Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 498–502, January 1985
Genetics

A synthetic *HIS4* regulatory element confers general amino acid control on the cytochrome *c* gene (*CYC1*) of yeast

(*HIS4* 5′ noncoding DNA/synthetic oligonucleotide/promoter fusions)

ALAN G. HINNEBUSCH*†, GIOVANNA LUCCHINI*‡, AND GERALD R. FINK*

*Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; †Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20205; and ‡Dipartimento di Biologia, Sezione di Genetica Microbiologia, Via Celoria 26, 20133 Milano, Italy

Contributed by Gerald R. Fink, September 24, 1984

ABSTRACT **Hybrid promoters constructed from upstream sequences of the yeast *HIS4* promoter and the downstream element of the yeast *CYC1* promoter place iso-1-cytochrome *c* (*CYC1*) expression under the general amino acid control, typical of *HIS4*. *HIS4* fragments that confer regulation contain at least one copy of the sequence T-G-A-C-T-C that is repeated at *HIS4* and other genes subject to the general control. A 14-base-pair synthetic oligonucleotide containing a single copy of the *HIS4* repeat places *CYC1* under the general control. Two copies of this oligonucleotide produce a derepressed level of expression nearly equivalent to that conferred by the largest *HIS4* 5′ noncoding fragments we examined and direct regulated expression of a set of transcripts with 5′ ends typical of the *CYC1* promoter. Comparison of the expression levels conferred by the short synthetic repeat and larger *HIS4* 5′ fragments reveals additional promoter elements required for maintaining efficient gene expression under repressing growth conditions.**

The regulatory sequences adjacent to the yeast *HIS3* and *HIS4* genes have been studied by functional analysis of mutations made *in vitro*. These two genes, similar to at least 26 others encoding amino acid biosynthetic enzymes, are under the control of a cross-pathway regulatory system known as general amino acid control. Starvation for any single amino acid leads to increased transcription of each of these genes (for review, see ref. 1). Deletion analyses of the *HIS4* (2, 3) and *HIS3* (4) promoters have identified a short nucleotide sequence, found non-tandemly repeated within several hundred base pairs (bp) upstream of these and other genes subject to the general control (5), that functions as a site for positive regulation of transcription.

The deletion analyses of *HIS3* and *HIS4* gave no direct evidence for the functional significance of the redundancy of the short regulatory sequence. In fact, a small deletion that removes just one of the *HIS3* repeats eliminates derepression completely. Moreover, deletion of two upstream copies of the repeat at *HIS4* does not prevent derepression, and only when a third more proximal repeat is also removed is derepression impaired. However, deletion of the *HIS4* upstream repeats results in a lower efficiency of expression, suggesting that these sequences play some role in *HIS4* promoter function.

Recent studies on hybrid promoters in yeast have provided new approaches to the dissection of the sequences involved in the control of transcription (6, 7). The analysis of the *CYC1* promoter has revealed a site designated UAS (upstream activation site) located in a region 275 bp upstream from the 5′ end of the transcript that is absolutely required for heme regulation of the *CYC1* gene (8). Insertion of a fragment containing the upstream sequences of the *GAL10* 5′ non-coding DNA in place of the *CYC1* UAS results in galactose induction of the *CYC1* transcription unit (7). These studies show that the *CYC1* control region can be divided into the UAS required for heme regulation and a downstream segment that determines the mRNA 5′ termini and, most probably, the sites of transcription initiation. Activation of the downstream promoter segment of *CYC1* is an efficient assay for heterologous sequences containing a UAS.

In this report, we examine the ability of the different *HIS4* repeats to confer general control on the downstream segment of the *CYC1* promoter. The chimeric promoters were constructed by fusing fragments from the *HIS4* upstream region or synthetic oligomers to a *CYC1* downstream region lacking its own UAS. The *CYC1* coding region was fused in frame to *lacZ*, so that the hybrid constructions could be assayed by measuring β-galactosidase activity.

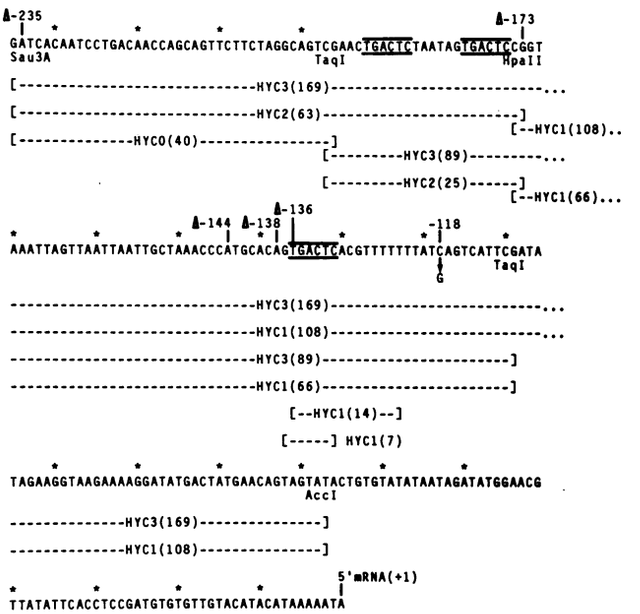

FIG. 1. Fragments of the *HIS4* 5′ non-coding region used in promoter fusions. The 236 bp upstream from the 5′ end of the transcript (+1) in *his4 Δ-235* are shown. The G residue at −236 is the upstream junction (at −589) of the 5′ deletions constructed by Donahue *et al.* (2). Three copies of the general control repeat are both overlined and underlined and the positions of deletions described previously (2, 3) are indicated. The first and last *HIS4* nucleotides in each fragment correspond to the positions of the open and closed brackets, respectively. Fragments are labeled according to the final designations of the resulting *HIS4–CYC1* hybrid promoters.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: bp, base pair(s); UAS, upstream activation site.

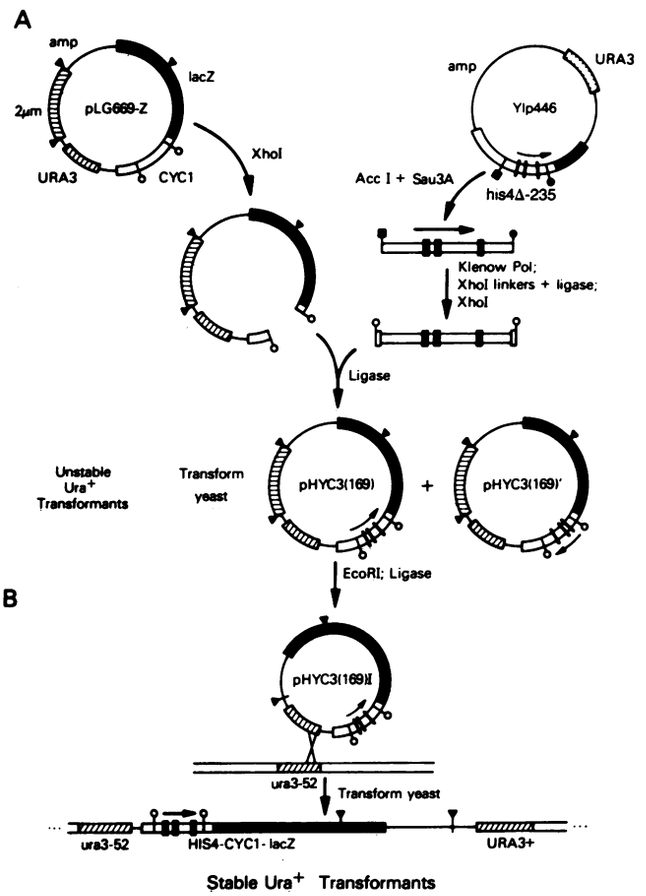

FIG. 2. Insertion of *HIS4* fragments upstream from the *CYC1* gene. Construction of *HYC3(169)* is depicted as a typical example. ■, *Sau*3A; ●, *Acc* I; ▼, *Eco*RI; ○, *Xho* I; the three vertical black bars in the *HIS4* region represent the short repeats. (*A*) Autonomously replicating plasmids. The −236 to +97 *HIS4 Sau*3A fragment from YIp446(2) was isolated by preparative gel electrophoresis and digested with *Acc* I in constructing *HYC3(169)*, with *Acc* I/*Hpa* II in constructing *HYC2(63)* and *HYC1(108)*, with *Taq* I in constructing *HYC3(89)* and *HYC0(40)*, and with *Taq* I/*Hpa* II in constructing *HYC2(25)* and *HYC1(66)*. *Xho* I octanucleotide linkers (5′ C-C-T-C-G-A-G-G 3′) were added as described (9), and the appropriate fragments were isolated by gel electrophoresis. Two pairs of complementary synthetic oligonucleotides were prepared (10) containing the *HIS4* sequences from the −136 region labeled *HYC1(14)* and *HYC1(7)* in Fig. 1. Additional residues were added to produce *Sal* I ends. The sequences of the oligomers are 5′ T-C-G-A-C-*T-G-A-C-T-C-A-G-T-T-T-T-T-G* 3′ and 3′ G-*A-C-T-G-A-G-T-G-C-A-A-A-A-A-C*-A-G-C-T 5′ for *HYC1(14)* and 5′ T-C-G-A-C-*G-T-G-A-C-T-C-G* 3′ and 3′ G-*C-A-C-T-G-A-G*-C-A-G-C-T 5′ for *HYC1(7)*. The italicized bases are those found in the *HIS4* sequence. Complementary oligomers were reannealed and treated with polynucleotide kinase as described (10). After ligation of *Xho* I linker containing fragments with the *Xho* I fragment of pLG699-Z (11), *Escherichia coli* transformants harboring recombinant plasmids were identified by colony hybridization (9) using the *HIS4 Sau*3A fragment as a probe. After ligation of the synthetic oligomers (with *Sal* I ends) to the pLG669-Z *Xho* I fragment, the ligation mixture was digested with *Xho* I to eliminate nonrecombinant plasmids from the transformation. The number and orientation of inserts were determined, whenever possible, by restriction enzyme digestions. For each plasmid chosen for analysis in yeast, the DNA sequence of the *CYC1–HIS4* junction was determined (12) by analyzing the −78 to −1078 *Nde* I/*Sal* I *CYC1* fragments (labeled at the *Nde* I site) in which the inserted *HIS4* DNA resides. A *ura3*$^-$ yeast strain was transformed with the autonomously replicating plasmids, selecting for Ura$^+$ transformants. (*B*) Nonreplicating plasmids were constructed by deleting the *Eco*RI fragment carrying the 2-μm replication origin from the autonomously replicating plasmids. Stable Ura$^+$ yeast transformants were obtained and analyzed by Southern blotting (13) to verify that a single copy of the plasmid was integrated as shown at the *URA3* locus. Integration at *URA3* was favored by transforming yeast with plasmid DNA cut at the unique *Sma* I site in the *URA3* sequence (14).

METHODS

The *HIS4* sequences inserted upstream of *CYC1* are indicated in Fig. 1. Plasmids were constructed as described in Fig. 2, and the resulting constructs are shown schematically in Fig. 3. Ura$^+$ transformants of yeast strains TD28 (*α ura3-52 ino1*), L1356 (*α gcn4-101 ura3-52*), and 9617-ID (*α gcd1-101 ura3-52*) containing autonomously replicating plasmids were obtained by the method of Hinnen *et al.* (15). Stable Ura$^+$ transformants containing integrated *HYC* plasmids and a regulatory mutation were obtained by tetrad analysis of sporulated diploids from crosses between stable transformants of TD28 (see Fig. 2) and untransformed regulatory mutants of the opposite mating type.

Transformants were grown to mid-logarithmic phase in minimal salts/dextrose (SD) medium (16) for repressing conditions and for 6–8 hr in SD medium/10 mM 3-aminotriazole for derepressing conditions (17). β-Galactosidase assays were performed as described (3). For S1 nuclease mapping, 25 μg of total RNA was hybridized with 0.3 pmol of the single-stranded *CYC1 Bam*HI/*Xho* I fragment of pLG669-Z (coordinates +81 to −78), 5′ end-labeled at the *Bam*HI site. Since the *Bam*HI site is within a linker at the *CYC1–lacZ* junction, RNA from endogenous *CYC1* does not hybridize with the probe (8). Isolation of RNA, preparation of the DNA probe, DNA·RNA hybridization, S1 nuclease digestion, and gel electrophoresis were all carried out as described (5).

RESULTS

Expression of the Hybrid Promoters Is Subject to General Amino Acid Control. The data in Fig. 3 show that neither the intact *CYC1*$^+$ promoter nor the truncated derivative *CYC1* Δ*Xho* derepresses in response to histidine starvation. In fact, we consistently observe a decrease in the specific activity of β-galactosidase directed by these promoters in response to histidine starvation. By contrast, an autonomous plasmid carrying the wild-type *HIS4* promoter, directing the expression of a *HIS4–lacZ* protein fusion (pRB84; ref 18), displays a clear increase in enzyme activity during histidine starvation. Likewise, derepression is evident for nearly all of the *HYC* promoter fusions, regardless of whether one, two, or three copies of the *HIS4* repeat are present. Two DNA segments containing no repeats [the *HYC0(40)* fragment and a segment of the *HIS4* protein coding sequence not shown] do not confer a starvation response to the *CYC1* downstream promoter element.

The *HYC1(7)* promoter, which consists of the synthetic consensus sequence 5′ G-T-G-A-C-T-C 3′ immediately flanked by the sequences added for cloning the fragment (Fig. 2), fails to exhibit derepression in either one or two copies. Given that *HYC2(25)* and *HYC1(14)* each suffice to confer regulation and yet share no sequences in common aside from the repeat, it seems likely that the linkers interfere in some way with the function of the repeat in *HYC1(7)*.

Constructs containing all three of the repeats show extremely efficient expression. However, neither the upstream [*HYC2(63)*] nor the downstream [*HYC1(108)*] half of the largest *HIS4* fragment functions efficiently under repressing conditions, and the upstream portion functions inefficiently under derepressing conditions as well. The fact that the downstream half can function efficiently in the absence of the upstream repeats under derepressed conditions but not under repressed conditions suggests that the sequence re-

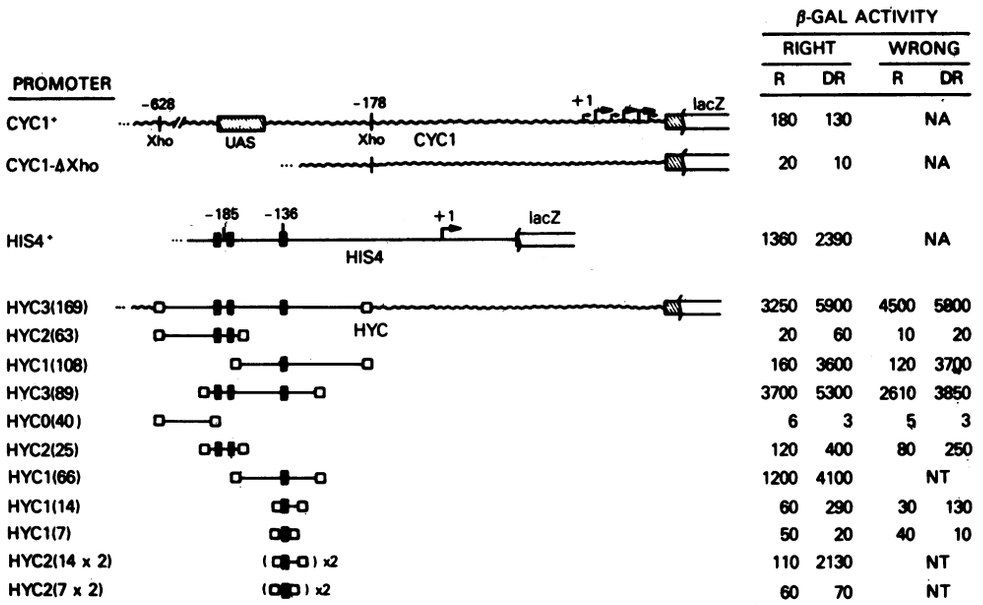

FIG. 3. Expression of promoter fusions on autonomously replicating plasmids in yeast. Depicted on the left are the promoter fusions. Black boxes signify the 5′ T-G-A-C-T-C 3′ repeat; open boxes are nucleotides added to permit ligation with *Xho* I staggered ends (see Fig. 2). The *HYC* promoters (for *HIS·CYC* hybrids) are designated according to the number of *HIS4* repeats (0, 1, 2, or 3) and the number of *HIS4* nucleotides in the construct (placed in parenthesis). Only the constructs in which the *HIS4* sequences are oriented at *CYC1* in the same direction (relative to transcription) that they occur at *HIS4* are shown, and enzyme activities under the column labeled "Right" refer to these constructs. Values under the "Wrong" column refer to the opposite orientation. R, repressing growth conditions; DR, derepressing conditions; NA, not applicable; NT, not tested. Activities shown are averages from three independent transformants and have standard errors of <20%. β-Galactosidase-specific activity is given in units of nmol·min^{-1}·mg^{-1}.

quirements for efficient expression differ in repressed and derepressed growth conditions. This point is further illustrated by the comparison of *HYC1(14)* and *HYC2(14×2)*, containing, respectively, one and two copies of the synthetic 14 bp containing the −136 repeat. The addition of the second copy of the repeat in tandem results in nearly a one order of magnitude increase in the level of derepressed expression but has little effect on the repressed level of expression.

Fig. 3 also shows that a number of the *HIS4* fragments function in the opposite orientation. This is best illustrated by *HYC1(108)*, which displays essentially the same magnitude of both repressed and derepressed expression, and a >10-fold derepression ratio in either orientation relative to *CYC1*. The fact that the levels of expression given by the various constructs are similar in either orientation argues that the gene expression we observe in these hybrid promoters is not the result of the novel junctions formed between the *HIS4*, *CYC1*, and linker DNA sequences.

We also examined the expression of the same hybrid promoters integrated into the genome in single copy at the *URA3* locus (see Fig. 2 for details). The levels of β-galactosidase measured for these strains are about one order of magnitude lower under both repressed and derepressed growth conditions than the values listed in Fig. 3 (data not shown). This reduction probably reflects the expected difference in gene dosage between transformants carrying high-copy autonomously replicating plasmids and transformants containing a single integrated copy of the same construct. The uniform reduction in all levels of expression indicates that the regulatory behavior of these promoters is similar whether stably integrated into the genome or replicating extrachromosomally.

Expression of the *HYC* Promoters Responds to General Control Regulatory Mutations. The *gcn4-101*§ mutation leads to low constitutive *HIS4* expression at 1/3rd to 1/4th the normal repressed level (3); the *gcd1-101* mutation results in high constitutive expression of *HIS4* (3, 17). We tested the *HYC* constructs for their response to these regulatory mutations by transforming strains carrying either the *gcn4-101* or the *gcd1-101* mutation with each of the autonomously replicating plasmids listed in Fig. 3. The expression of the plasmid-borne *HYC* constructs in these transformants is shown in Table 1. Similar studies with the integrated *HYC* constructs in *gcn4-101* and *gcd1-101* strains gave similar results (data not shown).

The *gcn4-101* mutation reduces the derepression ratio (DR:R) of all of the *HYC* constructs that are regulated in a

Table 1. Effect of regulatory mutations on *HYC* promoters

	Wild type		*gnc4-101*		*gcd1-101*	
Promoter	R	DR	R	DR	R	DR
CYC1⁺	180	130	3200	3280	160	150
CYC1-ΔXho	20	10	70	60	10	5
HYC3(169)	3250	5900	1410	1450	11,970	9,720
HYC2(63)	20	60	70	60	50	50
HYC1(108)	160	3600	180	110	7,380	7,910
HYC1(108)′	120	3700	25	40	7,520	8,950
HYC3(89)	3700	5300	4490	5160	13,380	10,505
HYC3(89)′	2600	3800	4440	4500	13,220	11,000
HYC2(25)	120	400	210	140	300	430
HYC2(25)′	80	250	110	90	230	290
HYC1(66)	1200	4100	720	870	11,270	8,160
HYC1(14)	60	290	70	100	950	1,470
HYC2(14×2)	110	2130	35	35	5,840	9,830
HYC1(7)	50	20	90	140	40	50
HYC2(7×2)	60	70	130	200	160	190

Data in the first two columns are from Fig. 3. R and DR refer to repressed and derepressed growth conditions as described in Fig. 3. Each activity is the average from three independent transformants. Constructs labeled with a prime carry the *HIS4* fragments oriented away from the direction of transcription.

§A new nomenclature for general control regulatory genes was adopted recently. The *gcn4-101* mutation was known previously as *aas3-1* (31); *gcd1-101* was known as *tra3-1* (17).

wild-type strain. With the exception of *HYC3(89)*, *HYC3(89)'*, and *HYC2(63)*, this is the result of reductions by a factor of 3–100 in the levels of expression in derepressing conditions. (The three exceptions are anomalous in that their expression becomes constitutive through an increase in repressed expression.) In several cases [*HYC3(169)*, *HYC(108)'*, *HYC1(66)*, and *HYC2(14×2)*], the expected severalfold reduction in expression in repressing conditions is also observed in the *gcn4*$^-$ strain. [An unexpected finding is that expression of *CYC1*$^+$ is increased by a factor of 10 by *gcn4-101*. This appears to be true for *CYC1-ΔXho* as well, although to a lesser extent (Table 1). The significance of this finding for general control is not understood at present.]

The *gcd1-101* mutation also greatly decreases the derepression ratio of all the regulated *HYC* constructs, in this case, as the result of 2.5- to 60-fold increases (relative to wild type) in the levels of expression in repressing conditions. These increases result in constitutive expression at levels even greater than the derepressed levels in wild type. The effect of the *gcd1-101* mutation is especially striking for the *HYC1(108)* and *HYC2(14×2)* constructs, which show 50-fold higher expression under repressing conditions in the mutant versus wild type. It is also remarkable that in the *gcd1*$^-$ strain, two copies of the synthetic oligonucleotide [*HYC2(14×2)*] give derepressed levels nearly as high as those constructs containing all three copies of the repeat and their flanking sequences.

The Hybrid Promoters Utilize *CYC1* mRNA 5′ Ends. S1 nuclease mapping of the 5′ ends of the transcripts directed by the *HYC* promoters (Fig. 4) shows that, in every case in which enough mRNA was produced to be detected, the identical 5′ end pattern described for the intact *CYC1*$^+$ gene (8, 19) was observed for the *HYC* promoters. In addition, the relative levels of the transcripts produced by the *HYC* constructs are consistent with the enzyme activities given in Fig. 3.

DISCUSSION

We have shown that DNA fragments from the *HIS4* 5′ noncoding region can substitute for the upstream regulatory region of the *CYC1* gene to promote expression of authentic *CYC1* mRNA subject to the general amino acid control. Fragments containing the repeat function as regulatory elements when placed in either orientation with respect to the truncated *CYC1* promoter located downstream. These hybrid constructions provide important information on the role of the repeats in both regulation and promoter efficiency. All fragments that confer general control contain at least one copy of the short repeated sequence 5′ T-G-A-C-T-C 3′. This result is especially striking in the fusion containing a small synthetic *HIS4* fragment of only 14 bp, which contains 1 copy of the repeat and only 8 bp from the surrounding sequences. This shows clearly that the −136 repeat is sufficient for derepression to occur. The results obtained with the *HYC2(25)* construct indicate that the −185 repeat pair can also confer regulation in the absence of the −136 repeat, demonstrating functional redundancy in the *HIS4* regulatory region.

A simple doubling of the *HYC(14)* oligonucleotide produces a striking increase in derepressed expression to a level nearly equivalent to that found in the *HYC* constructs containing three copies of the repeat. This dramatic difference between one and two copies of the oligonucleotide suggests that the number of repeats is a key to efficient derepressed expression. However, there are two observations that seem to be at odds with this simple conclusion. First is the high level of derepression achieved in *HYC1(66)*, which ostensibly contains only one copy of the repeat. This derepression level could be explained if this segment actually contains another sequence that can function as a repeat. In fact, a sequence at −119, 5′ T-C-A-G-T-C 3′, resembles the consensus sequence 5′ T-G-A-C-T-C 3′. This site is also the location of a substitution mutation to 5′ T-G-A-G-T-C 3′ (see Fig. 1) that restores *HIS4* derepression in a strain lacking a canonical copy of the repeat at *HIS4* (2, 3). It is possible that this variant repeat, while not sufficient by itself, functions in the wild-type *HIS4* promoter in concert with other repeats and accounts for the efficient expression of *HYC1(66)*. An alternative explanation to account for the great difference in efficiency between *HYC1(66)* and *HYC1(14)* is that there are additional sequences present in *HYC1(66)*, unrelated to the repeats, that are required for high-level derepression. The second anomaly is that *HYC2(25)* contains two copies of the

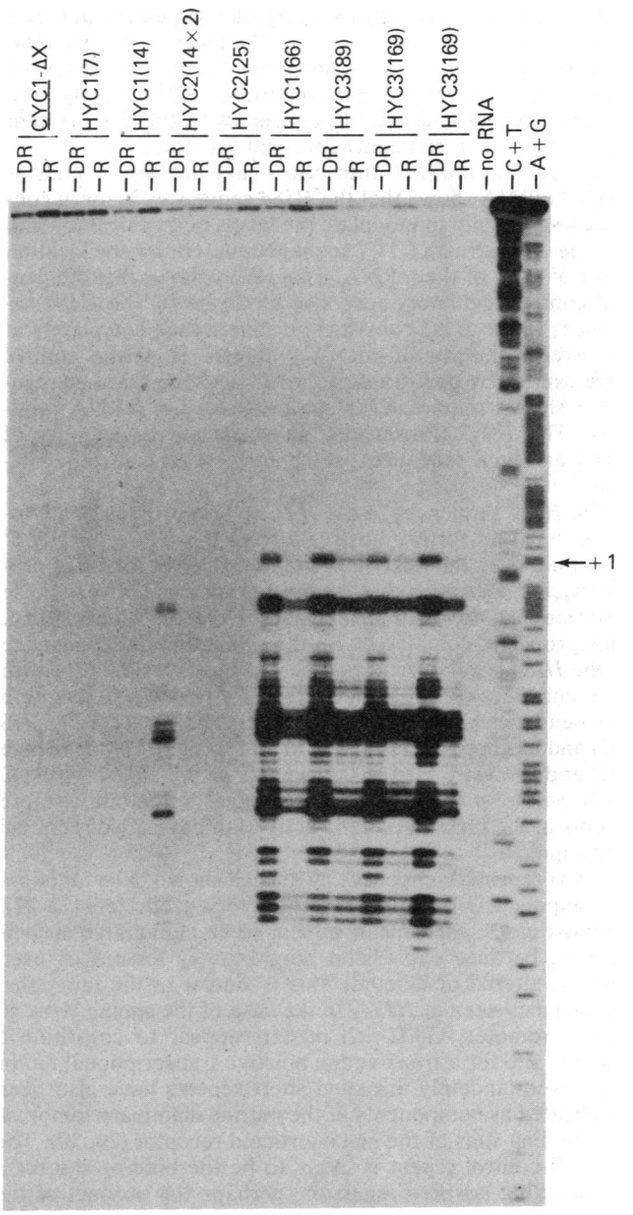

FIG. 4. S1 nuclease mapping of *HYC* promoted transcripts in total RNA isolated from yeast transformants carrying the indicated constructs on autonomously replicating plasmids, grown under repressing (R) and derepressing (DR) conditions. A+G and C+T are Maxam–Gilbert sequence reactions on the labeled probe; "no RNA" refers to a mock hybridization done without RNA; +1 is the position in the DNA sequence expected for the largest of the S1 nuclease protected fragments from the *CYC1*$^+$ promoter (1, 8).

repeat and yet functions inefficiently. It is possible that the low levels of expression observed on derepression of this construct are a result of the close proximity of the repeats or the character of the sequences flanking the repeats.

The sequences required to maintain the repressed level of expression are less well-defined than those required for derepression. The fact that two copies of the synthetic oligomer produce low repressed levels suggests that there are other sequences required to maintain repressed expression. A comparison of *HYC1(66)* and *HYC1(14)* suggests that such sequences reside in the DNA immediately flanking the −136 repeat. In addition, promoter fusions lacking the upstream repeats function less efficiently than those containing all three repeats [compare *HYC1(108)* with *HYC3(169)* and *HYC1(66)* with *HYC3(89)*]. The same observation was made previously for 5′ deletions that eliminate the upstream repeats (2, 3). These findings suggest that sequences immediately 3′ to the upstream repeats, at the junction between the promoter half-fragments, contribute to the efficiency of expression under repressed conditions. Alternatively, the upstream repeats may act in concert with the downstream repeat to form an efficient repressed promoter.

The fact that the *HYC* transcripts have the same 5′ ends as $CYC1^+$ mRNA shows that the *HIS4* sequences in these constructs serve only to modulate the levels of transcription and that the downstream *CYC1* sequences determine the location of the 5′ ends of the mRNA. This result shows that the general control regulatory response mediated by the *HIS4* sequences in the *HYC* constructs is determined completely at the level of the rate of mRNA synthesis. If general control were exerted by post-transcriptional mechanisms, then regulation should require *HIS4* sequences in the mRNA products. The *HYC* transcripts, although composed only of *CYC1* and *lacZ* sequences, still show general control regulation.

The *HIS4* sequences in the *HYC* hybrid promoters are located >100 bp further upstream from the *CYC1* mRNA 5′ ends than from the mRNA 5′ end in the wild-type *HIS4* promoter, demonstrating that the precise spacing between these upstream and downstream promoter elements is not critical. This property, combined with the orientation independence of the *HIS4* regulatory sequences, suggests a similarity with viral enhancer elements (see ref. 20 for review). Orientation independence is also a characteristic of the yeast *CYC1* UAS (32) and has been reported for the MTV regulatory sequence (21) and the G+C-rich 21-bp repeats of the simian virus 40 early promoter (22). This feature suggests that one or more events in the process of gene activation have a bidirectional consequence.

Short repeated sequences have been shown to be functional components of a number of eukaryotic promoters (22–27). In most cases, the repeats appear to be cumulative in their effects, because expression progressively lessens as each copy is altered or deleted. This is similar to the functional redundancy seen at *HIS4*. In the case of the simian virus 40 early promoter, G+C-rich repeats appear to constitute a binding site for a *trans*-acting positive transcriptional factor (28). Non-tandemly arranged short repeats have also been implicated as components of the murine mammary tumor virus binding sites of the glucocorticoid receptor (29, 30). The general control repeat is likely to be the binding site for a *trans*-acting positive regulator, perhaps the product of the *GCN4* gene (3, 31). If so, sequence repetition could provide for cooperative binding or might simply increase the local concentration of binding sites.

We thank Leonard Guarente for his advice, gift of pLG669-Z, and helpful comments on the manuscript; Mark Zoller, Tom Atkinson, and Michael Smith for their efforts in the synthesis of the oligomers; Catherine Chen for help on enzyme assays and strain construction; and Mary Thorne for preparation of the manuscript. This work was supported in part by National Institutes of Health Grants CA34429 and GM31802 to G.R.F., an American Cancer Society Research Professor of Genetics. A.G.H. is grateful for support as a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

1. Jones, E. W. & Fink, G. R. (1982) in *The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression*, eds. Strathern, J. N., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 181–300.
2. Donahue, T. F., Daves, R. S., Lucchini, G. & Fink, G. R. (1983) *Cell* **32**, 89–98.
3. Lucchini, G., Hinnebusch, A. G., Chen, C. & Fink, G. R. (1984) *J. Mol. Cell. Biol.* **4**, 1326–1333.
4. Struhl, K. (1982) *Nature (London)* **300**, 284–287.
5. Hinnebusch, A. G. & Fink, G. R. (1983) *J. Biol. Chem.* **258**, 5238–5247.
6. Beier, D. R. & Young, E. T. (1982) *Nature (London)* **300**, 724–728.
7. Guarente, L., Yocum, R. R. & Gifford, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7410–7414.
8. Guarente, L. & Mason, T. (1983) *Cell* **32**, 1279–1286.
9. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 390–401.
10. Edge, M., Greene, A. M., Heathcliffe, G. R., Meacock, P. A., Schuch, W., Scanlon, D. B., Atkinson, T. C., Newton, C. R. & Markham, A. F. (1981) *Nature (London)* **292**, 756–762.
11. Guarente, L. & Ptashne, M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2199–2203.
12. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
13. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
14. Orr-Weaver, T., Szostak, J. & Rothstein, R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6354–6358.
15. Hinnen, A., Hicks, J. B. & Fink, G. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1929–1933.
16. Sherman, F., Fink, G. R. & Lawrence, C. W. (1974) *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 61–64.
17. Wolfner, M., Yep, D., Messenguy, F. & Fink, G. R. (1975) *J. Mol. Biol.* **96**, 273–290.
18. Silverman, S. J., Rose, M., Botstein, D. & Fink, G. R. (1982) *Mol. Cell. Biol.* **2**, 1212–1219.
19. Faye, G., Leung, D. W., Tatchell, K., Hall, B. D. & Smith, M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2258–2262.
20. Khoury, G. & Gruss, P. (1983) *Cell* **33**, 313–314.
21. Chandler, V. L., Maler, B. A. & Yamamoto, K. R. (1983) *Cell* **33**, 489–499.
22. Everret, R. D., Baty, D. & Chambon, P. (1983) *Nucleic Acids Res.* **11**, 2447–2464.
23. Fromm, M. & Berg, P. (1983) *J. Mol. Appl. Genet.* **1**, 457–481.
24. Dierks, P., van Ooyen, A., Cochran, M. D., Dobkin, C., Reiser, J. & Weissmann, C. (1983) *Cell* **32**, 695–706.
25. McKnight, S. L. & Kingsbury, R. (1982) *Science* **217**, 316–324.
26. Macken, S. & Roizman, B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4917–4921.
27. Hearing, P. & Shenk, T. (1983) *Cell* **33**, 695–703.
28. Dynan, W. S. & Tjian, R. (1983) *Cell* **35**, 79–87.
29. Scheidereit, C., Geisse, S., Westphal, H. M. & Beato, M. (1983) *Nature (London)* **304**, 749–752.
30. Payvar, F., DeFranco, D., Firestone, G. L., Edgar, B., Wrange, O., Okret, S., Gustafsson, J. & Yamamoto, K. R. (1983) *Cell* **35**, 381–392.
31. Hinnebusch, A. G. & Fink, G. R. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5374–5378.
32. Guarente, L. & Hoar, E. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7860–7864.