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A Nucleosome-Positioning Sequence Is Required for GCN4 To Activate Transcription in the Absence of a TATA Element

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In the *gal-his3* hybrid promoter *his3*-GG1, the yeast upstream activator protein GCN4 stimulates transcription when bound at the position normally occupied by the TATA element. This TATA-independent activation by GCN4 requires two additional elements in the *gal* enhancer region that are distinct from those involved in normal galactose induction. Both additional elements appear to be functionally distinct from a classical TATA element because they cannot be replaced by the TFIID-binding sequence TATAAA. One of these elements, termed Q, is essential for GCN4-activated transcription and contains the sequence GTCAC CCG, which overlaps (but is distinct from) a GAL4 binding site. Surprisingly, relatively small increases in the distance between Q and the GCN4 binding site significantly reduce the level of transcription. The Q element specifically interacts with a yeast protein (Q-binding protein [QBP]) that may be equivalent to Y, a protein that binds at a sequence that forms a constraint to nucleosome positioning. Analysis of various deletion mutants indicates that the sequence requirements for binding by QBP in vitro are indistinguishable from those necessary for Q activity in vivo, strongly suggesting that QBP is required for the function of this TATA-independent promoter. These results support the view that transcriptional activation can occur by an alternative mechanism in which the TATA-binding factor TFIID either is not required or is not directly bound to DNA. In addition, they suggest a potential role of nucleosome positioning for the activity of a promoter.

Most eucaryotic promoters transcribed by RNA polymerase II contain a TATA element, consensus sequence TATAAA, that is important for both the rate and accuracy of transcription in vivo and in vitro (for reviews, see references 15 and 43). TATA elements function by binding TFIID, a protein generally believed to be part of the basic transcription machinery that includes RNA polymerase II as well as a variety of other factors (4, 28). Extensive mutagenesis of a yeast *his3* TATA element indicates that the DNA sequence requirements for function in vivo are rather stringent (7, 17) and that levels of transcription in vivo are strongly correlated with levels of TFIID-dependent transcription in vitro (47). However, TFIID can functionally interact with some A+ T-rich sequences that differ from the consensus (16, 35). TATA-dependent transcription can be strongly stimulated by activator proteins that bind specifically to promoter elements upstream of the TATA sequence, and it has been suggested this might be due to a direct interaction between upstream activator proteins and TFIID (21, 32, 41).Yeast upstream activator proteins function in a variety of eucaryotic organisms (12, 23, 26, 45), mammalian and invertebrate upstream activator proteins function in yeasts (13, 31, 33, 36, 42), and yeast and mammalian TFIID are functionally interchangeable for TATA-dependent transcription in vitro (5, 6, 47), thus indicating a common mechanism for transcriptional activation in all eucaryotic cells.

Despite the relatively stringent sequence requirements for a functional TATA element, some eucaryotic promoters lack sequences that resemble TATAAA, which suggests the possibility that TFIID is not universally required for transcription by RNA polymerase II. In previous work, we have described several promoters in which the proximal element required for transcription may not interact with TFIID. First, transcription dependent on T_C, the proximal TATA-like element required for constitutive initiation from the +1 site in the wild-type *his3* promoter, cannot be induced by the GCN4 or GAL4 activator protein (40). Moreover, in yeast nuclear extracts capable of carrying out accurate TFIID-dependent initiation (25), T_C-dependent transcription from the +1 site is not observed (30). Second, a wide variety of DNA sequences, some of which do not support TFIID-dependent transcription in vitro, can substitute for the required TATA element in a *gal-his3* hybrid promoter (35). In both of these cases, however, the presumptive protein acting at the proximal promoter element is unknown, and the possibility remains that this protein is TFIID.

Previously, we showed that GCN4, a yeast protein that normally activates transcription when bound upstream of a TATA element, can stimulate transcription when its binding site replaces the required TATA element of a *gal-his3* hybrid promoter (8). In the chimeric promoter *his3*-GG1, in which GCN4 acts through the proximal promoter element, transcription depends on the GCN4 acidic activation region (20) and is initiated from wild-type mRNA start sites. However, unlike equivalent *gal-his3* hybrid promoters that contain a classical TATA sequence as the proximal element, transcription initiates preferentially at +1 rather than +12. This initiation pattern is normally observed in T_C-dependent transcription that is uninducible by GCN4 or GAL4 (40). Moreover, transcription from *his3*-GG1 occurs in glucose medium, conditions that inhibit the binding of GAL4 activator protein to its target sequences (14, 34) and hence prevent expression from equivalent *gal-his3* promoters (7, 17, 37). These unusual properties of *his3*-GG1, combined with the proximity of the GCN4 binding site to the mRNA initiation region and the apparent absence of a functional TATA element, suggest that transcription from this promoter may occur in the absence of TFIID (8). The yeast *trp3* promoter, which lacks a conventional TATA-like sequence but con-

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tains a GCN4 binding site at a proximal location (1, 48), might represent a natural version of *his3*-GG1.

Although GCN4-stimulated transcription in *his3*-GG1 does not require a standard TATA element or GAL4 activator protein, it does require an element(s) (previously called Q) found within the 365-base-pair (bp) fragment containing the *gal1-10* regulatory region (8). In addition to the four GAL4 binding sites (2, 14), the *gal* region contains other *cis*-acting elements, any of which could provide the Q function. These include an unmapped element(s) involved in catabolite repression in glucose medium (38), several negative control elements that repress transcription in the absence of galactose (46), two positive elements that permit constitutive low-level expression in the absence of GAL4 (11), and a sequence near a GAL4 target site that binds a protein called Y (10). Although the transcriptional role of Y is unknown, the Y binding site forms a constraint to nucleosome positioning, thus raising the possibility that Y mediates its effects through changes in chromatin structure (10).

In this report, we characterize the elements necessary for GCN4 to activate transcription in the absence of a conventional TATA element and identify a protein from fractionated yeast extracts that binds specifically to one of these sequences. In addition, we address the possible role of TFIID by inserting a wild-type TATA element at various locations within the *his3*-GG1 promoter. The results are discussed in terms of the role of TFIID in transcriptional initiation.

MATERIALS AND METHODS

Construction of deletion and insertion mutations. To facilitate the construction of deletions of *his3*-GG1 (8), the *Bam*HI site within the *ded1* structural gene (39) was mutated to AGATCC by site-directed mutagenesis (24). The resulting molecule, Sc5085, still encodes the wild-type *ded1* protein yet contains a unique *Bam*HI site at position −447 of the *his3* gene (39). For the set of 5′ deletions into the *gal* fragment, *Bam*HI-cut *his3*-GG1 DNA was digested with *Bal*31 nuclease, blunted with the Klenow fragment of DNA polymerase I, ligated to *Bam*HI linkers, and cleaved with *Bam*HI and *Kpn*I. For the set of 3′ deletions (except for *his3*-GG111, which derived from *his3*-GG1), *Eco*RI-cleaved *his3*-GG155 DNA (lacks sequences upstream of 649 in the *gal* region to −447 of *his3*) was treated with *Bal*31 nuclease, blunted with the Klenow fragment, ligated to *Eco*RI linkers, and cleaved with *Eco*RI and *Bam*HI. For both sets of deletions, the resulting fragments were cloned into pTZ18 (Pharmacia) for sequencing and then recloned back into *his3*-GG1. Internal deletions were constructed by combining appropriate 5′ and 3′ deletions via filled-in *Bam*HI and *Eco*RI sites; hence, they all contain an insertion of the sequence GGAATTGATC at the deletion site.

To insert the Q element at various positions upstream of the GCN4 binding site, the sequence GGGAATTCGGATC CGGCTGTCACCCGCGAGCTCAGATCTG (underlined region representing sequences from the *gal* fragment) was converted to the double-stranded form by mutually primed synthesis (29) and cloned into pTZ18 as an *Eco*RI-*Sac*I fragment. The resulting molecule was cleaved at the *Bam*HI sites in the oligonucleotide and the polylinker, and the relevant fragment was inserted in both orientations into the *Bam*HI sites of the *his3*-GG1 derivatives. To insert a functional TATA element upstream of the GCN4 binding site (*his3*-GG206T derivatives), the degenerate oligonucleotide GGATCCT(A/T/G)TAAAGTAGATCT was treated as described above and cloned as *Bam*HI-*Bgl*II fragments into *his3*-GG206. Molecules *his3*-GG5T, *his3*-GG280T, and *his3*-GG282T were constructed by inserting the appropriate oligonucleotide containing the functional *his3* T_R element into the *Eco*RI site of the parental molecule (see Fig. 5 and 8). The molecule *his3*-GG282lex was constructed by inserting a symmetrical 32-bp oligonucleotide containing the LexA binding site (8) into the *Eco*RI site of *his3*-GG282.

Phenotypic analysis. DNAs representing the various deletions of *his3*-GG1 were introduced by gene replacement into KY322, a strain that constitutively expresses GCN4 protein as a result of removal of the translational control sequences in GCN4 mRNA (19, 44). KY322 was constructed by replacing the wild-type *gcn4* allele in strain KY320 (7) with p139 DNA (obtained from A. Hinnebusch). The resulting strains were examined for their level of *his3* expression by their ability to grow in glucose minimal medium containing 10 mM aminotriazole (AT). As shown previously, growth in the presence of AT is directly related to the level of *his3* mRNA (7, 18). The levels of *his3* and *ded1* mRNAs from glucose-grown cells were determined by using oligonucleotide probes as described previously (7, 9).

Identification and partial purification of QBP. A crude yeast extract was prepared from the protease-deficient diploid strain BJ926 by a procedure developed by C. R. Wobbe. Briefly, cells were grown to late log phase in rich medium containing glucose, treated with lyticase to form spheroplasts, and then disrupted by treatment with a Dounce homogenizer. The extract was made to 0.3 M ammonium sulfate and cleared by centrifugation, after which the DNA was removed by precipitation with polymin P. The resulting protein was precipitated with 70% ammonium sulfate, resuspended, and then dialyzed in HEPES-NaOH (pH 7.5)–100 mM KCl–10% glycerol–1 mM EDTA–1 mM dithiothreitol–1 mM phenylmethylsulfonyl fluoride–0.4 μg of pepstatin A per ml. The protein was chromatographed on a phosphocellulose column at a protein/resin ratio of 10 mg/ml and then eluted with a 100 mM to 1 M gradient of KCl. Q-binding protein (QBP)-containing fractions, as determined by band shift assays, were chromatographed further on an S200 fast protein liquid chromatography sizing column to yield a protein preparation representing approximately a 1,000-fold purification of QBP from the crude extract.

Band shift assays. Approximately 5-ng samples of DNA probes labeled at their 5′ ends with ^{32}P were combined with 200 ng of the S200-purified Q protein fraction in 15 μl of buffer containing 20 mM Tris hydrochloride (pH 7.9), 25 mM KCl, 4 mM $MgCl_2$, 1 mM dithiothreitol, 200 μg of bovine serum albumin per ml, and 160 μg of poly(dI-dC) per ml and incubated at room temperature for 15 min. After addition of 5 μl of the same buffer containing 20% glycerol, the products were separated on a 7% native polyacrylamide gel containing 100 mM Tris-borate (pH 8.3).

DNase I footprinting. Approximately 600 ng of the QBP fraction eluted from the S200 column was incubated with 1 ng of the appropriate *gal* DNA fragment (5′ labeled at one end with ^{32}P) and 1 μg of poly(dI-dC) competitor DNA for 20 min on ice in binding buffer (12.5 mM HEPES-KOH [pH 7.6], 6.25 mM $MgCl_2$, 0.5 mM EDTA, 5% glycerol, 5% polyvinyl alcohol, 50 mM KCl). After treatment with 2 to 16 μg of DNase I for 1 min at room temperature in the presence of 2.5 mM $CaCl_2$ and 8 mM $MgCl_2$, the resulting products were electrophoretically separated on a 6% denaturing acrylamide gel in parallel with DNA sequencing ladders.

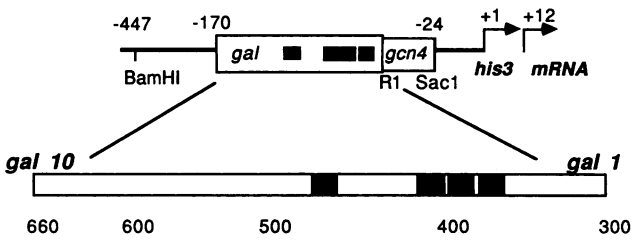

FIG. 1. Structure of *his3*-GG1. *his3*-GG1 (8) contains components from *pet56-his3* (39) and the *gal1-10* regulatory region (22). The *gal* box represents the *Dde*I-*Sau*3A fragment from 299 to 660 of the *gal1-10* promoter region, containing four GAL4 binding sites (black boxes) (2, 14). This region has been fused between positions −170 and −24 of the *his3* promoter (39) and is flanked by an *Eco*RI-*Sac*I oligonucleotide of 26 bp that contains the wild-type GCN4 binding site. The center of the GCN4 binding site is 41 bp from the +1 initiation site, from which transcription of *his3*-GG1 begins.

RESULTS

Localization of the Q element. In the *his3*-GG1 promoter, GCN4 activates transcription when its binding site is located at the position normally occupied by the TATA element (8; Fig. 1). Unlike equivalent *gal-his3* promoters in which transcription depends on a functional TATA element, occurs only in galactose medium, and is initiated almost exclusively at the +12 site (7, 17, 35, 37), transcription from *his3*-GG1 occurs in glucose medium and initiates preferentially at the +1 site (8). This TATA-independent activation by GCN4 requires an additional element(s), termed Q, that resides within the 365-bp fragment from the *gal1-10* upstream regulatory region (8).

To characterize the sequence requirements for Q function, we generated a series of *his3*-GG1 derivatives containing 5′, 3′, or internal deletions of the *gal* fragment (Fig. 2). DNAs from these deleted derivatives were introduced into yeast strain KY322 by gene replacement. The phenotypes of the resulting strains were analyzed by their ability to grow in glucose medium containing 10 mM AT, a competitive inhibitor of the *his3* gene (Table 1; Fig. 3). As shown previously, growth in the presence of AT is directly related to the level of *his3* mRNA (7, 18). For some derivatives, this was directly confirmed by determining *his3* mRNA levels and transcriptional initiation sites (Fig. 4).

Starting from the *Bam*HI site at position −447 of the *his3* gene, we generated a set of 5′ deletions that removed increasing amounts of the *gal* region. In comparison with *his3*-GG1, deletions that removed sequences up to position 393 of the *gal* region (*his3*-GG245) did not significantly affect growth on 10 mM AT. In contrast, deletion of an additional two bases to position 391 (*his3*-GG244) severely reduced growth, and deletions with endpoints further downstream failed to grow. As expected, a derivative containing the Q element (*his3*-GG228) resulted in significant levels of *his3* RNA, whereas a more deleted derivative lacking Q (*his3*-GG206) did not (Fig. 4, lanes 4 and 5). Moreover, the *his3*-GG228 allele behaved indistinguishably from *his3*-GG1 in that transcription occurred at a similar level (about two- to threefold less than the basal level obtained from the wild-type *his3* promoter), did not require GAL4 protein (lane 3), and was initiated preferentially from the +1 site (8). Thus, the 5′ boundary of the Q element is located between positions 391 and 393.

A set of successive 3′ deletions into the *gal* sequences was generated, starting from the *Eco*RI site that defines the boundary between the oligonucleotide containing the GCN4 binding site and the *gal* DNA fragment. Although a deletion of 12 bp to position 311 (*his3*-GG348) did not affect the phenotype, a deletion of only 22 bp to position 321 (*his3*-GG282) substantially reduced cell growth on 10 mM AT and the level of *his3* RNA (Fig. 4, lane 6). Further deletion of *gal* sequences also resulted in reduced cell growth, and in one case (*his3*-GG111) growth was completely eliminated. One interpretation of these results is that transcription from *his3*-GG1 requires another element distinct from Q whose 3′ boundary is located between positions 311 and 321. However, because 3′ deletions also bring Q closer to the GCN4 site, effects on growth may result from altered spacing between the sites rather than from deletion of specific sequences.

To define more accurately the 3′ boundary of Q, small internal regions between positions 367 and 399 of the *gal* fragment were replaced by a 12-bp sequence so that the spacing between the GCN4 and Q sites was not dramatically changed. One of these derivatives, *his3*-GG310 (deleted between positions 391 to 399), prevented growth on 10 mM AT, thus indicating that the Q element is necessary even in the context of the entire *gal* region. In contrast, five derivatives including *his3*-GG309 (contains the 12-bp sequence inserted between positions 383 and 386) did not affect the growth rate; this result indicates that the 3′ boundary of Q is upstream of 386 and that the region between 367 and 386 is functionally unimportant. These results, combined with those of the 5′ deletions, indicate that Q is defined primarily by the sequence GTCACCCG that is positioned between bases 386 and 394.

A second element is required for maximal GCN4-dependent transcription. As mentioned above, a small 3′ deletion (*his3*-GG282) reduced expression, suggesting the possibility of a second element that includes *gal* nucleotides between 311 and 321. Two experiments were carried out to eliminate the alternative hypothesis that the phenotypic effects of these deletions result from altered spacing (Fig. 2). First, a short insertion replacing the *gal* sequences between positions 321 and 318 was made (*his3*-GG346). Second, the *gal* sequences deleted in *his3*-GG282 were replaced by a 32-bp fragment containing the *Escherichia coli lexA* operator (*his3*-GG282lex). In both cases, *his3* expression was significantly reduced in comparison with *his3*-GG1 expression even though the spacing between the GCN4 and Q sites was very similar. Thus, a second element, now termed Z, located just upstream of position 310 is required for optimal GCN4 and Q-dependent transcription. Interestingly, this Z element is flanked on its downstream side by a stretch of seven T residues. Although it is unknown whether the T residues have any significant role, this T stretch is similar to that found following the GCN4 binding site (18).

Spacing but not orientation of Q with respect to the GCN4 site influences the level of transcription. In addition to internal deletions of *his3*-GG1, we generated two internal duplications. Interestingly, an internal duplication that contains an additional 22 bp (*his3*-GG314) resulted in a diminished growth rate, whereas a derivative in which the spacing was increased to 35 bp (*his3*-GG313) failed to grow in 10 mM AT and showed very low levels of *his3* RNA (Fig. 4, lane 7). These observations suggested that spacing between Q and the GCN4 binding site might be crucial for function.

To examine the effects of spacing more explicitly, we inserted an oligonucleotide that contained *gal* sequences from 385 to 397 at the *Bam*HI sites of several 5′ deletion

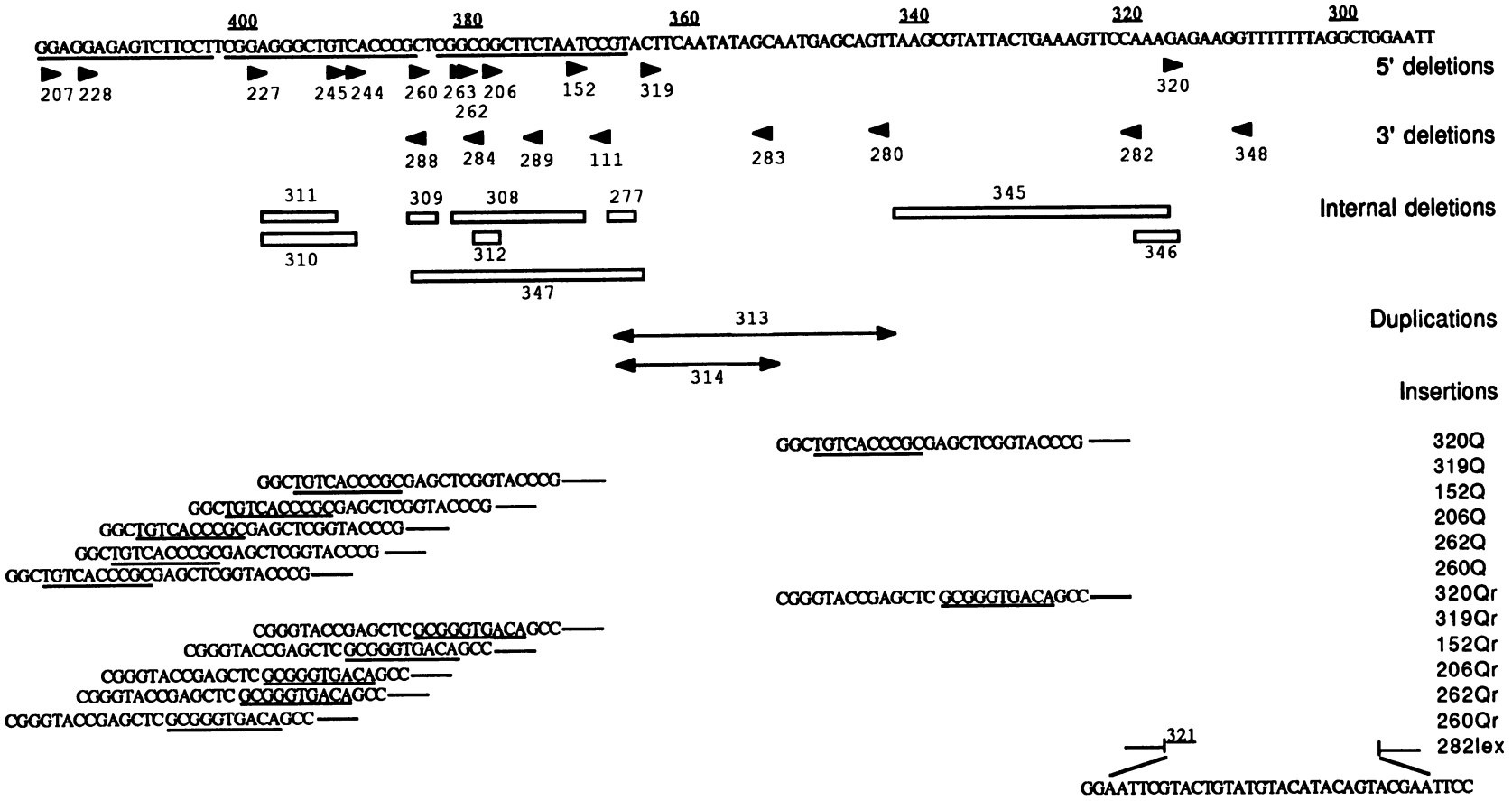

FIG. 2. Structures of *his3*-GG1 derivatives. The *gal1-10* regulatory region from 419 to the *Eco*RI site found at its junction with the GCN4 binding site oligonucleotide at 299 (22) is shown along with the positions of the four GAL4 binding sites (underlined) (2, 14). The endpoints and directions of 5′ and 3′ *his3*-GG deletion derivatives are shown with arrows. Sequences removed in the alleles with internal deletions are indicated with open boxes, and sequences added in the alleles with internal duplications are indicated with double-arrowed lines. Oligonucleotides containing the Q element in the correct (Q) or incorrect (Qr) orientation or the *lexA* operator (lex) are indicated by the relevant DNA sequence fused to *gal* sequences (dash) at the indicated insertion site.

TABLE 1. Sequences and phenotypes of *his3*-GG1 derivatives[a]

his3-GG allele	Sequence	Endpoint within *gal1-10*	Phenotype
5′ deletions			
155	ggatccgCGCTTCGCTG	649	+++
156	ggatccGTGAAGACGA	437	+++
209	ggatccgGAGGACGCGG	429	+++
207	ggatccgGGAGGAGAGA	419	+++
228	ggatccGGAGAGTCTTC	416	+++
227	ggatccGAGGGATGTCA	400	+++
245	ggatccgGTCACCCGCT	393	+++
244	ggatccgCACCCGCTCG	391	+
260	ggatccgGCTCGGCGGC	386	−
263	ggatccgGGCGGCTTCT	382	−
262	ggatccGCGGCTTCTAA	381	−
206	ggatccgGGCTTCTAAT	379	−
152	ggatccgATCCGTACTT	371	−
3′ deletions			
111	ggaattccGATTAGAAGC	369	−
283	ggaattcCTATTGAAGTA	354	+
280	ggaattcCTGCTCATTGC	344	+
282	ggaattccGGAACTTTCA	320	+
348	ggaattCCTTCTCTTTGG	311	+++
Internal deletions			
277	TCTAATCGgaattgatccgGTACTTC	369–367	+++
308	CCGCTCGGaattgatccgATCCGAT	381–371	+++
309	TCACCCGgaattgatccGCGGCTTC	386–381	+++
310	CTTCGGggaattgatccgCACCCGC	399–391	+
311	CTTCGGggaattgatccgGTCACCC	399–393	+++
312	CCGCTCGGaattgatccgGGCTTCT	379–381	+++
345	AATGAGCAGgaattgatccgAGAGAA	344–318	+
346	AAAGTTCCggaattgatccgAGAGAA	320–318	+
347	GACACCCggaattgatccgACTTCA	386–365	+++
Duplications			
279	ggaattccaattccTAGCCTAA	Insertion of 6 bp at 299	+++
313	GCAGgaattgatccgGTA...CAGTTAA	Duplicated 343–367	−
314	AATAGgaattgataagGTA...ATAGCAA	Duplicated 353–367	+

[a] For each derivative, the *gal1-10* sequences are capitalized, linker sequences are in lowercase, and sequences inserted or duplicated are underlined (for *his3*-GG313 and *his*-GG314, the internal regions of the duplicated sequences are indicated by dots). 5′ deletions were made from the *Bam*HI site at −447 of *his3*, and 3′ deletions were made from the *Eco*RI site marking the boundary between *gal* sequences and the GCN4 binding site. All 3′ deletions as well as the internal deletions that were constructed from them (with the exception of *his3*-GG111) were constructed from *his3*-GG155, which lacks sequences upstream of 649 in *gal1-10* to −447 of *his3*. The phenotypes listed represent rates of growth on minimal plates containing 10 mM AT: +++, has wild-type growth; ++, grows slightly slower than wild type; +, grows very slowly; −, does not grow.

mutants that lacked the Q element and hence failed to activate transcription (Table 2; Fig. 3). The Q element can function bidirectionally because derivatives containing the oligonucleotide in the correct (e.g., *his3*-GG206Q) or reverse (e.g., *his3*-GG206Qr) orientation conferred *his3* expression. Interestingly, activation provided by the Q oligonucleotide was less efficient as the distance to the GCN4 binding site (and the initiation region) was increased. This pattern held for molecules with Q in either orientation with the sole exception of *his3*-GG152Qr, whose level of expression was lower than expected. Insertion of the Q oligonucleotide at position 318 (*his3*-GG320Q and Qr) did not result in activation, presumably because of the absence of the Z element in the *gal* region. In comparison with *his3*-GG1, all derivatives with a similar or reduced separation of the Q and GCN4 sites conferred equivalent levels of *his3* expression; in the case of *his3*-GG319Q, this was confirmed by RNA measurements (Fig. 4, lane 8). However, an increased separation of 23 bp

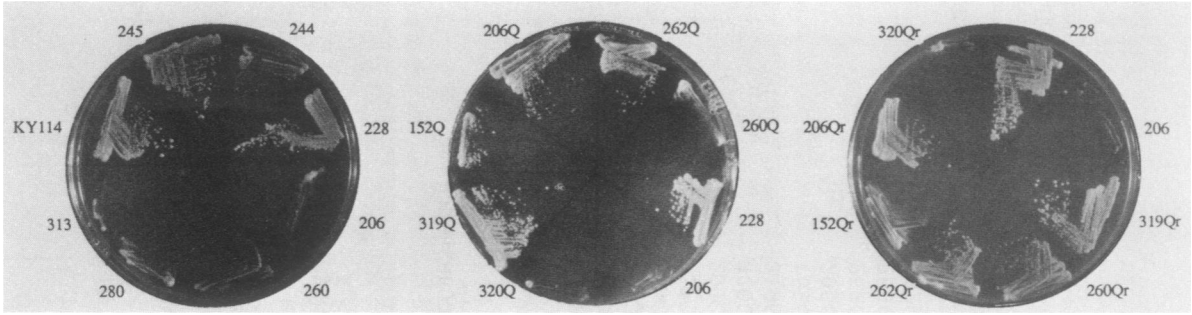

FIG. 3. Growth of strains containing *his3*-GG alleles. Yeast strain KY322 cells containing the indicated *his3*-GG alleles were streaked onto minimal plates containing 10 mM AT and 2% glucose and grown at 30°C for 5 days. Yeast strain KY114, which contains a wild-type *his3* gene, was included as a positive control.

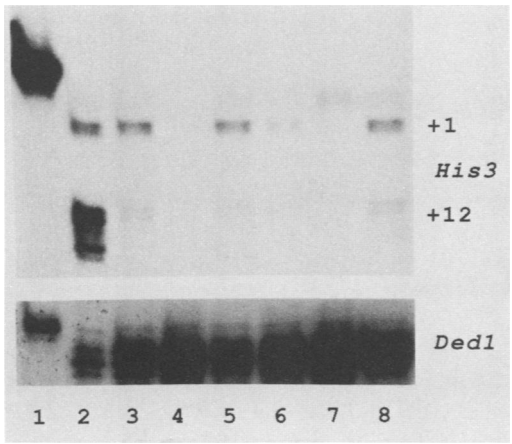

FIG. 4. RNA analysis. RNA was prepared from GCN4-expressing cells (KY322 derivatives for lanes 4 to 8 and derivatives with YCp88-GCN4 for lanes 2 and 3) containing the various *his3* alleles that were grown in YP medium with glucose. For each sample, 25 μg of total RNA was hybridized to *his3* and *ded1* 5'-end-labeled oligonucleotide probes and then digested with S1 nuclease. Lanes: 1, probes alone; 2, *his3* wild type (strain KY114); 3, *his3*-GG228 (in a *gal4* disrupted derivative of KY320); 4, *his3*-GG206; 5, *his3*-GG228; 6, *his3*-GG282; 7, *his3*-GG313; 8, *his3*-GG319Q. Different exposure times of the identical autoradiogram are shown for the regions corresponding to the *ded1* and *his3* transcripts. As indicated by the intensity of the *ded1* control band, about two- to threefold less RNA was loaded in lane 2 than in the other lanes.

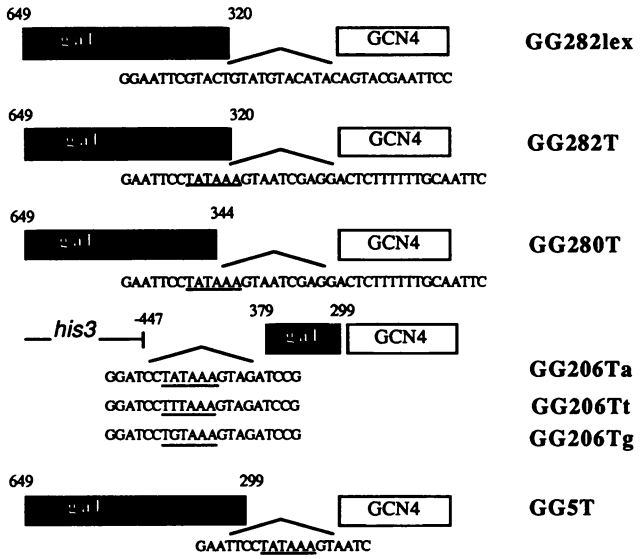

FIG. 5. Insertion of a functional TATA element upstream of the GCN4 binding site. For each derivative, the sequence of the inserted oligonucleotide containing the *his3* T_R element (TATAAA underlined) or the *lexA* operator is shown with respect to the position of *gal* DNA sequences (black box) and the GCN4 binding site (open box). In the case of *his3*-GG5T, the GCN4 binding site is located 13 bp closer to the *his3* initiation region (8).

from the wild type (*his3*-GG262Q) resulted in a dramatically reduced growth rate, similar to that seen for *his3*-GG314, in which the increased spacing was the result of sequence duplication. This finding also suggests that Q is completely ineffective when displaced more than 35 bp upstream as in *his3*-GG313.

Insertion of a functional TATA element upstream of the GCN4 binding site does not restore transcription. Although it seemed unlikely that *his3*-GG1 contained a functional TATA element (8), the possibility remained that Q or some other sequence interacted with a TATA-binding factor and that the spatial relationship between GCN4 and a TATA-binding protein could be reversed. To test this directly, we first inserted oligonucleotides which contained the wild-type T_R sequence (TATAAA; 7), an anomalous T_R sequence that will activate transcription with GCN4 but not GAL4 (TTTAAA; 17), or a nonfunctional sequence (TGTAAA) into the *Bam*HI site of *his3*-GG206 which lacks Q (Fig. 5; Table 1). In the same position that the Q oligonucleotide was completely functional, none of the TATA sequences permitted GCN4-activated transcription. Therefore, Q cannot be a binding site for TFIID or a functional equivalent.

TABLE 2. Sequences and phenotypes of GG derivatives[a]

GG allele	Sequence	Distance to:		Phenotype
		+1	GCN4	
260Q	GGCTGTCACCCGCgagctcggtacccgggatccgGCTCGGCG	144	103	+
262Q	GGCTGTCACCCGCgagctcggtacccgggatccGCGGCTTCT	139	98	++
206Q	GGCTGTCACCCGCgagctcggtacccgggatccgGGCTTCTA	137	96	++
152Q	GGCTGTCACCCGCgagctcggtacccgggatccgATCCGTAC	129	88	+++
319Q	GGCTGTCACCCGCgagctcggtacccgggatccgACTTCAATA	123	82	+++
320Q	GGCTGTCACCCGCgagctcggtacccgggatccgAGAGAAGG	87	46	−
260Qr	cgggtaccgagctcGCGGGTGACAGCCggatccgGCTCGGCG	132	91	++
262Qr	cgggtaccgagctcGCGGGTGACAGCCggatccGCGGCTTCT	126	85	+++
206Qr	cgggtaccgagctcGCGGGTGACAGCCggatccgGGCTTCTA	125	84	+++
152Qr	cgggtaccgagctcGCGGGTGACAGCCggatccgATCCGTAC	119	78	+
319Qr	cgggtaccgagctcGCGGGTGACAGCCggatccgACTTCAATA	113	72	+++
320Qr	cgggtaccgagctcGCGGGTGACAGCCggatccgAGAGAAGG	65	24	−
228	gGAGAGAGTCTTCCTTCGGAGGGCTGTCACCCGCTC	127	86	+++
206	gGGCTTCTAAT	NA	NA	−

[a] The Q-containing oligonucleotide with *gal* sequences from 397 to 385 was inserted as a *Bam*HI fragment into the *Bam*HI site of the indicated 5'-deleted *his3*-GG derivatives in either the correct or reverse orientation to generate Q or Qr alleles, respectively. The sequence surrounding the Q oligonucleotide is shown with linker sequences in lowercase. Distance to +1 is from the underlined nucleotide. Phenotype refers to growth on 10 mM AT as defined in the footnote to Table 1 and shown in Fig. 3. NA, Not applicable.

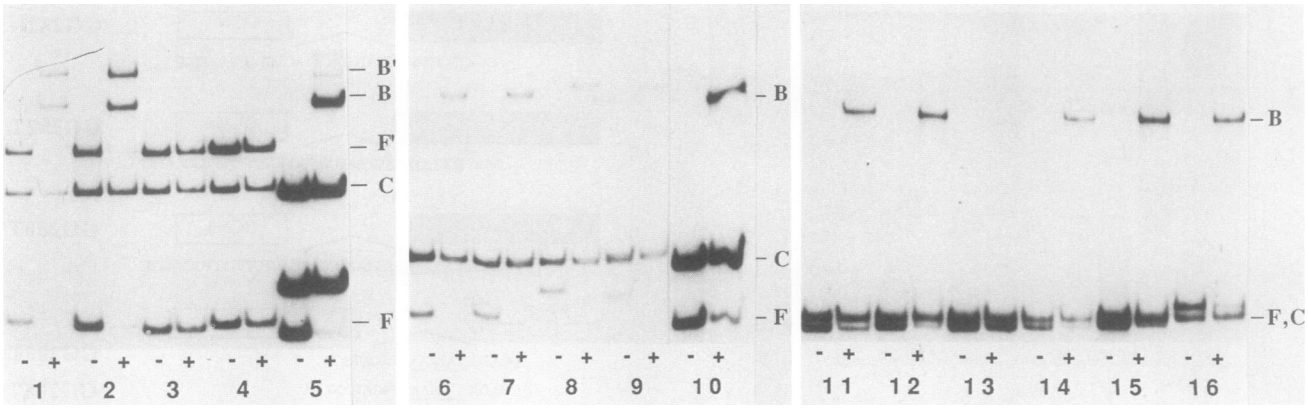

FIG. 6. DNA sequence requirements for QBP, as determined by band shift assay to locate the QBP binding site within the *gal* fragment. DNA fragments from 5′, 3′, and internally deleted derivatives were separated by electrophoresis in a native polyacrylamide gel after incubation with (+) or without (−) S200-purified QBP. *his3* DNA from downstream of the *Eco*RI site was included as a control for specificity with the 5′ deletions; the *Hin*PI-*Bam*HI fragment containing *gal* and *pet56* sequences was included with the 3′ and internal deletions. Positions of bands corresponding to the DNA fragments of interest (F), control DNA fragments (C), and protein-DNA complexes (B) are indicated (electrophoretic mobilities for individual derivatives differ slightly as a result of variability in DNA fragment lengths caused). Some of the 5′ deletions were only partially digested with *Eco*RI (producing bands designated F′), thus accounting for the appearance of an additional shifted band (designated B′). The additional unshifted band in lane 5 is due to the structure of *his3*-GG111, which was derived from a different molecule than the other derivatives. Lanes: 1, *his3*-GG227; 2, *his3*-GG245; 3, *his3*-GG260; 4, *his3*-GG244; 5, *his3*-GG111; 6, *his3*-GG284; 7, *his3*-GG289; 8, *his3*-GG280; 9, *his3*-GG283; 10, *his3*-GG288; 11, *his3*-GG313; 12, *his3*-GG312; 13, *his3*-GG310; 14, *his3*-GG311; 15, *his3*-GG309; 16, *his3*-GG308.

In a related experiment, we inserted an oligonucleotide containing the wild-type T_R sequence at the *Eco*RI sites of *his3*-GG282 and *his3*-GG280, derivatives that lack the Z element. The resulting derivatives, *his3*-GG282T and *his3*-GG280T, were phenotypically indistinguishable from the parental molecules and *his3*-GG282lex, indicating that the Z element cannot be replaced by a TATA element at a similar position within the *his3*-GG1 promoter. The oligonucleotide clearly contains a functional TATA element because it permits normal GAL4-dependent activation when inserted in place of the GCN4 site (data not shown).

Finally, we have generated *his3*-GG5T, a derivative in which it appears that GCN4 bound just downstream of a functional TATA element can actually repress transcription. Specifically, this promoter shows a very low level of GAL4-activated expression (evidenced by a failure to grow in galactose medium containing AT) despite the fact that it contains the *gal* enhancer and wild-type T_R element at the identical positions as found in highly active *gal-his3* promoters (7, 17). This apparent repression depends on GCN4 because it is not observed in a derivative of *his3*-GG5T that contains a point mutation in the GCN4 binding site. Presumably, this repression reflects the inability of TFIID to bind the TATA element as a result of steric interference caused by GCN4 binding to an overlapping target site.

Identification of a QBP. A crude yeast cell extract was fractionated on a phosphocellulose column to search for a protein that bound Q and thus might be a potential mediator of Q-induced expression. Three fractions eluting with salt concentrations of approximately 230, 260, and 600 mM KCl contained proteins that shifted the electrophoretic mobility of a *gal* DNA fragment (nucleotides 400 to 299) containing the Q element. The fraction eluting at 600 mM KCl bound in a Q-specific fashion and was further chromatographed on a Sephacryl S200 column, where it eluted with an apparent molecular weight of approximately 80,000. The other two fractions bound upstream sequences that did not correspond to the Q or to the Z element.

To further delineate the binding specificity of the QBP, deletions of the *gal* region used in the in vivo analyses were examined as substrates in the band shift assay (Fig. 6). DNA fragments with 5′ deletions up to and including *his3*-GG245 (position 393) were shifted by QBP, whereas DNA from *his3*-GG244 (position 391) was very weakly bound and further 5′ deletions such as *his3*-GG260 (position 386) were not bound. DNAs from the set of 3′ deletions all bound QBP, defining the 3′ boundary of the QBP site upstream of bp 385 (*his3*-GG288). All of the internal *gal* deletions were shifted by QBP with the exception of *his3*-GG310, which also did not have Q activity in vivo. QBP did not bind a DNA fragment between the *Bam*HI site at −447 and the *Hin*PI site in *gal* (position 475), indicating that there is only one QBP site within the entire *gal* region. In addition, the oligonucleotide that showed Q activity in vivo was also bound by QBP. This binding was competed for by the Q site but not by an oligonucleotide containing the sequence TATAAA (Fig. 7). Thus, specific DNA binding by QBP precisely parallels the ability of the Q element to allow GCN4 to stimulate transcription.

The binding site of QBP was examined further by DNase I footprinting (Fig. 8). On the noncoding strand, QBP protected a region of 27 bp from 372 to 398 from digestion DNase I digestion, whereas on the coding strand protection was observed over the 24-bp region from 378 to 402. For both strands, the protected sequences completely include those required for QBP binding in the band shift studies and for Q activity in vivo. The QBP recognition site in the *gal* enhancer significantly overlaps a GAL4 binding site (2, 14). However, the *his3*-GG245 derivative eliminates half of the GAL4 binding site but does not affect the QBP site.

QBP and Y protein have very similar DNA-binding specificities. A previously identified protein, termed Y, yields a DNase I footprint over the same GAL4 binding site that is very similar to that observed with QBP (10). To determine whether QBP and Y are the same protein, we examined a set of three Y-binding oligonucleotides that contain sequences

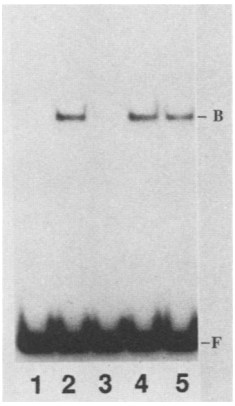

FIG. 7. QBP binding to Q oligonucleotide, as determined by band shift assay of labeled Q oligonucleotide in the presence or absence of unlabeled Q or TATA oligonucleotides. The Q oligonucleotide was cloned as an *Eco*RI-*Sac*I fragment in pTZ18, and a labeled *Eco*RI-*Hin*dIII fragment was used in the band shift assay. Lanes: 1, no protein added; 2, QBP added; 3, QBP and 200 ng of Q oligonucleotide added; 4 and 5, QBP plus 200 and 400 ng, respectively, of TATAAA oligonucleotide added. Positions of the free (F) and protein-bound (B) oligonucleotides are indicated.

from the *act1*, *trp5*, and 35S RNA genes (kindly provided by Dan Chasman and Roger Kornberg) for their ability to compete for binding to QBP (Fig. 9). All three oligonucleotides inhibited the binding of QBP to the *gal* sequence. Moreover, the order of affinities for these oligonucleotides for QBP was the same as that for Y: 35S > *trp5* > *act1* (D. Chasman and R. Kornberg, personal communication). This result indicates that QBP and Y have indistinguishable binding specificities and thus may be the same protein. The discrepancy between the apparent molecular weights of QBP (80,000) and Y (127,000; Chasman and Kornberg, personal communication) could be due to proteolysis of QBP in our preparation.

DISCUSSION

Elements of the *his3*-GG1 promoter. In addition to the GCN4 binding site, transcription from *his3*-GG1 requires the Q element defined primarily by the sequence GTCACCCG between nucleotides 386 and 394 of the *gal* regulatory region and a second element, called Z, that includes sequences between positions 311 and 321. Although these two elements may be sufficient for *his3*-GG1 promoter function, the possibility of an additional element(s) cannot be excluded. However, the deletion analysis restricts the location of such an element to the region between bases 320 and 366.

Is TFIID required for transcription from *his3*-GG1? It is commonly believed that the TATA-binding protein TFIID is part of the basic RNA polymerase II machinery and hence universally required for transcription from all promoters. Even in promoters lacking a typical TATA sequence (consensus TATAAA), TFIID could play an essential role because of its ability to functionally interact with nonconsensus sequences (16, 35). Alternatively, it is possible that upstream activator proteins could increase the ability of TFIID to function from sites that otherwise would be considered poor TATA elements. Thus, the absence of an apparent TATA element in a promoter does not exclude a role for TFIID, especially considering the difficulties in predicting whether a given sequence is a good or bad substrate for TFIID (35).

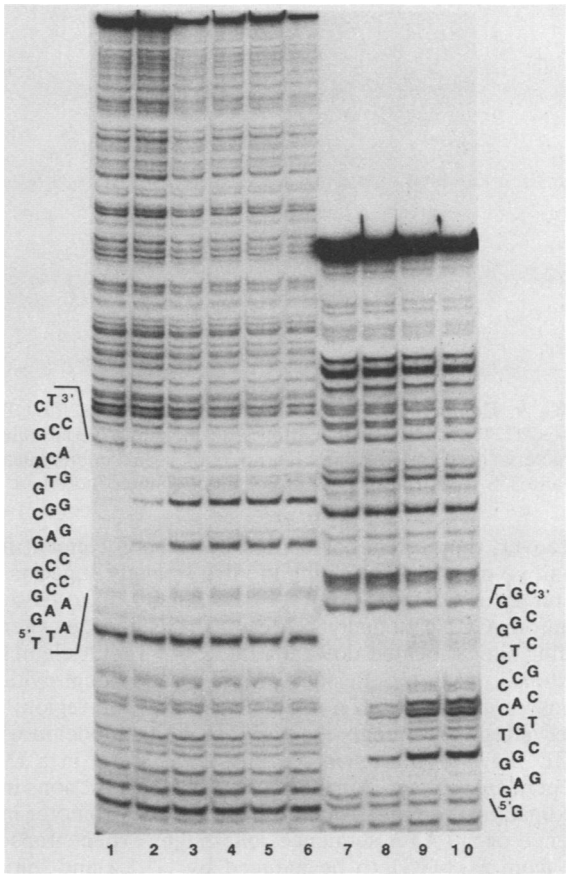

FIG. 8. DNase I footprint of QBP on *gal1-10*. The *Eco*RI-*Bam*HI fragment of *his3*-GG155 was 5′ labeled at the *Eco*RI site, incubated with sequential fourfold dilutions of QBP (lanes 1 to 5) or without QBP (lane 6), and treated with DNase I. Lanes 7 to 10 represent a similar treatment for the opposite strand, using the *Bam*HI-*Sac*I fragment of *his3*-GG228 labeled at the *Bam*HI site. Sequence ladders were run in parallel with the DNase I-treated fragments. The sequence of the region protected by QBP is indicated.

We argued previously that transcription from *his3*-GG1 might not involve TFIID (8). First, GCN4 can activate transcription when bound very close to the initiation site, a position that would seemingly prevent TFIID from binding to the proximal promoter element (8). Second, a large number of equivalent promoters containing mutated TATA elements in place of the GCN4 binding site are inactive (7, 17), thus arguing against the possibility of cryptic TATA elements either in the *gal* DNA fragment or downstream of position −24 in the *his3* promoter. Third, even in the case of equivalent promoters containing functional TATA elements, transcription is never observed in glucose medium (17, 35). However, more complex models invoking TFIID could be imagined. For example, GCN4 bound at the proximal position could attract TFIID to the promoter by a protein-protein interaction; i.e., TFIID would be functionally important even though not bound directly to DNA. Alternatively, the *gal* region could contain a novel TATA element that functions when upstream of a GCN4 binding site but not downstream of four GAL4 binding sites.

The observations reported here make it extremely unlikely that Q and Z are cryptic TATA elements because they bear no similarity to TATAAA and cannot be replaced by a wild-type TATA sequence at the comparable position.

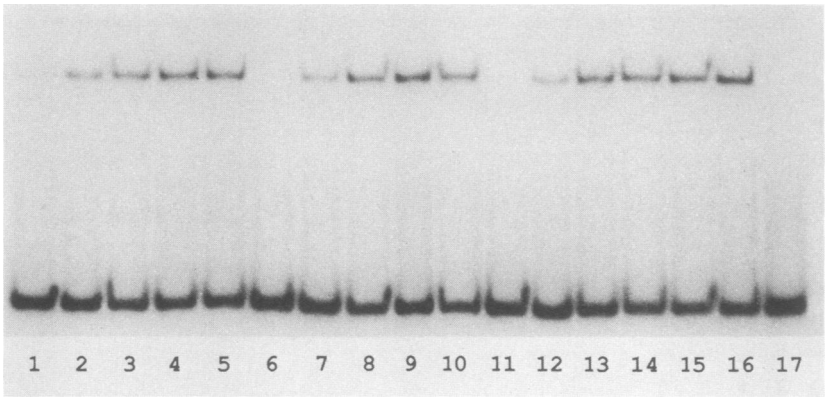

FIG. 9. Competition of Y-binding oligonucleotides for QBP. A band shift assay with the end-labeled *Bam*HI-to-*Eco*RI fragment of *his3*-GG227 and QBP was performed, including serial dilutions of competing double-stranded oligonucleotides obtained from and characterized by Chasman and Kornberg (personal communication). From left to right, 20 ng to 2.0 pg of *act1* (lanes 1 to 5), *trp5* (lanes 6 to 10), and 35S RNA (lanes 11 to 15) or no oligonucleotide (lane 16) was added. Lane 17 shows the probe DNA in the absence of QBP.

Moreover, the perfect correlation between Q element function in vivo and QBP binding in vitro strongly suggests that the target of Q is QBP, not TFIID. Finally, in at least one situation, GCN4 actually represses transcription when its binding site is located downstream of the TATA element in a *gal-his3* promoter. Besides providing additional evidence against a cryptic TATA element in the *gal* region, this observation is seemingly inconsistent with a model in which TFIID plays a role by interacting with GCN4 in a DNA-independent manner. Furthermore, if GCN4 functions in the proximal position by attracting TFIID to the promoter in the absence of a TATA sequence, one might expect transcription from *his3*-GG1 to be induced by GAL4 and initiated preferentially from the +12 site. Thus, we favor the idea that transcription from *his3*-GG1 does not involve TFIID. However, we cannot exclude the possibility that TFIID functions in a novel manner that does not involve sequence-specific binding to DNA and that results in completely different properties from normal TFIID-dependent transcription such as selection of initiation sites, failure to respond to GAL4, and requirement for Q.

Potential molecular mechanisms. QBP binds specifically to DNA fragments that contain a functional Q element, and it also footprints over Q, thus strongly suggesting that QBP is required for transcription from the *his3*-GG1 promoter. However, we cannot exclude the possibility that the Q element acts by binding some other protein that has yet to be identified. The DNA-binding properties of QBP strongly resemble those of a previously described protein called Y (10; Chasman and Kornberg, personal communication). Specifically, QBP and Y yield very similar DNase I footprints in the *gal* regulatory region and show the same order of binding affinities on a set of target sites. Thus, it is highly probable, though not definitely established, that QBP and Y are the same protein.

The DNA sequence recognized by Y forms a constraint to nucleosome positioning, although a direct role for Y protein in chromatin structural alterations has yet to be established (10). The results here establish a functional role for the Q element which coincides with the nucleosome positioning sequence. Thus, if QBP and Y are the same protein and if QBP is directly responsible for activation through the Q element, then changes in the local chromatin structure induced by QBP might allow GCN4 to stimulate transcription in the absence of a TATA element. To put it another way, the TFIID requirement for GCN4-induced transcription might be bypassed by an appropriate structure in the promoter region. For example, if the critical role of TFIID is to directly or indirectly enable the melting of DNA near the mRNA initiation site, perhaps the process might be facilitated by the appropriate positioning of nucleosomes. In this regard, it is interesting that the level of transcription is strongly influenced by relatively minor alterations in the distance between the QBP and GCN4 binding sites.

It has been proposed that acidic transcriptional activation regions function by directly contacting some component of the basic transcription machinery (9, 20, 27, 41). A variety of observations have implicated TFIID (21, 32, 41) or RNA polymerase II (3) as the target of upstream activator proteins, although any of the basic transcription factors are also obvious candidates (4). If TFIID is not involved in transcription from the *his3*-GG1 promoter, the requirement for the GCN4 acidic activation region (8) would disfavor the view that TFIID is the functionally important target. This view would not exclude potential interactions between TFIID and upstream activator proteins but rather would suggest at least one other essential role for the acidic activation region.

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