Proc. Natl. Acad. Sci. USA
Vol. 78, No. 4, pp. 2199–2203, April 1981
Biochemistry

Fusion of *Escherichia coli lacZ* to the cytochrome *c* gene of *Saccharomyces cerevisiae*

(*lacZ* gene fusion/*in vitro* recombination/yeast transformation/*CYC1* gene/yeast promoter)

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Contributed by Mark Ptashne, December 31, 1980

ABSTRACT **Hybrid genes between the *Escherichia coli lacZ* gene and the iso-1-cytochrome *c* (*CYC1*) gene of *Saccharomyces cerevisiae* were constructed by recombination *in vitro*. Each of the hybrid genes encodes a chimeric protein with a cytochrome *c* moiety at the amino terminus and an active β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) moiety at the carboxy terminus. When these hybrids are introduced into *S. cerevisiae* on plasmid vectors, they direct synthesis of β-galactosidase. β-Galactosidase levels directed by one such plasmid display the pattern of regulation normally seen for cytochrome *c* (i.e., a reduction of synthesis in cells grown in glucose). This plasmid contains one codon of *CYC1* fused to *lacZ*, and the fused gene is preceded by the 1100 nucleotides that lie upstream from *CYC1*. An analysis of deletions in the upstream DNA suggests that sequences required for efficient transcription initiation of *CYC1* lie within the DNA segment 250–700 base pairs upstream from the start of the *CYC1* coding sequence. This region is at least 130 base pairs upstream from the "Hogness box" sequence that precedes the *CYC1* coding sequence.**

A genetic analysis of the promoter of the yeast gene *CYC1*, which encodes the iso-1-cytochrome *c* of *Saccharomyces cerevisiae*, has been initiated. This gene lies in yeast nuclear DNA and is transcribed by RNA polymerase II. The approach taken makes use of a genetic tool that has been widely employed in the study of prokaryotic genes: fusion of the *Escherichia coli lacZ* gene to the promoter under study. Once fused to *lacZ*, expression of a promoter can be monitored by convenient selections and screenings that identify bacterial colonies producing different levels of β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) (for review, see ref. 1). Fusions of this kind have greatly facilitated the isolation of mutations affecting transcription initiation in bacteria (2, 3).

It is shown herein that the *lacZ* fusion method can readily be applied to *S. cerevisiae*. *S. cerevisiae* has no endogenous β-galactosidase activity that would interfere with assays for activity encoded by *lacZ*. Several yeast genes have been cloned into *E. coli* plasmid vectors, which greatly simplifies the construction of fusions (see below). A yeast transformation system (4) allows a *lacZ* fusion constructed *in vitro* to be introduced into *S. cerevisiae* and its activity to be monitored *in vivo*.

The DNA coding and 5′ flanking sequences of *CYC1* have been determined (5). *CYC1* is regulated at the transcriptional level by the carbon source in the growth medium (6). For example, cytochrome *c* (and its mRNA) is about 4–7 times more abundant in cells growing in medium containing raffinose (or nonglycolytic carbon sources) than in medium supplemented with glucose. Detailed genetic analysis of *CYC1* by Sherman *et al.* has identified mutations that effect translation initiation on *CYC1* mRNA (7), but mutations have yet to be identified that effect transcriptional regulation.

A series of *E. coli* plasmids that allow DNA regions encoding the amino terminus of any protein to be fused in register to a large 3′-end fragment of *lacZ* has been described (8). The resulting hybrid gene contains information to encode a hybrid protein with β-galactosidase activity. In this report, we describe the use of this technique to construct several plasmids bearing hybrid *CYC1-lacZ* genes. These plasmids also carry selectable markers and origins of replication for both *E. coli* and *S. cerevisiae*. One hybrid contains four nucleotides of *CYC1* coding DNA preceded by about 1100 nucleotides that normally are upstream of *CYC1* in the yeast chromosome. This hybrid gene directs the expression of β-galactosidase in *S. cerevisiae*. These levels of β-galactosidase are repressed by glucose. Moreover, deletion of a portion of the *CYC1* 5′ flanking DNA from this plasmid severely reduces the levels of β-galactosidase expressed in *S. cerevisiae*. The deleted DNA extends from 250 to 700 base pairs upstream from the start of the *CYC1* coding sequence. These results suggest that sequences involved in transcription initiation of *CYC1* lie within the region 250–700 base pairs upstream from the start of the coding sequence. Another hybrid contains 47 codons of *CYC1* coding DNA and about 300 nucleotides of DNA normally upstream of *CYC1*. This hybrid directs expression of β-galactosidase in *S. cerevisiae*, and the levels are not repressed by glucose (see *Discussion*). In addition, the expression in yeast of a heterologous gene, the histone *H2A* gene of the sea urchin *Strongylocentrotus purpuratus*, was probed by first fusing it to *lacZ*. In this case, we detected no β-galactosidase synthesis in yeast, which suggested that the *S. purpuratus* promoter does not function in yeast.

MATERIALS AND METHODS

Strains. The *E. coli* strain LG90 (F^-, Δ*lac pro*) (8) and the *S. cerevisiae* strain DB745 (Adel-100, Leu2-3, Leu2-112, URA3-52) (constructed in the lab of D. Botstein) were used in this study. DNA constructions and *E. coli* transformation were done as described (8). Yeast transformation was performed as described (4).

Media. Yeast were typically grown in 1% yeast extract/2% bacto-peptone and subcultured in minimal medium (7 mg of yeast nitrogen base without amino acids, 20 mg of sugar, and 0.04 mg of adenine and leucine per ml) for assays. Plates for yeast transformations contained the above minimal medium.

β-Galactosidase Assay. *In culture.* Yeast cultures (5 ml) were grown to an OD_{600} of 1.0 in minimal medium containing either glucose or raffinose. Cells were spun down and assayed by one of two methods: (*i*) The pellets were resuspended in 1 M sorbitol with 1% Glusulase (Endo Laboratories, New York). Spheroplasts were washed three times with sorbitol and lysed in a

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buffer containing 10 mM Tris (pH = 7.0), 50 mM 2-mercaptoethanol, and 0.1% Triton X 100. Extracts were then assayed for *o*-nitrophenol-β-D-galactoside-hydrolyzing activity in Z buffer (9). Protein concentrations were determined by the method of Bradford (10).

(*ii*) The pellets were resuspended in 1 ml of Z buffer, treated with 0.05 ml of chloroform and 0.02 ml of 0.1% $NaDodSO_4$, and vortexed. Cells were then assayed as described (9).

E. coli β-galactosidase levels were assayed as described (9).

On plates. Minimal-medium plates were buffered with 0.2 M 2-(*N*-morpholino) ethanesulfonic acid at pH 6.1 and supplemented with 0.2 mg of 5-bromo-4-chloro-3-indolyl-β-D-galactoside per ml.

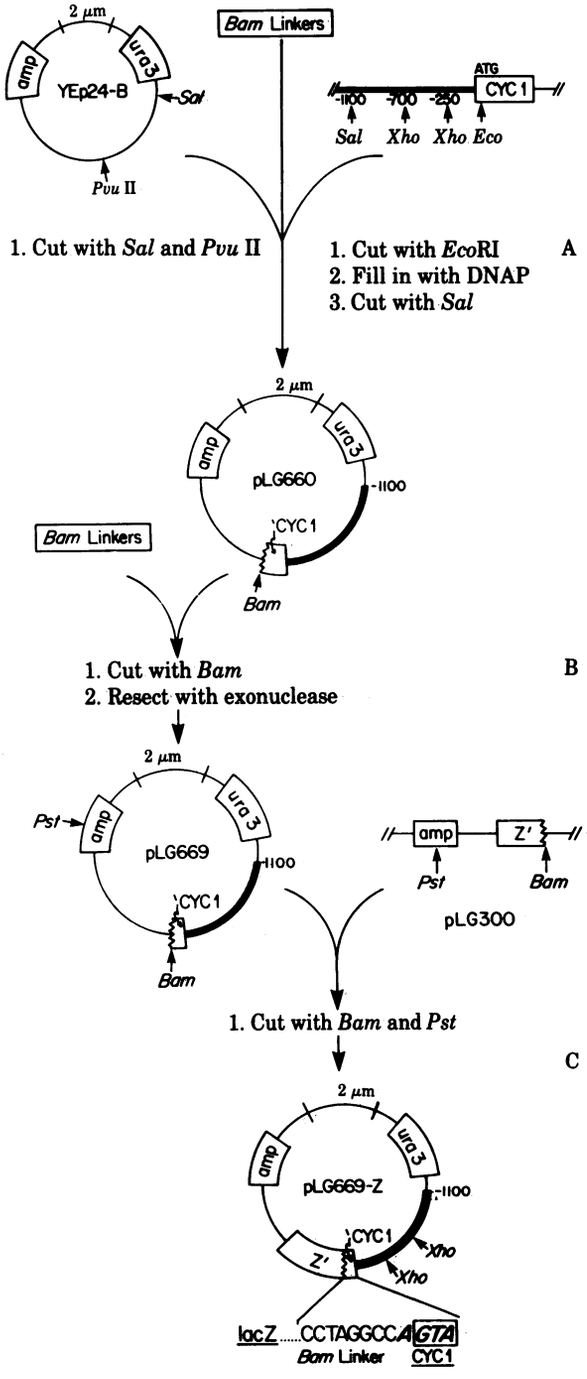

FIG. 1. (*A*) A fragment of DNA was prepared from pYe*CYC1* (heavy line) that extended from a *Sal* I end 1100 base pairs upstream from the start of *CYC1* to an *Eco*RI end, which had been filled in with DNA polymerase (DNAP; DNA nucleotidyltransferase, EC 2.7.7.7) (11) 11 nucleotides into the *CYC1* coding sequence. This fragment was ligated into a backbone from YEp24-B (whose *Bam*HI site had been destroyed by filling in with DNA polymerase) with *Sal* I and *Pvu* II (flush) ends. In the same ligation, a *Bam*HI linker (12) was inserted between the filled-in *Eco*RI end from pYe*CYC1* and the *Pvu* II end from YEp24-B. The resulting plasmid, pLG660, contains approximately 1100 nucleotides of *CYC1* 5′ flanking DNA (thick line) and 10 base pairs of *CYC1* 5′ coding DNA. (*B*) The amount of *CYC1* coding DNA was reduced by cutting pLG660 with *Bam*HI, resecting with the exonuclease *Bal* I 31 as described (8), and reclosing in the presence of *Bam*HI linkers. One resulting plasmid, pLG669, contains but four nucleotides (A-T-G-A) of *CYC1* coding DNA [as determined by Maxam–Gilbert sequence determination (13)]. (*C*) *CYC1* was fused in frame to *lacZ*. pLG669 was cut with *Bam*HI and *Pst* I, as was pLG300. Joining of the *Bam*HI ends of pLG669 and pLG300 generates a *CYC1-lacZ* fused gene, whereas joining of the *Pst* I ends regenerates the *amp* gene. The resulting plasmid, pLG669-Z, was shown to contain a *Bam*HI site at the *CYC1-lacZ* junction, verifying that the *Bam*HI ends of pLG669 and pLG300 were joined precisely. pLG669-Z carries approximately 1100 base pairs of *CYC1* 5′ flanking DNA and the first four base pairs of the *CYC1* coding sequence fused in frame to *lacZ*. The *CYC1* flanking sequence contains two sites sensitive to the restriction endonuclease *Xho* I at positions about 250–700 nucleotides upstream from the start of *CYC1*. Deletion of this fragment yields pLG670-Z.

RESULTS

Construction of Fused Genes. A DNA fragment encoding a large carboxy-terminal region of β-galactosidase was fused *in vitro* to DNA encoding the amino terminus of cytochrome *c* as shown in Figs. 1 and 2. Two plasmids that contain *CYC1-lacZ* fusions in the proper reading frame were constructed. The first, pLG669-Z, contains one codon of *CYC1* fused to *lacZ*, and the fused gene is preceded by about 1100 nucleotides of DNA that naturally precede *CYC1*. The second plasmid, pLGHY2, contains 47 codons of the *CYC1* gene fused to *lacZ*. This fused gene is preceded by about 300 nucleotides of DNA that naturally precede the *CYC1* gene in the yeast chromosome. Each plasmid also contains an *E. coli* selectable marker (amp^R), an origin of replication in *E. coli* (from pBR322), a *S. cerevisiae* selectable marker (*URA3*), and a yeast origin of replication (from the 2-μm plasmid circle) (14).

In a parallel experiment, *lacZ* was fused to the cloned histone *H2A* gene of the sea urchin *S. purpuratos* (15). This construction is shown in Fig. 3. Plasmid pLGHS2 carries 95 codons of *H2A* fused to *lacZ*, and the fused gene is preceded by about 1200 nucleotides of DNA that lie to the 5′ side of *H2A* in the *S. purpuratus* chromosome. This plasmid also contains the *E. coli* and *S. cerevisiae* selectable markers and origins of replication described above.

Levels of β-Galactosidase Directed by *CYC1-lacZ* Fused Genes. In the *E. coli* strain LG90, plasmids pLGHY2 and pLG669-Z directed the synthesis of about 100 units (Fig. 4) and 10 units of β-galactosidase, respectively, whereas pLGHS2 (plasmid carrying *S. purpuratus H2A-lacZ*) failed to direct detectable expression. This suggests that the yeast DNA fragments of pLGHY2 and pLG669-Z may contain sequences that function as promoters in *E. coli*. These plasmids were introduced into the yeast strain DB745 by transformation and selection of URA^+. Transformants were unstable in the absence of URA^+ selection, segregating URA^- colonies at a frequency of about 20%. This is consistent with the idea that pLGHY2, pLG669-Z, and pLGHS2 exist as plasmids in yeast (14).

β-Galactosidase activity in the yeast strain DB745 was assayed, and the results are shown in Fig. 4. pLG669-Z directed the synthesis of 100 units of β-galactosidase in glucose-grown cells and 375 units in raffinose-grown cells. These β-galactosidase levels follow the pattern of regulation that has been shown to occur for *CYC1* transcription (6). One hundred units of β-

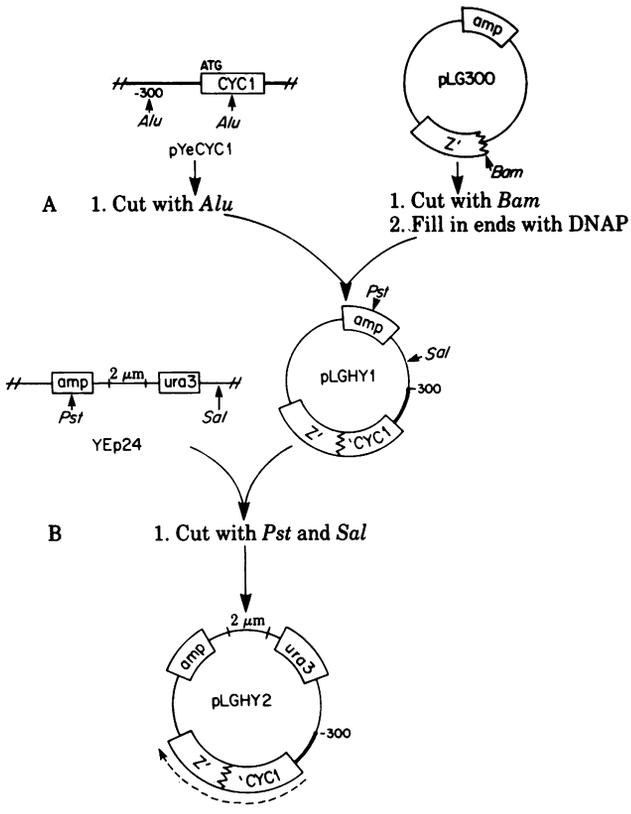

FIG. 2. (*A*) An *Alu* I fragment from pYe*CYC1* containing about 300 nucleotides to the 5′ side of *CYC1* (heavy line) and 47 codons at the 5′ end of *CYC1* (5) was ligated to pLG300 (8), which had been cut with *Bam*HI and rendered flush-ended with DNA polymerase (11). pYe*CYC1* bears a 2.5-kb fragment of yeast DNA containing *CYC1* inserted between the *Bam*HI and *Hin*dIII sites of pBR322 (5). pLG300 contains a single *Bam*HI cut, which exposes a large 3′-end region of *lacZ* (8). This *lacZ* 3′-end fragment contains information to encode active β-galactosidase if it is transcribed and translated. (This fragment actually contains a short region of *lacI* fused in frame to *lacZ*.) Joining the *Alu* I end that is 47 codons into *CYC1* to the filled-in *Bam*HI end creates a *CYC1-lacZ* in-frame hybrid gene preceded by about 300 nucleotides that naturally flank *CYC1*. *E. coli* strain LG90 (Δ*lac*) was transformed with the ligation mix and plated on plates containing the dye 5-bromo-4-chloro-3-indolyl-β-D-galactoside. Dark blue, pale blue, and white colonies appeared, whereas only white colonies were observed if the *Alu* I fragment had been omitted from the ligation mix. The dark blue clones bore plasmids containing the *Alu* I fragment inserted into pLG300 to create a *CYC1-lacZ* fused gene (pLGHY1), and the pale blue clones bore plasmids with the *Alu* I fragment inserted in the opposite orientation. Precise joining of the fragments was verified by the regeneration of the *Bam*HI restriction site. DNAP, DNA polymerase. (*B*) The fused gene was transferred to a plasmid YEp24 (14) bearing the yeast selectable marker *URA3* and the yeast 2-μm circle replicon. pLGHY1 was cut with *Pst* I and *Sal* I and transferred to a YEp24 backbone, which had been digested likewise. Joining of the *Pst* I ends of pLGHY1 and YEp24 regenerates the *amp* gene. This step requires a *Pst* I partial digest of YEp24 as two additional *Pst* I sites lie on the plasmid (one in *URA3* and one in the 2μm segment). The structure of the resulting plasmid, pLGHY2 was verified by restriction enzyme analysis. The arrow indicates the direction of transcription of the fused gene.

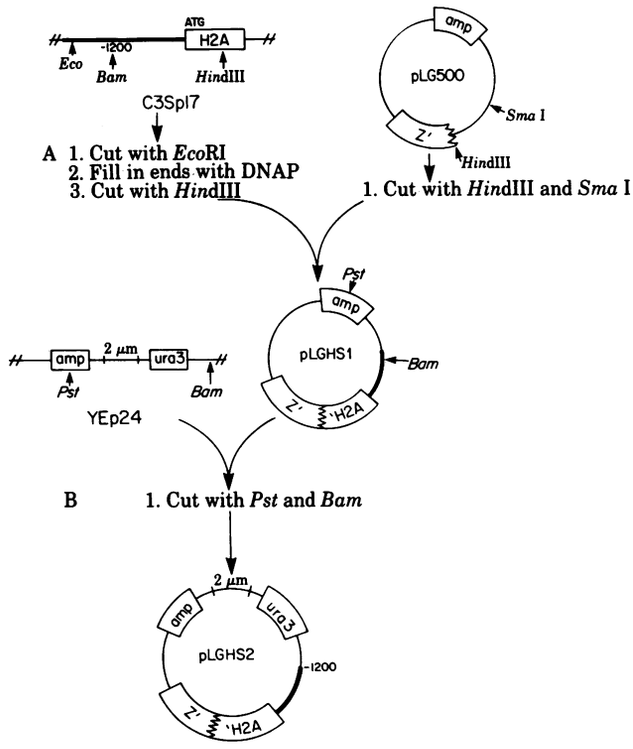

FIG. 3. (*A*) A C3Sp17 fragment bearing a filled-in *Eco*RI end and a *Hin*dIII end was inserted into a backbone derived from pLG500 that bore a *Sma* I (flush) end and a *Hin*dIII end to give pLGHS1. C3Sp17 bears a 1.9-kb *Eco*RI fragment containing the sea urchin histone genes *H3* and *H2A* in an *amp*^{R} backbone (15). The *Eco*RI–*Hin*dIII fragment derived from this plasmid carries 95 codons of *H2A* preceded by about 1700 bases of *S. purpuratus* DNA (heavy line). pLG500 bears a unique *Hin*dIII site that exposes a large 3′-end region of *lacZ*. Joining of the *Hin*dIII ends creates a *H2A-lacZ* hybrid gene with an approximately 1700-base pair region from *S. purpuratus* flanking the H2A gene to the 5′ side. Regeneration of the *Hin*dIII site in pLGHS2 verifies that the *Hin*dIII ends of C3Sp17 and pLG500 were joined precisely. DNAP, DNA polymerase. (*B*) This hybrid gene was transferred on a *Bam*HI–*Pst* I fragment to a YEp24 backbone with similar ends to yield pLGHS2.

galactosidase (if one assumes the same specific activity as that of the wild-type enzyme) corresponds to about 0.2% total cell protein (see Fig. 4 legend) and is comparable to the amount of cytochrome *c* typically found in *S. cerevisiae* (16). pLGHY2 directed levels of β-galactosidase of about 100 units in cells grown in glucose or raffinose.

β-Galactosidase activity in yeast colonies harboring either pLGHY2 or pLG669-Z could be monitored on plates containing the chromogenic substrate 5′bromo-4-chloro-3-indolyl-β-D-galactosidase (17). pLGHS2 failed to give rise to a detectable level of the enzyme regardless of the carbon source in the growth medium. Thus, it is likely that the upstream region of *S. purpuratus H2A* cannot function as a promoter in yeast. However, the possibility that the lack of activity of this hybrid is due to effects on mRNA stability or translatability has not been excluded.

A Deletion That Reduces Expression of a *CYC1-lacZ* Fused Gene in *S. cerevisiae*. An analysis of the *CYC1* promoter has been initiated using these *lacZ* gene fusions. Because the level of β-galactosidase directed by pLG669-Z was regulated by the carbon source in the growth medium, it seemed likely that the activity resulted from transcription initiation at the *CYC1* promoter. A deletion was constructed in pLG669-Z DNA extending from the *Xho* I site 250 nucleotides upstream from the start of the *CYC1* coding sequence to the *Xho* I site about 700 nucleotides upstream from the gene start (Figs. 2 and 4). This deletion lies entirely within a region of DNA that normally flanks *CYC1* in the yeast chromosome. The level of β-galactosidase directed in *S. cerevisiae* by the deletion derivative pLG670-Z was reduced to about 1/50th of that directed by pLG669-Z (Fig. 4). Furthermore, the basal level of expression in pLG670-Z was not regulated by the carbon source in the

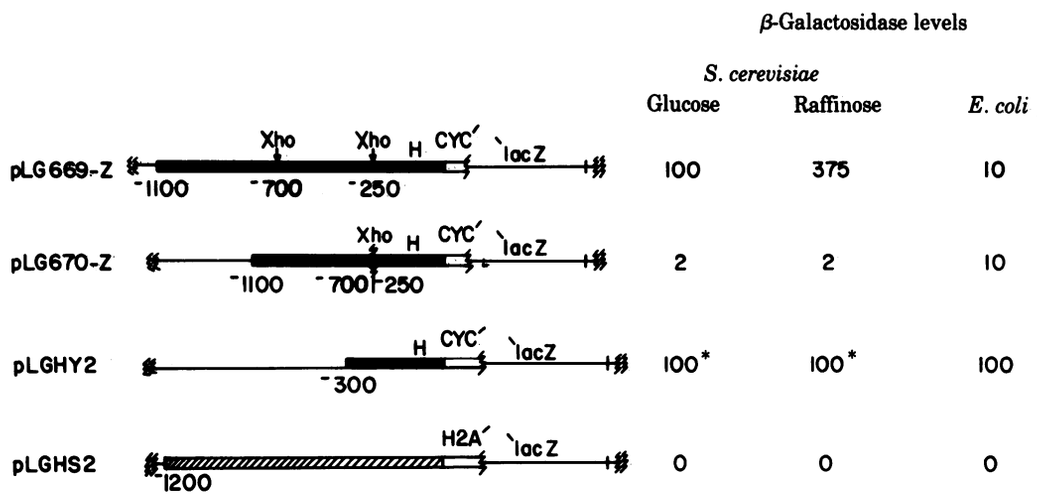

FIG. 4. The thick bars represent sequences from *S. cerevisiae* (for pLG669-Z, pLG670-Z, and pLGHY2) or *S. purpuratas* (pLGHS2). Dark regions are 5′ flanking sequences of *CYC1*, white regions are coding sequences for *CYC1* or *H2A*, and the hatched region is the 5′ flank of *H2A*. *S. cerevisiae* cells (DB745) were grown and assayed by method *ii*. *E. coli* cells (LG90) were grown in M-9 minimal media (9), supplemented with glucose and proline, and assayed as described (9). All units are expressed as described (9). The difference in β-galactosidase levels in *E. coli* between pLG669-Z and pLGHY2 may derive from a difference in translation initiation efficiency at the ATG codon at the start of *CYC1*.

* β-Galactosidase levels were also determined by method *i*. When so assayed, the extract contained about 600 units/mg of protein (1 unit = the amount of enzyme that hydrolyzes 10^{-9} mol of *o*-nitrophenol-β-D-galactoside per min at 28°C). Because pure β-galactosidase has a specific activity of 3×10^5 units/mg of protein (9), the amount of β-galactosidase in the cell extract is about 0.2% of total cell protein. This assumes that the chimeric protein has the same specific activity as wild-type. When assayed in this way, a given number of cells displayed the same *o*-nitrophenol-β-D-galactoside-hydrolyzing activity as when assayed by method *ii*. Thus, the 100 units of activity determined by method *ii* corresponds to an amount of enzyme that is roughly 0.2% of total cell protein.

growth medium. The deletion has no effect on the levels of β-galactosidase made in *E. coli* (Fig. 4).

DISCUSSION

The construction of two hybrid genes between *S. cerevisiae CYC1* and *E. coli lacZ* has been described. These genes are carried on plasmids that replicate autonomously in *S. cerevisiae* and *E. coli*. Both plasmids direct the synthesis of active β-galactosidase in *S. cerevisiae*.

The first of these hybrid genes, carried on pLG669-Z, contains one codon of *CYC1* and about 1100 nucleotides that normally precede *CYC1* in the yeast chromosome (Figs. 1 and 4). We believe that this β-galactosidase expression results from transcription initiation at the *CYC1* promoter for the following reasons. β-Galactosidase synthesis was not elicited by fusion of *lacZ* to a DNA fragment encoding the amino terminus of the histone *H2A* of the sea urchin *S. purpuratus*. Moreover, the levels of β-galactosidase directed by pLG669-Z are repressed by glucose (Fig. 4), which is typically seen for cytochrome *c*. The possibility that this repression is due to a change in plasmid copy number has not explicitly been eliminated. This seems unlikely, however, because other plasmids that direct the synthesis of β-galactosidase in yeast do not show the repression (see below). Because pLG669-Z contains only the first four nucleotides of *CYC1*, it appears that the *CYC1* promoter lies entirely to the 5′ side of the gene (unless a sequence distal to the fusion point fortuitously replaces an essential element of the promoter).

The second hybrid gene, carried on pLGHY2, directs levels of β-galactosidase comparable to those directed by pLG669-Z in glucose-grown cells but fails to direct elevated levels of the enzyme in raffinose-grown cells (Fig. 4). One way in which this plasmid differs from pLG669-Z is that yeast flanking DNA includes only the 300 base pairs immediately preceding *CYC1* (as opposed to 1100 base pairs in pLG669-Z) (Fig. 2). It is possible that a region greater than 300 nucleotides upstream from *CYC1* is responsible for the stimulation of transcription in raffinose-grown cells, whereas the unstimulated promoter lies within 300 nucleotides of the gene. A second possibility is that the observed β-galactosidase activity is due to the presence of a large region of *CYC1* (47 codons). This coding sequence also could specify a promoter that is not repressed by glucose. A third possibility is that the abutting of *E. coli* sequences 300 base pairs from *CYC1* may inactivate the *CYC1* promoter and introduce a sequence in *E. coli* DNA that fortuitously functions as a promoter in yeast and gives rise to the observed β-galactosidase activity.

An analysis of the *CYC1* promoter has been initiated utilizing pLG669-Z. A 450-base pair deletion lying entirely within the 1100-base pair region that naturally flanks *CYC1* abolishes promoter activity (2% of pLG669-Z levels). This result suggests that one element of the *CYC1* promoter lies within the region covered by this deletion: the segment of DNA 250–700 base pairs upstream from the start of the *CYC1* coding sequence. This element is at least 130 base pairs upstream from the "Hogness (T-A-T-A) box"* consensus sequence, which is about 120 nucleotides to the 5′ side of the start of the *CYC1* coding sequence (5). The possibility is raised that two components of the *CYC1* promoter are separated by several hundred base pairs in the yeast chromosome. Because the 450-base pair deletion exerts no effect on the levels of β-galactosidase directed by the plasmid in *E. coli*, the *CYC1* promoter is probably at least in part different from the promoter that functions in *E. coli*.

The plasmid that contains the 450-base pair deletion, pLG670-Z, is a general vector for constructing precise gene fusions to *lacZ* for analysis in *S. cerevisiae*. It contains a yeast marker (*URA3*) and origin of replication (from the 2-μm plasmid circle) as well as an *E. coli* marker (*amp*) and origin of replication (from pBR322). Moreover, this plasmid carries a *lacZ* gene that

* Hogness box (T-A-T-A box) is a consensus sequence found upstream from several eukaryotic genes which is hypothesized to be an element of an RNA polymerase II promoter.

is missing a functional promoter in yeast, and a unique *Bam* restriction site into which gene fragments can be cloned and directly fused to *lacZ*.

The *in vitro lacZ* fusion approach described here and the *in vivo* approach described in the accompanying paper by Rose *et al.* (18) will expedite a more detailed analysis of *CYC1* transcriptional control. Mutagenesis can be directed to a small region of interest (i.e., a region to the 5′ side of a gene). After transformation of *S. cerevisiae* by mutagenized DNA, plates containing the dye 5-bromo-4-chloro-3-indolyl-β-D-galactoside can be used to identify plasmids with mutations in the *CYC1* promoter. Plasmids of interest can then be transferrred to *E. coli* for structural or sequence analysis. Also, the fusion is an aid in the search for mutations unlinked to *CYC1* that affect *CYC1* regulation. This search is complicated by mutations that alter cofactor (heme) biosynthesis or attachment if cytochrome *c* is the assayed product.

Note Added in Proof. Faye *et al.* (19) have found that transcription of *CYC1* begins at multiple sites downstream from the "Hogness box" referred to above. The most proximal site is about 30 base pairs downstream. This means that the promoter element we have identified is at least 160 base pairs upstream from the nearest transcriptional start.

We are grateful to B. Hall for providing the *CYC1* clone, to D. Leung, D. Montgomery, and M. Smith for providing restriction enzyme data, to D. Botstein for providing YEp24 and DB745, and to M. Rose, M. Casadaban, and D. Botstein for making their results available prior to publication. We also thank A. Johnson and R. Yocum for comments on the manuscript. L.G. is a Postdoctoral Fellow of the Jane Coffin Childs Memorial Fund for Medical Research. This research was supported by grants from the National Science Foundation and the National Institutes of Health to M.P.

1. Bassford, P., Beckwith, J., Berman, M., Brickman, E., Casadaban, M., Guarente, L., Saint-Girous, I., Sarthy, A., Schwartz, M., Shuman, H. & Silhavy, T. (1978) in *The Operon*, eds. Miller, J. H. & Reznikoff, W. S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 245–261.
2. Berman, M. & Beckwith, J. (1979) *J. Mol. Biol.* **130,** 303–315.
3. Ptashne, M., Jeffrey, A., Johnson, A. D., Maurer, R., Meyer, B. J., Pabo, C. O., Roberts, T. M. & Sauer, R. T. (1980) *Cell* **19,** 1–11.
4. Hinnen, A., Hicks, J. & Fink, J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1929–1933.
5. Smith, M., Leung, D., Gillam, S., Astell, C., Montgomery, D. & Hall, B. (1979) *Cell* **16,** 753–761.
6. Zitomer, R., Montgomery, D., Nichols, D. & Hall, B. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3627–3631.
7. Sherman, F., Stewart, J. & Schweingruber, A. (1980) *Cell* **20,** 215–222.
8. Guarente, L., Lauer, G., Roberts, T. M. & Ptashne, M. (1980) *Cell* **20,** 543–553.
9. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 352–355.
10. Bradford, M. (1976) *Anal. Biochem.* **72,** 248–254.
11. Backman, K., Ptashne, M. & Gilbert, W. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4174–4178.
12. Sheller, R., Dickerson, R., Boyer, H., Riggs, H. & Itakura, K. (1977) *Science* **196,** 177–180.
13. Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
14. Botstein, D., Falco, C., Stewart, S., Brennan, M., Scherer, S., Stinchomb, D., Struhl, K. & Davis, R. (1979) *Gene* **8,** 17–24.
15. Sures, I., Lowry, J. & Kedes, L. (1978) *Cell* **15,** 1033–1043.
16. Sherman, F. & Stewart, J. (1971) *Annu. Rev. Genet.* **5,** 257–296.
17. Davies, J. & Jacob, F. (1968) *J. Mol. Biol.* **36,** 413.
18. Rose, M., Casadaban, M. & Botstein, D. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2460–2464.
19. Faye, G., Leung, D. W., Tatchell, K., Hall, B. D. & Smith, M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2258–2262.