exclude a more generalized function served by the presence of many dispersed RNA polymerase III promoters. Whether a limited group or a subset of each of the SINE families has evolved or taken over some previous cellular function is unknown. The insertion of hundreds of thousands of repetitive DNA elements into new genomic locations over the past 45 Myr must have had major effects on the structure and evolution of the genome.

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Negative control at a distance mediates catabolite repression in yeast

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In prokaryotic organisms, the control of gene expression is mediated by regulatory proteins that activate or repress transcription[1,2]. However, the molecular mechanisms of positive and negative control are different. In terms of negative control, repressor proteins bind to sites located within the promoter region and as a consequence sterically interfere with functional binding by RNA polymerase. Here, I examine the properties of a regulatory sequence that specifies catabolite (glucose) repression in the yeast *Saccharomyces cerevisiae*. Specifically, a DNA segment containing this regulatory site was fused upstream of the intact *his3* promoter region and structural gene at several locations. Normally, *his3* expression in these derivatives occurs at a basal level which can be induced by conditions of amino-acid starvation. However, in glucose medium, the catabolite regulatory sequence overrides the normal *his3* promoter elements and reduces transcription both in normal and starvation conditions. The implication from these results is that in contrast to catabolite repression in *Escherichia coli*, which is mediated by catabolite-activating protein (CAP)[3], catabolite repression in yeast occurs by a negative control mechanism involving a putative repressor protein. The observation that this regulatory site exerts its repressing effects even when located upstream of an intact promoter region suggests that repression in yeast is not mediated by steric interference between regulatory proteins and the transcriptional apparatus.

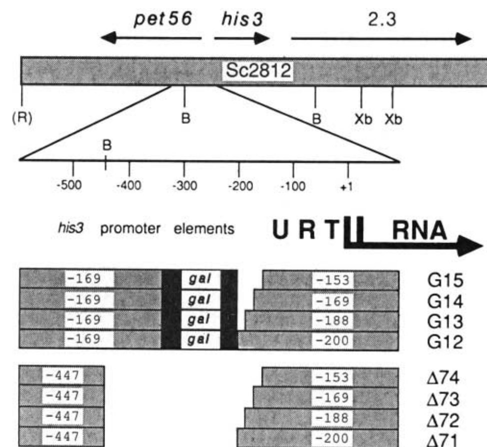

Fig. 1 Experimental design. All the DNA molecules derive from YIp5-Sc2812, which contains a 6.1-kilobase (kb) *Eco*RI-*Sal*I *his3* segment (shaded bar at top) cloned in the *ura3*$^+$ integrating vector YIp5 (refs 13, 22). The locations and orientations of the *his3*, *pet56* and 2.3-kb transcripts are shown as arrows above the shaded bar, and restriction sites are indicated by vertical lines: R, mutated *Eco*RI site; B, *Bam*HI; Xb, *Xba*I; S, *Sal*I. Beneath the bar is an expanded view of the *his3* promoter region, which includes the upstream promoter element (U), the positive regulatory site (R), the TATA promoter element (T), and the RNA initiation sites located at positions +1 and +12 (solid vertical lines)[13,16]. The derivatives that were tested in the present work (numbered at the right-hand side) were constructed by joining DNA segments containing the *his3* promoter region and structural gene (right side) to upstream segments in which the *gal1,10* element was either present or absent (left side). The promoter-containing DNA fragments have varying amounts of upstream sequences and they are bounded by *Eco*RI linkers (details of the method to be published elsewhere). The precise endpoints (indicated within the shaded bars and listed in Table 1) were determined by dideoxy DNA sequencing[23] of appropriate fragments cloned into the single-stranded vector mp8 (ref. 24). To create *gal-his3* fusions, the *his3* promoter segments were joined via the *Eco*RI cohesive ends to a segment containing the 365-bp *gal1,10* region (solid box) and *his3* sequences upstream of nucleotide −169 (ref. 13) (not drawn to scale). The control molecules lack the *gal* region, but they contain *his3* sequences upstream of −447. These derivatives, which effectively have an *Eco*RI site at the normal position of the *Bam*HI site, are derived from YIp5-Sc3319 (ref. 13). To assess the phenotypes of the derivatives, DNA molecules were cleaved with *Xba*I and introduced into KY117 (relevant genotype *ura3*-52, *his3*-Δ200, *GAL*$^+$) by selecting for Ura$^+$ transformants. As the *his3*-Δ200 allele deletes all sequences between −178 and +874 (unpublished results), the only *his3* sequences in the resulting strains arise from integration of the transforming DNA. The 1.7-kb *Bam*HI-generated DNA fragment was used as a hybridization probe to prove that the transformants resulted from integration of a single molecule into the *his3* locus; it was also used to measure RNA levels (see Fig. 2).

Certain yeast genes are transcribed at reduced levels when the sole carbon source in the medium is glucose as opposed to other compounds which are less directly metabolized[4-7]. This glucose effect (catabolite repression) is usually observed for genes involved in the metabolism of poor carbon sources, the rationale being that such gene products are not needed when cells are metabolizing glucose[8].

The *gal7*, *gal1* and *gal10* genes, which convert exogeneous galactose into glucose 1-phosphate, are highly induced in galactose-containing medium, and they are also subject to catabolite

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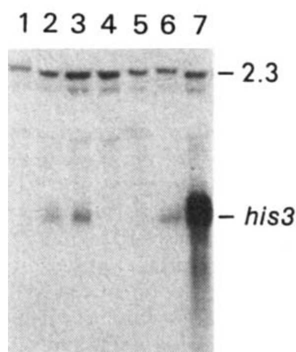

Fig. 2 RNA levels. Strains were grown in YP medium (1% yeast extract, 2% peptone) containing either 2% glucose or 2% galactose as the sole source of carbon. RNA was extracted from half of a 30-ml culture collected in the exponential phase of growth. Lanes 1-6 represent 50 μg total RNA from the following strains grown in glucose medium: the untransformed parent KY117 (lane 1); control molecule, −200 endpoint (lane 2); control molecule, −153 endpoint (lane 3); *gal-his3* fusion, −188 endpoint (lane 4); *gal-his3* fusion, −153 endpoint (lane 5); the wild-type strain KY114 (lane 6). Lane 7 contains 50 μg RNA from a strain with Sc3357 grown in galactose medium. All RNAs were separated electrophoretically in a 1.7% agarose gel containing 6% formaldehyde, then transferred to nitrocellulose. The hybridization probe was ^{32}P-labelled Sc2676 DNA (corresponding to the 1.7-kb *Bam*HI fragment) prepared by nick-translation; this probe is homologous to the entire *his3* gene, and also to 330 bp of a 2.3-kb mRNA species, which provides an internal control. Details of the method have been described previously[14].

repression[9]. Studies of the divergently transcribed *gal1* and *gal10* genes indicate that the regulatory sites specifying catabolite repression and galactose induction are located several hundred base pairs (bp) upstream from the RNA initiation site[10-13]. This location is well upstream from the TATA sequences which are necessary, but not sufficient, for transcriptional initiation. Furthermore, when the upstream promoter elements of the *cyc1* and *his3* genes are replaced by a 365-bp fragment from the *gal1,10* region, these unrelated genes are now subject to both catabolite repression and galactose induction[10,13].

Note, however, that in these fusions, the *gal* region is fused to DNA segments that by themselves are transcriptionally inactive because they contain only the TATA element. Thus, it is impossible to determine whether the lack of expression in glucose medium is due to a 'failure to activate' or to bona fide repression. Moreover, this problem is complicated by the fact that it is impossible to observe repression in the absence of transcription. In previous *gal* experiments[9-12], catabolite repression was mesured in medium containing both glucose and galactose because transcriptional activation occurs only in galactose medium. Thus, in this and other examples of catabolite repression, it is difficult to distinguish the effects of activation from those of repression.

In the present work, I fuse the 365-bp *gal10* segment to several *his3* segments containing the entire promoter region. In glucose-containing medium, the wild-type *his3* gene is transcribed at a basal level (1-2 messenger RNA molecules per cell) which is typical for yeast genes in general[14]. This basal level is not affected by the source of carbon in the medium[13]. Extensive deletion analysis has defined the *his3* promoter elements that are necessary and sufficient for this basal level of transcription: these include an upstream promoter element located 112-126 nucleotides upstream from the RNA initiation site (nucleotides −112 to −126) and a TATA element located between −32 and −52 (refs 15-17). In other words, the entire promoter region necessary for normal levels of *his3* transcription is contained within the 130-bp region immediately preceding the RNA coding sequences.

Thus, in this experimental scheme, catabolite repression is analysed under cicrumstances in which transcriptional activation is achieved by a fully functional and heterologous promoter. In addition, by measuring *his3* levels in glucose medium, the repressing effects conferred by the *gal* segment can be separated from activating effects.

Table 1 Structure and phenotypes of *his3* derivatives

DNA fragment	*his3* allele	Upstream endpoint	Downstream endpoint	IGP dehydratase activity Glucose	Galactose
Sc3355	G12	*GAL*	−200	0.2	12
Sc3357	G13	*GAL*	−188	0.2	16
Sc3379	G14	*GAL*	−169	0.2	12
Sc3377	G15	*GAL*	−153	0.2	14
Sc3404	Δ71	−447	−200	1.0	0.9
Sc3405	Δ72	−447	−188	1.1	1.0
Sc3406	Δ73	−447	−169	1.0	1.1
Sc3387	Δ74	−447	−153	1.1	1.0
Sc3366	Δ75	−447	−131	1.0	1.0
Wild type	-	-	-	1.0	1.0

All DNA fragments were derived from Sc2812, and their corresponding *his3* allele numbers are prefaced by a G for *gal-his3* fusions or by a Δ for internal deletions. The upstream and downstream endpoints are listed (see Fig. 1 for details). Strains were grown in YP medium (1% yeast extract, 2% peptone) containing 2% of the carbon source to be tested (glucose or galactose). IGP dehydratase enzymes activities were determined by standard methods[21] on chloroform-permeabilized cells from 15-ml cultures. Assays were performed in duplicate and values were normalized to the number of cells assayed. Activity levels are presented relative to that of wild-type cells (defined as 1.0); the error ~±10%.

The DNA molecules used in these experiments were derived from YIp5-Sc2812, which contains the *ura3* selectable markers and a 6.1-kilobase (kb) *his3* DNA fragment (Fig. 1). The *gal* segment was fused to the *his3* gene at positions −200, −188, −169 and −153. As controls, the identical *his3* DNA segments were fused to position −447 instead of the *gal* DNA fragment; this position is located within the *pet56* structural gene, and the adjacent sequences do not contain an upstream promoter element[17]. By selecting for *ura3*$^+$ transformants, the *gal-his3* fusions and the control DNAs were integrated in single copy exactly at the normal *his3* chromosomal location (see Fig. 1 legend). The resulting strains were grown in appropriate medium and then assayed for *his3* expression either by assessing the level of IGP dehydratase (the *his3* gene product) (Table 1), or by determining *his3* RNA levels (Fig. 2).

When the strains were grown in glucose medium, the *gal-his3* fusions produced only 20% as much IGP dehydratase as both the control molecules and was the wild-type *his3* gene (Table 1). When the same strains were grown in galactose medium, *his3* was expressed at an extremely high level, which was indistinguishable from that observed with previous *gal-his3* fusions in which the *gal* element replaced the *his3* upstream element[13]. To prove that this 'glucose effect' occurs at the level of transcription, two of the *gal-his3* fusions and their respective control molecules were analysed for *his3* RNA production. As shown in Fig. 2, the *gal-his3* fusions (lanes 4, 5) produced undetectable quantities of RNA, whereas the control molecules (lanes 2, 3) produced similar levels to the wild-type strain (lane 6).

Table 2 Detailed phenotypes

his3 allele	Upstream endpoint	Downstream endpoint	IGP dehydratase activity Glucose	Glucose +AT	Raffinose	Raffinose +AT
G13	*GAL*	−188	0.2	0.9	1.1	3.2
G15	*GAL*	−153	0.2	0.7	1.0	2.9
Δ72	−447	−188	1.0	3.1	1.0	3.4
Δ74	−447	−153	1.1	3.0	1.1	3.1
Wild type	-	-	1.0	3.0	1.0	3.3

The *his3* allele numbers and the upstream and downstream endpoints are described in the text and in the legend to Table 1. Strains were grown in minimal medium containing 2% of the carbon source to be tested (glucose or raffinose). For conditions of amino-acid starvation, cells were collected 6-8 h after addition of aminotriazole (AT) to 10 mM. IGP dehydratase activities were determined as described in Table 1.

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To prove that these effects are due specifically to catabolite repression of the intact *his3* promoter, two of the *gal-his3* fusions and their respective control molecules were analysed in more detail (Table 2). First, in the *gal-his3* fusions, the reduction of *his3* expression was not observed in raffinose medium, conditions in which catabolite repression does not occur[5-9]. This result indicates that the effects are caused specifically by catabolite repression. Second, in the control molecules, *his3* was expressed at the wild-type basal level in glucose, raffinose or galactose medium. This reaffirms that the *his3* sequences are fully competent for *his3* expression[15-17], and that the glucose effects are due specifically to the 365-bp *gal* segment. Third, *his3* expression in all derivatives tested was induced during conditions of amino-acid starvation. This reaffirms previous observations that *his3* expression in *gal-his3* fusions depends specifically on *his3* promoter sequences[13]. This induced level is clearly subject to catabolite repression because in glucose medium the *gal-his3* fusions were expressed at only 20-30% of the level of expression of control molecules, whereas in raffinose medium, the fusions behaved similarly to the controls. Thus, catabolite repression of the *his3* gene is observed even when the *gal* regulatory site lies upstream of a functionally intact promoter region.

In many forms of regulation, RNA levels of a particular gene are higher in one circumstance than in another. However, it is impossible from such physiological phenomenology to determine whether this gene is subject to positive or negative control. For example, the term catabolite repression defines the lower levels of expression that are observed when glucose is the carbon source[8]. However, in *E. coli*, catabolite repression, despite its name, represents a positive control mechanism because the CAP regulatory protein activates transcription[3]. This activation requires cyclic AMP as a cofactor which binds to the CAP protein. Thus, as cells grown in glucose medium contain less intracellular cyclic AMP than cells grown in poorer carbon sources, catabolite repression is actually a consequence of the lack of a functional activator protein rather than the presence of a repressor protein.

The experiments reported here suggest strongly that catabolite repression in yeast indeed occurs by a negative control mechanism. The control region in the *gal* segment overrides the normal *his3* promoter elements and greatly reduces trancription. Such a result cannot be explained by the lack of a functional activator protein because if this were the case, normal levels of *his3* expression would be expected. Indeed, normal levels are observed in raffinose medium, conditions in which catabolite repression does not occur. Thus, catabolite repression is probably mediated by a repressor protein that recognizes sites in the *gal* segment and in analogous regions for other genes under similar control.

The experiments described here also demonstrate that catabolite repression and galactose activation are separable properties of the *gal* regulatory region. In particular, catabolite repression of *his3* expression is observed in the absence of galactose. This suggests strongly that these two regulatory phenomena are mediated, at least in part, by different DNA sequences in the *gal* segment. This notion is supported by deletion mutations of the *gal* segment which are defective in glucose repression yet are fully functional in terms of galactose activation[12]. Note that the catabolite repression described here represents a 5-fold effect, whereas previous experiments (comparing cells grown in galactose with those grown in galactose plus glucose) indicated a 20-fold effect[9,10]. This apparent difference probably results from inducer exclusion effects (cells prefer to transport glucose in preference to galactose)[9,10] and effects on the binding of the *gal4* activator protein[18]. The experimental design used in the present work clearly distinguishes repression from these other effects, which both represent incomplete activation.

Prokaryotic repressor proteins inhibit transcriptional initiation by binding to sites that overlap the promoter region and thus interfere with functional RNA polymerase binding[1,2]. Similarly, the purified simian virus 40 T-antigen binds to sites with which transcription factors are known to interact[19]. In a somewhat different situation, the *E. coli* LexA protein represses galactose induction in yeast cells when its cognate operator site is located at various positions between the *gal* element and the TATA box[20]. This effect is not observed when the *lexA* site is placed close to but upstream from the *gal* element.

In contrast, the catabolite repression site in the *gal* region exerts its effects even when located upstream of an intact promoter region. The *gal* site also appears to reduce expression when upstream of the *cyc1* promoter, although this is complicated by the fact that *cyc1* expression itself is subject to catabolite repression[25]. In the derivatives examined here, the critical regulatory region is located ~100-150 bp upstream of the *his3* upstream promoter element[12,17]. Because this is a rather large distance, and because the *his3* elements are intact, it seems probable that the mechanism of repression does not involve steric competition between the presumptive regulatory protein and the transcriptional apparatus.

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Nuclear extracts from globin-synthesizing cells enhance globin transcription *in vitro*

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***In vitro* transcription studies of cloned messenger RNA-coding genes have yielded considerable information regarding the sequence elements and protein factors involved in transcription initiation and RNA processing (reviewed in ref. 1). Fractionation of whole-cell[2], S-100 protein[3] and nuclear extracts[4] reveals the existence of both general class II[5-9] and gene-specific[8,10,11] transcription initiation factors. Because the soluble *in vitro* transcription systems prepared from cells in culture are largely nonspecific for the origin of the template DNA[1,12], they are highly suited to**

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