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Genetics

Deletion mapping a eukaryotic promoter

(gene expression/gene regulation/yeast *his3* gene/transcription/recombinant DNA)

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ABSTRACT **The phenotypes of 24 mutants that successively delete DNA sequences adjacent to the 5′ end of the *Saccharomyces cerevisiae* (yeast) *his3* structural gene are described. Deletions retaining >155 base pairs before the mRNA coding sequences are phenotypically indistinguishable from the wild-type *his3* allele. Deletions having end points between 113 and 65 base pairs before the transcription initiation site express *his3* at reduced levels. Mutations retaining <45 base pairs are indistinguishable from null alleles of the *his3* locus. These results indicate (*i*) that a sequence(s) located 113–155 base pairs upstream from the transcribed region is necessary for wild-type expression and (*ii*) that the T-A-T-A box (a sequence in front of most eukaryotic genes) is not sufficient for wild-type promoter function. Thus, the yeast *his3* promoter region appears large when compared with prokaryotic promoters, suggesting that it may be more complex than a simple site of interaction between RNA polymerase and DNA.**

A promoter is the genetic element necessary for maximal potential expression of a particular structural gene. It is distinguished from the structural gene itself and from elements that regulate the expression of the gene (1). Experimentally, it is defined by *cis*-dominant mutations closely linked to the structural gene that alter the basal level of expression independently of gene regulation (2). *Escherichia coli* promoters have been characterized molecularly by comparing the structures and functions of wild-type and promoter-mutant DNAs both *in vivo* and *in vitro* (for review, see ref. 3). *In vitro* systems using purified *E. coli* RNA polymerase and purified DNA templates mimic the phenotypes *in vivo* of wild-type and mutant genes (4, 5). The base pairs altered in many promoter mutants are those that interact specifically with RNA polymerase (6, 7). Thus, the general view is that *E. coli* promoters are regions of DNA that specifically bind RNA polymerase such that transcription of the structural gene is correctly initiated.

What is the molecular nature of a eukaryotic promoter? Is it simply an RNA polymerase binding site analogous to a prokaryotic promoter? Or is it a more complex structure that involves specific interactions with histones and nonhistone proteins found associated in chromatin?

These questions have been approached by cloning individual intact eukaryotic structural genes, isolating and physically characterizing mutated derivatives, and assaying their "phenotypes." The phenotypes of cloned DNAs have been determined by transformation back into the native organism (8–10), by microinjection into frog oocytes (11), and by transcription *in vitro* (12, 13). Although derivatives behaving in altered ways have been obtained, interpretation of these experiments is not simple. First, in the microinjection and *in vitro* assay systems, the phenotypes are determined under nonphysiological conditions. Second, it has been difficult to distinguish effects on gene expression from those on gene regulation and DNA replication (9, 10). Third, because only a small number of deletion mutations have been tested, it is unclear whether their phenotypes are due to the absence of a particular DNA sequence or to effects of the fused sequences at and around the novel joint. Thus, to analyze a promoter, it is important to obtain a set of derivatives that can be related to each other easily and to use a simple experimental system that mimics physiological conditions.

The experiments discussed here were designed to determine the minimum contiguous DNA sequence necessary for a functional eukaryotic promoter. A series of 24 deletion mutants that successively remove DNA sequences adjacent to the 5′ end of the mRNA coding region of the *Saccharomyces cerevisiae* (yeast) *his3* gene are described. The deletion mutant DNAs are introduced back into yeast cells. In some of the experiments, the transformed yeast cells contain one to three copies of the transforming DNA replicating autonomously. In others, the cells contain one copy of the transforming DNA integrated at the normal chromosomal location for *his3*. Thus, the deletion mutant DNAs are analyzed in their native physiological environment.

MATERIALS AND METHODS

The eight deletions described previously all derive from λgt4-Sc2601, a hybrid containing the intact *his3* gene flanked by 2.5 and 6.9 kilobases (kb) of yeast DNA (see Figs. 1 and 2) (14). Most were formed by an *in vivo* "illegitimate" recombination event mediated by the bacteriophage λ *int* gene; these have one common deletion end point at the λ attachment site (*att*). This property not only permits accurate physical mapping by simple restriction endonuclease cleavage but also results in the fusion of the identical λ sequence to different points in the *his3* gene (14). Unfortunately, *his3* end points of these *int*-mediated deletion mutants are not located randomly because the recombination event depends on partial sequence homology between *att* and yeast DNA (8). Therefore, I developed a new vector (λgt9) that retains the possibility of obtaining *int*-mediated deletions and also prevents re-isolation of those described already.

λgt9-Sc2601 (Fig. 1*D*) was constructed by ligating the appropriate partial *Eco*RI cleavage products of λgt-λC′ (Fig. 1*B*; ref. 15) and λgt4-Sc2601 (Fig. 1*C*; ref. 14). The key feature of this phage is that *int* and *att* are inverted with respect to *his3*. Thus, the partial sequence homology favoring *int*-mediated deletion formation will occur between the inverted *att* and the *his3* sequences. In fact, hot spots for deletions of λgt9-Sc2601 predicted from the *his3* sequence (8) should occur at a number of sites between 44 and 300 base pairs (bp) upstream from the transcribed region.

Deletion mutations that have end points within this region were isolated as follows. Thirty-six independent stocks of λgt9-Sc2601 were treated with EDTA to kill selectively the parent

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Abbreviations: kb, kilobase(s); bp, base pair(s).

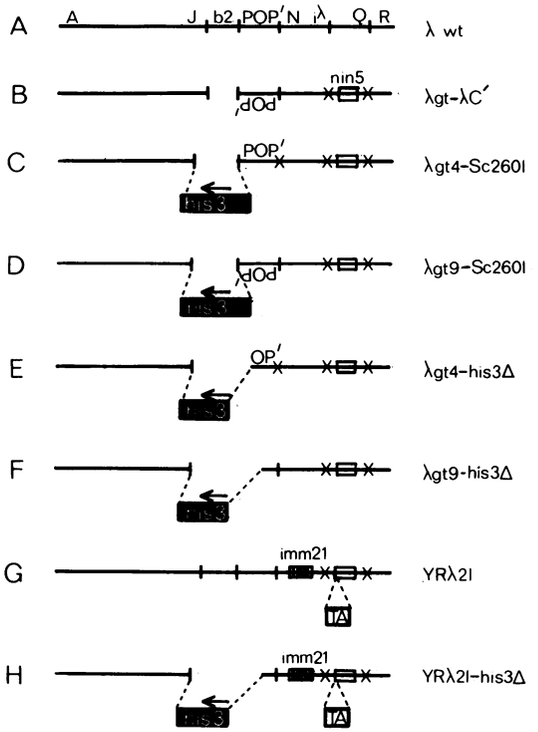

FIG. 1. (*A*) Wild-type λ. *Eco*RI sites are indicated by vertical lines, phage genes are indicated above the horizontal line, and λ attachment site is indicated by POP'. (*B*) λgt-λC'. Mutated *Eco*RI sites (×), *nin5* deletion (□), *Eco*RI B-fragment deletion, and *Eco*RI C-fragment inversion are indicated (14). (*C*) λgt4-Sc2601 contains the left arm from λ*plac5*, a 10.1-kb *Eco*RI insertion of *his3* DNA (■, direction of transcription indicated; see Fig. 2) (13). (*D*) λgt9-Sc2601. (*E*) *int*-mediated deletion of λgt4-Sc2601 that has one end point at *att* (13). (*F*) Deletion of λgt9-Sc2601 (λ end point varies). (*G*) YRλ21 contains the *imm*21 substitution (■) and a 1.4-kb *Eco*RI fragment containing *trp1* and *ars1* cloned at the *Sst* II site near *nin5* (box marked TA). (*H*) Recombinant of phages *F* and *G* used for transformation of yeast.

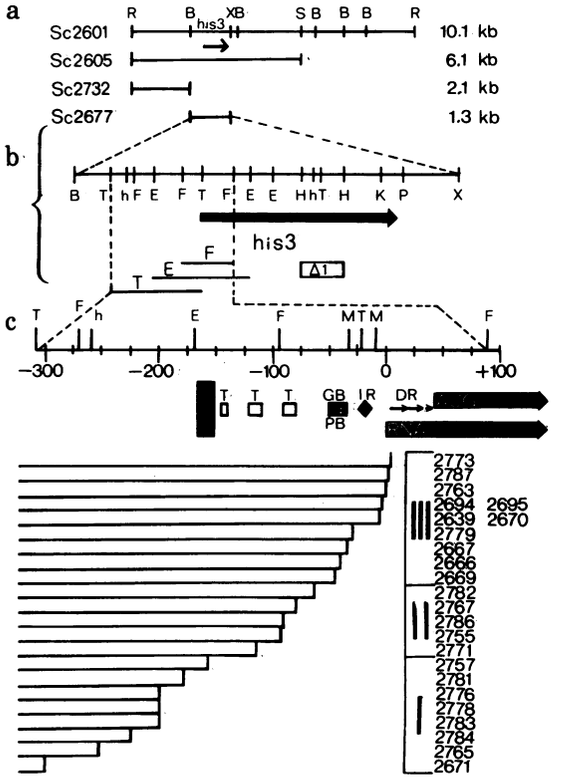

FIG. 2. *his3* deletions. The *his3* region is drawn in the inverted orientation with respect to Fig. 1 and to previous publications (8, 13). This has been done so that *his3* transcription proceeds from left to right (indicated by arrow). All drawings are to scale. (*a*) Sc2601, the 10.1-kb *Eco*RI fragment containing the *his3* gene (18). *Bam*HI (B), *Eco*RI (R), *Xho* I (X), and *Sal* I (S) sites (vertical lines) and mapping positions of various subcloned fragments. (*b*) Expanded view of Sc2677. Additional sites include *Taq* I (T), *Hha* I (h), *Hin*fI (F), *Hae* III (E), *Hin*dIII (H), *Kpn* I (K), and *Pst* I (P). Positions of fragments (F, E, T) used for nuclease S1 mapping experiments and the *his3*-Δ1 deletion are indicated. (*c*) Expanded view of 5'-flanking region of *his3*. The coordinate scale is defined such that positive numbers indicate transcribed nucleotides, negative numbers indicate nontranscribed nucleotides, and zero indicates the 5' terminus of the stable *his3* mRNA (8). Also shown are the positions of two *Mbo* II (M) sites, the start of translation (box containing AUG), the start of transcription (box containing RNA), the 36-bp direct repeat in the mRNA leader (DR), the 12-bp palindrome (IR), the Goldberg–Hogness T-A-T-A-A-A-T-A box (GB) (19), the Pribnow box used for expression in *E. coli* (PB), and runs of T residues 7, 11, and 9 bases long. The nucleotide sequence of the coding strand from position −159 ± 1 to the translation initiating AUG codon is 5'-G-G-T-A-C-A-C-T-C-T-A-T-A-T-T-T-T-T-T-A-T-G-C-C-T-C-G-G-T-A-A-T-G-A-T-T-T-T-C-A-T-T-T-T-T-T-T-T-T-T-T-C-C-C-C-T-A-G-C-G-G-A-T-G-A-C-T-C-T-T-T-T-T-T-T-T-T-T-C-T-T-A-G-C-G-A-T-T-G-G-C-A-T-T-A-T-C-A-C-A-T-A-A-T-G-A-A-T-T-A-T-A-C-A-T-T-A-T-A-T-A-T-A-A-A-C-T-A-A-T-G-T-G-A-T-T-T-C-T-T-C-G-A-A-G-A-A-T-A-T-A-C-T-A-A-A-A-A-A-T-G-A-G-C-A-G-G-C-A-A-G-A-T-A-A-A-C-G-A-A-G-G-C-A-A-G-A-T-A-A-A-C-G-A-A-G-G-C-A-A-A-G-A-T-G-3' (8). The transcriptional (localized to ±1 bp) and translational initiating nucleotides are underlined. DNA sequence data are not available for nucleotides upstream from −160 (black box). The end points of individual deletion mutants are diagrammed below. Deletion mutants are divided into three classes: wild-type (class I), partially functional (class II), and nonfunctional (class III).

phage (14). The survivors were screened for the presence of an intact *his3* structural gene by the lytic "plaque without a lawn" assay (16). Conditions were chosen so that the parental phage scored negative. Ten presumptive deletion mutants from each original stock were picked and screened for sequences homologous to YIp5-Sc2732 (ref. 17; Fig. 2) by plaque-filter hybridization (20). Those phages showing homology have yeast DNA sequences located at least 400 bp adjacent to the 5' end of the structural gene; these were discarded. Those not showing homology have deletion end points <400 bp from *his3*. One such phage from each original stock was selected for further study (designated Sc2755-Sc2790). *Hin*fI, *Hae* III, and *Hha* I DNA fragments from each phage were tested for hybridization with a labeled probe of Sc2677 DNA by the method of Southern (21). This analysis locates deletion end points with respect to the *Hin*fI, *Hae* III, and *Hha* I sites located between nucleotides −260 and −95 (Fig. 2). The 21 mutants having end points to the left of the *Hha* I site were discarded. The deletion end points of the remaining 15 mutants were mapped to a resolution of ±2 bp by the modification of the heteroduplex-nuclease S1 procedure described previously (8, 14). This involved determining the length of a ^{32}P-labeled single strand of the three DNA fragments (Fig. 2) protected from nuclease S1 digestion following hybridization by a given deletion mutant DNA. Knowing the *his3* end point makes it possible to map the end point in λ DNA (within 100 bp) by simply cleaving the deletion DNAs with *Sal* I or *Hin*dIII. Surprisingly, none of the deletion mutants appear to have been generated by *int*-mediated recombination because they lack a small *Hin*dIII DNA fragment of predicted length. Such a fragment would be produced by cleavage at the *Hin*dIII site in the *his3* structural gene and the site at 0.570 in λ DNA (≈200 bp from *att*).

To test the phenotypes *in vivo*, each deletion allele was transferred to vectors containing the yeast *trp1* gene and *ars1*, an element that permits autonomous replication in yeast cells in

the absence of significant recombination with the host genome (17, 22). First, each λ*his3* deletion phage was crossed with λ*imm*21 *trp1 ars1 nin*5. This phage contains the 1.4-kb *Eco*RI fragment with *trp1* and *ars1* cloned at the *Sst* II site at 0.824 on the λ map; it was derived from a phage constructed by and obtained from T. St. John. The *his3 trp1* recombinants were selected as a "plaque without a lawn" on a λ lysogen of *his*B463 (16) and tested for *trp1* function by lytic complementation of an *Escherichia coli trpC* auxotroph (22). Second, the *his3*-containing *Sal* I DNA fragments from some of the λ*his3* deletions were cloned into the plasmid vector YPp7 (17).

Hybrid DNA molecules were introduced into yeast strain SC3 (relevant genotype *his3*-Δ1 *trp1*-289) by selecting transformants with the vector-coded gene *TRP1*. The *his3*-Δ1 allele is a 150-bp deletion within the structural gene (23). The *ars1* transformants behave typically in that the autonomously replicating hybrid molecules are mitotically unstable in the absence of Trp^+ selection (22). Such transformants contain an average of one to three hybrid molecules per cell (17, 22). The transformants were tested for their ability to grow in the absence of histidine. In cases in which the transformants did not grow in the absence of histidine, *his3* sequences on the transforming DNA were shown to be present in the transformed strain by the ability to obtain His^+ recombinants with the genomic allele at the expected frequency (22).

For class I derivatives and for the nondeleted derivative Sc2605, it was desirable to ensure that the strains contain only one copy of the transforming DNA per cell. This was achieved by selecting strains that were mitotically stable for the *TRP1* character (22). The majority of the resulting strains have one copy of the transforming DNA integrated into the chromosome at the *his3* locus. It is possible that this integration event could produce a wild-type *HIS3* gene by recombination between *his3*-Δ1 and the deletion mutation on the transforming DNA. Such a wild-type recombinant should occur infrequently. Of 7.5 kb of homology between the transforming DNA and the normal *his3* locus, only 400 bp are located between the deletion mutations. For example, when class II or class III derivatives are integrated in this manner, none of the resulting strains behave as wild-type *his3* recombinants. However, to rule out the possibility of wild-type recombinants for each class I derivative, four independent mitotically stable *TRP1* colonies were isolated; all showed identical phenotypes.

The basal level of *his3* expression is sufficient for cells to grow at wild-type rates in the absence of histidine. Strains that have the wild-type *his3* allele express *his3* at the basal level regardless of the presence or absence of histidine in the growth medium (8, 24). Thus, strains having *his3* mutations that grow at reduced rates in the absence of histidine express *his3* at less than the basal level. However, the level of *his3* expression can be regulated. When cells are starved for histidine (or any of a number of other amino acids), the level of *his3* mRNA is increased 5- to 10-fold (8). Physiological conditions that can cause this regulatory effect may be achieved in strains having mutations that express reduced levels of *his3* or by supplementing the medium with 10 mM aminotriazole (a competitive inhibitor of the *his3* gene product). Cells expressing *his3* at the basal level fail to grow in the presence of aminotriazole (24). Thus, growth in the presence of this inhibitor assays derivatives for their ability to reach maximal *his3* expression levels and for their ability to regulate the gene properly.

RESULTS

Isolation and Mapping *his3* Deletion Mutants. The *Saccharomyces cerevisiae* (yeast) *his3* gene codes for the histidine biosynthetic enzyme imidazoleglycerol-phosphate dehydratase (25). A λ*his3* phage was isolated by virtue of its ability to allow an *E. coli* histidine auxotroph to grow in the absence of histidine (18). By using a heteroduplex-nuclease S1 protection technique, the 5′ end of the *his3* mRNA was accurately mapped with respect to the cloned fragment (8). It is always difficult to prove that the 5′ end of the mRNA represents the site of transcriptional initiation. However, there is no evidence for intervening sequences in the gene or for mRNA precursors despite attempts to find them (8).

Spontaneously arising deletion mutants of a λ*his3* hybrid (λgt4-Sc2601) were isolated and physically characterized (14). Eight mutants that retained the intact *his3* structural gene but deleted sequences adjacent to the 5′ end were tested for their *in vivo* phenotype in yeast cells (8). Deletions that retain less than 45 bp upstream from transcribed sequences fail to make *his3* mRNA and are *cis*-cominant; they behave indistinguishably from classically defined promoter mutations. A deletion retaining 300 bp upstream from the mRNA coding region is phenotypically similar to the wild-type gene. Therefore, the *his3* promoter includes sequences located between 44 and 300 bp before the region encoding the 5′ end of the message.

Additional mutants are necessary to locate the *his3* promoter more precisely. To prevent re-isolation of mutants described before, these new mutants were derived from a different λ*his3* hybrid (λgt9-Sc2601). Thirty-six independent deletions, all containing the intact structural gene and no more than 400 adjacent nucleotides at the 5′ end, were isolated. These were mapped crudely by restriction endonuclease cleavage and those having end points <260 bp from the structural gene were mapped precisely by a heteroduplex-nuclease S1 technique (Table 1).

Phenotypes of Deletion Mutants. To test the phenotypes *in vivo*, the deletion alleles were transferred to *ars1* vectors capable of autonomous replication in yeast cells in the absence of significant recombination with the host genome (17, 22). This was accomplished either by bacteriophage λ cross (resulting in YRλ21 hybrids) or by subcloning *his3*-containing *Sal* I-generated DNA fragments into the plasmid vector YRp7. Hybrid molecules were introduced into yeast strain SC3 (relevant genotype *ura3*-52 *trp1*-289 *his3*-Δ1 by selecting transformants having the vector-coded gene *TRP1*. The his3 phenotypes were determined by measuring the growth rates of the transformants in the absence of histidine. *Ars1* hybrids containing a wild-type *his3* gene grow with a doubling time of 3 hr.

The phenotypes of the mutants fall into three classes. The first class includes seven deletions that have end points between 155 and 290 bp upstream from mRNA coding sequences; these grow at the wild-type rate. Further, their growth rates in the presence of aminotriazole, a competitive inhibitor of yeast imidazoleglycerol-phosphate dehydratase (the *his3* gene product), are indistinguishable from those of strains that have the wild-type allele. However, deletion alleles of the other two classes have break points <115 bp from the transcribed sequences and do not show wild-type phentotypes. Mutations containing 60–115 bp (class II) grow at reduced rates in the absence of histidine and not at all in the presence of aminotriazole. Those having break points <45 bp from the mRNA coding region (class III) do not grow at all (these include deletions analyzed previously).

To show that the apparent wild-type phenotypes of class I deletions are not due to the presence of more than one copy of each allele per cell, the autonomously replicating molecules were integrated into the chromosome at the *his3* locus by mitotic recombination under conditions nonselective for *his3* expression. The resulting strains have one copy per cell of the *his3* deletion allele to be tested; their growth properties are indistinguishable from strains having one chromosomal copy of the wild-type *his3* gene. When class II and class III derivatives

Table 1. Mapping coordinates and phenotypes of deletion mutations

his3 derivatives			Deletion end points		Growth	
YRλ21	YRp7	Allele	*his3*	λ	−his	+AT
		λgt4-Sc2601 derivatives				
Sc2694	Sc2713	Δ2	−4 ± 2	0.633	−	−
Sc2695	Sc2714	Δ3	−4 ± 2	0.633	−	−
Sc2639	Sc2715	Δ4	−8	att	−	−
Sc2670	Sc2738	Δ5	−8	att	−	−
Sc2667	Sc2735	Δ6	−35	att	−	−
Sc2666	Sc2734	Δ7	−39	att	−	−
Sc2669	Sc2737	Δ8	−43	att	−	−
Sc2671	Sc2716		−300 ± 5	att	3	+
		λgt9-Sc2601 derivatives				
Sc2773	Sc2862	Δ9	+6 ± 2	0.566	−	−
Sc2787	Sc2866	Δ10	+4 ± 2	0.550	−	−
Sc2763	Sc2859	Δ11	+2 ± 2	0.560	−	−
Sc2779	NT	Δ12	−29 ± 2	0.566	−	−
Sc2782	Sc2864	Δ13	−60 ± 2	0.566	9	−
Sc2767	Sc2860	Δ14	−78 ± 2	0.568	5	−
Sc2786	Sc2865	Δ15	−90 ± 2	0.592	4	−
Sc2755	Sc2858	Δ16	−92 ± 2	0.566	5	−
Sc2771	Sc2861	Δ17	−113 ± 3	0.564	5	−
Sc2757	NT		−155 ± 3	0.544	3	+
Sc2765	NT		−250 ± 5	0.548	3	+
Sc2776	NT		−205 ± 5	0.550	3	+
Sc2778	NT		−255 ± 5	0.560	3	+
Sc2781	NT		−185 ± 5	0.550	3	+
Sc2783	NT		−205 ± 5	0.558	3	+
Sc2784	NT		−220 ± 5	0.560	3	+
Sc2601	Sc2605		No deletion		3	+

All derivatives that did not confer wild-type phenotypes were given *his3* deletion allele numbers. Negative coordinates of *his3* end points indicate distance from the start of the mRNA transcript; see Fig. 2. λ end points were determined by using a standard map. −his, growth rate in the absence of histidine, determined as doubling time in hr. +AT, ability to grow in the presence of 10 mM aminotriazole, determined by colony formation. NT, not tested.

are integrated by the same method, the resulting strains fail to express *his3* at the wild-type rate. As expected, class III derivatives confer a His^- phenotype, while class II derivatives permit cells to grow at reduced rates in the absence of histidine (the absolute growth rates have not been determined).

DISCUSSION

The basal level of *his3* expression is sufficient for yeast cells to grow at wild-type rates in the absence of histidine (8, 18). Analysis of 24 deleted derivatives indicates that the minimum contiguous DNA sequence necessary for wild-type function includes 115–155 nontranscribed bp adjacent to the region encoding the 5′ end of the mRNA. The 16 deletions that do not confer wild-type phenotypes are promoter mutations by the classical definition (1, 2). All map outside the structural gene yet reduce the basal level of expression. The seven mutated derivatives of λgt4-Sc2601 have been examined in more detail; all are *cis*-dominant and do not produce detectable mRNA (8).

Mapping the *his3* promoter with λ*his3* deletions depends on the assumption that the λ sequences fused to *his3* do not have variable effects on expression. Each mutant has a unique DNA sequence at the novel joint. It is difficult to assess the potential effects of individual λ*his3* fusions without analysis of equivalent *his3* deletions that have different fused sequences. Knowledge of the precise DNA sequences of the derivatives in question cannot establish the effects of individual novel joints. Nevertheless, it is unlikely that the mutations described here produce bizarre and unrepresentative phenotypes. Sequential deletion into the *his3* promoter region decreases expression from wild-type levels, to lower basal levels, to nondetectable levels. Thus, even though each derivative has a unique novel joint and many have different fusion sequences, the phenotypes produce a consistent pattern.

The most striking finding is that this eukaryotic promoter region is apparently quite large when compared with prokaryotic promoters. In *E. coli*, the key elements (the Pribnow box and the −35 sequence) are both located <45 bp upstream from the site of mRNA initiation (for review, see ref. 3). In addition to being necessary for promotion, this 45-bp region seems to be sufficient. For example, chemical probing experiments indicate that RNA polymerase does not contact the DNA template farther than 45 bp upstream from the initiation site (26) and deletion mutants containing <50 bp before the mRNA encoding sequences retain full promoter function (3, 7, 26, 27). In fact, because the yeast *his3* gene is expressed in *E. coli* under conditions requiring a promoter near the structural gene (8, 14, 16, 25), the deletions described here can be used to directly compare a prokaryotic and a eukaryotic promoter. As expected, those mutations retaining >50 bp upstream from the relevant Pribnow box all express *his3* in *E. coli*.

Yeast RNA polymerase II must interact with DNA at the site of transcriptional initiation. However, because this polymerase is roughly the same size as the *E. coli* enzyme (28, 29), a simple protein–DNA interaction is likely to involve only 40 or 50 bp. This strongly suggests that the *his3* promoter is not a simple RNA polymerase binding site in a manner similar to prokaryotic promoters.

There are two classes of models that explain how a eukaryotic promoter could act at a distance. The first class is that RNA polymerase II has a radically different mechanism from the *E. coli* enzyme. For example, the transcriptionally competent enzyme (*i*) might be a multimer, (*ii*) could bind first to a distant site and then move in some manner, or (*iii*) might require factors that interact with polymerase and distant sites. The second class is that the structure of the DNA template *in vivo* plays an important role. In yeast, as in other eukaryotes, DNA, histones, and other proteins are associated into a chromatin structure. Distance effects on promoter function could be explained by any of the following: (*i*) a requirement for correct phasing of nucleosomes before polymerase binding (30), (*ii*) a compact or higher order DNA structure that effectively brings distanct sequences closer to the site of transcriptional initiation, or (*iii*) interaction of specifically bound nonhistone proteins to RNA polymerase.

The *his3* deletion mutants described here are of particular interest because they form a related set and because their phenotypes are determined under physiological conditions. They define the minimum promoter to include 115–155 bp flanking the end of the 5′-transcribed sequences. This analysis identifies a sequence 115–155 nucleotides upstream from the mRNA coding sequences as necessary, but it has no bearing on other promoter elements; these must be defined by mutation. It does confirm previous results (8) indicating that the conserved sequence in front of eukaryotic genes (the Goldberg–Hogness T-A-T-A box; ref. 19) is not sufficient for *his3* promoter function. This seems to conflict with results obtained by *in vitro* transcription studies indicating that a 20-bp region of adenovirus DNA containing the conserved sequence is sufficient for correct initiation (12). Thus, despite various suggestions (11, 12, 19), there is little evidence that bears on the importance or the role of this conserved sequence with respect to promoter function. To understand the molecular nature of promoter mutants, it will

be necessary to determine the biochemical reason why they are defective. In this regard, the deletions that are only partially defective may prove illuminating.

In this paper, I have analyzed promoter elements independently from regulatory elements. The derivatives that delete at least one promoter element (i.e., lower the basal level of expression) may or may not delete regulatory elements. In this regard, the regulatory properties of class II (partially functional) deletions will prove interesting. However, the fact that *his3* alleles retaining >155 bp upstream from the mRNA coding region grow at wild-type rates in the presence of aminotriazole strongly suggests that all the elements necessary for proper regulation are located in the same region as the promoter.

Note Added in Proof. C. Benoist and P. Chambon have obtained similar results with the early promoter of simian virus 40 (31).

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