The Type of Basal Promoter Determines the Regulated or Constitutive Mode of Transcription in the Common Control Region of the Yeast Gene Pair GCY1/RIO1*

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The yeast genes, GCY1 and RIO1, are transcribed divergently from the 869-base pair intergenic region. GCY1 is inducible by galactose about 25-fold due to Gal4p-binding to a single UAS_{GAL}, whereas RIO1 is constitutively expressed. GCY1 has a TATA box obeying the consensus TATAAA, whereas the RIO1 5′-upstream region lacks such a motif. In vitro mutagenesis of the TATA motif of GCY1, on the one hand, and introduction of a TATA-element into the promoter of RIO1, on the other hand, as well as inversion of the intergenic region have revealed that transcription of GCY1 and RIO1 is only regulated by Gal4p when a consensus TATA motif is included in their core promoters but not in its absence. The data imply that only transcription complexes that assemble at a consensus TATA box are compatible with specific transactivators, such as Gal4p. As a result, the adjacent gene is subject to regulated expression. By contrast, if a consensus TATA sequence is absent, the initiation complex does not respond to regulatory transcription factors, and consequently, the respective gene is constitutively transcribed. On the other hand, we show that two blocks of homo-oligomeric (dA·dT) sequences do not function as boundary sequences that might confine regulatory action of Gal4p to GCY1.

The yeast genome is densely packed with genetic information, and consequently, distances between two genes are relatively short. Frequently, adjacent genes are transcribed divergently from the same intergenic control region. In some cases, the mode of their expression differs, e.g. one gene is expressed in a regulated fashion, whereas the other one is constitutively transcribed. One such example is HIS3 and PET56; HIS3 is induced by a shortage of amino acids, whereas expression of PET56 is unregulated (1). TATA boxes and initiator elements are targets of basal transcription factors (reviewed in Ref. 2). These elements are sufficient for basal transcription. However, specific transactivators, which bind to cis-acting upstream elements, are required for regulated gene expression (reviewed in Ref. 3). Regulatory transcription factors act in both orientations and at long distances. Therefore, it seems to be contrary that transactivator-binding to an intergenic region exclusively influences expression of one gene, whereas the divergently transcribed gene is constitutively expressed. The maintenance of individual gene expression is controversially discussed. Analyses of the HIS3 promoter resulted in the hypothesis that two distinct types of TATA boxes exist: constitutive elements that assemble the basal initiation complex independent of upstream-binding transactivators, and regulatory TATA motifs following the consensus “TATAAA” that are responsive to upstream regulatory elements (4, 5). It was proposed that there are several TATA-binding proteins that associate with either type of TATA motif (4, 6, 7). A heterogeneity of TATA-binding proteins could not yet be found in yeast, and it has been shown that TBP binds to both types of TATA-elements (8, 9). However, recent results reveal that multiple TATA-binding protein associated factors (TAFs)3 exist which modulate the properties of TFIIID and dictate the specificity of the initiation complex by transmitting the signals from specific transactivators to the basal transcription machinery (10).

The 869-bp intergenic region of the genes GCY1 and RIO1 represents an example of divergently transcribed genes. GCY1 (galactose-inducible crystallin-like yeast protein, YOR120w) encodes an aldo/keto reductase of unknown substrate specificity and shows particularly high homology to prostaglandin F synthetase. Expression of GCY1 is inducible about 25-fold by growth on galactose as carbon source (11). This induction is due to Gal4p binding to a single UAS_{GAL} in the intergenic region. RIO1 (right open reading frame, YOR119c), which codes for an auxiliary protein, is constitutively expressed. As regulatory factors, such as Gal4p, function in both orientations and at long distances, the question arises of why expression of RIO1 is not influenced by Gal4p. Therefore, a mechanism must exist that ensures an individual mode of gene expression and that confines the regulatory action of the specific transactivator, Gal4p, to transcription of GCY1. We found two blocks of oligo(dA·dT) tracts 230 and 195 bp 5′ upstream of the initiation site of RIO1 in the intergenic region. These sequences are discussed as targets of datin, a protein possibly associated with the nuclear scaffold (12). In this case, such sequences could act as boundaries, i.e. they might act as repressors in such a manner that they prevent the effect of farther upstream binding transactivators on the expression of the respective gene. As the UAS_{GAL} is located 5′ of the oligo(dA·dT) tracts with respect to the initiation site of RIO1, it appears feasible that the oligo(dA·dT) sequences prevent transmission of the Gal4p-dependent activation potential on the transcription of RIO1. On the other hand, these tracts are described as UAS elements for the expression of constitutive genes. Either the oligo(dA·dT) tracts exclude the assembly of nucleosomes by virtue of their stiff DNA structure (13, 14) or T-rich element-binding factors stimulate transcription (15). Thus, these sequences could exert an activating function on the expression of RIO1, particularly as they occur in conjunction with a presumptive Rap1p-binding sequence.

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1 The abbreviations used are: TAF, TATA-binding protein associated factor; bp, base pair(s).
site, a constellation which has been found to synergistically activate gene expression (16). The importance of these sequences was analyzed by deleting these blocks.

The most obvious difference between both genes consists of the type of basal promoters: a consensus TATA box (TATAAA) occurs in the upstream region of GCY1, whereas such an element is absent from the promoter of RIO1. To test whether the basal promoter dictates the mode of gene expression, we changed the TATA element of GCY1 to a nonconsensus sequence and introduced a consensus TATA box into the promoter region of RIO1. As soon as a consensus TATA element was present in the core promoter, the respective gene was transcribed under the control of Gal4p, whereas in its absence, gene expression was constitutive. These results imply that it depends on the type of basal promoter whether interactions between specific transcription factors and the basal transcription machinery are productive or not.

MATERIALS AND METHODS

Plasmids and Strains—pBluescript M13 KS (±) vectors (Stratagene, Heidelberg), respectively. The intergenic region of GCY1 and RIO1 with a N-terminal coding region of both genes was ligated either as EcoRI-BamHI or as BamHI-EcoRI fragments (11) to the polylinker of pBluescript for in vitro mutagenesis. All constructs were then fused in the respective orientation to the bacterial lacZ gene of the low copy yeast expression vector pYLZ6 (19). The reporter gene lacked promoter and translational initiation codon. Analyses of gene expression were performed in the yeast strain W303-1A (18) that had been transformed by the respective constructs (wild-type constructs: pGCY, and pRIO; mutant constructs: pGCY-TATAA; pRIO-TATAA, pRIOΔDA, pRIOΔAVU-Tp, pRIOΔAVU-Tp, pRIOΔmut-PGCY, pRIO-SM, pRIO-BN, pGSCY-SM, and pGSCY-BN). Primer extension reactions of RIO1 promoter-lacZ fusion constructs were performed using the multiplexic yeast expression vector pYLZ6 (19) harboring the same wild-type and some of the mutant constructs as above (detailed in the legends).

Oligonucleotide-directed Mutagenesis—Site-specific in vitro mutagenesis was performed as described by Kunkel et al. (17, 20). Mutations were verified by restriction analysis and by nucleotide sequencing of the respective constructs (21). Oligonucleotide 5′-GATAATATAAAAATTG-G-3′ was used as a mutagenic primer to introduce a consensus TATA sequence into the RIO1 promoter. Oligonucleotide 5′-TGGGCGTATAAGACCGGG-3′ served to mutate the TATA box of GCY1. Restriction sites for enzymes producing compatible ends, flanking the TATA box of GCY1, and the TATA-reshaping sequence of RIO1, respectively, were introduced into the intergenic region using oligonucleotides 5′-CGGC-AGTAAAAGATGTCTTACCCCGGGGTATAAAACCGCTGAGTAC-GAGATTTCC-3′, and 5′-GTCCTTAGATCTACCTATCAGATTATATTATGATGGCCTTAAAGTTTTTCATC-3′. They contained restriction sites for the enzymes SmaI plus BsrFI and NruI plus MscI, respectively. By these means, the intergenic region could be inverted once including and once excluding the TATA sequence of GCY1.

Recombinant DNA Methods—A synthetic UASgal was inserted into the unique XhoI restriction site of the intergenic region of GCY1 and RIO1. A HindIII restriction site in this element then allowed the deletion of a 220-bp fragment containing the oligo(dA-dT) tracts and the presumptive Rap1p-binding site.

RNA Isolation and Primer Extension—Yeast cells in midlog phase were harvested, washed in water, and resuspended in RNA extraction buffer (0.15 M NaCl, 50 mM Tris/HCl, pH 7.5, 5 mM EDTA, 5% SDS). 1 volume of glass beads (Braun, Melsungen) and 1 volume of phenol/ chloroform were added, and the cells were vortexed (5 min). Glass beads were washed with 1 volume of RNA extraction buffer, and the combined supernatants were centrifuged (20,000 × g, 5 min). After extracting the aqueous phase twice with phenol/chloroform, RNA and DNA were precipitated by ethanol (20,000 × g, 20 min). The RNA/DNA pellet was resuspended in TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 7.4). LiCl added to a final concentration of 3.0 M, incubated on ice for 2–5 h, and RNA precipitated by centrifugation. Primer extensions were performed according to Maniatis et al. (22). 70–100 μg of total RNA was denatured in 10 mM Tris/HCl, pH 7.2, 5.6 M NaCl, 20 mM EDTA, 2 units/μl RNase inhibitor (Boehringer Mannheim), and 50% formamide at 80 °C for 15 min and hybridized with 5′ radioactively labeled oligonucleotides (1–2 × 10⁶ cpm) at 45 °C for 1.5 h and at 37 °C for 1.5 h. After precipitation of the annealed probe, reverse transcription was performed with reverse transcriptase from avian myeloblastosis virus (Boehringer Mannheim) according to the manufacturer instructions. RNA was hydrolyzed in 0.09 M NaOH at 60 °C for 1 h. cDNA was recovered by ethanol precipitation, resuspended in TE buffer, and analyzed on denaturing sequencing gels; 5′-ends of the transcripts were identified by nucleotide sequencing (21) of the respective regions using the same oligonucleotides and the reaction mixtures in adjacent lanes. Transcriptional start sites of GCY1 wildtype mRNA were determined using the oligonucleotide 5′-GGGATCCCGTGCCGATGAT-3′ (position +40 to +64 of the terminal coding region of GCY1), whereas the oligonucleotide 5′-GGAGGATCCTGGGCTCCAGTAGT-3′ (positions +139 to +155 of the N-terminal coding region of the lacZ gene) served to identify those of GCY1- or RIO1lacZ hybrid RNA.

Analyses of Gene Expression in Yeast—The yeast expression vector pYLZ6 was used for determining the β-galactosidase activities of the respective promoter/lacZ fusion constructs (19). Cells were harvested in midlog phase and broken by vortexing with glass beads (Braun, Melsungen). β-Galactosidase assays were performed at 30 °C with ONPG (o-nitrophenyl-β-D-galactopyranoside) as substrate (23). Activities are given as units per milligram of total protein. One unit is defined as the amount of enzyme that hydrolyzes 1 nmol ONPG/min at 30 °C. All activity values have been corrected for background levels produced by the vector pYLZ6 (0.5–0.8 units/mg).

Miscellaneous Procedures—Yeast transformations were performed as described by Keszenman-Pereyra and Hieda (24). Protein contents were determined according to the method described by Bradford (25). Other molecular methods were performed according to standard procedures (22) or as recommended by the manufacturer.

RESULTS

The intergenic region of GCY1 and RIO1 harbors several sequences which follow the consensus of various cis-acting elements (Fig. 1). A UASgal is responsible for the galactose-induced expression of GCY1 (11), and 160 bp upstream of the transcriptional initiation sites of GCY1 (position –215, relative to the translational start codon), a functional binding site for the general regulatory protein, Reb1p, is found (26). We show that the basal promoter of GCY1 contains a consensus TATA box, whereas RIO1 is a TATA-less gene. In the 5′-upstream region of RIO1, a presumptive Rap1p-binding site is located adjacent to two blocks of oligo(dA-dT) tracts.

The TATA Box Is the Sole Basal Promoter Element of GCY1—The promoter region of GCY1 contains a perfect match to the consensus TATA sequence TATAAA. To test functional-
TABLE I

| Plasmid            | \(\beta\)-Galactosidase activities in units/mg |
|--------------------|-----------------------------------------------|
|                    | Glc   | Gal   | Gro/EtOH |
| pGCY (TATAAA)      | 13.7±2 | 338.0±25.0 | 25.0±2.1  |
| pGCY-TC (TATAAG)   | 1.1±0.3 | 4.8±0.8   | 0.8±0.1   |

Values given represent the mean of activities derived from three to five independent yeast transformant clones; maximum deviations from the average are given. Glc, glucose; Gal, galactose; Gro/EtOH, glycerol/ethanol.

Introduction of a consensus TATA box into the RIO1 promoter leads to activation by Gal4p

Values given represent the mean of activities derived from three to five independent yeast transformant clones; maximum deviations from the average were 10–20%. The blank activity caused by the vector alone (usually 0.5 units/mg) has been subtracted from all values. Glc, glucose; Gal, galactose; Gro/EtOH, glycerol/ethanol.

| Plasmid | TATAAG | TATAAA (T\(_{R1}\)) |
|---------|--------|---------------------|
|         | Glc    | Gal    | Gro/EtOH |
| pRIO    | 1.6    | 2.9    | 1.3       |
| pRIO\(\Delta\)A | 1.7    | 3.2    | 1.7       |
| pRIO\(\Delta\)AVU | 1.7    | 9.3    | 1.8       |

The activities of the RIO1 promoter-lacZ fusion constructs pRIO-T\(_R\) exhibited an induction by galactose as carbon source by a factor of about 4. This value seemed to be more moderate compared with the induced expression of GCY1 by galactose (factor of 25 compared with growth on glucose). Considering the distances between the UAS\(_GAL\), and basal promoter elements, we found that this UAS is more than 100 bp farther away from the transcriptional start site of RIO1 than from those of GCY1. We deleted the oligo(dA-dT) blocks-containing fragment (220 bp) from the intergenic region (Fig. 3B). Although this deletion had no effect on expression from the TATA-less RIO1 promoter (compare pRIO with pRIO\(\Delta\)A), the TATA-containing RIO1 promoter (pRIO\(\Delta\)A-T\(_R\)) was induced by galactose by a factor of nearly 8 (compare pRIO\(\Delta\)A-T\(_R\) on glucose with galactose, Table II). This shows that the UAS\(_GAL\) is, in fact, subject to an evident positional effect. Surprisingly, the inducibility of RIO1 by galactose increased further to 95-fold (i.e. 12.3-fold over the construct pRIO\(\Delta\)A-T\(_R\) after introducing a second UAS\(_GAL\) (pRIO\(\Delta\)A\(\Delta\)U-T\(_R\)) indicating synergism of both UAS\(_GAL\) (Fig. 3C). Expression of GCY1, however, was not influenced by the insertion of an additional UAS\(_GAL\). The latter observation conforms with results showing that a second UAS\(_GAL\) does not influence the inducibility of a gene significantly that, by nature, already has one such element, e.g. GAL80 or MEL1 (28).

Introduction of a consensus TATA box into the promoter of RIO1 created an additional basal promoter element. Therefore, it was not surprising that basal expression of RIO1 was increased by a factor of 2.4 as well. These results indicate that a canonic TATA sequence alleviates the rate-limiting step in
transcription relative to a TATA-less promoter. It remains to be shown whether assembly of the transcriptional initiation complex, transcriptional (re-)initiation or promoter escape is affected. Increase of mRNA synthesis due to the introduction of a consensus TATA box was corroborated by primer extension reactions performed with total RNA of the respective yeast strains. The natural transcriptional start site of both genes (pGCYmut, pRIOmut) (Table III). Nevertheless, the expression potential of Gal4p.

Inversion of the Intergenic Region between GCY1 and RIO1—The above results indicated that the basal transcription complex that recognizes a consensus TATA box constitutes an operon-like unit with regulatory transactivators and, thus, allows regulated gene expression. On the other hand, no productive interaction is established between specific transcription factors and the general transcription machinery that assembles on basal promoters that lack such a sequence. To corroborate the data obtained above, we introduced restriction sites into the intergenic region in the flanks of the promoter regions of both genes (pGCYmut, pRIOmut) in such a manner that we could invert the control region, once including the TATA box of RIO1 (Fig. 5). By these means, we were able to eliminate positional effects of basal promoters and upstream activating factors on the one hand, and on the other hand, we could analyze the importance of the constellation between transactivator and the type of basal promoter. β-galactosidase activities of both promoter-lacZ fusion constructs decreased by about one-half of the wild-type activities by introducing the restriction sites (pGCYmut, pRIOmut) (Table III). Nevertheless, the mode of regulated or constitutive transcription remained unaffected for both genes. When the intergenic region was inverted excluding the TATA-sequence of GCY1, the mode of gene expression remained essentially the same as in the original constellation (pGCY-SM, pRIO-SM). GCY1 was still inducible by galactose though to a lesser extent as in the wild-type promoter. These results confirm that the dA-dT blocks, which are promoter-proximal in this construct, do not delimit the activation potential of Gal4p. RIO1, however, continued to be expressed constitutively, although the UASgal was then closer to the initiation site of RIO1 than to the transcriptional start sites of GCY1.

As soon as the intergenic region was inverted, including the
Basal Promoter Determines Mode of Transcription

Fig. 5. Inversion of the intergenic region of GCY1 and RIO1. A, introduction of restriction sites for BbrI and SmaI flanking the consensus TATA box of GCY1 (GCYmut) and for MscI and NruI flanking the TATA-resembling sequence of RIO1 (RIOmut). B, the intergenic region of GCY1 and RIO1 was inverted excluding the TATA-motif by restriction digest with SmaI and MscI (GCY-SM, RIO-SM). C, restriction with BbrI and NruI inverted the intergenic region including this sequence (GCY-BN and RIO-BN). All constructs were fused to either GCY1/lacZ or RIO1/lacZ, and β-galactosidase activities were determined separately in yeast transformants of the four resulting constructs (see Table III).

Table III  
β-Galactosidase activities of GCY1- and RIO1/lacZ fusions after inversion of the intergenic region

| Construct | β-Galactosidase activities in units/mg |  |
|-----------|---------------------------------------|---|
| GCY/lacZ  | Glc  | Gal  | Gro/EtOH | Glc  | Gal  | Gro/EtOH |
| G  | 4.6  | 1613 | 7.6  | 0.8  | 1.3  | 1.0  |
| Bb  | 6.9  | 476  | 8.9  | 0.9  | 2.3  | 2.3  |
| Nru  | 1.1  | 2.9  | 1.2  | 6.7  | 68.3 | 12.9 |

Values given represent the mean of activities derived from three to five independent yeast transformant clones; maximum deviations from the average were 10–20%. Glc, glucose; Gal, galactose; Gro/EtOH, glycerol/ethanol.

- **Construct** indicates the type of restriction enzymes used (GCY-SM, RIO-SM).
- **G** represents the presence of a consensus TATA box in the promoter of GCY1.
- **B** indicates inversion of the intergenic region excluding the TATA motif of GCY1 (pGCY-SM, pRIO-SM).
- **Bb** refers to the presence of the intergenic region including the TATA element (pGCY-BN, pRIO-BN); see also Fig. 5.

TATA-sequence, the mode of gene expression changed. mRNA synthesis of GCY1 was no longer inducible by galactose, whereas transcription of RIO1 was regulated by Gal4p (pGCY-BN, pRIO-BN). In the construct pRIO-BN, the same initiation sites were used as in the point mutation construct pRIO-TR (Fig. 6). These findings suggest that the basal transcription complex that assembles on a consensus TATA box and specific regulator proteins are able to build up a compatible unit, and as a result, expression of the respective gene is under regulated control. In contrast, if a promoter lacks such a motif, no productive interactions of basal transcription factors with a specific transactivator seem to occur.

**DISCUSSION**

Above experiments demonstrate that individual gene expression in yeast is guaranteed by the type of basal promoters despite short intergenic regions. One type of promoter is concerned with regulated transcription, whereas the other one directs constitutive expression independent of an upstream located UAS. Regulated transcription seems to depend on a consensus TATA element. Comparison with other Gal4p regulated genes shows that GAL1, GAL2, GAL3, GAL7, and GAL80 have bona fide TATA sequences that obey the consensus TATAAA or TATATA and are positioned between 50 and 140 bp 5′ of the most distal transcriptional initiation site. Surprisingly, GAL10, although transcription is strongly inducible by galactose, dispenses with a canonical TATA box. Rather, non-canonical sequences, TATTAA or TATAAG, are present (29, 30). Possibly, one or both of these sequences function as a weak TATA element, whereas TATAGA found in the 5′-flank of the RIO1 promoter does not. Weak interactions of TBP with non-conventional TATA elements may be stabilized by additional interactions with promoter sequences or with basic transcription factors (31, 32). These considerations are, however, hampered by the fact that functional tests of core promoter elements are lacking in most instances.

Transcription analysis of RIO1 has revealed that a TATA box is absent from the RIO1 promoter. In agreement with the above hypothesis, we have furthermore shown that transcription of this gene is not significantly influenced by Gal4p, even if the region containing the presumptive Rap1p-binding site and the oligo(dA:dT) tracts are deleted, thereby decreasing the distance of the UASgal to the initiation sites of RIO1. This indicates that interactions between the transcription machinery, which binds to core promoters lacking a consensus TATA element, and specific transactivators are much weaker than those established between the basal transcription complex recruited to a consensus TATA motif and specific regulatory DNA-binding proteins, such as Gal4p. Binding of TBP/TFIID to a TATA-less promoter could be too transient to allow response to specific transactivators (9). Mutation of the consensus TATA box of the GCY1 gene does not abolish the inducibility by galactose completely, but the decrease of induction by this carbon source is drastic, confirming the above conclusion. In addition, these results demonstrate that the TATA box is functional and most likely the sole core promoter element of GCY1. Usage of the same initiation sites for basal as well as for induced expression verifies that the TATA element is responsible for both modes of expression. This points to the existence of two different control mechanisms in yeast; the basal expression of some genes, such as HIS3 and GAL80, seems to be directed by a basal promoter element that is distinct from the one responding to regulated transcription (4, 27). In contrast, other genes like GCY1 harbor just one cis-acting element in their core promoter, the TATA element, which mediates basal as well as regulated transcrip-
tion. Possibly, there are several classes of basal transcription factors, some of which recognize promoter elements that confer only basal transcription, and the other class binds to a consensus TATA box and directs basal expression from this site as long as a specific transactivator is absent from upstream binding sites or is blocked by the binding of a repressor. Recent data imply that, besides their role as coactivators, TAFs may act as core promoter recognition factors as well. Together with TBP these proteins constitute distinct TFIIID complexes that assemble on different types of basal promoter elements (33).

We have shown that expression of RIO1 becomes inducible by galactose by introducing a consensus TATA box. Thereby, it is unimportant whether this sequence is introduced by a point mutation or is posed there by inversion of the intergenic region. In both cases, the same initiation sites are used. These transcriptional start sites are used more frequently than the initiation site of the constitutive wild-type promoter. In contrast, when the intergenic region is inverted excluding the TATA sequence, the mode of expression is principally maintained, i.e. expression of GCY1 is still controlled by Gal4p, whereas transcription of RIO1 remains constitutive. These results suggest that the type of basal promoter determines whether interactions between specific transactivators and the general transcription machinery are productive or not. In the case of a consensus TATA element, the basal transcription factors are able to interact with regulatory factors, and as a consequence, the respective gene is expressed in a regulated way. In contrast, basal transcription complexes that assemble on TATA-less promoters cannot respond to the signals of an upstream-binding transactivator. Apparently, only one TATA-binding protein exists in yeast and binds to promoters that contain a conventional TATA motif as well as to promoters that lack such a sequence (8, 9). But data accumulate, suggesting that different preinitiation complexes exist. Different TAFs are required to support transcription mediated by distinct specific transactivators in vitro (34). In mammalian cells, two types of TFIIID complexes were found: D-TFIIID, which is responsive to specific transactivators, and B-TFIIID, which does not respond to regulatory proteins (35). Recent results indicate that distinct general transcription complexes exist in yeast as well (36–38). The existence of complexes of TBP with different subsets of TAFs, dependent on the type or context of the promoter, could provide an explanation for the fact that some basal promoters (e.g. that of GCY1) respond to specific transactivators, whereas others (e.g. that of RIO1) do not. This could mean that some TAFs transmit the effect of specific transactivators to the general transcription machinery that assembles on promoters containing a consensus TATA box, whereas a different subset of TAFs is responsible for transcriptional initiation on promoters lacking a TATA element. However, recent results imply that, in vivo, TAFs might not be generally required for transcriptional activation (31, 39). Alternatively, the mediator complex, a component of the RNA-polymerase II holoenzyme, which is indispensable for regulated gene expression, might serve as coactivator (40, 41). For instance, Gal11p, a component of the mediator, is essential for regulated expression of GAL80, but not required for initiator-dependent constitutive transcription of this gene (42). The mechanism that distinguishes between TATA-containing and TATA-less promoters remains to be elucidated.

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REFERENCES
1. Struhl, K. (1985) Nucleic Acids Res. 13, 8587–8601
2. Roeder, R. G. (1991) Trends Biochem. Sci. 16, 402–408
3. Guarente, L. (1988) Cell 52, 303–305
4. Struhl, K. (1986) Mol. Cell. Biol. 6, 3847–3853
5. Ponticielli, A. S., and Struhl, K. (1990) Mol. Cell. Biol. 10, 2832–2839
6. Chen, W., and Struhl, K. (1995) Proc. Natl. Acad. Sci. U. S. A. 82, 2691–2695
7. Harbury, P. A. B., and Struhl, K. (1989) Mol. Cell. Biol. 9, 5298–5304
8. Hahn, S., Buratowski, S., Sharp, P. A., and Guarente, L. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 5718–5722
9. Cormack, B. F., and Struhl, K. (1992) Cell 70, 685–696
10. Dynlacht, B. D., Hoey, T., and Tjian, R. (1991) Cell 66, 563–576
11. Magdolen, V., Oechsner, U., Trommler, P., and Bandlow, W. (1990) Gene 90, 105–114
12. Winter, E., and Varshavsky, A. (1989) EMBO J. 8, 1867–1877
13. Struhl, K. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 8419–8423
14. Iyer, V., and Struhl, K. (1995) EMBO J. 14, 2570–2579
15. Loer, N. F., Buchman, A. R., and Kornberg, R. D. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 486–490
16. Rotenberg, M. O., and Woolford, J. L., Jr. (1986) Mol. Cell. Biol. 6, 674–687
17. Kunkel, T. A., Roberts, J. D., and Zaks, R. A. (1987) Methods Enzymol. 154, 367–382
18. Crivelline, M. D., Wu, M., and Tzagoloff, A. (1988) J. Biol. Chem. 263, 14323–14333
19. Hermann, H., Hacker, U., Bandlow, W., and Magdolen, V. (1992) Gene 119, 137–141
20. Kunkel, T. A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 488–492
21. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
22. Chen, E., and Seeburg, P. H. (1985) DNA 4, 165–170
23. Guarente, L. (1983) Methods Enzymol. 101, 181–191
24. Kezempen-Pereyra, D., and Hieda, K. (1988) Curr. Genet. 13, 21–23
25. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
26. Angermayer, M., and Bandlow, W. (1997) Mol. Gen. Genet., in press
27. Kuratsu, H., Ohishi, T., and Fukasawa, T. (1994) Mol. Cell. Biol. 14, 6619–6628
28. Bram, R. J., Lue, N. F., and Kornberg, R. D. (1986) EMBO J. 5, 603–608
29. Johnstone, M. T., and Davis, R. W. (1984) Mol. Cell. Biol. 4, 1440–1448
30. Yocum, R. R., Hanley, S., West, R., and Ptashne, M. (1984) Mol. Cell. Biol. 4, 1984–1998
31. Moqtederi, Z., Bai, Y., Poon, D., Weil, P. A., and Struhl, K. (1996) Nature 383, 168–191
32. Struhl, K. (1996) Cell 84, 179–182
33. Verrijzer, C. P., Chen, J. L., Yokomori, K., and Tjian, R. (1995) Cell 81, 1115–1125
34. Chen, J. L., Attardi, L. D., Verrijzer, C. P., Yokomori, K., and Tjian, R. (1994) Cell 79, 93–105
35. Timmers, H. T. M., Meyers, R. E., and Sharp, P. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 8140–8144
36. Collart, M. A., and Struhl, K. (1994) Genes Dev. 8, 525–537
37. Poon, D., Campbell, A. M., Bai, Y., and Weil, P. A. (1994) J. Biol. Chem. 269, 23115–23140
38. Auhle, D. T., Hansen, K. E., Mueller, C. G. F., Lane, W. S., Thorner, J., and Hahn, S. (1994) Genes Dev. 8, 1920–1934
39. Walker, S. S., Reese, J. C., Apone, L. M., and Green, M. R. (1996) Nature 383, 185–188
40. Thompson, C. M., Kulesa, A. J., Chao, D. M., and Yeung, R. A. (1993) Cell 73, 1361–1375
41. Kim, Y., Bjorklund, S., Li, Y., Sayre, M. H., and Kornberg, R. D. (1994) Cell 77, 599–608
42. Sakurai, H., Ohishi, T., and Fukasawa, T. (1996) FEBS Lett. 39, 113–119