MOLECULAR AND CELLULAR BIOLOGY, Dec. 1995, p. 7059–7066 Vol. 15, No. 12
0270-7306/95/$04.00+0

Mechanism of Differential Utilization of the *his3* T_R and T_C TATA Elements

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Received 7 August 1995/Returned for modification 21 September 1995/Accepted 28 September 1995

The yeast *his3* promoter region contains two TATA elements, T_C and T_R, that are differentially utilized in constitutive *his3* transcription and Gcn4-activated *his3* transcription. T_R contains the canonical TATAAA sequence, whereas T_C is an extended region that lacks a conventional TATA sequence and does not support transcription in vitro. Surprisingly, differential *his3* TATA-element utilization does not depend on specific properties of activator proteins but, rather, is determined by the overall level of *his3* transcription. At low levels of transcription, the upstream T_C is preferentially utilized, even though it is inherently a much weaker TATA element than T_R. The TATA elements are utilized equally at intermediate levels, whereas T_R is strongly preferred at high levels of transcription. This characteristic behavior can be recreated by replacing T_C with moderately functional derivatives of a conventional TATA element, suggesting that T_C is a collection of weak TATA elements. Analysis of promoters containing two biochemically defined TATA elements indicates that differential utilization occurs when the upstream TATA element is weaker than the downstream element. In other situations, the upstream TATA element is preferentially utilized in a manner that is independent of the overall level of transcription. Thus, in promoters containing multiple TATA elements, relative utilization not only depends on the quality and arrangement of the TATA elements but can vary with the overall level of transcriptional stimulation. We suggest that differential TATA utilization results from the combination of an intrinsic preference for the upstream element and functional saturation of weak TATA elements at low levels of transcriptional stimulation.

Most yeast promoters contain TATA elements that are recognized by the TATA-binding protein (TBP) a component of the general transcription factor TFIID (43). Unlike the situation in other eukaryotic organisms, yeast TATA elements are located at various positions (40 to 100 bp) upstream of the initiation site; consequently, they are responsible for the general location, but not the precise selection, of initiation sites. Detailed mutational analysis of yeast TATA elements indicates that the optimal sequence is TATAAA(A/T)(A/T) and that functional activity of TATA elements in vivo is strongly correlated with the level of TBP-dependent transcription in vitro (8, 17, 46). Native yeast TATA elements strongly resemble, but are often not identical to, the optimal TATA sequence, and these variations can affect the TBP interaction and promoter strength. In addition, some yeast promoters do not appear to have conventional TATA elements, but they require TBP for transcriptional activity in vivo (11). It is presumed that TBP binds to such TATA-less promoters in a manner that is not sequence specific and that is stabilized by protein-protein interactions.

Some yeast promoters contain multiple TATA elements (28, 37, 42), a situation that poses the issues of relative utilization and potential competition. In the simplest view, TATA elements would act independently in accord with their relative abilities to interact productively with TBP. While relative quality of TATA elements is clearly important, it is not sufficient to explain relative utilization. First, there is evidence for preferential utilization of TATA elements that are located proximally to upstream promoter elements (28, 32). Second, a variety of experiments suggest that yeast cells may contain functionally distinct TATA elements (17, 28, 33, 37, 42). For example, deletion of the consensus TATA element in the *his3*, *his4*, and *ura3* promoters blocks activation by Gcn4 and Ppr1 but does not affect constitutive transcription (33, 37, 42). Third, competition between Ty and *his4* promoter elements has been described for the *his4-912*δ allele (19), and it can be affected by mutant derivatives of TBP (12). It is also possible that the location or quality of the relevant initiator elements might influence TATA element utilization.

Detailed mutational analysis of the yeast *his3* promoter region has defined two TATA elements. T_R, the downstream element, contains a canonical TATA sequence (TATATAAA at positions −47 to −40), and it directs initiation almost exclusively from the +13 start site (42). As expected for a conventional TATA element, T_R is highly sensitive to point mutations (8, 17), and it supports efficient TBP-dependent transcription in vitro (35, 46). In contrast, T_C, which is responsible for transcription from the +1 initiation site, does not have a sequence resembling the consensus binding site for TBP (29, 42). Unlike conventional TATA elements, T_C maps to an extended region (−54 to −83) that is remarkably insensitive to single and multiple point mutations (29), and it does not support TBP-dependent transcription in vitro (35). Nevertheless, despite the absence of an efficient TBP recognition sequence, TBP is required for T_C-dependent transcription in vivo (11).

An interesting feature of the *his3* promoter region is that T_C and T_R are differentially utilized under conditions of constitutive transcription and Gcn4-activated transcription. Under conditions of constitutive *his3* transcription (defined by growth in rich medium or in *gcn4* deletion strains), transcription is initiated with equal efficiencies from the +1 and +13 sites (42). In this situation, T_C and T_R are utilized with approximately equal efficiencies (29), a surprising observation given that T_R efficiently supports transcription in vitro whereas T_C does not (35). In contrast, Gcn4-activated transcription is almost exclu-

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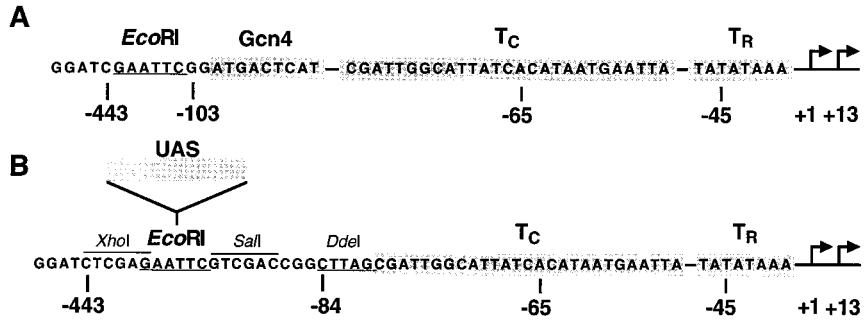

FIG. 1. *his3* promoter derivatives. The nucleotide sequence (numbered with respect to the +1 initiation site) and positions of the promoter elements (shaded) are indicated. (A) The promoter for testing Gcn4 activation contains an optimal Gcn4 binding site upstream of T_C and T_R; sequences between −103 and −443 are replaced by an *Eco*RI site. (B) The promoter for testing other activators contains the indicated sequence and restriction sites in place of *his3* sequences between −84 and −443. Oligonucleotides containing activator binding sites (upstream activating sequence [UAS]) were cloned into the *Eco*RI site.

sively T_R dependent and initiated from the +13 site; Gcn4 can efficiently activate transcription from the +1 site but only if T_R is moved further upstream (35, 42). Similarly, in *gal-his3* hybrid promoters, Gal4 activation is almost exclusively T_R dependent, and the preferential +13 transcription is not affected by the distance between the Gal4 binding sites and the *his3* TATA region (41). From these and other observations, we suggested that T_C and T_R are mechanistically distinct, with T_R but not T_C being able to respond to acidic activators. A remarkably similar set of observations on the *ura3* promoter has been interpreted in the same manner (37).

In this study, we performed a molecular analysis of the parameters that govern differential utilization of the *his3* TATA elements. This study was initially motivated by the idea that the functional differences between the *his3* TATA elements could be exploited to distinguish among transcriptional activators on the basis of their relative stimulation of T_R-dependent transcription and T_C-dependent transcription. However, our results strongly argue against our original hypothesis that T_C and T_R are mechanistically distinct and, instead, suggest that differential TATA element utilization is determined primarily by the overall level of transcriptional stimulation, not by specific functional properties of activator proteins.

MATERIALS AND METHODS

***his3* promoter derivatives.** For experiments involving the analysis of Gcn4 derivatives (Fig. 2, 4, and 5), the promoter derivative was *his3*-Δ*101*,189 (45), which contains an optimal Gcn4 binding site as the only functional element upstream of T_R and T_C (Fig. 1A). To analyze transcriptional stimulation by different yeast activators, we first generated a related promoter in which the Gcn4 binding site was replaced by an oligonucleotide containing an *Xho*I, an *Eco*RI, and a *Sal*I sites (Fig. 1B). Oligonucleotides corresponding to binding sites for a variety of known yeast transcriptional activators were then cloned into the *Eco*RI site. For each transcriptional activator, the corresponding DNA sequence represents either a binding site from a native yeast promoter or an optimal binding sequence defined by sequence comparison or functional analysis (Table 1).

For the TATA reversal and duplication experiments (Fig. 6), we first constructed a derivative in which T_R, flanked by an upstream *Eco*RI site and a downstream *Sac*I site, was located just downstream of the optimal Gcn4 binding site. This was done by PCR amplification of the *his3* promoter region with CGCGAATTCTATATAAAGTAATGTGAGCTCGCG (the *Eco*RI and *Sac*I sites are underlined) and an oligonucleotide annealing at position +950 as primers, digesting the resulting product with *Kpn*I, and replacing the corresponding region between the blunted *Sal*I site and the *Kpn*I site of the construct with a single Gcn4 binding site upstream of T_C and T_R (Fig. 1B). To generate the promoter in which the order of T_C and T_R was reversed, a blunted *Dde*I-*Eco*RI fragment containing T_C was cloned into the blunted *Sac*I site of the initial derivative. To generate the promoter containing a tandem duplication of T_R, a blunted *Eco*RI-*Sac*I fragment containing T_R was cloned into the blunted *Sac*I site.

For the T_C replacement experiments (Fig. 7), the starting molecule contained the minimal T_R sequence (TATAAA instead of TATATAAA) described previously (8). The *Dde*I-*Eco*RI fragment that defines T_C (29) was replaced by a degenerate oligonucleotide that represents mutated T_R derivatives. The sequence TATAAAG within the degenerate oligonucleotide was synthesized such that each position consisted of 80% wild-type nucleotide and 20% of an equimolar mixture of the other three nucleotides. The resulting molecules were sequenced, and selected derivatives were chosen for analysis.

Yeast strains. For all experiments involving Gcn4 activation, the *his3* promoter constructs were introduced into yeast strain KY321 (relevant genotype, *ura3-52*

TABLE 1. Transcriptional activators

Activator	Binding site[a]	Promoter	Medium[b]	Reference
Bas2	GGTAAATTAGTTAATTAATT	*HIS4*	YP-dextrose, no P_i	44
Hap1	TGGCCGGGGTTTACGGACGATGA	*CYC1*	YP-dextrose + DP IX	34
Leu3	CCGGGACCGG	*LEU1*	SD, no leucine	13
Rap1	ACACCCAGACATC	Consensus	YP-dextrose	25
Reb1	CTGTCACCCGGCC	*ACT1*	YP-dextrose	6
Abf1	ATCACTTCGGACG	*PHO2*	YP-dextrose	3
$(dA\text{-}dT)_n$	$(T)_{42}CCG$[c]	Homopolymer	YP-dextrose	22
Hap2-5	CGTTGATTGGTGGA	*CYC1*	YP-lactate	30
Ppr1	TTCGGTAATCTCCGAA	*URA3*	CAA, no uracil	37
Put3	TCGGGAAGCCAACTCCGA	*PUT2*	SD, no NH_4, + proline	38
Gcn4	GATGACTCATT	Optimal	SD + AT	18
Gal4	CGGAGGACAGTACTCCG	Consensus	YP-galactose	5
Hsf	CTAGAAGCTTCTAGA	*TKS2*	YP-dextrose with a 39°C shift	39
Ace1	GATGCGTCTTTTCCGCTGAACCGTTCCAGCAAAAAAGACTA	*CUP1*	SD + 400 μM $CuSO_4$	14

[a] Top strand sequence with flanking *Eco*RI sites not shown.
[b] YP, yeast extract-peptone; DP IX, 100 ng of deuteroporphyrin IX per ml; AT, 10 mM aminotriazole; CAA, Casamino Acids; SC, synthetic complete; SD, synthetic minimal. All media contain glucose unless otherwise indicated.
[c] Poly(dA-dT) stimulates transcription but does not appear to bind an activator protein.

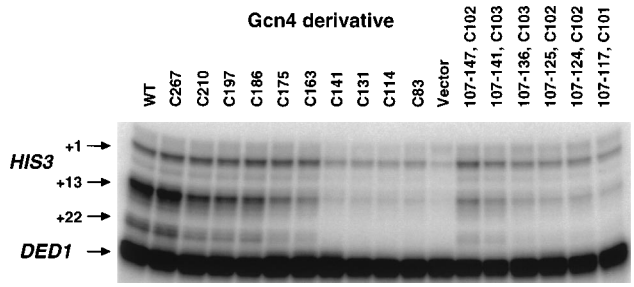

FIG. 2. Stimulation of T_C and T_R by Gcn4 derivatives with various portions of the transcriptional activation domain. RNAs from strains containing the indicated Gcn4 derivatives were subjected to quantitative S1 analysis; the positions of the *his3* (+1, +13, and +22) and *ded1* transcripts are indicated. N-terminally truncated Gcn4 derivatives are indicated by the number of C-terminal amino acids that remain (e.g., C83), whereas internal deletions are indicated by the residues that are fused to the C-terminal DNA-binding domain (e.g., 107-147, C102). WT, wild type.

his3-Δ200 gcn4-Δ1), a *gcn4-Δ1* derivative of KY320 (8), by replacement of the chromosomal *his3* locus. The resulting strains were then transformed by *URA3* centromeric plasmids that expressed Gcn4 derivatives under the control of the *DED1* promoter. The Gcn4 derivatives contained either deletions of different portions of the transcriptional activation region (20, 21) or amino acid substitutions in the bZIP DNA-binding domain (36). The resulting transformants were grown in glucose medium containing Casamino Acids but lacking uracil. To vary Gcn4 levels, the appropriate yeast strain was transformed by a centromeric plasmid that expresses Gcn4 from a tightly regulated, copper-inducible promoter (22); the resulting strain was grown in glucose synthetic complete medium lacking uracil and induced by adding $CuSO_4$ (0 to 400 μM) for 45 min. For the experiments involving the effects of other activator proteins on differential TATA element utilization (Fig. 3), the *his3* promoter constructs were introduced into KY2002 (25) by gene replacement. The resulting strains were grown under conditions appropriate for transcriptional induction by the activator (Table 1).

Transcriptional analysis. Total RNA was prepared from logarithmically growing cells and quantitated by A_{260}. Twenty micrograms of each RNA sample was hybridized to completion with a 10- to 100-fold excess of ^{32}P-labelled *HIS3* and *DED1* oligonucleotides and treated with S1 nuclease as described previously (22). *HIS3* RNA levels were quantitated with respect to the *DED1* internal control by PhosphorImage analysis (Molecular Dynamics); the values are accurate to ±15%.

RESULTS

Relative TATA-element utilization is affected by the quality of the Gcn4 activation domain. Previous results indicate that the acidic activators Gcn4 and Gal4 stimulate transcription almost exclusively through T_R (35, 41, 42). To determine the relationship between the quality of an acidic activation domain and relative utilization of the *his3* TATA elements, we examined a large number of Gcn4 derivatives for their ability to stimulate transcription from the +1 and +13 initiation sites (initiation from +22 behaves similarly to initiation from +13 but occurs at a much lower level and is ignored in this study). These Gcn4 derivatives contain the bZIP DNA-binding domain but lack (either in the N terminus or internally) various portions of the acidic activation domain (20, 21). To simplify the analysis, these Gcn4 derivatives were assayed on a modified *his3* promoter containing an optimal Gcn4 binding site as the only functional element upstream of the *his3* TATA region (Fig. 1A).

As shown in Fig. 2, N terminally deleted derivatives lacking the previously defined activation domain (e.g., C83, C114, C131, or C141) stimulate transcription slightly above the level observed for an isogenic *gcn4* deletion strain. Unexpectedly, this weak stimulation occurs primarily through the +1 site, indicative of preference for T_C over T_R. In contrast, derivatives containing progressively larger portions of the activation domain show an increasing preference for stimulation through +13. The same qualitative behavior is observed with a set of Gcn4 derivatives with internal deletions of the activation domain (Fig. 2). Thus, weak Gcn4 activators preferentially utilize T_C and initiate transcription from +1, whereas increasing activation function progressively leads to a strong preference for T_R-dependent transcription that is initiated from +13.

Effects of different activator proteins on *his3* TATA-element utilization. The hypothesis that T_R and T_C are functionally distinct TATA elements suggested that transcriptional activators could be mechanistically distinguished by their preferences for T_R- or T_C-dependent transcription. To examine this possibility, we assayed *his3* transcription from a set of promoters in which oligonucleotides corresponding to binding sites for a variety of yeast transcriptional activators (Table 1) were cloned upstream of T_C and T_R (Fig. 1B). The activator proteins stimulate *his3* transcription to different extents above the level of the control promoter lacking an upstream element (Fig. 3). Interestingly, *his3* initiation stimulated by the various activators shows a clear pattern. Activators promoting a low level of transcription (e.g., Leu3 and Hap1) show more initiation from +1 than from +13, indicating that they preferentially utilize T_C over T_R. Activators stimulating intermediate levels of transcription (e.g., Abf1, Rap1, and Hap2-5) have similar levels of +1 transcription and +13 transcription, indicating comparable utilization of T_C and T_R. Strong activators (e.g., Gal4, Gcn4, and Hsf) function primarily through T_R, as evidenced by the strong preference for initiation from +13. These observations indicate that the preferential use of T_R with increasing levels of activation is a general phenomenon and is not restricted to acidic activation domains.

Relative TATA element utilization depends on the overall level of transcriptional stimulation. An attractive explanation for the above results (Fig. 2 and 3) is that the mere act of DNA binding by an activator results in weak stimulation of T_C-dependent transcription whereas T_R is specifically responsive to the function of the activation domain. Because activator binding sites are very close to T_C, it is plausible that binding of a protein to these sites might locally disrupt the chromatin structure, thereby making T_C preferentially accessible to TBP and causing an increase in +1 transcription. In this view, the +13/+1 ratio would be diagnostic of the quality of the activation domain of a transcription factor.

To test this idea, we examined a set of Gcn4 derivatives with point mutations on the DNA-binding surface (36). Because these derivatives contain an intact Gcn4 activation domain, they should not affect the strong preference for +13 initiation characteristic of Gcn4. In contrast to this prediction, all mutants conferring reduced *his3* levels show a decrease in the

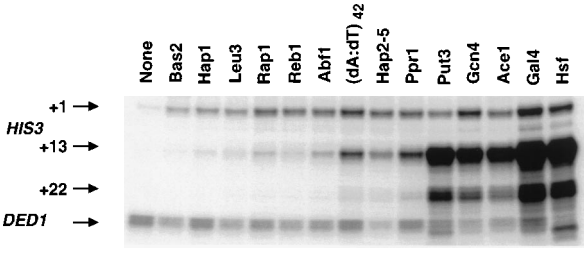

FIG. 3. Stimulation of T_C and T_R by various yeast activators. RNAs from strains containing *his3* promoters with binding sites for the indicated transcription factors (Table 1; Fig. 1B) were subjected to quantitative S1 analysis; the positions of the *his3* (+1, +13, and +22) and *ded1* transcripts are indicated. When strains were grown in conditions inducing for the activator (Table 1), analysis of the control promoter lacking the binding site showed no change in its transcription under the same growth conditions. The specific activity of the *ded1* probe relative to *his3* is reduced by 50% in this experiment.

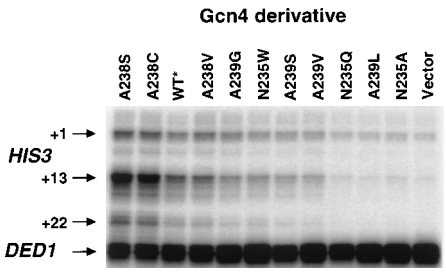

FIG. 4. Effect of Gcn4 DNA-binding activity on utilization of the *his3* TATA elements. RNAs from strains containing the indicated Gcn4 derivatives containing mutations on the DNA-binding surface were subjected to quantitative S1 analysis; the positions of the *his3* (+1, +13, and +22) and *ded1* transcripts are indicated. All Gcn4 derivatives in this experiment lack the 3′ untranslated region (indicated by an asterisk in the case of wild-type [WT] Gcn4), resulting in slightly less Gcn4 function presumably because of the reduced stability of *GCN4* mRNA (36).

ratio of the +13 transcripts to +1 transcripts (Fig. 4). Moreover, some mutants (for reasons unclear) show increased *his3* transcription and an even greater preference for T_R, as indicated by the abnormally high +13/+1 ratio. These results indicate that differential TATA-element utilization does not specifically depend on the nature of the transcriptional activation domain.

Taken together, the results in Fig. 2 to 4 strongly suggest that differential utilization of the *his3* TATA elements is determined primarily by the overall level of transcriptional stimulation. To confirm this conclusion without relying on a comparison between native and mutant proteins, we tested whether the characteristic behavior of the *his3* TATA elements could be reproduced simply by varying the levels of Gcn4. This was accomplished by using a strain in which Gcn4 was expressed from a copper-inducible promoter such that Gcn4 levels could be controlled as a function of exogenously added $CuSO_4$ (22). As shown in Fig. 5, *his3* TATA utilization varies in the expected manner as a function of Gcn4 levels: a preference for +1 at low levels, equal utilization of +1 and +13 at moderate levels, and a strong preference for +13 at high levels.

Proximity to the activator affects differential TATA-element utilization. In vitro, *his3* transcription is mediated almost exclusively through T_R (35), presumably because T_R contains a consensus TATA sequence that is efficiently recognized by TBP whereas T_C does not. The inherent superiority of T_R in promoting TBP-dependent transcription in vitro is likely to account for the preferential utilization of T_R in vivo, which occurs under conditions of efficient *his3* transcription. The surprising observation is that, under conditions of low *his3* transcription in vivo, T_C is preferentially utilized, even though it is inherently a much weaker element than T_R. This result could be due either to mechanistic distinctions between T_R and T_C or to the relative proximity of T_C to the activator binding site.

To distinguish between these possibilities, we examined several Gcn4 derivatives for their ability to stimulate transcription from an artificial promoter in which the order of the *his3* TATA elements was reversed such that T_R was placed at the location normally occupied by T_C and vice versa (Fig. 6A). Under conditions of both low levels and high levels of Gcn4 activation, almost all *his3* transcription initiates from +1 (Fig. 6B), indicating preferential use of T_R. In fact, it is unclear if the low level of +13 initiation is dependent on T_C and/or T_R. Thus, under conditions of low *his3* transcription, T_C is preferentially utilized in the wild-type arrangement of TATA elements, whereas T_R is preferentially utilized when the order of the elements is reversed. This observation suggests that preferential stimulation of T_C is due primarily to its relative proximity to the activator, not an inherent mechanistic property.

To determine if Gcn4 derivatives have an intrinsic preference for the upstream TATA element in the *his3* promoter, we assayed transcription from a promoter in which the T_C region was precisely replaced by an oligonucleotide containing T_R and its flanking sequences (Fig. 6A). Although the tandemly duplicated T_R elements are identical, Gcn4-activated transcription initiated from +1 occurs at a level approximately 70% higher than the level of transcription initiated from +13 (Fig. 6B). Interestingly, the +1/+13 initiation ratios are similar at all levels of Gcn4 activation. These results suggest that Gcn4 has an intrinsic, albeit quantitatively modest, preference for utilizing the upstream TATA element in the *his3* promoter. However, this intrinsic preference for the upstream TATA element does not explain differential utilization of T_C and T_R as a function of the overall level of *his3* transcription.

T_C can be functionally replaced by a moderately active derivative of T_R. The defining functional property of T_C is that its utilization relative to T_R varies according to the overall level of *his3* transcription. Unlike conventional TATA elements, T_C is an extended sequence that is remarkably insensitive to mutations (29) and is unable to support TBP-dependent transcription in vitro (35). Because the mechanism of T_C-dependent transcription is poorly understood, we examined whether T_C could be functionally replaced by a TATA element with defined biochemical properties.

Previously, we characterized a large set of mutated T_R derivatives for their ability to support transcription in vitro and in vivo (8, 17, 46). By using a degenerate oligonucleotide, a library of mutated T_R derivatives was cloned upstream of the actual T_R sequence in the *his3* promoter such that the derivative replaced T_C and could support initiation from +1 (Fig. 7A). The resulting promoters were analyzed for the pattern of transcriptional initiation in the presence of Gcn4 derivatives that weakly (C83) or strongly (C267) activate transcription (Fig. 7B).

As expected from the tandem T_R duplication (Fig. 6B), the upstream TATA element is preferentially utilized under conditions of low or high *his3* transcription if it is a good target for TBP (e.g., TATAAAG or TATAAAC). Also as expected, upstream T_R derivatives that behave as weak TATA elements are strongly disfavored by both activator derivatives. However, the behavior of T_C appears to be mimicked by a variety of upstream TATA elements (e.g., CATAAA or TATAAC) that support a modest level of TBP-dependent transcription in vivo

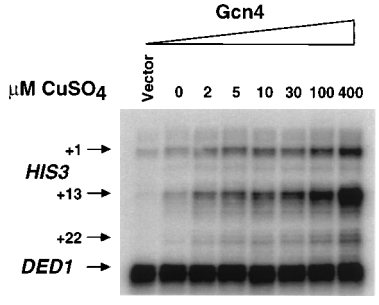

FIG. 5. Effect of Gcn4 concentration on utilization of the *his3* TATA elements. A strain expressing Gcn4 from a copper-inducible promoter was grown at the indicated (micromolar) concentrations of $CuSO_4$, and RNAs were subjected to quantitative S1 analysis; the positions of the *his3* (+1, +13, and +22) and *ded1* transcripts are indicated.

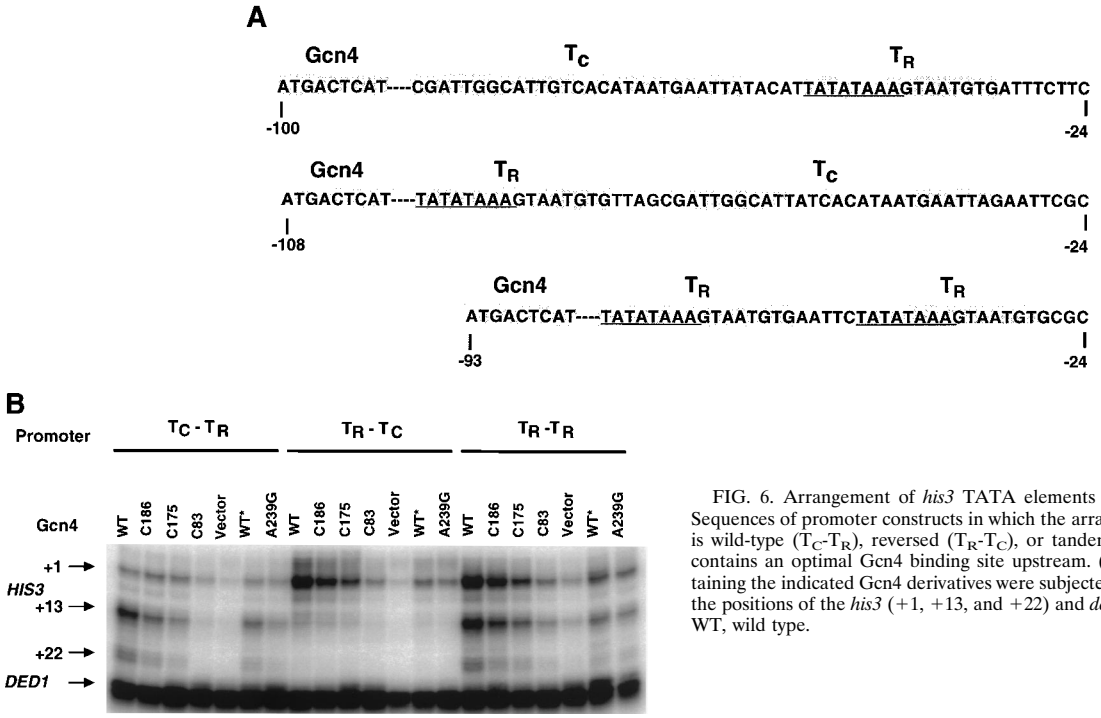

FIG. 6. Arrangement of *his3* TATA elements affects their utilization. (A) Sequences of promoter constructs in which the arrangement of TATA elements is wild-type (T_C-T_R), reversed (T_R-T_C), or tandemly duplicated (T_R-T_R) and contains an optimal Gcn4 binding site upstream. (B) RNAs from strains containing the indicated Gcn4 derivatives were subjected to quantitative S1 analysis; the positions of the *his3* (+1, +13, and +22) and *ded1* transcripts are indicated. WT, wild type.

and in vitro. In these promoters, the upstream element is preferentially utilized at low levels of activation, but the downstream element is preferentially utilized at high levels of activation.

Figure 8 shows a more detailed analysis of representative promoters activated by a series of Gcn4 derivatives that stimulate different levels of *his3* transcription. The sequences CATAAA and TATAAC behave indistinguishably from T_C; when located upstream of T_R, they are preferentially utilized at low levels of *his3* transcription and are increasingly disfavored as *his3* transcription is stimulated to higher levels. The sequence CATAAC, which contains two T_C-like mutations, is a very weak TATA element that is disfavored even at the lowest levels of activation. Conversely, an upstream sequence that is a good TATA sequence (TATAAA) is preferentially utilized over T_R even at the highest levels of activation. Thus, in terms of the defining functional property of differential TATA-element utilization as a function of *his3* transcription levels, T_C is indistinguishable from biochemically defined TATA sequences of moderate function.

Differential utilization occurs with certain arrangements of TATA elements. The relative utilization of TATA elements (as defined by the +13/+1 initiation ratio) for representative promoters activated by a series of Gcn4 derivatives is quantitatively summarized in Fig. 9. When T_C and its functional replacements (CATAAA and TATAAC) are located upstream of T_R, they are preferentially utilized at low levels of *his3* transcription and are increasingly disfavored at higher levels. Similarly, although very weak TATA elements (e.g., CATAAC and TCTAAA) are disfavored under all conditions, the +13/+1 ratio increases significantly in accord with overall levels of *his3* transcription. Conversely, the +13/+1 ratio does not vary as a function of *his3* transcription in the promoters in which T_R is tandemly duplicated or the order of T_R and T_C is reversed.

To gain more information about the parameters that govern differential TATA element utilization, we examined a promoter in which the moderately functional TATA element CATAAA is tandemly duplicated (Fig. 8). As is the case with the promoter containing tandemly duplicated T_R elements, the upstream TATA element is preferentially utilized, and the +13/+1 ratio does not significantly vary as a function of Gcn4 activation (Fig. 9). A similar result occurs in a promoter containing tandemly duplicated TATAAC sequences. Taken together, these observations suggest that differential TATA element utilization occurs when the upstream TATA element is weaker than the downstream element.

DISCUSSION

T_C is likely to be a collection of weak, overlapping TATA elements. On the basis of several observations (see the introduction), we previously suggested that T_C and T_R function by distinct molecular mechanisms. However, results presented in this article strongly argue against the hypothesis that T_C and T_R are functionally distinct. Most convincingly, in terms of differential TATA-element utilization, T_C can be functionally replaced by several T_R derivatives that support a moderate level of TBP-dependent transcription in vitro (Fig. 7 and 8). T_R derivatives that support high or low levels of transcription in vitro behave differently from T_C. Thus, the ability of a T_R derivative to mimic the behavior of T_C depends on its level of function, not a special sequence(s). In agreement with this view, preferential utilization of T_C under conditions of low *his3* transcription is not observed if the order of T_C and T_R is reversed (Fig. 6); hence, it is due primarily to the proximal location of T_C to the activator binding site, not a distinct functional property.

Although mutational analysis suggests that T_C has an unusual structure (29), our present results indicate that T_C is functionally equivalent to a conventional TATA element of moderate quality. To resolve this apparent discrepancy, we suggest that T_C is a collection of conventional TATA elements,

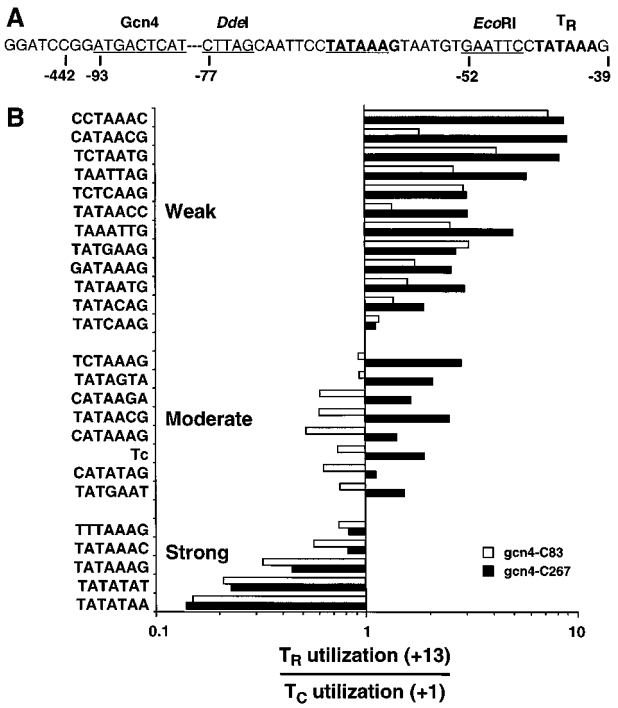

FIG. 7. Replacement of T_C by biochemically defined TATA elements. (A) Promoter with an optimal Gcn4 binding site upstream of tandem minimal T_R elements (TATAAA instead of TATATAAA) flanked by indicated restriction sites. The degenerate oligonucleotide containing T_R (mutated bases are underlined) was cloned between the *Dde*I and *Eco*RI sites at the position originally occupied by T_C. (B) Relative utilization of the two TATA elements at low and high levels of activation. The ratio of +13 transcription to +1 transcription, which reflect utilization of T_R and utilization of T_C, respectively, is plotted on a log scale for each promoter derivative (indicated by the sequence of the upstream TATA element, with T_C representing the natural sequence in the *his3* promoter) in conjunction with a Gcn4 derivative stimulating low (gcn4-C83) or high (gcn4-C267) levels of transcription. Upstream TATA sequences are grouped according to weak, moderate, and strong elements as defined by the level of +1 transcription in this experiment and, if previously examined (46), by their transcriptional activity in vitro. A value of 1 represents equal initiation from +1 and +13, values of <1 indicate preferential initiation from +1, and values of >1 indicate preferential initiation from +13.

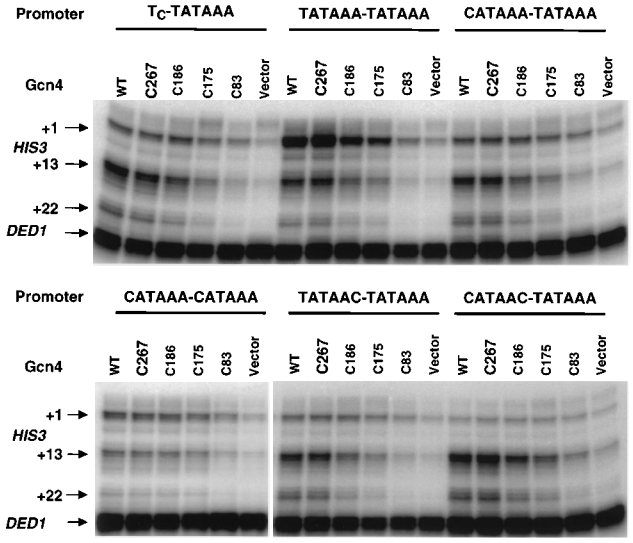

FIG. 8. Relative utilization of biochemically defined TATA elements in modified *his3* promoters. RNAs from strains containing the indicated *his3* promoters (indicated by upstream and downstream TATA elements) and Gcn4 derivatives were subjected to quantitative S1 analysis; the positions of the *his3* (+1, +13, and +22) and *ded1* transcripts are indicated. WT, wild type.

each of which interacts very weakly with TBP. In this view, TBP interacts at several distinct positions within the T_C region, and T_C-dependent transcription represents the sum of the productive events. Although the relationship between DNA sequence and transcriptional activity is poorly understood for weak TATA elements, the T_C region contains several sequences with four or five matches to the consensus TATA hexanucleotide: TATCAC (−69 to −64), CATAAT (−64 to −59), AATGAA (−61 to −56), and TATACA (−54 to −49). This hypothesis of multiple, but very weak, TATA elements explains why T_C maps to a large region and is unusually insensitive to mutations. However, it is less clear why T_C appears functionally inactive in vitro (35) whereas the T_R derivatives that behave like T_C in vivo have detectable activity in vitro (46). Although this apparent inconsistency may simply reflect differences in the in vitro assays, it is also possible that TBP interactions with the extended T_C region might be stabilized in vivo by chromatin or auxiliary transcription factors.

Differential utilization of tandem TATA elements depends on overall transcriptional activity as well as the arrangement and quality of the individual elements. To explain the phenomenon of differential utilization of the *his3* T_R and T_C elements, we proposed that transcription complexes nucleated at T_R, but not T_C, can respond to acidic activators (8, 17, 29, 35, 42). Although this model was attractive and was consistent with all available data, results presented in Fig. 2 to 5 indicate that it is incorrect. Instead, differential TATA-element utilization in the *his3* promoter region depends on the overall level of

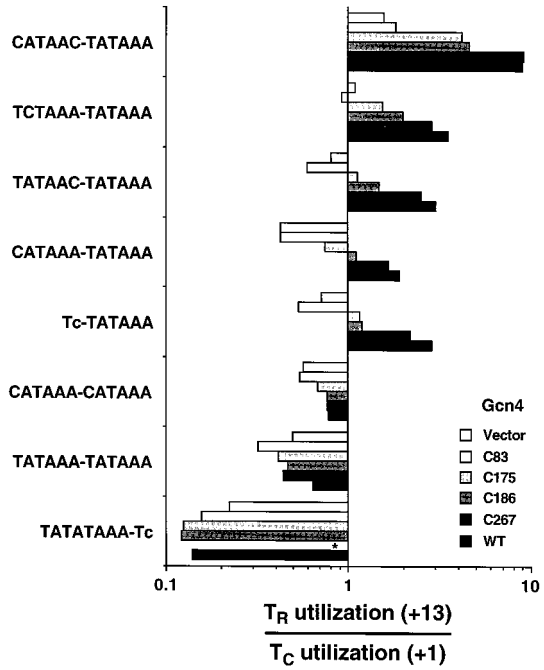

FIG. 9. Relative utilization of two TATA elements as a function of Gcn4 activation. The ratio of +13 transcription to +1 transcription, which reflect utilization of T_R and utilization of T_C, respectively, is plotted on a log scale for each promoter derivative in conjunction with the indicated Gcn4 derivative (the data are from Fig. 8 except for T_R-T_C [Fig. 6B]). Asterisk, not done. Promoters showing differential utilization have different +13/+1 ratios as a function of transcriptional activation.

transcription. The same *his3* initiation pattern is observed when the transcription level is varied as a function of the specific activator protein, the functional quality of the activation domain, the affinity of the activator to its target site, or the level of the activator protein. In all cases, T_C is preferentially utilized at low levels of *his3* transcription, but T_R becomes increasingly preferred as the level of *his3* transcription becomes higher.

The observation that differential TATA-element utilization does not depend on any specific functional property of the activator protein provides additional evidence that T_C and T_R are not inherently different in their mechanisms of action. Furthermore, it suggests that differential utilization reflects properties of the TATA elements, not the activator proteins. In agreement with this view, only a subset of TATA elements can functionally replace T_C in terms of differential utilization. Finally, the fact that the ratio of +13 to +22 transcripts, which both depend on T_R, does not significantly change under any experimental condition argues that differential utilization does not involve the inherent properties of the initiator element or the distance between the TATA and initiator elements.

Differential TATA element utilization as a function of overall *his3* transcription levels occurs when the upstream element (T_C or an appropriate T_R derivative) is moderately functional and the downstream element (T_R) is highly functional. A similar situation occurs when the upstream element is very weak; although initiation from +1 is always less than that from +13, the +13/+1 ratio increases in accord with overall *his3* transcription levels. However, when the upstream element is as functional as or more functional than the downstream element (T_R-T_R, T_R-T_C, or T_C-T_C promoter), relative utilization of TATA elements is not significantly affected by the level of *his3* transcription. Thus, differential TATA-element utilization appears to require a particular arrangement of TATA elements.

Given the new results in this article, we propose a modified hypothesis for differential *his3* TATA element utilization that invokes two concepts. First, in a promoter region containing two TATA elements, there is an intrinsic preference to utilize the upstream element. Second, initiation that depends on weak TATA elements reaches saturation at a low level of overall transcriptional stimulation.

The combined effects of preferential utilization of upstream TATA elements and functional saturation of weak TATA elements can account for essentially all of the observations in this and previous papers. At low levels of transcription, the intrinsic preference for upstream TATA elements results in T_C (or its functional equivalent) being preferentially utilized over T_R, even though it is the weaker TATA element. As *his3* transcription is increased, T_C-dependent initiation reaches a maximal level, which reflects its inherent capacity, and all further stimulation occurs from T_R. When the upstream element is very weak, the same pattern of differential utilization occurs, except that the level of +1 initiation is saturated at a lower level. In contrast, when the upstream TATA element is highly functional, it is intrinsically preferred over the downstream element, but its activity is not saturated before that of the downstream element. In this case, the +13/+1 ratio will depend on the relative quality of the TATA elements but will not vary with the overall level of transcription.

Molecular interpretations. Recruitment of TBP to the promoter is a major rate-limiting step for *his3* transcription in vivo. Kinetic analysis indicates that accessibility of TBP to the *his3* promoter region is slow but can be accelerated by the Gcn4 activator (24). Moreover, direct recruitment of TBP to the *his3* promoter by physically connecting TBP to a heterologous DNA-binding domain activates transcription (7, 23). In agreement with these observations on the *his3* promoter, mutations on the DNA-binding surface of TBP that impair TATA-element binding in vitro can cause a specific defect in the response to activator proteins in vivo (1, 27). Another activation-defective TBP derivative fails to interact with TFIIA (40), a factor that stabilizes the TBP-TATA element interaction (4, 16, 26). Taken together, these results strongly suggest that an efficient TBP-TATA interaction is particularly important when transcription is initiated at a high level.

From these considerations, we suggest a molecular interpretation of the phenomenon of differential TATA-element utilization. Under conditions of low *his3* transcription, TBP transiently associates with the promoter, thereby leading to a small number of productive transcription complexes. Because of proximity of their binding sites, the activator preferentially stabilizes the interaction of TBP with T_C, such that the +1/+13 ratio is higher than expected on the basis of the relative qualities of the TATA elements. In this situation, the TATA elements are largely unoccupied by TBP, and there is no competition between events at T_R and T_C.

Under conditions of moderate activation, there is increased recruitment of TBP to the promoter, but the TATA elements are only partially occupied. Although the activator inherently prefers to utilize the upstream TATA element, T_C-dependent initiation reaches a limit that depends on the inherent stability of the transcription complex assembled at a weak TATA element. As the activator cannot increase recruitment to T_C beyond a certain point, it increases recruitment to T_R; thus, higher levels of overall transcription are associated with increased T_R utilization and +13 transcription. The shift from T_C utilization to T_R utilization could reflect either independent events at these TATA elements or sliding of an individual TBP molecule from T_C to T_R. In this situation, TATA-element utilization can be viewed as a kinetic competition between complexes assembled at T_R and T_C.

Conditions of high activation resemble those of moderate activation, except that recruitment to T_R is very efficient. In the extreme case, T_R might be completely occupied by a TBP molecule that is part of a stable transcription complex that initiates transcription at a maximal rate. As it is very unlikely that productive transcription complexes can assemble simultaneously on T_R and T_C elements in the same DNA molecule, situations in which TBP is efficiently recruited to the *his3* promoter region are likely to result in steric competition between complexes assembled at T_R and T_C.

Although the above molecular interpretation is plausible and consistent with the experimental observations, direct biochemical evidence is lacking. In addition, the interpretation makes the simplifying assumption that activators function by a common mechanism involving increased recruitment of TBP to the promoter. While evidence presented here (Fig. 2 to 5) and elsewhere (7, 23, 24) supports this assumption in the case of the *his3* promoter, mechanistic differences among activator proteins and promoters are likely.

There are proteins that differentially affect utilization of the *his3* TATA element but are apparently unrelated to the process of transcriptional activation. The NOT complex, which appears to consist of at least four proteins (NOT1 to -4), negatively regulates the transcription of many genes and preferentially represses T_C-dependent transcription (10). Strains containing *not* mutations show increased initiation from +1 but not from +13. Conversely, *mot1* mutant strains show decreased initiation from +1 but appear normal for initiation from +13 (9, 15, 31). Mot1 is an ATP-dependent inhibitor of TBP binding to the TATA element, and it negatively regulates transcription of many genes (2). These proteins might influence the choice of TATA element at which the initiation com-

plex is formed by altering the inherent preference for the upstream element and/or the relative utilization of weak and strong TATA elements.

Generality of the phenomenon. Although they are less well described, there are several phenomena that formally resemble differential utilization of the *his3* TATA elements. First, the *ura3* promoter region is remarkably similar to that of *his3* (37). It contains two TATA elements (defined as T_C and T_R) in the same arrangement, the consensus TATA element is required for Ppr1-activated transcription but not constitutive transcription, and Ppr1 preferentially stimulates transcription from downstream initiation sites. Second, the *his4* promoter contains a single consensus TATA element that is required for high-level activation (by Gcn4 and moderately functional derivatives) but not for low-level activation (by Bas1/Bas2 and weak Gcn4 derivatives) (33). Although the proximal element required for low-level activation was not defined, there are potential weak TATA elements located upstream of the consensus TATA element that might function similarly to T_C. Third, several *cyc1* promoter derivatives containing tandem, functional TATA elements show a strong preference for utilizing the upstream element (28). While this observation was interpreted as evidence for distinct types of TATA elements (28), we favor the view that it reflects another example of differential utilization. More generally, many yeast promoter regions contain multiple TATA elements, and it seems likely that the phenomenon of differential TATA-element utilization will apply in some of these cases.

ACKNOWLEDGMENTS

This work was supported by grants to K.S. from the National Institutes of Health (GM30186) and from the Lucille Markey Trust.

REFERENCES

1. **Arndt, K. M., S. Ricupero-Hovasse, and F. Winston.** 1995. TBP mutants defective in activated transcription *in vivo*. EMBO J. **14:**1490–1497.
2. **Auble, D. T., K. E. Hansen, C. G. F. Mueller, W. S. Lane, J. Thorner, and S. Hahn.** 1994. Mot1, a global repressor of RNA polymerase II transcription, inhibits TBP binding to DNA by an ATP-dependent mechanism. Genes Dev. **8:**1920–1934.
3. **Buchman, A. R., and R. D. Kornberg.** 1990. A yeast ARS-binding protein activates transcription synergistically in combination with other weak activating factors. Mol. Cell. Biol. **10:**887–897.
4. **Buratowski, S., S. Hahn, L. Guarente, and P. A. Sharp.** 1989. Five intermediate complexes in transcription initiation by RNA polymerase II. Cell **56:** 549–561.
5. **Carey, M., H. Kakidani, J. Leatherwood, F. Mostashari, and M. Ptashne.** 1989. An amino-terminal fragment of GAL4 binds DNA as a dimer. J. Mol. Biol. **209:**423–432.
6. **Chasman, D. I., N. F. Lue, A. R. Buchman, J. W. LaPointe, Y. Lorch, and R. D. Kornberg.** 1990. A yeast protein that influences the chromatin structure of UAS_G and functions as a powerful auxiliary gene activator. Genes Dev. **4:**503–514.
7. **Chatterjee, S., and K. Struhl.** 1995. Connecting a promoter-bound protein to the TATA-binding protein overrides the need for a transcriptional activation region. Nature (London) **374:**820–822.
8. **Chen, W., and K. Struhl.** 1988. Saturation mutagenesis of a yeast *his3* TATA element: genetic evidence for a specific TATA-binding protein. Proc. Natl. Acad. Sci. USA **85:**2691–2695.
9. **Collart, M. A.** Personal communication.
10. **Collart, M. A., and K. Struhl.** 1994. *NOT1*(*CDC39*), *NOT2*(*CDC36*), *NOT3*, and *NOT4* encode a global negative regulator of transcription that differentially affects TATA element utilization. Genes Dev. **8:**525–537.
11. **Cormack, B. P., and K. Struhl.** 1992. The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. Cell **69:** 685–696.
12. **Eisenmann, D. M., C. Dollard, and F. Winston.** 1989. *SPT15*, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation in vivo. Cell **58:**1183–1191.
13. **Frieden, P., and P. Schimmel.** 1987. LEU3 of *Saccharomyces cerevisiae* encodes a factor for control of RNA levels of a group of leucine-specific genes. Mol. Cell. Biol. **7:**2708–2717.
14. **Furst, P., S. Hu, R. Hackett, and D. Hamer.** 1988. Copper activates metallothionein gene transcription by altering the conformation of a specific DNA binding protein. Cell **55:**705–717.
15. **Hahn, S.** Personal communication.
16. **Hahn, S., S. Buratowski, P. A. Sharp, and L. Guarente.** 1989. Identification of a yeast protein homologous in function to the mammalian general transcription factor, TFIIA. EMBO J. **8:**3379–3382.
17. **Harbury, P. A. B., and K. Struhl.** 1989. Functional distinctions between yeast TATA elements. Mol. Cell. Biol. **9:**5298–5304.
18. **Hill, D. E., I. A. Hope, J. P. Macke, and K. Struhl.** 1986. Saturation mutagenesis of the yeast *HIS3* regulatory site: requirements for transcriptional induction and for binding by GCN4 activator protein. Science **234:**451–457.
19. **Hirschman, J. E., K. J. Durbin, and F. Winston.** 1988. Genetic evidence for promoter competition in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**4608–4615.
20. **Hope, I. A., S. Mahadevan, and K. Struhl.** 1988. Structural and functional characterization of the short acidic transcriptional activation region of yeast GCN4 protein. Nature (London) **333:**635–640.
21. **Hope, I. A., and K. Struhl.** 1986. Functional dissection of a eukaryotic transcriptional activator protein, GCN4 of yeast. Cell **46:**885–894.
22. **Iyer, V., and K. Struhl.** 1995. Poly(dA:dT), a ubiquitous promoter element that stimulates transcription via its intrinsic structure. EMBO J. **14:**2570–2579.
23. **Klages, N., and M. Strubin.** 1995. Stimulation of RNA polymerase II transcription initiation by recruitment of TBP in vivo. Nature (London) **374:**822–823.
24. **Klein, C., and K. Struhl.** 1994. Increased recruitment of TATA-binding protein to the promoter by transcriptional activation domains *in vivo*. Science **266:**280–282.
25. **Klein, C., and K. Struhl.** 1994. Protein kinase A mediates growth-regulated expression of yeast ribosomal protein genes by modulating RAP1 transcriptional activity. Mol. Cell. Biol. **14:**1920–1928.
26. **Lee, D. K., J. Dejong, S. Hashimoto, M. Horikoshi, and R. G. Roeder.** 1992. TFIIA induces conformational changes in TFIID via interactions with the basic repeat. Mol. Cell. Biol. **12:**5189–5196.
27. **Lee, M., and K. Struhl.** 1995. Mutations on the DNA-binding surface of TATA-binding protein can specifically impair the response to acidic activators in vivo. Mol. Cell. Biol. **15:**5461–5469.
28. **Li, W.-Z., and F. Sherman.** 1991. Two types of TATA elements for the *CYC1* gene of the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**666–676.
29. **Mahadevan, S., and K. Struhl.** 1990. T_C, an unusual promoter element required for constitutive transcription of the yeast *his3* gene. Mol. Cell. Biol. **10:**4447–4455.
30. **McNabb, D. S., Y. Y. Xing, and L. Guarente.** 1995. Cloning of yeast HAP5—a novel subunit of a heterotrimeric complex required for CCAAT binding. Genes Dev. **9:**47–58.
31. **Moqtaderi, Z., and K. Struhl.** Unpublished observations.
32. **Nagawa, F., and G. R. Fink.** 1985. The relationship between the "TATA" sequence and transcription initiation sites at the *HIS4* gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **82:**8557–8561.
33. **Pellman, D., M. E. McLaughlin, and G. R. Fink.** 1990. TATA-dependent and TATA-independent transcription at the *HIS4* gene of yeast. Nature (London) **348:**82–85.
34. **Pfeifer, K., K.-S. Kim, S. Kogan, and L. Guarente.** 1989. Functional dissection and sequence of yeast HAP1 activator. Cell **56:**291–301.
35. **Ponticelli, A. S., and K. Struhl.** 1990. Analysis of *Saccharomyces cerevisiae his3* transcription in vitro: biochemical support for multiple mechanisms of transcription. Mol. Cell. Biol. **10:**2832–2839.
36. **Pu, W. T., and K. Struhl.** 1991. Highly conserved residues in the bZIP domain of yeast GCN4 are not essential for DNA binding. Mol. Cell. Biol. **11:**4918–4926.
37. **Roy, A., F. Exinger, and R. Losson.** 1990. *cis*- and *trans*-acting regulatory elements of the yeast *URA3* promoter. Mol. Cell. Biol. **10:**5257–5270.
38. **Siddiqui, A. H., and M. C. Brandriss.** 1989. The *Saccharomyces cerevisiae* PUT3 activator protein associates with proline-specific upstream activation sequences. Mol. Cell. Biol. **9:**4706–4712.
39. **Sorger, P. K., M. J. Lewis, and H. R. B. Pelham.** 1987. Heat shock factor is regulated differently in yeast and HeLa cells. Nature (London) **329:**81–84.
40. **Stargell, L. A., and K. Struhl.** 1995. The TBP-TFIIA interaction in the response to acidic activators *in vivo*. Science **269:**75–78.
41. **Struhl, K.** 1984. Genetic properties and chromatin structure of the yeast *gal* regulatory element; an enhancer-like sequence. Proc. Natl. Acad. Sci. USA **81:**7865–7869.
42. **Struhl, K.** 1986. Constitutive and inducible *Saccharomyces cerevisiae* promoters: evidence for two distinct molecular mechanisms. Mol. Cell. Biol. **6:**3847–3853.
43. **Struhl, K.** 1995. Yeast transcriptional regulatory mechanisms. Annu. Rev. Genet. **29:**651–674.
44. **Tice-Baldwin, K., G. Fink, and K. T. Arndt.** 1989. BAS1 has a Myb motif and activates *HIS4* transcription only in combination with BAS2. Science **246:** 931–935.
45. **Tzamarias, D., and K. Struhl.** 1994. Functional dissection of the yeast Cyc8-Tup1 transcriptional corepressor complex. Nature (London) **369:** 758–761.
46. **Wobbe, C. R., and K. Struhl.** 1990. Yeast and human TATA-binding proteins have nearly identical DNA sequence requirements for transcription in vitro. Mol. Cell. Biol. **10:**3859–3867.