Increased Recruitment of TATA-Binding Protein to the Promoter by Transcriptional Activation Domains in Vivo

Charles Klein and Kevin Struhl*

The rate at which the TATA-binding protein (TBP) interacts with the TATA element and promotes transcription by RNA polymerase II was determined in yeast cells. A TBP derivative with altered TATA-element specificity was rapidly induced, and transcription from promoters with appropriately mutated TATA elements was measured. Without a functional activator protein, basal transcription was observed only after a lag of several hours. In contrast, GCN4-activated transcription occurred rapidly upon induction of the TBP derivative. These results suggest that accessibility of TBP to the chromatin template in vivo is rate limiting and that activation domains increase recruitment of TBP to the promoter.

Interaction of the TATA-binding protein (TBP) with the TATA promoter element is the first step in transcriptional initiation by RNA polymerase II (*1*). In vitro, the ability of TBP (or the multiprotein complex TFIID) to bind the TATA element and initiate transcription is strongly repressed by nucleosomes (*2*, *3*). In chromatin reconstitution experiments, TBP or TFIID cannot compete with nucleosomes for template occupancy, whereas TBP added before or concomitantly with histones can access DNA and support TATA-dependent transcription. A similar competition occurs when DNA is injected into fertilized *Xenopus* eggs; transcription is not observed unless TBP is preincubated with DNA or chromatin assembly is disrupted by the addition of an excess of nonspecific competitor DNA (*4*). The inability of TBP to bind nucleosomal templates probably reflects the unusual structure of the TBP-TATA element complex (*3*, *5*) and differs from the properties of some other DNA-binding transcription factors such as glucocorticoid receptor and Gal4 (*6*, *7*).

Although nucleosomes severely repress basal TATA-dependent transcription in vitro, preinitiation complex formation can occur on assembled chromatin templates in the presence of an activator protein bound to its cognate site (*7*, *8*). Moreover, accessibility of TBP to nucleosomal templates in vitro can be increased by histone acetylation or by the Swi-Snf complex, parameters that are associated with transcriptional activation in vivo (*3*). These biochemical experiments suggest that accessibility of TBP to chromatin templates is a central regulatory step for transcriptional initiation. However, the physiological relevance of these experiments has yet to be established. Conversely, whereas evidence suggests that chromatin structure plays a dynamic and important role in transcriptional regulation in vivo (*9*), the rate at which TBP can be productively recruited to nucleosomal templates has not been determined.

To study TBP recruitment to chromatin templates in vivo, we manipulated TBP expression in a manner analogous to that of the experiments in vitro while maintaining normal physiological conditions. We wished to rapidly induce TBP from nondetectable to wild-type levels without substantially altering cellular metabolism. Because TBP is required for cell growth, it was necessary to induce a genetically marked TBP derivative (TBP^{m3}) whose activity could be distinguished from that of wild-type TBP by its ability to permit transcription from promoters containing a mutated (TGTAAA) TATA element (*10*) (Fig. 1, A and B). Thus, transcription from TGTAAA-containing promoters at various times after induction of TBP^{m3} reflects the rate at which TBP productively associates with the chromatin template in vivo.

For rapid induction, we used a copper-inducible promoter that is dependent on the Ace1 transcription factor rather than a Gal4-dependent promoter that is inducible by galactose. Copper induction involves a peripheral aspect of cellular metabolism and occurs by a simple mechanism, the copper-dependent folding of the DNA-binding domain of the Ace1 transcription factor (*11*). In contrast, galactose induction involves a change in carbon metabolism, which is a central aspect of yeast cell physiology, and occurs by a complex and unknown mechanism (*12*). By fusing an Ace1 binding site to the *HIS3* TATA region (*13*), we generated a tightly regulated, copper-inducible promoter (Sc3451; Fig. 1A). When this promoter was used to control expression of the *HIS3* gene, RNA was not detected in the absence of copper (Fig. 1B), and *HIS3* expression was sufficiently low that cells were unable to grow in the absence of histidine. Addition of 500 μM copper sulfate resulted in high levels of *HIS3* RNA within 10 min and maximal levels within 20 min (Fig. 1B), but had no effect on cell growth. When this promoter controlled the expression of an epitope-tagged version of TBP^{m3}, copper induction led to a substantial increase in protein levels within 30 min and maximal levels within 60 min (Fig. 2, A to D). Under conditions of maximal induction, the level of TBP^{m3} is comparable to that of the endogenous TBP.

For investigation of TBP function at a

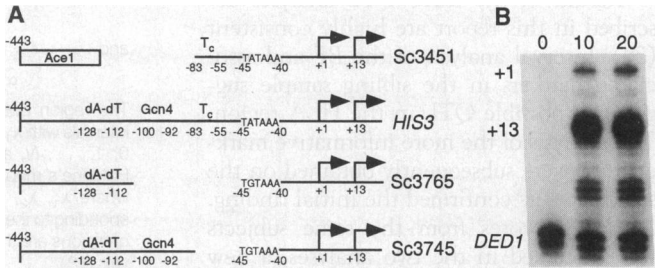

Fig. 1. (**A**) Structures of the promoters. The wild-type *HIS3* promoter contains a poly(dA)·poly(dT) upstream element, a Gcn4 binding site, a nonconsensus TATA element (T_C) that is required for initiation from the +1 site but responds weakly to transcriptional activator proteins, and a conventional TATA sequence (TATAAA) that strongly responds to transcriptional activators and mediates transcription primarily from the +13 site (*23*). Sc3765 and Sc3745 are derivatives of the *HIS3* promoter that contain a TGTAAA TATA sequence, are deleted for T_C, and either lack or possess the Gcn4 binding site (*14*). Sc3451, a *his3* allele containing a tightly regulated, copper-inducible promoter, was generated by insertion of an oligonucleotide containing an Ace1 binding site (TAAGTCTTTTTT-GCTGGAACGGTTGAGCGGAAAAGACGCATC) derived from the *CUP1* promoter into the Eco RI site of Ylp55-Sc3370 (*24*). To regulate the levels of the altered-specificity TBP molecule (TBP^{m3}) and to distinguish it from endogenous TBP, a fragment of Sc3451 containing this copper-inducible promoter up to the ATG initiation codon (*HIS3* nucleotides −548 to +22) was generated by polymerase chain reaction amplification and fused to a derivative of TBP^{m3} containing the simian virus 40 nuclear localization signal and the HA1 epitope from influenza virus at the NH_2-terminus. This construct was cloned into pRS314, a centromeric *TRP1* plasmid (*25*). (**B**) Induction kinetics in response to copper addition. Quantitative S1 analysis of *HIS3* (+1 and +13 transcripts) and *DED1* (the internal control) RNAs (*28*) in a derivative of strain KY320 containing Sc3451 at the normal *HIS3* chromosomal locus at 0, 10, and 20 min after copper induction.

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

*To whom correspondence should be addressed.

basal, TATA-dependent promoter, TBP^{m3} was induced in a strain containing Sc3765, a *HIS3* allele with a TGTAAA TATA element but without a binding site for the Gcn4 activator protein (*14*) (Fig. 1A). In the absence of copper, *HIS3* transcript levels were comparable to those found in the control strain without TBP^{m3} (Fig. 2A). Upon copper induction, no change in *HIS3* RNA levels was observed at 30 min, and there was only a very slight (16%) increase at 60 min even though the level of TBP^{m3} was maximal at this time (Figs. 2A and 3). Transcription that was dependent on TBP^{m3} increased at later times after copper induction (44% at 2 hours and twofold at 4 hours) up to a threefold increase at steady state (*10*). Thus, there is a substantial lag period of roughly 2 hours between the synthesis of TBP^{m3} and its ability to function at basal, TATA-dependent promoters. This observation is analogous to in vitro experiments in which TBP does not function at basal promoters in the context of preassembled nucleosomal templates (*2*, *3*).

Because acidic activators allow TBP to initiate transcription on nucleosomal templates in vitro, they might be expected to reduce the lag time of TBP utilization. To evaluate the function of an acidic activator in vivo, we induced TBP^{m3} in a strain containing Sc3745 (Fig. 1A), a *HIS3* promoter with a binding site for the Gcn4 transcriptional activator upstream of a TGTAAA TATA element (*14*). In the absence of Gcn4, the kinetics of *HIS3* transcription were indistinguishable from those obtained with the promoter lacking the Gcn4 binding site (Figs. 2B and 3). However, in the presence of constitutively expressed Gcn4 (Figs. 2C and 3), *HIS3* RNA levels rapidly increased upon copper induction (2.3-fold at 30 min and 3.2-fold at 60 min) in a manner that was kinetically similar to the increase in TBP^{m3} levels. Thus, in the presence of an acidic activator protein, TBP can rapidly and productively bind to the chromatin template in vivo.

A similar experiment was done in a strain that constitutively expressed gcn4-C131 (*15*), a deleted derivative that contains the intact DNA-binding and dimerization domain but lacks the acidic activation domain (Figs. 2D and 3). Upon induction of TBP^{m3}, the activation-defective Gcn4 derivative showed a lag in *HIS3* transcriptional initiation that was kinetically similar to that observed without Gcn4 (Fig. 2B) or the Gcn4 binding site (Figs. 2A and 3). Thus, the Gcn4 acidic activation domain is required to overcome the lag between synthesis of TBP^{m3} and its ability to support transcription from TGTAAA promoters.

Our results (Fig. 3) suggest that recruitment of TBP to the promoter is rate limiting in vivo and that acidic activation domains increase the rate of this step as part of the mechanism by which they stimulate transcription. Although technical considerations make it difficult to directly measure TBP^{m3} binding to the TGTAAA sequence in vivo (*16*), we doubt that the several hour lag observed in the absence of an activator reflects a rate-limiting step that occurs after TBP binding (for example, recruitment of TFIIB). In vitro, TBP binding is sufficient for template commitment (*1*), and the available evidence argues against a kinetic lag in steps subsequent to TBP binding either in the absence or presence of an acidic activator (*17*). Even in experiments which suggest that activation domains function at later steps, the observed effects were at the level of complex stability, not kinetics of formation (*18*). In vivo, photofootprinting of the yeast *GAL1,10* promot-

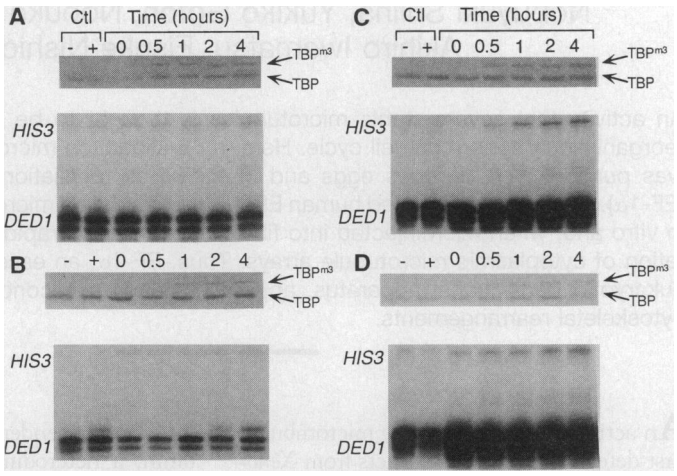

Fig. 2. *HIS3* transcription from TGTAAA promoters upon copper-induction of TBP^{m3}. For each experimental situation, strains contain the *TRP1* centromeric plasmid that expresses the TBP^{m3} derivative from copper-inducible promoter and the indicated *HIS3* and *GCN4* alleles (**A**) Sc3765, *GCN4*, (**B**) Sc3745, *gcn4*-Δ1, (**C**) Sc3745,*gcn4*-Δ1,YCp88-GCN4, and (**D**) Sc3745, *gcn4*-Δ1, YCp88-gcn4-C131 (*14*, *15*); the *gcn4*-Δ1 allele lacks the entire *GCN4* protein-coding region. All yeast strains are derived from KY320 (*26*) and were grown to mid-log phase [typically at an absorbance at 600 nm (A_{600}) of 0.3] in synthetic complete medium, which contains a trace amount (approximately 150 nM) (*27*) of copper. Copper induction was achieved by addition of copper sulfate to a final concentration of 500 μM, and portions of cells were harvested before and at various times (in hours) after the addition of copper. Cells were frozen in liquid nitrogen until all samples were harvested, after which RNA and protein were extracted. Control lanes refer to the relevant strain lacking the TBP^{m3} expression plasmid grown for 12 hours in the presence (+) or absence (−) of 500 μM copper sulfate. The top part of each panel represents a protein immunoblot with the positions of TBP^{m3} and wild-type TBP (as an internal control) indicated. Total protein (50 μg) from each sample was separated by SDS–polyacrylamide gel electrophoresis, electroblotted onto nitrocellulose, and probed with a 1:1000 dilution of a polyclonal antibody to TBP. The bottom part of each panel represents a quantitative S1 analysis of *HIS3* and *DED1* RNAs, which was performed as described (*28*). Because all the *his3* promoters lack T_C, essentially no transcription is initiated from the +1 site; hence, the *HIS3* band represents transcription from the +13 site. An equal amount of unlabeled *DED1* probe was added to the reactions shown in (A), (B), and (D); thus, for a given amount of *HIS3* RNA (relative to *DED1* control), bands in (C) are twofold less intense than those in other panels.

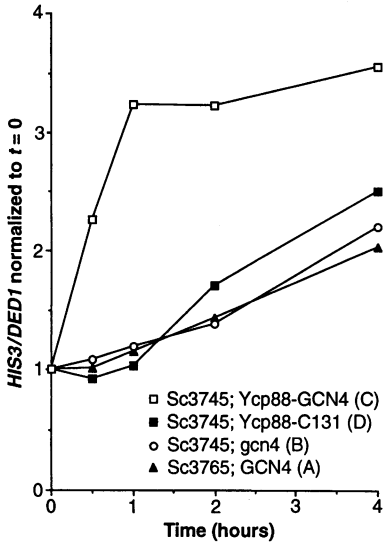

Fig. 3. Kinetics of *HIS3* transcription from TGTAAA promoters upon copper-induction of TBP^{m3}. For each experimental situation (labeled according to the *his3* allele and Gcn4 derivative), data points correspond to *HIS3* RNA levels (normalized to the *DED1* internal control) that are relative to the zero time point (defined as 1.0 and that represents transcription mediated by wild-type TBP at the relevant TGTAAA promoter) as a function of time after addition of copper sulfate to the medium. The fold increase in relative *HIS3* RNA levels upon copper induction for each strain represents TBP^{m3}-dependent transcription. At the zero time points and at steady-state synthesis of TBP^{m3}, *HIS3* RNA levels are fourfold higher under conditions of Gcn4 activation (Fig. 2C) as compared to basal conditions (Fig. 2, A, B, and D). At early time points after copper induction, the apparent fold activation by Gcn4 is higher, a reflection of the differential kinetic effect of TBP^{m3} in the presence or absence of a functional activation domain. RNA levels were quantitated by PhosphorImager (Molecular Dynamics) analysis and represent the average of three independent experiments (one of which is shown in Fig. 2); the error for each determination is ±10%.

er indicates a transcription-dependent change at the TATA element that probably reflects TBP binding (*19*). Finally, our results are consistent with and support the physiological relevance of biochemical experiments indicating that TBP binds very weakly to nucleosomal templates in the absence of a transcriptional activator protein.

The lag between synthesis of TBP^{m3} and its ability to support basal transcription takes several hours, the approximate length of a single cell cycle. It is possible that, in the absence of an activator, recruitment of TBP to the promoter might occur only at a specific time of the cell cycle. In this regard, DNA replication–coupled chromatin assembly has been associated with a variety of transcriptional events in vivo and in vitro (*20*). Alternatively, recruitment under these conditions might be stochastic but simply slow in comparison to the situation at the Gcn4-activated promoter.

Our results do not distinguish between potential mechanisms by which activation domains increase recruitment of TBP to promoters in vivo. Acidic activation domains and the Swi-Snf complex, which is involved in the activation process, cause changes in chromatin structure that occur in the absence of a functional TATA element and transcription (*21*). Perhaps these structural changes increase the accessibility of TBP to the promoter. Increased recruitment also may reflect direct interactions of activation domains to TBP or associated factors (TAFs) that are components of the TFIID complex (*22*). In this view, different classes of activation domains might function in a fundamentally similar manner even though they may interact with different components of the TFIID complex. These models are not incompatible and indeed may be synergistic in explaining how activation domains increase recruitment of TBP to the promoter. Finally, although our results strongly implicate TBP recruitment as a critical step in the transcriptional activation process in vivo, they do not exclude the importance of other steps implicated from in vitro experiments such as recruitment of TFIIB or other general factors (*18*).

REFERENCES AND NOTES

1. R. C. Conaway and J. W. Conaway, *Annu. Rev. Biochem.* **62**, 161 (1993); L. Zawel and D. Reinberg, Eds., *Initiation of Transcription by RNA Polymerase II: A Multi-Step Process* (Academic Press, San Diego, 1993); S. Buratowski, *Cell* **77**, 1 (1994).
2. J. L. Workman and R. G. Roeder, *Cell* **51**, 613 (1987).
3. A. N. Imbalzano, H. Kwon, H. M. R. Green, R. E. Kingston, *Nature* **370**, 481 (1994).
4. M.-N. Prioleau, J. Huet, A. Sentenac, M. Mechali, *Cell* **77**, 439 (1994).
5. Y. Kim, J. H. Geiger, S. Hahn, P. B. Sigler, *Nature* **365**, 512 (1993); J. L. Kim, D. B. Nikolov, S. K. Burley, *ibid*, p. 520.
6. B. Pina, U. Bruggemeier, M. Beato, *Cell* **60**, 719 (1990); R. H. Morse, *Science* **262**, 1563 (1993).
7. I. C. A. Taylor, J. L. Workman, T. J. Schuetz, R. E. Kingston, *Genes Dev.* **5**, 1285 (1991).
8. J. L. Workman, R. G. Roeder, R. E. Kingston, *EMBO J.* **9**, 1299 (1990); P. J. Laybourn and J. T. Kadonaga, *Science* **254**, 238 (1991); G. E. Croston, P. J. Laybourn, S. M. Paranjape, J. T. Kadonaga, *Genes Dev.* **6**, 2270 (1992).
9. M. Grunstein, *Annu. Rev. Cell Biol.* **6**, 643 (1990); G. Felsenfeld, *Nature* **355**, 219 (1992); G. E. Croston and J. T. Kadonaga, *Curr. Opin. Cell Biol.* **5**, 417 (1993); K. Struhl, *Curr. Biol.* **3**, 220 (1993); J. L. Workman and A. R. Buchman, *Trends Biochem. Sci.* **18**, 90 (1993); A. P. Wolffe, *Cell* **77**, 13 (1994).
10. M. Strubin and K. Struhl, *Cell* **68**, 721 (1992); C. Klein and K. Struhl, unpublished data.
11. P. Furst, S. Hu, R. Hackett, D. Hamer, *Cell* **55**, 705 (1988).
12. M. Johnston, *Microbiol. Rev.* **51**, 458 (1987).
13. The Ace1 binding site was fused to the Eco RI site of Sc3370, a *his3* allele that does not support growth in the absence of histidine. The unusually low levels of *his3* expression in Sc3370 may reflect the presence of a Sac I linker that lies just upstream of −85.
14. P. A. B. Harbury and K. Struhl, *Mol. Cell. Biol.* **9**, 5298 (1989).
15. I. A. Hope and K. Struhl, *Cell* **46**, 885 (1986).
16. Attempts to demonstrate TBP binding to the TGTAAA sequence by photofootprinting in vivo were unsuccessful. This is not surprising given that the interaction between TBP^{m3} and TGTAAA is considerably weaker than the interaction between wild-type TBP and a canonical TATAAA sequence (*10*). However, although TBP^{m3} binding to TGTAAA is reduced in comparison to wild-type TBP binding to the optimal TATAAA, it occurs at a level that is comparable to wild-type TBP bound to TATA elements in some natural yeast promoters (*10*). Moreover, the fact that TBP^{m3} can function immediately in the presence of an activator indicates that there is no inherent rate limitation in the binding of TBP^{m3} to TGTAAA sequences. Thus, the experimental situation simulates true in vivo situations.
17. M. W. Van Dyke, M. Sawadogo, R. G. Roeder, *Mol. Cell. Biol.* **9**, 342 (1989); W. Wang, J. D. Gralla, M. Carey, *Genes Dev.* **6**, 1716 (1992); T. Chi and M. Carey, *Mol. Cell. Biol.* **13**, 7045 (1993).
18. Y.-S. Lin and M. R. Green, *Cell* **64**, 971 (1991); B. Choy and M. R. Green, *Nature* **366**, 531 (1993).
19. S. B. Selleck and J. Majors, *Nature* **325**, 173 (1987).
20. G. Almouzni and A. P. Wolffe, *Genes Dev.* **7**, 2033 (1993); R. T. Kamakaka, M. Bulger, J. T. Kadonaga, *ibid.*, p. 1779; O. M. Aparicio and D. E. Gottschling, *ibid.* **8**, 1133 (1994).
21. J. D. Axelrod and J. Majors, *ibid.* **7**, 857 (1993); J. N. Hirschhorn, S. A. Brown, C. D. Clark, F. Winston, *ibid.* **6**, 2288 (1992).
22. C. J. Ingles, M. Shales, W. D. Cress, S. J. Triezenberg, J. Greenblatt, *Nature* **351**, 588 (1991); K. F. Stringer, C. J. Ingles, J. Greenblatt, *ibid.* **345**, 783 (1990); T. Hoey *et al.*, *Cell* **72**, 247 (1993); J. A. Goodrich, T. Hoey, C. J. Thut, A. Admon, R. Tjian, *ibid.* **75**, 519 (1993).
23. K. Struhl, *Mol. Cel. Biol.* **6**, 3847 (1986).
24. ——— and D. E. Hill, *ibid.* **7**, 104 (1987).
25. R. S. Sikorski and P. Hieter, *Genetics* **122**, 19 (1989).
26. W. Chen and K. Struhl, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2691 (1988).
27. M. A. Greco, D. I. Hrab, W. Magner, D. J. Kosman, *J. Bacteriol.* **172**, 317 (1990).
28. W. Chen, S. Tabor, K. Struhl, *Cell* **50**, 1047 (1987).

29. We thank V. Iyer for strains and DNAs, S. Buratowski for antibodies, and A. Berk, S. Buratowski, J. Gralla, R. Kingston, R. Roeder, and L. Stargell for fruitful discussions. Supported by a research grant to K.S. from the National Institutes of Health (GM30186).

6 July 1994; accepted 24 August 1994

Microtubule Severing by Elongation Factor 1α

Nobuyuki Shiina, Yukiko Gotoh, Nobuko Kubomura, Akihiro Iwamatsu, Eisuke Nishida*

An activity that severs stable microtubules is thought to be involved in microtubule reorganization during the cell cycle. Here, a 48-kilodalton microtubule-severing protein was purified from *Xenopus* eggs and identified as translational elongation factor 1α (EF-1α). Bacterially expressed human EF-1α also displayed microtubule-severing activity in vitro and, when microinjected into fibroblasts, induced rapid and transient fragmentation of cytoplasmic microtubule arrays. Thus, EF-1α, an essential component of the eukaryotic translational apparatus, appears to have a second role as a regulator of cytoskeletal rearrangements.

An activity that severs stable microtubules, first detected in mitotic extracts from *Xenopus laevis* eggs (*1*), is thought to participate in the microtubule rearrangements that occur between interphase and mitosis (*1–3*). Microtubule severing also occurs during flagellar excision in the green algae *Chlamydomonas* (*4*). Two microtubule-severing factors have been purified: p56, which severs microtubules slowly in an adenosine triphosphate (ATP)–independent manner (*3*), and katanin, a heterodimeric protein that severs and disassembles microtubules in an ATP-dependent manner (*5*).

We purified from *Xenopus* egg extracts a third protein of molecular weight 48,000 that can rapidly sever taxol-stabilized fluorescent microtubules in vitro (Fig. 1) (*6*). Phosphocellulose chromatography of the extracts generated two peaks of microtubule-severing activity. The activity in flow-through fractions had been previously purified and designated p56 (*3*). Immunoblot analysis with an antibody to p56 indicated that the activity in the adsorbed fraction was not due to p56. The adsorbed fraction severed microtubules only in the

N. Shiina, Y. Gotoh, E. Nishida, Department of Genetics and Molecular Biology, Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan.
N. Kubomura and A. Iwamatsu, Kirin Brewery Company Limited, Central Laboratories for Key Technology, Yokohama, Kanagawa 236, Japan.

*To whom correspondence should be addressed.