., and Pugh, B. F. (2002) \textit{Mol. Cell} 10, 871–882
17. Campbell, K. M., Ranallo, R. T., Stargell, L. A., and Lumb, K. J. (2000) \textit{Biochemistry} 39, 2633–2638
18. Blair, W. S., and Cullen, B. R. (1997) \textit{Mol. Cell} 17, 2888–2896
19. Huisenga, K. L., and Pugh, B. F. (2004) \textit{Mol. Cell} 13, 573–585
20. Poon, D., Schneider, S., Wang, C. K., Yamamoto, T., Horikoshi, M., Roeder, R. G., and Weil, P. A. (1991) \textit{Mol. Cell} 11, 4809–4821
21. Kim, Y., Geiger, J. H., Hahn, S., and Sigler, P. B. (1993) \textit{Nature} 365, 512–520
22. Kim, J. L., Nikolov, D. B., and Burley, S. K. (1993) \textit{Nature} 365, 520–527
23. Eisenmann, D. M., Arndt, K. M., Ricupero, S. L., Rooney, J. W., and Winston, F. (1992) \textit{Genes Dev.} 6, 1319–1331
24. Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. R., Candau, R., Obha, R., Owen-Hughes, T., Allis, C. D., Winston, F., Berger, S. L., and Workman, J. L. (1997) \textit{Genes Dev.} 11, 1640–1650
25. Chasman, D. I., and Pugh, B. F. (1996) \textit{J. Biol. Chem.} 270, 13850–13859
26. Liu, D., Ishima, R., Tong, K., Bagby, S., Kokubo, T., Muhandiram, D. R., Kay, L. E., Nakatani, Y., and Ikura, M. (1998) \textit{Cell} 94, 573–583
27. Lee, M., and Struhl, K. (2001) \textit{Genetics} 158, 87–93
28. Darst, R. P., Dasgupta, A., Zhu, C., Hsu, J. Y., Vroom, A., Muldrow, T., and Auble, D. T. (2003) \textit{J. Biol. Chem.} 278, 13216–13226
