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Genetics

Genetic properties and chromatin structure of the yeast *gal* regulatory element: An enhancer-like sequence

(gene regulation/promoters/transcription/yeast genetics/enhancer elements)

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ABSTRACT **DNA molecules created by fusing a 365-base-pair segment of yeast DNA encoding the galactose-regulated upstream promoter element (*gal*) to a set of derivatives that systematically delete sequences upstream from the *his3* gene are introduced in single copy back into the yeast genome precisely at the *his3* locus and then assayed for transcription. Fusions of the *gal* regulatory element to *his3* derivatives containing all normal mRNA coding sequences but lacking essentially the entire promoter region fail to express *his3* under any growth conditions. Fusions to derivatives lacking the *his3* upstream promoter element but containing the "TATA box" place *his3* expression under *gal* control—i.e., extremely high RNA levels in galactose-containing medium and essentially no *his3* RNA in glucose-containing medium. However, of the two normal *his3* initiation sites, only the downstream one is activated by the *gal* element. In fusions of this type, neither the orientation of the *gal* element nor the distance between the element and the *his3* TATA box affects the level or the initiation points of transcription. However, the *gal* element does not influence transcription when placed 100 or 300 base pairs downstream from the normal mRNA start sites. Fusions to derivatives containing the entire *his3* promoter region restore the basal level of *his3* transcription in glucose-grown cells, and both transcriptional initiation sites are used. Furthermore, RNA levels in galactose-grown cells, although somewhat higher than in glucose-grown cells, are significantly below the fully induced level. The distance from *his3* coding sequences does not affect RNA levels, suggesting that specific sequences, possibly corresponding to the *his3* upstream promoter element, reduce the ability of the *gal* element to activate transcription. Analysis of chromatin from some of these strains indicates a DNase I-hypersensitive site(s) in the middle of the *gal* element. However, this structural feature is not correlated with transcriptional initiation because it is found when cells are grown in glucose medium and also in derivatives lacking a TATA box. Thus, the *gal* upstream element possesses most, but not all, of the properties of viral and cellular enhancer sequences of higher eukaryotes. In addition, it appears that the *his3* and *gal* upstream sequences represent two distinct classes of promoter elements, which activate transcription from different initiation sites.**

Transcription of most eukaryotic structural genes depends on DNA sequences located >100 base pairs (bp) from the initiation site of RNA synthesis (1–10). Such sequences (termed upstream promoter elements, modulators, enhancers, potentiators, upstream activator sequences) are not found in prokaryotic organisms. In general, these upstream regions are necessary but not sufficient for maximal levels of transcription; the highly conserved TATA element located 25–30 bp upstream from the initiation site is also required. Viral and cellular enhancer elements have several striking, but as yet inexplicable, properties (5–10). They are functional when located at various distances from either the TATA box or the start of transcription, even as far away as hundreds (and perhaps thousands) of base pairs. Furthermore, these elements can work in either orientation and also when located downstream from the transcriptional initiation site. In some cases, enhancer sequences are also regulatory sites—i.e., they activate transcription only under certain physiological conditions, such as in response to hormones (8), or only in specific cell types (9, 10). From these properties, it is popularly supposed that enhancer sequences are the critical elements that regulate gene expression during normal and abnormal development of multicellular organisms.

In the unicellular eukaryote *Saccharomyces cerevisiae* (yeast), transcription depends on "TATA box" sequences as well as upstream regions that have no fixed positional relationship to the initiation site or to the TATA box (3, 4, 11–14). Some of these upstream regions serve as regulatory sites, although some do not. One example of an upstream promoter/regulatory site is the region between the divergently transcribed *gal1* and *gal10* genes. In wild-type yeast strains, both genes are expressed at extremely high levels (about 50 molecules per cell) when cells are grown in medium containing galactose as a source of carbon and extremely low levels (≈0.1 molecule per cell) when cells are grown in glucose-containing medium (15). A 365-bp fragment from this region confers galactose inducibility and glucose (catabolite) repressibility when fused to the β-galactosidase gene of *Escherichia coli* (13). Furthermore, this segment containing the *gal* upstream regulatory element is functional only in the presence of a TATA box and mRNA initiation region (from the yeast *cyc1* gene).

This paper characterizes the genetic properties and chromatin structure of the *gal* upstream regulatory element by fusing it to a set of DNA segments used previously to define the promoter elements necessary for *his3* expression (4, 11). The properties of the *gal* element are compared both to mammalian enhancer sequences and to the *his3* upstream promoter element. Additionally, interactions between the *gal* and *his3* upstream elements are examined.

MATERIALS AND METHODS

The DNA fragment containing the *gal* regulatory site described previously (13) was derived from λgt-Sc481 (15), which was obtained from Tom St. John. Yeast strains used in these experiments were KY117 (*a ura3*-52 *trp1*-Δ1 *lys2*-801am *ade2*-101oc *his3*-Δ200 GAL^+) and KY114 (same as KY117 except $his3^+$); these were created by and obtained from Michael Fasullo and Phil Hieter. The procedures for DNA manipulations, yeast transformation, RNA analysis, and nuclei isolation have been described in earlier work (16–19). To analyze DNase I-hypersensitive sites, DNA purified after light DNase I treatment of nuclei [40 ng/ml, final con-

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

centration for 10 min at 37°C, which produced DNA of average double-stranded length of 10–20 kilobases (kb)] was cleaved with *Xho* I, transferred to nitrocellulose after electrophoresis in agarose, and hybridized to a radioactive probe containing sequences from the *Xho* I site to nucleotide −32.

RESULTS

Fusion of the *gal* Upstream Regulatory Site to Sequentially Deleted Derivatives of the *his3* Gene. A systematically derived set of *his3* DNA segments, each containing the intact mRNA coding region but different amounts of upstream sequences, was fused to the *gal* regulatory site (Fig. 1). The fusions can be arranged into three classes depending on the *his3* promoter elements present in a given derivative. Class I fusions contain both the *his3* upstream promoter element and the TATA box element, class II fusions lack the *his3* upstream region but contain the TATA box, and class III fusions lack both *his3* promoter elements. The three types of fusions correspond to the three phenotypic classes of deletion mutants used originally to define the *his3* promoter elements (4, 11).

Each of the resulting promoter fusions was introduced back into the yeast genome at the *his3* locus such that there was one copy of the new allele per haploid cell. These strains were grown in broth containing glucose or galactose as the sole source of carbon and then assayed for *his3* transcription. The results of this experiment are shown in Fig. 2, and the quantitation of *his3* RNA (in molecules per cell) is presented in Table 1.

When the *gal* regulatory site is fused to *his3* derivatives lacking both promoter elements (class III fusions), *his3* expression is barely detected under both growth conditions. The level of 0.1 molecule per cell is roughly equivalent to the levels of the *gal7,10,1* genes in repressing conditions (glucose medium) (15).

When the *gal* regulatory site is fused to derivatives containing the *his3* TATA box (class II), *his3* becomes regulated just like the *gal7,10,1* genes. *his3* transcripts are present only at 0.1 copy per cell when cells are grown in glucose medium, but they are induced to ≈50 copies per cell when cells are grown in galactose medium. Analysis of the 5′ ends of these transcripts (Fig. 3) gives a surprising result. In wild-type strains, *his3* transcription is initiated about equally at two sites designated +1 and +13 (17). However, in the fusions, almost all of the transcription that occurs in galactose-grown cells is initiated at the +13 site.

In fusions containing both *his3* promoter elements (class I), *his3* expression in glucose medium approximates the level found for the wild-type gene, 1 or 2 mRNA molecules per cell (16). In galactose medium, transcripts are present at between 6 and 9 molecules per cell. This level represents a reduction by a factor of about 6 when compared to class II derivatives, but about a 4-fold induction over the basal level observed in glucose medium. Analysis of the initiation points (Fig. 3) indicates that transcription in glucose-grown cells begins at both normal locations and that the induced levels observed in galactose-grown cells are accounted for by transcription from the +13 site.

The *gal* Regulatory Site Activates Transcription When Present in Either Orientation with Respect to the *his3* Gene. A DNA segment containing the *gal* regulatory site was fused in each of the two possible orientations to a *his3* derivative containing the TATA box but not the upstream element (Fig. 1). These two molecules, Sc3305 and Sc3311, behave indistinguishably. Like other class II fusions, they express *his3* at 0.1 molecule per cell in glucose medium and 50 molecules per cell in galactose medium (data not shown). Furthermore, the 500-fold induction in galactose-grown cells is due almost completely to initiation at the +13 site (Fig. 3).

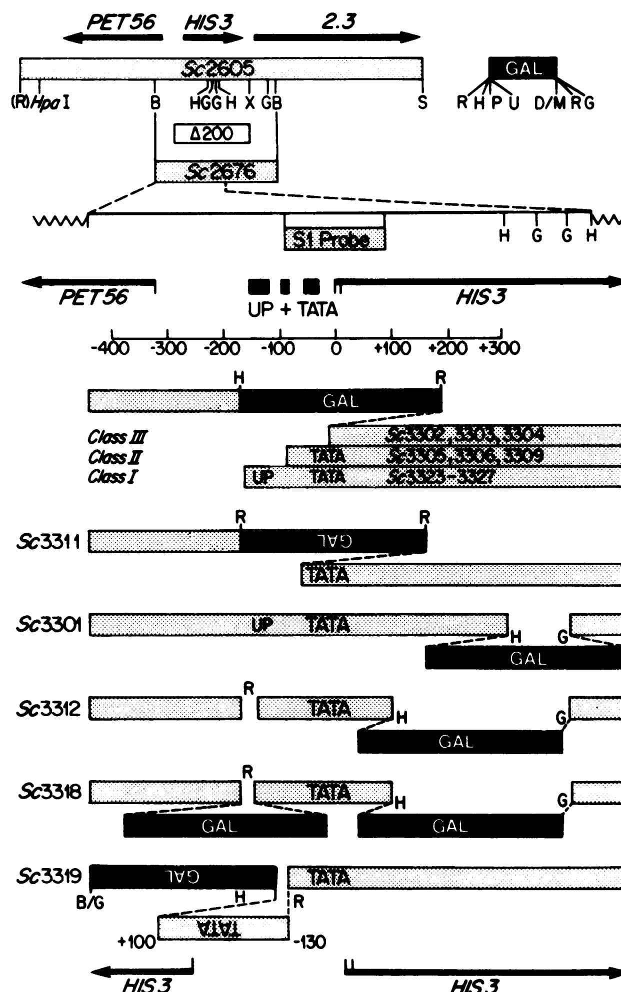

FIG. 1. Experimental design. All of the DNA molecules derive from YIp5-Sc2605, which contains the basic 6.1-kb *Eco*RI–*Sal* I *his3* DNA segment Sc2605 (11, 18) indicated as a gray bar cloned into the *ura3*$^+$ integrating vector YIp5 (18). The unique *Eco*RI site was then mutated. The locations and orientations of the *his3*, *pet56*, and 2.3-kb transcripts are shown as arrows above the gray bar, and restriction endonuclease sites are shown below as vertical lines. DNA molecules were cleaved with *Hpa* I and introduced into KY117, a strain containing the *his3*-Δ200 allele (unshaded box), which deletes all sequences between *his3* nucleotide −178 and 25 bp prior to the *Xho* I site (unpublished results). Standard hybridization methods using the purified 1.7-kb *Bam*HI fragment Sc2676 as a probe were used to prove that the transformants resulted from integration of a single DNA copy into the *his3* locus; this probe was also used for measuring RNA levels. An expanded view of a 950-bp subregion of Sc2676 is shown below. The locations of the probe for S1 nuclease mapping, the *his3* upstream element (UP), the *his3* regulatory site (+), TATA box (TATA), and the +1 and +13 initiation sites (vertical lines) are indicated (11, 19). The structures of the DNA molecules are diagrammed below the coordinate scale. The restriction sites connecting the *his3* sequences (gray) to the *gal* sequences (black) are indicated. The *gal* fragment shown in the top right corner of the figure was obtained by cloning the 365-bp *Sau*3A–*Dde* I fragment (13) of λgt-Sc481 (15) into the *Bam*HI–*Sma* I-cleaved pUC8 DNA (the *Dde* I end was made flush with DNA polymerase I). The orientation in particular molecules is indicated by inversion of the lettering. Abbreviation for restriction sites are R (*Eco*RI), B (*Bam*HI), H (*Hin*dIII), G (*Bgl* II), X (*Xho* I), S (*Sal* I), P (*Pst* I), U (*Sau*3A site at the end of the *gal* fragment), and D/M (junction between *Dde* I site at the end of *gal* and *Sma* I site of pUC8).

The *gal* Regulatory Site Does Not Activate Transcription When Located Downstream from the *his3* Initiation Site. When the *gal* regulatory site is placed downstream from the *his3* RNA initiation sites at position +330 (Sc3301) or +100

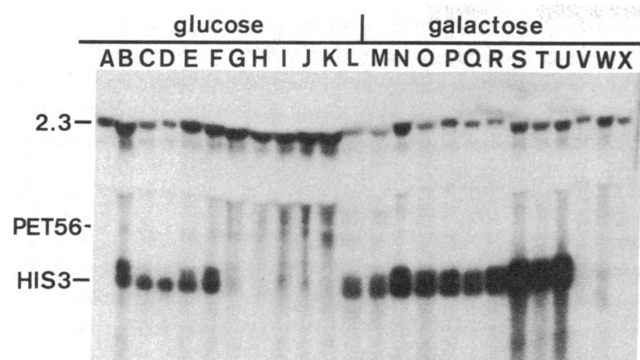

FIG. 2. RNA levels. Electrophoretically separated total RNAs from the strains containing the following *his3* alleles grown in glucose (40 μg each for lanes A–L) or in galactose (20 μg each for lanes M–X) were hybridized to nick-translated Sc2676 DNA: Δ200 (lanes A and X); class I alleles Sc3323 (lanes B and N), Sc3324 (lanes C and O), Sc3325 (lanes D and P), Sc3326 (lanes E and Q), Sc3327 (lanes F and R); class II alleles Sc3309 (lanes G and S), Sc3306 (lanes H and T), Sc3305 (lanes I and U); class III alleles Sc3304 (lanes J and V), Sc3302 (lanes K and W), wild type (lanes L and M). The mobilities of bands representing the 2.3-kb, *pet56* (1.2 kb), and *his3* (0.7 kb) RNAs are indicated (16).

(Sc3312), *his3* transcription is not detected in glucose or in galactose medium (Fig. 4, lanes A, B, F, and G). To prove that this lack of transcription is not simply a result of an unstable RNA species due to the presence of the *gal* element in the middle of the *his3* structural gene, a second *gal* element was placed upstream of the relevant part of YIp5-Sc3312 (see Fig. 1). Strains containing this new molecule, YIp5-Sc3318, produce *his3* RNA only when grown in galactose medium (Fig. 4, lanes C and H). However, in galactose medium, the RNA is more heterogeneous in size and about 1/10th as abundant when compared to class II derivatives, and in glucose medium, RNA levels are below the detection limit (0.03 molecule per cell). Probably, transcription of this allele is fully inducible in galactose medium, but the apparent levels are lower due to instability of the RNA product. Thus, transcription of the *his3* allele containing the *gal* element within the structural gene can be induced in galactose medium, but only if the regulatory element is located 5′ to the start of transcription.

Table 1. Structures and phenotypes of *gal–his3* fusions

Fragment	Allele	Class	End point	Glucose	Galactose
Sc3302	G1	III	−8	0.1	0.1
Sc3303	G2	III	−20	0.1	0.1
Sc3304	G3	III	−32	0.1	0.1
Sc3305	G4	II	−52	0.1	48
Sc3306	G5	II	−77	0.1	40
Sc3309	G6	II	−131	0.1	54
Sc3327	G7	I	−249	2	9
Sc3326	G8	I	−326	2	7
Sc3325	G9	I	−353	2	7
Sc3324	G10	I	−383	2	8
Sc3323	G11	I	−385	1	5
his3$^+$				2	2
gal1,10^{+}*				0.1	50

The leftmost columns list the DNA fragments and *his3* allele numbers of the various fusions. For each derivative, the fusion class (see text and Fig. 1), *his3* end point, and RNA levels (expressed as molecules per cell) are indicated. The *his3* RNA levels were levels of the 2.3-kb RNA in the same lane (Fig. 2). The absolute levels were calculated with respect to the wild-type *his3* levels (2 molecules per cell) determined previously (16).

*Wild-type *gal1,gal10* genes; the levels were determined in ref. 15.

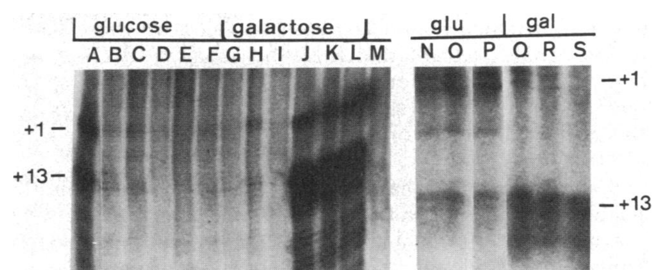

FIG. 3. Transcriptional initiation sites. RNA samples prepared from strains containing the following *his3* alleles were hybridized to the ^{32}P-labeled *Hin*fI fragment shown in Fig. 1 and treated with S1 nuclease as described (17): wild type (lane A); class III alleles Sc3302 (lanes B and G), Sc3303 (lane H), Sc3304 (lanes C and I); class II alleles Sc3305 (lanes D and J), Sc3311 (lanes E and K), Sc3309 (lanes F and L); class I alleles Sc3323 (lanes N and Q), Sc3325 (lanes O and R), and Sc3327 (lanes P and S). Lane M represents the control of purified yeast tRNA. Samples shown in lanes A–F and N–P were prepared from glucose-grown cells, whereas samples in lanes G–L and Q–S were from galactose-grown cells. Lanes A–M and N–S represent two separate experiments. The positions of the +1 transcript (91 bases in length) (17) and +13 transcript (78 bases in length) are shown.

Sequences That Inhibit Transcriptional Activation by the *gal* Regulatory Site. The observation that class II fusions are maximally expressed in galactose medium, whereas class I fusions show reduced expression, suggests that a specific sequence (defined to be between −131 and −249) inhibits activation by the *gal* regulatory element. The alternative explanation—namely, that the *gal* element is simply less effective at longer distances from the TATA box—seems less likely because varying the distance within a given class of fusions has little, if any, phenotypic effect. Two examples described below also indicate that inhibition of *gal* activation depends upon specific sequences and not on distance *per se*.

pet56, a nuclear gene important for mitochondrial function (unpublished results), is located about 300 bp upstream of *his3*, and it is transcribed in the opposite direction (16). In the experiments involving fusions to sequentially deleted derivatives of the *his3* gene (see Figs. 1 and 2), the *gal* regulatory site is also upstream of *pet56*, ≈200 bp upstream from the structural gene and 100 bp upstream from a likely TATA box sequence (unpublished results). In other words, the DNA segment containing the *gal* regulatory element, which normally activates both the *gal1* and *gal10* genes, has been placed upstream of the diversely transcribed genes *his3* and *pet56*. Nevertheless, in derivatives such as Sc3309, *his3* expression is induced 500-fold in galactose medium, whereas the 1.2-kb *pet56* RNA is not induced at all.

Another situation in which the *gal* regulatory element does not activate transcription occurs in the derivative Sc3319, in which *his3* is expressed at the basal level in both growth me-

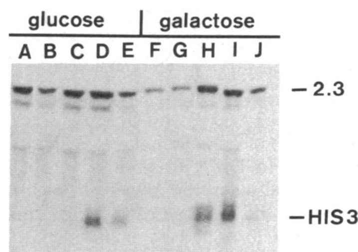

FIG. 4. RNA levels in strains with *gal* located downstream from *his3*: RNA samples from Sc3301 (lanes A and F), Sc3312 (lanes B and G), Sc3318 (lanes C and H), Sc3319 (lanes D and I), and wild type (lanes E and J) grown in glucose or galactose. The positions of the 2.3-kb and *his3* RNAs are indicated.

dia (Fig. 4, lanes D and I). It is equivalent to the class II fusion Sc3305, except that a 230-bp segment of DNA has been inserted between *gal* and *his3* mRNA coding sequences. This 230-bp segment contains *his3* sequences between −131 and +100 oriented in the opposite direction from the test *his3* gene.

It should be noted that in both examples of inhibition, the *gal* element is closer to the relevant TATA box than in cases in which transcriptional activation is observed, thus strongly supporting the idea that specific sequences are involved.

Chromatin Structure of the *gal* Element and Its Relationship to Transcriptional Activation. In nuclear chromatin, the simian virus 40 enhancer element is hypersensitive to DNase I digestion, and this structural feature is correlated to transcriptional activation (20). The chromatin structure of the *gal* element was analyzed in strains containing Sc3304 (a class III fusion) or Sc3305 (class II fusion) that were grown in glucose or galactose medium. Specifically, nuclei were digested lightly with DNase I and hypersensitive sites of cleavage were mapped with respect to the *Xho* I site just past the 3′ end of the *his3* structural gene. As shown in Fig. 5, hybridization bands are observed that correspond to cleavage within the *gal* element as well as cleavage further upstream. However, these hypersensitive sites are observed in both growth media and in both strains tested. Moreover, the *gal* regulatory site at its normal location on chromosome II is hypersensitive to DNase I in both glucose and galactose media (data not shown). Thus, although the *gal* regulatory site is hypersensitive to DNase I cleavage, this feature of chromatin structure does not correlate with its ability to activate transcription either as a function of physiological conditions or the presence of another required genetic element, the TATA box.

DISCUSSION

Comparison of the *gal* Upstream Regulatory Element to Enhancer Sequences. A major conclusion of this paper is that the *gal* upstream regulatory element possesses many properties of enhancer elements in higher eukaryotes. Similar findings and conclusions were obtained for the upstream element responsible for heme control of the yeast *cyc1* gene (21). (*i*) The *gal* regulatory site, when fused to either the *cyc1* (14) or *his3* gene, activates transcription at normal initiation sites, but only when cells are grown in medium containing galactose as a source of carbon. (*ii*) The *gal* element is necessary but not sufficient for activation of the *cyc1* (14) or *his3* promoter. In the fusions described here, *his3* sequences between −32 and −52 are also required. This region coincides exactly with the TATA box promoter element necessary for normal *his3* expression (11). (*iii*) The *gal* element can be located at different distances from the TATA box, yet transcription initiates at the same position. (*iv*) The *gal* element activates transcription even when located hundreds of base pairs upstream from the initiation site of RNA synthesis. Deletion analysis (22) indicates that the *gal* element is located centrally within the 365-bp segment; thus it is about 325 bp from the RNA start point in Sc3309, a class II fusion that activates to the maximal level. Even when *gal* is located >600 bp away, such as in Sc3323, there is some activation in galactose-grown cells. (*v*) The *gal* element functions equally well in either orientation with respect to the *his3* coding region, and in these cases it initiates at the same position. This result is not surprising because the 365-bp *gal* segment used here is normally located upstream of both *gal1* and *gal10*, genes that are induced by galactose (15). Thus, it is not clear whether there are two *gal* elements each activating a single gene or if there is one element functioning bidirectionally. In principle, this issue is not easy to resolve.

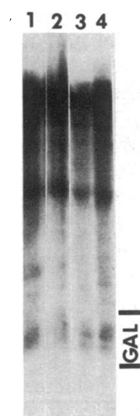

FIG. 5. DNase I hypersensitive sites: chromatin from strains containing Sc3304 (lanes 1 and 3) and Sc3305 (lanes 2 and 4) grown in glucose (lanes 1 and 2) or galactose (lanes 3 and 4). The hybridization bands correspond to DNA fragments whose length is determined by the distance between the *Xho* I site past the 3′ end of the *his3* structural gene and the hypersensitive site. The lengths of all of these fragments were determined by using size standards derived from bacteriophage DNA. The region indicative of cleavage within the *gal* element (corresponding to DNA fragments 1.0–1.4 kb in length) is indicated by horizontal lines. In some experiments, the hypersensitivity within the *gal* region appears to be due to cleavage at two or three closely spaced sites. These hypersensitive sites are dependent on DNase I, as they are not observed in naked DNA (data not shown).

The yeast *gal* element differs from enhancer sequences in at least two ways. First, it does not appear to activate transcription even when located only 100 bp downstream from the initiation site. In these cases, it is unlikely that the *gal* element is too far from the initiation site. However, it is possible that some region between the TATA box and +100 could block activation. In one attempt to test this hypothesis, Sc3319, the *gal* element, was placed upstream and proximal from one copy of the *his3* gene and downstream and distal from another copy. The result was that the downstream *his3* copy was not subject to activation in galactose medium. However, this negative result may be due to blocking by sequences upstream of the TATA box or it may be a consequence of the inverted repeat nature of the *his3* genes; thus, it does not constitute a definitive test. A second way in which the *gal* element differs from the simian virus 40 enhancer sequence involves nuclease hypersensitivity in chromatin (20). Although the element contains a DNase I-hypersensitive site, this structural feature does not correlate with transcriptional activation; it is observed in both growth media and in the presence or absence of a TATA box. Possibly, there is always a protein(s) bound to the *gal* element (thus causing the nuclease hypersensitivity) but that transcriptional activation either requires a conformation change of this protein or the presence of another protein.

Two Classes of Upstream Elements in Yeast. The unusual and distinctive feature of the *his3*, *gal*, and other upstream elements is that they act at a long and variable distance from the TATA box and the transcriptional initiation site. This property suggests that upstream elements may activate transcription by a common mechanism, although the presumptive proteins that interact with the different elements may recognize different DNA sequences.

However, the implication from the results presented here is that the *his3* and *gal* upstream elements represent two different classes. In particular, the *his3* upstream element activates transcription from +1 and +13, whereas the *gal* element activates transcription only at +13, even though both elements require the same TATA box region for transcription. Another way to view this phenomenon is that the *his3* gene has two kinds of promoters that are defined by the +1

and +13 start site. In this view, the *his3* upstream element activates both kinds of promoters, whereas the *gal* element activates only the +13 type. A similar phenomenon has been observed in some revertants of deletion mutations that lack the *his3* upstream promoter element; these initiate transcription only at the +13 site (unpublished results).

The upstream elements differ in other ways. The *gal* element is a regulatory site that, depending on physiological conditions, causes extremely high transcription rates or essentially no transcription at all. In this way, it resembles enhancer elements, because these generally derive from genes that are activated to high levels, but only under specific environmental or developmental conditions. Indeed, most eukaryotic genes under current study are of this type. On the other hand, the *his3* element is responsible only for the basal, unregulated levels found for average genes that are expressed all the time.

Domains of Action for Upstream Promoter Sequences. Given that upstream promoter/regulatory elements such as *gal* act at a long and variable distance, cells must have a mechanism to prevent such elements from activating transcription of nearby, unrelated genes. Otherwise, genes on a given eukaryotic chromosome would be subject to the control mechanisms of all upstream elements on the same chromosome. One possible solution to this problem is to propose that upstream elements activate transcription over a limited range and that unrelated genes are too far away. However, in yeast the genes are packed closely together, and there are undoubtedly countless examples of divergently transcribed genes, such as *his3* and *pet56*, that are regulated in totally different ways (the *gal1,10* region is a notable exception to this rule).

A more attractive solution is that specific sequences block transcriptional activation by upstream elements—i.e., sequences defining gene domains that are refractory to outside influences. A domain is defined formally as a unit of autonomous function, although its biochemical basis might involve structural features of chromatin. There are several examples presented here in which specific sequences inhibit transcription dependent upon the *gal* regulatory site. Of particular interest is the example in which the "inhibitory sequence" corresponds roughly in location to the *his3* upstream promoter element. The nucleotide sequence of this region contains many tracts of poly-(dA)·(poly)dT and thus is similar to spacer regions found between genes. A simple hypothesis is that these spacer regions mark the domain boundaries and also serve as upstream promoter elements for typical genes such as *his3*. To achieve transcription levels above this basal level, special enhancer-like elements responding to regulatory proteins are necessary.

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