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Constitutive and Inducible *Saccharomyces cerevisiae* Promoters: Evidence for Two Distinct Molecular Mechanisms

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his3 **and** ***pet56*** **are adjacent** ***Saccharomyces cerevisiae*** **genes that are transcribed in opposite directions from initiation sites that are separated by 200 base pairs. Under normal growth conditions, in which** ***his3*** **and** ***pet56*** **are transcribed at similar basal levels, a poly(dA-dT) sequence located between the genes serves as the upstream promoter element for both. In contrast,** ***his3*** **but not** ***pet56*** **transcription is induced during conditions of amino acid starvation, even though the critical regulatory site is located upstream of both respective TATA regions. Moreover, only one of the two normal** ***his3*** **initiation sites is subject to induction. From genetic and biochemical evidence, I suggest that the** ***his3-pet56*** **intergenic region contains constitutive and inducible promoters with different properties. In particular, two classes of TATA elements, constitutive (T_c) and regulatory (T_r), can be distinguished by their ability to respond to upstream regulatory elements, by their effects on the selection of initiation sites, and by their physical structure in nuclear chromatin. Constitutive and inducible** ***his3*** **transcription is mediated by distinct promoters representing each class, whereas** ***pet56*** **transcription is mediated by a constitutive promoter. Molecular mechanisms for these different kinds of** ***S. cerevisiae*** **promoters are proposed.**

Saccharomyces cerevisiae promoters are composed of upstream, TATA, and initiator elements that are necessary for the accuracy, amount, and regulation of transcriptional initiation (reviewed in references 10 and 36). Upstream elements, which resemble mammalian enhancers (11, 33), are required for transcription, and they usually determine the particular regulatory properties of a promoter (1, 7, 8, 12, 13, 28, 29). Upstream elements for coregulated genes are similar in DNA sequence (7, 30), whereas those of unrelated genes are different. In several cases, these elements have been shown to be specific DNA-binding sites for transcriptional regulatory proteins (2, 9, 17, 19). TATA elements (consensus sequence TATAAA) are present in essentially all yeast promoters, although the distances from their respective mRNA initiation sites range between 40 and 120 base pairs (bp). These elements are required for gene expression (12, 27, 29) and have been presumed to have a general role in the process of transcription. In higher eucaryotic organisms, proteins that bind specifically to TATA sequences have been identified (6, 23). The initiator element, located near the mRNA start site, has little effect on the overall RNA level, but it determines where transcription begins (5, 14, 20, 21).

In the natural *S. cerevisiae* genome, *his3* and *pet56* are adjacent genes that perform unrelated functions (35). *his3* encodes imidazoleglycerolphosphate dehydratase, a histidine biosynthetic enzyme, and *pet56* is essential for mitochondrial function. These genes are expressed at similar basal levels under normal growth conditions (38) and are transcribed divergently from initiation sites that are separated by 191 bp (35) (Fig. 1). Although each gene has its own TATA element, a 17-bp region of poly(dA-dT) located between the genes serves as the upstream promoter element for both (34). Thus, this constitutive element acts bidirectionally to activate transcription of two unrelated genes.

Under conditions of amino acid starvation, transcription of the *his3* gene is induced threefold over the basal level, whereas *pet56* transcription remains at its basal level (38). Maximal induction depends on two copies of an upstream regulatory site that are located upstream of the *his3* and *pet56* TATA sequences and on either side of the poly(dA-dT) sequence necessary for constitutive transcription (30, 37; Struhl and Hill, Mol. Cell. Biol., in press) (Fig. 1). The *his3*-proximal copy is required for induction and by itself is sufficient to confer partial induction. The distal copy is necessary only for full induction and is inactive in the absence of the proximal copy. Coordinate induction of *his3* and other amino acid-biosynthetic genes is mediated by the *gcn4* protein, which binds specifically to the *his3*-proximal regulatory site and to promoter regions of other coregulated genes (17).

Under normal growth conditions, *his3* transcription is initiated at equal frequency from two major sites, defined as +1 and +12 (35) (Fig. 1). This pattern of initiation sites is observed even when the *his3* upstream and TATA elements are replaced by the analogous *ded1* promoter sequences (5). However, the normal initiation pattern is not observed in a wild-type strain when *his3* transcription is induced during conditions of amino acid starvation. Instead, transcription from +1 remains at the normal basal level, whereas transcription from +12 is induced about fivefold (37). In addition, transcription from +22, normally a minor initiation site, is also induced. The same selectivity of *his3* initiation sites has been observed in strains in which the *gal1,10* enhancer-like sequence is fused to the *his3* promoter region at various positions (33) and in *his3*-Δ*13* strains, in which transcription depends on *ope* suppressor mutations (22). In all these cases of selectivity, *his3* transcription is subject to some form of regulation.

In this paper, I describe genetic and biochemical experiments that address two questions. First, why are *pet56* and *his3* not coordinately induced during starvation conditions even though the regulatory sites, which function bidirectionally (16) and at variable distances from mRNA start sites (16, 30), are located upstream of both TATA elements? Second, what is the basis for selectivity of initiation sites during

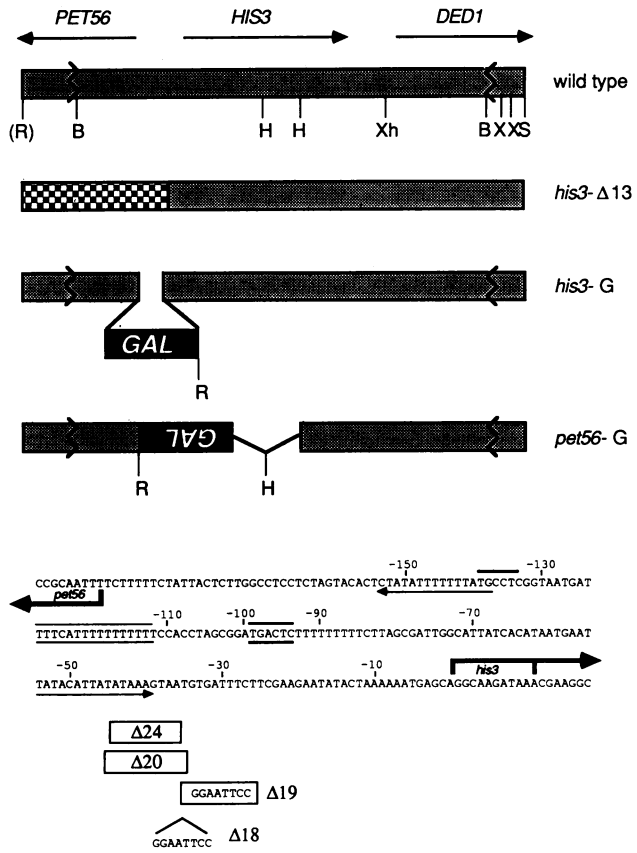

FIG. 1. Structure of the *pet56-his3-ded1* region in wild-type and mutant strains and DNA sequence of the *his3-pet56* promoter region. The shaded bar at the top represents a 6.1-kb *Eco*RI-*Sal*I fragment (with a mutated *Eco*RI site) that contains the intact *pet56*, *his3*, and *ded1* genes (35). The location and orientation of the transcripts are indicated by arrows above the shaded bar, and restriction endonuclease cleavage sites are indicated as vertical lines below the bar (R, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; Xh, *Xho*I; X, *Xba*I; S, *Sal*I). The drawing is to scale for the 1,765 bp between the *Bam*HI sites. The structures of *his3-Δ13* as well as *gal-his3* (*his3*-G) and *gal-pet56* (*pet56*-G) fusions are shown, with the solid bar indicating the 365-bp *gal* segment (33) and the checkered bar indicating the IS*1* and bacteriophage λ sequences fused to position −66 of the *his3* gene (22). The bottom half of the figure shows the nucleotide sequence of the *his3* coding strand of the *his3-pet56* promoter region. The coordinates above the sequence are determined with respect to the upstream-most *his3* initiation site, which is defined as +1. Features of the DNA sequence include the *his3* and *pet56* initiation sites (thick arrows) (34), the poly(dA-dT) upstream element for constitutive expression (thin lines above and below the sequence) (34), the TGACTC sequence that is critical for *his3* induction (thick lines above and below the sequence) (31, 38), the related sequence that affects the maximal level of *his3* induction (thick line above the sequence), and the *his3* and *pet56* TATA regions (thin, directional arrows) (29, 34). The extents of various *his3* deletions are shown as open boxes below the sequence. The *his3-Δ18* and *-Δ19* alleles also contain an insertion of 8 bp of *Eco*RI linker as indicated (5).

inducible but not constitutive *his3* transcription? From the results, I suggest that the constitutive and inducible promoters for *his3* and *pet56* expression are qualitatively different and that they exemplify two distinct mechanisms of transcriptional initiation.

MATERIALS AND METHODS

Genetic manipulations. The genotypes of the *S. cerevisiae* strains used in these experiments are listed in Table 1. Generally, these strains were grown at 30°C in liquid suspension or on 2% agar plates (Difco Laboratories, Detroit, Mich.) in YPD broth or in minimal medium containing 2% glucose and appropriate supplements (22). In some experiments, 2% galactose was used as the carbon source instead of glucose. Strains containing the *gcd1-101* mutation are temperature sensitive for growth (43) and were propagated at 23°C.

The procedures for constructing hybrid DNA molecules have been described previously (32). All the molecules contain a 6.1-kilobase (kb) segment of yeast chromosomal DNA with the entire *pet56-his3-ded1* gene region (33, 35) cloned in the *ura3*$^+$ vector YIp5 (39) or YIp55. YIp55 was constructed by cloning a 1.1-kb *Hin*dIII fragment containing the *ura3* gene (24) into vector pUC8 (42). The structures and DNA sequences of some of the hybrid molecules have been described elsewhere (5, 22, 29, 33). The *gal-pet56* fusions were generated by inserting Sc3296 (an *Eco*RI-*Hin*dIII fragment containing the *gal* upstream regulatory site) (33) be-

TABLE 1. *S. cerevisiae* strains

Strain	Relevant genotype	Reference or source[a]
KY114	**a** *ura3-52 ade2-101 trp1-Δ1 lys2-801 HIS3 GAL*$^+$	33
KY117	**a** *ura3-52 ade2-101 trp1-Δ1 lys2-801 his3-Δ200 GAL*$^+$	33
KY466	α *ura3-52 trp1-Δ1 lys2-801 his3-Δ200 gcd1-101*	TW
KY475	α *ura3-52 HIS3*$^+$ *gcd1-101*	DH
KY66	α *ura3-52 trp1-289 his3-Δ200 can1* (YRλ21-Sc2782; *TRP1 his3-Δ13*)	22
KY516	KY66 *ope1-1*	22
KY522	KY66 *ope2-1*	22
KY523	KY66 *ope3-1*	22
KY255	KY117 (YIp5-Sc3305; *URA3 his3-G4*)	33
KY257	KY117 (YIp5-Sc3309; *URA3 his3-G6*)	33
KY314	KY117 (YIp5-Sc3393; *URA3 pet56-G1*)	TW
KY315	KY117 (YIp5-Sc3392; *URA3 pet56-G2*)	TW
KY316	KY117 (YIp5-Sc3391; *URA3 pet56-G3*)	TW
KY163	**a** *ura3-52 trp1-289 ade2-1 his3-Δ20*	30
KY166	**a** *ura3-52 trp1-289 ade2-1 his3-Δ24*	30
KY321	KY466 except *his3-Δ20*	TW
KY322	KY466 except *his3-Δ24*	TW
KY122	KY117 except *his3-Δ18*	5
KY125	KY117 except *his3-Δ19*	5
KY323	KY466 except *his3-Δ18*	TW
KY324	KY466 except *his3-Δ19*	TW
KY56	α *ura3-52 trp1-289 his3-Δ200 can1* (YRλ21-Sc2757; *TRP1 his3*-Sc2757)	28
KY60	α *ura3-52 trp1-289 his3-Δ200 can1* (YRλ21-Sc2771; *TRP1 his3-Δ17*)	28

[a] TW, Strains constructed in this work; DH, strains constructed in my laboratory by David Hill.

tween artificial *Eco*RI sites at positions −22, −66, and −86 with respect to the *pet56* mRNA start site and the *Hin*dIII site at +515 of the *his3* gene (34, 35). The DNA fragments corresponding to *his3-Δ20* and *his3-Δ24* (Sc3101 and Sc3112) (30) were recloned into YIp55. YIp5 or YIp55 hybrid DNAs were introduced into yeast cells so that they replaced the chromosomal *his3* locus as described previously (5, 35).

RNA analysis. The procedures for isolation of RNA, synthesis and purification of 5′-end-labeled probes, DNA-RNA hybridization, nuclease S1 treatment, and product analysis by gel electrophoresis have been described in previous work (5, 22, 34, 35). Conditions of amino acid starvation were achieved by using strains containing the *gcd1-101* allele. This mutation abolishes the translational control of *gcn4* mRNA and results in a constitutively high level of GCN4 protein (15, 40). As this situation causes induced levels of all amino acid-biosynthetic genes even during growth in broth, it is equivalent to conditions of amino acid starvation.

Chromatin analysis. The mapping of micrococcal nuclease cleavage sites in chromatin has been described previously (22, 31). Osmotically lysed spheroplasts were incubated with an appropriate concentration of enzyme for 10 min at 37°C and then deproteinized by phenol extraction and ethanol precipitation. For the analysis shown in Fig. 5, the DNA was cleaved with *Hin*dIII, separated in 2% agarose, transferred to nitrocellulose, and challenged for hybridization with

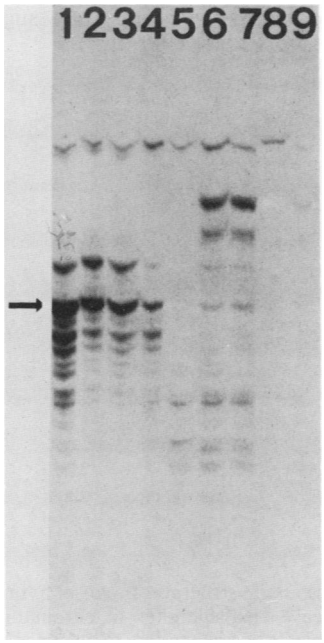

FIG. 2 *pet56* transcription is uninducible by the *his3* or *gal* upstream elements. Lanes 1 through 4 represent transcription of the wild-type *pet56* gene under normal (lanes 1 and 2) and inducing (lanes 3 and 4) conditions in *GCN4* (lanes 1 and 3) or *gcn4* (lanes 2 and 4) strains (KY114 and KY484, respectively). Lanes 5 and 6 are strains KY315 and KY316 (*pet56 G2* and *G3* alleles, respectively) grown in glucose medium, and lanes 7 through 9 are strains KY316, KY315, and KY314 (containing the *G3*, *G2*, and *G1* alleles, respectively) grown in galactose medium. The *pet56* endpoints of the *gal-pet56* fusions are −86 (*G3*), −66 (*G2*), and −22 (*G1*). Each lane contained 50 μg of total RNA. The arrow indicates the position of the major *pet56* transcript. The extra bands in lanes 6 and 7 are due to readthrough transcription which is initiated upstream of the *pet56* coding sequences.

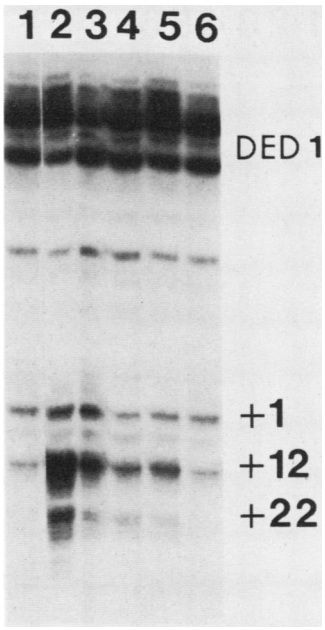

FIG. 3 *his3* mutations that reduce induction and the selectivity of initiation sites. Transcription of the wild-type *his3* gene in normal (KY114, lane 1) and inducing (KY475, lane 2) conditions, the *his3-Δ20* allele in normal (KY163, lane 3) and inducing (KY321, lane 4) conditions, and the *his3-Δ24* allele in normal (KY166, lane 6) and inducing conditions (KY322, lane 5). The positions of the *his3* +1, +12, and +22 mRNAs and the *ded1* control are shown.

^{32}P-Sc3231 DNA prepared by nick translation. The probe extends from the *Hin*dIII site at +328 to an *Eco*RI linker at −136 of the *his3* gene; the band representing cleavage at the TATA element is 370 ± 5 bp in length (22, 35). For the analysis of the *gal-his3* fusions (see Fig. 5), the procedure was essentially the same except that the DNA was cleaved with *Xho*I and then separated in 0.7% agarose. This change was made because the *Hin*dIII site within the *gal* element complicated the analysis. The probe extends from the *Xho*I site at +880 to the *Eco*RI linker at −136, and the band representing cleavage at the TATA region is now approximately 925 bp in length. As the relevant fragment is significantly larger than in the experiments with *Hin*dIII as a marker, the micrococcal nuclease concentration was decreased by a factor of 2.

RESULTS

***pet56* transcription is uninducible by the *his3* or *gal* regulatory element.** *pet56* transcription is not induced during conditions of amino acid starvation (38) (Fig. 2), even though the critical regulatory sites are located upstream of the TATA element (34, 35). To determine whether this lack of inducibility was due to properties of the *his3* regulatory sequences (e.g., the *his3*-proximal copy is identical to the consensus and is capable of binding GCN4 protein, whereas the *pet56*-proximal copy is imperfect and is unable to bind GCN4 protein) (17), the *gal* regulatory element was fused to the *pet56* promoter region at several positions (Fig. 1). Unlike fusions between the identical *gal* DNA segment and the *cyc1* (13) or *his3* (33) promoters, *pet56* transcription was not induced when the cells were grown in galactose medium (Fig. 2). This result cannot be explained by failure to include *pet56* TATA sequences in the fusions. Previous deletion

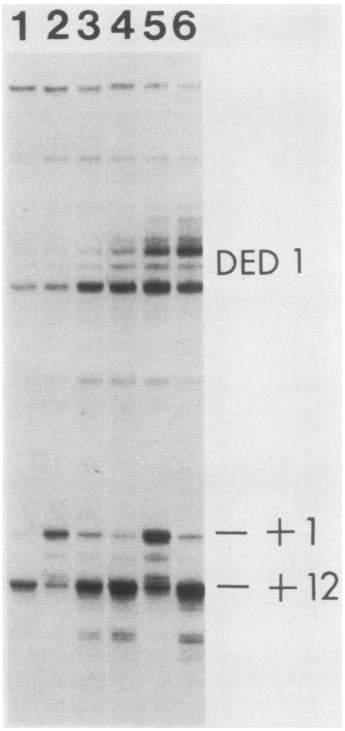

FIG. 4. *his3* mutations that abolish selectivity of initiation sites without affecting inducibility. Analysis of wild-type (lanes 1 and 4), *his3-Δ18* (lanes 2 and 5), and *his3-Δ19* (lanes 3 and 6) under inducing conditions. Lanes 1 through 3, Strains KY114, KY122, and KY125, respectively, grown in the presence of 10 mM aminotriazole; lanes 4 through 6, inducing conditions were achieved by using a *gcd1-101* genetic background (strains KY475, KY323, and KY324, respectively). The positions of the *his3* +1, +12, and +22 mRNAs and the *ded1* transcript are shown. Induction of both transcripts in *his3-Δ18* is evident from their band intensities relative to the *ded1* control. See Fig. 3 for a comparison with normal *his3* mRNA levels.

analysis of the *pet56* promoter (34) indicates that *pet56-G2*, which includes the sequences TATAGA and CATAAA, contains a functional TATA element and that *pet56-G3* contains the entire *pet56* promoter region. Thus, the *pet56* transcript, like the *his3* transcript initiating at +1, was not activated by two different upstream regulatory elements. This indicates that the differences between the constitutive and inducible promoters cannot be explained solely by the upstream elements.

Small deletions in the *his3* TATA region that reduce inducibility and selectivity. In the original description of mutations that prevent the induction of *his3* enzyme activity in response to amino acid starvation, two classes were obtained (30). One class removed the TGACTC upstream regulatory element, which is now known to bind the *gcn4* positive regulatory protein, whereas the other class deleted sequences in the TATA region (Fig. 1). Deletions between the TATA and TGACTC sequences had no effect on *his3* induction. Although the more extensive TATA deletions also lowered the basal level of *his3* expression, others had little effect (30). Transcriptional analysis of two of these mutations (*his3-Δ24*, which removes sequences between −35 and −44, and *his3-Δ20*, which deletes the region between −34 and −46) confirmed that they significantly lowered the induced level but did not affect the constitutive level (Fig. 3). Moreover, in contrast to the wild-type gene, the levels of the +1 and +12 transcripts were similar during normal and starvation conditions. This result cannot be due to a spacing effect. First, equivalently sized deletions between the TATA and initiation region do not influence the choice of initiation sites (5). Second, the +22 transcript was not inducible in *his3-Δ20* or *his3-Δ24* even though its distance from the TATA region equaled that of the wild-type gene between the +12 transcript and the TATA region. These results suggest that differences in the TATA region account for differences between constitutive and regulatory expression.

Selectivity of initiation eliminated by moving the TATA region 8 bp upstream. Although the pattern of *his3* transcriptional initiation can be affected by mutations in the TATA region, it is also possible that the selective utilization of the +1 and +12 sites depends on the properties of the initiation sites themselves. This possibility was addressed by examining the transcriptional patterns of mutations that alter the spacing between the TATA and initiation region (Fig. 1). In *his3-Δ18*, the distance was increased by 8 bp, and in *his3-Δ19*, the distance was decreased by 3 bp. When these strains were examined in normal growth conditions, the level of transcription and the initiation pattern were indistinguishable from those of the wild-type strain (5).

When *his3-Δ19* cells were subjected to starvation conditions, the pattern of transcriptional initiation resembled that observed in the wild-type strain (Fig. 4). In contrast, *his3-Δ18* cells induced transcription equally at the +1 and +12 sites, although the overall level of induction was unchanged (Fig. 4). This indicates that transcription from the +1 site has the potential to be induced and thus suggests that the

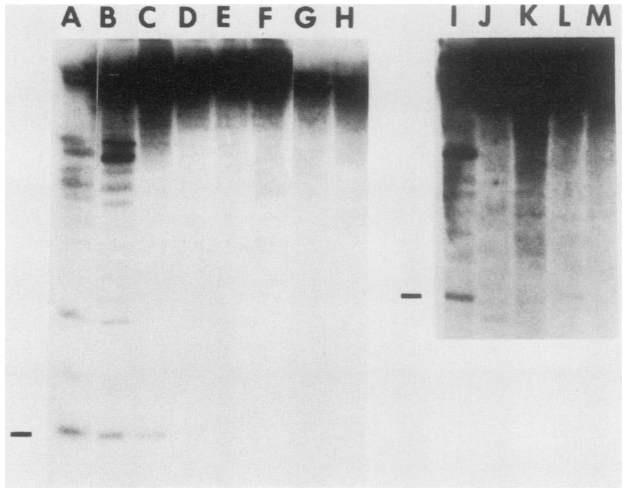

FIG. 5. *his3* chromatin structure. Bands of hybridization indicate micrococcal nuclease cleavage sites in chromatin, and the black lines indicate the bands corresponding to cleavage at the *his3* TATA region (22, 31). Lanes A through H: Strains were grown in minimal medium, and the nuclease-sensitive sites were mapped with respect to the *Hin*dIII site at +328: A, KY114 (wild-type *his3* gene); B, KY475 (wild-type *his3* gene in a *gcd1-101* background; i.e., inducing conditions); C, KY56 (*his3*-Sc2757, deleted to −155); D, KY60 (*his3-Δ17*, deleted to −115); E, KY66 (*his3-Δ13*, deleted to −66); F, KY516 (*his3-Δ13 ope1-1*); G, KY522 (*his3-Δ13 ope2-1*); H, KY523 (*his3-Δ13 ope3-1*). Lanes I through M, Chromatin prepared from *gal-his3* fusions: I, KY114 (wild-type *his3* gene); J and L, KY255 (*his3-G4*); K and M, KY257 (*his3-G6*). Strains in lanes I through K were grown in galactose medium, whereas strains in lanes L and M were grown in glucose medium. For chromatin preparations, the nuclease-sensitive sites were mapped with respect to the *Xho*I site at +880 (see Materials and Methods).

initiation region itself does not confer any specificity with regard to constitutive versus regulatory expression. Moreover, it strongly suggests that selectivity of initiation is strongly affected by the distance between the mRNA initiation sites and the TATA region.

Chromatin structural changes in the TATA region associated with constitutive and regulatory expression. In nuclear chromatin, the TATA region of the wild-type *his3* gene is preferentially cleaved by micrococcal nuclease (31). To correlate this structural feature with *his3* transcription, the relative sensitivity of the *his3* TATA region to micrococcal nuclease was measured in nuclear chromatin prepared from various strains (Fig. 5). First, the nuclease sensitivity of the wild-type locus was indistinguishable under normal and starvation conditions (31). Second, TATA sensitivity was observed in a deletion mutant containing all the elements necessary for proper *his3* expression but lacking sequences upstream of −158. Third, deletion mutants that retained the TATA region but lacked the poly(dA-dT) upstream element did not show nuclease sensitivity. This observation reflects a feature of chromatin structure, because control experiments indicated that the TATA region in purified *his3* mutant DNAs was equally sensitive to micrococcal nuclease (data not shown). Fourth, *his3-Δ13* strains never showed TATA sensitivity even in the presence of *ope* suppressor mutations, which confer wild-type transcription levels (22). Fifth, when the *gal* upstream regulatory site replaced the *his3* upstream promoter region, nuclease sensitivity was not observed in galactose medium, conditions causing extremely high levels of *his3* expression, or in glucose medium, in which transcription levels are undetectable.

These results indicate that nuclease sensitivity at the TATA region is correlated with the presence of the poly(dA-dT) sequence that serves as the upstream promoter element necessary for constitutive transcription. It is not correlated with transcription per se, and it is observed even when constitutive and inducible modes of *his3* expression are occurring simultaneously. Thus, these experiments provide evidence for a structural change at the TATA region that distinguishes constitutive expression from regulated expression.

DISCUSSION

Constitutive and inducible yeast promoters. This paper provides evidence for two classes of *S. cerevisiae* promoters, which are designated constitutive and inducible. In the *his3-pet56* intergenic region, the constitutive promoters are defined by the *his3* +1 and the *pet56* transcripts, and the inducible promoter(s) is defined by the +12 and +22 transcripts. Induction of *his3* expression by the *his3* or *gal* upstream regulatory sequences or by *ope*-mediated suppression clearly involves different DNA sequences (25, 36, 39, 44) and different activator proteins (2, 9, 17). Nevertheless, the fact that the same initiation pattern is observed suggests that the basic mechanism of transcriptional activation is similar. On the other hand, the clear difference in the initiation pattern observed during constitutive *his3* expression suggests that the poly(dA-dT) sequence behaves in a functionally distinct manner from the upstream regulatory elements.

Evidence for two classes of TATA elements. While different upstream elements are associated with constitutive and inducible transcription in the *his3-pet56* region, their effects on the utilization of initiation sites are unlikely to be direct. Three different upstream regulatory elements confer the same pattern of *his3* transcription, and systematic analysis of *gal-his3* fusions indicates that the initiation pattern does not depend on the orientation or location of the *gal* element with respect to the rest of the *his3* promoter elements (33). For the constitutive *his3* promoter, equal utilization of the +1 and +12 sites is observed with either the *his3* or the *ded1* (5) poly(dA-dT) upstream element and is independent of position (29). Thus, constitutive and inducible yeast promoters must differ in other ways besides the upstream elements.

Three separate lines of evidence strongly suggest that constitutive and inducible promoters contain different classes of TATA elements. First, small deletions in the *his3* TATA region reduced the inducibility and selectivity of initiation without affecting constitutive transcription. This indicates that specific sequences in the TATA region are essential for the inducible but not the constitutive *his3* promoter. Second, the *his3* +1 transcript was inducible when the distance to the TATA element was increased by 8 bp. This indicates that differential utilization of the +1 and +12 transcripts does not depend on specific sequences within the initiation region. Moreover, it supports the view that the TATA region is important for the selective utilization of initiation sites, especially in light of previous results that initiation at a particular site requires a TATA element located an appropriate distance upstream (5, 14, 20, 21). Third, nuclease sensitivity at the *his3* TATA region was not correlated with transcription per se but rather with the presence of the poly(dA-dT) sequence and the +1 transcript. Although the structural basis and molecular mechanism involved in micrococcal nuclease sensitivity of the *his3* TATA region are unknown, the experiments provide biochemical evidence that constitutive and inducible *his3* promoters are associated with different chromatin structures at the TATA region.

Properties of regulatory and constitutive TATA elements. From the results presented above, I propose two classes of TATA elements that correspond to the constitutive and inducible *his3* promoters. The functional distinction is that regulatory TATA elements (T_R) are active in the presence of essentially any upstream regulatory sequence, whereas constitutive TATA elements (T_C) are not. With these definitions, the transcriptional initiation patterns in the *his3-pet56* region are explained as diagrammed in Fig. 6. First, the *his3* promoter contains a T_R and a T_C element(s), whereas the *pet56* promoter contains only a T_C element. This explains why *his3* transcription was inducible by the *his3* and *gal* upstream regulatory elements and why *pet56* transcription was uninducible by either element. Second, *his3-Δ20* and *his3-Δ24* delete the T_R element (thus preventing induction) but retain the T_C element(s) (thus permitting basal-level expression). These mutations provide direct evidence for the existence and location of the T_R element. Third, more extensive deletions of the TATA region (for example, *his3-Δ38*, which remove *his3* sequences between −35 and −83) confer extremely low basal transcription levels (29), presumably because they lack all potential T_R and T_C elements. Fourth, preferential utilization of the +12 initiation site during induction occurs because T_R, which is required for induction, is too close to the +1 initiation site. This accounts for the inducibility of the +1 transcript in *his3-Δ18*, the derivative in which the distance between T_R and the +1 transcript, normally 45 bp, was increased to 53 bp. Although the minimal acceptable distance between TATA elements and initiation sites has not been determined precisely and may differ among promoters, the distances involved here are in excellent accord with those determined in previous exper-

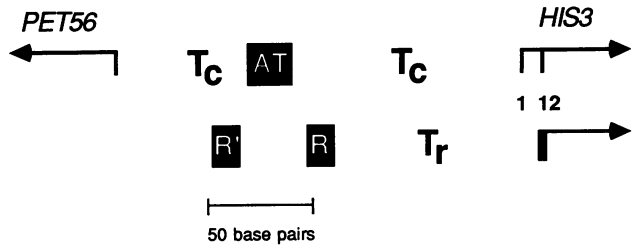

FIG. 6. Model for the constitutive and inducible promoters in the *his3-pet56* intergenic region. The top line indicates the poly(dA-dT) element necessary for the constitutive transcription of both the *pet56* and *his3* genes as well as the T_C elements necessary for the individual genes. Constitutive transcription results in equal initiation from the *his3* +1 and +12 sites and the major *pet56* site. The bottom line indicates the *his3* upstream regulatory elements and the T_R element necessary for transcriptional induction as a function of amino acid starvation. The proximal regulatory site (R) is absolutely essential for induction and by itself is partially sufficient (Struhl and Hill, in press). The distal site (R') is inactive by itself, but is important to achieve the maximal level of induction. Transcription from the inducible promoter occurs at a higher level (indicated by the thickness of the vertical line) and is initiated primarily at the +12 transcript (the +22 transcript is ignored in this diagram). The drawing is to scale. See text.

iments (5, 14, 20, 21). Fifth, the equal utilization of the +1 and +12 transcripts in the constitutive mode of expression is explained by the T_C element(s) being located upstream of T_R and hence far enough away from the +1 site to permit transcription.

The functional distinction between T_R and T_C elements strongly suggests that they are defined by different DNA sequences. Both mutations that delete the *his3* T_R element destroy the only perfect TATAAA sequence in the promoter region (nucleotides −45 to −40). Moreover, additional deletion mutations indicate that T_R is entirely included between −35 and −45, a location that coincides with the TATAAA sequence, and most point mutations of TATAAA prevent induction (Chen and Struhl, unpublished results). From the sequences of the T_R mutants and previous deletions of the TATA region (29), T_C maps somewhere between −45 and −83, a region that contains several TATAAA-like sequences, the best of which is TATACA (nucleotides −54 to −49). The *pet56* gene does not contain any perfect TATAAA sequences, although both TATAGA and CATAAA (nucleotides −40 to −35 and −50 to −45 with respect to the *pet56* initiation site) are found in the region implicated as being functionally important (34). Thus, TATAAA may define a T_R element, while certain related sequences may constitute T_C elements.

The functional distinctions and potential sequence differences between T_R and T_C suggest that these elements are targets for separate DNA-binding proteins that are necessary for transcription. If so, the distinct initiation patterns would reflect the distance between the mRNA start sites and the different binding sites. To explain the functional distinctions between these putative proteins, a T_R protein could contain a region that interacts with the "transcriptional activation" regions of an upstream activator protein such as *gcn4* (18) or *gal4* (3), whereas a T_C protein would lack such a region.

Generality of the model. In addition to the example described here, selectivity of initiation sites has also been observed during transcriptional induction of the yeast *suc2* (4) and *ura3* (24) genes and the *Neurospora crassa qa2* gene (41). Although the promoter elements that are necessary for constitutive and inducible expression and for selectivity of initiation sites have not been determined in these cases, the transcriptional patterns of these other genes can be readily explained by the model proposed for *his3* and *pet56* expression. For example, at appropriate positions of both the *ura3* (25) and *suc2* (26) promoters, there are poly(dA-dT) sequences similar in length and quality to the one between the *his3* and *pet56* genes, and there is a single presumptive T_R element as well as potential T_C elements.

Why does *S. cerevisiae* have two classes of promoters? In the case of *suc2*, the selectivity has important biological consequences because the inducible transcript encodes the secreted form of invertase, whereas the constitutive transcript encodes the intracellular form (4). Although the *ura3* transcripts may encode separate proteins from different reading frames, the significance of the short non-*ura3* peptide is unknown (24). For *qa2*, the significance of the selectivity is unclear because the different transcripts encode an identical protein (41). However, the model provides a sensible rationale for the organization of the *his3-pet56* region, as it permits closely packed and divergently transcribed genes to be regulated independently. This is particularly important for eucaryotic organisms because of their reliance on bidirectional upstream elements that can act at long and variable distances from the initiation site.

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