MOLECULAR AND CELLULAR BIOLOGY, June 1988, p. 2275–2279
0270-7306/88/062275-05$02.00/0

Vol. 8, No. 6

Oxygen-Dependent Upstream Activation Sites of *Saccharomyces cerevisiae* Cytochrome *c* Genes Are Related Forms of the Same Sequence

M. ESPERANZA CERDAN† AND RICHARD S. ZITOMER*

Department of Biological Sciences, State University of New York at Albany, Albany, New York 12222

Received 21 December 1987/Accepted 9 February 1988

In *Saccharomyces cerevisiae*, the two genes, *CYC1* and *CYC7*, that encode the isoforms of cytochrome *c* are expressed at different levels. Oxygen regulation is mediated by the expression of the *CYP1* gene, and the *CYP1* protein interacts with both *CYC1* upstream activation sequence 1 (UAS1) and *CYC7* UAS_o. In this study, the homology between the *CYP1*-binding sites of both genes was investigated. The most noticeable difference between the *CYC1* and *CYC7* UASs is the presence of GC base pairs at the same positions in a repeated sequence in *CYC7* compared with CG base pairs in *CYC1*. Directed mutagenesis changing these GC residues to CG residues in *CYC7* led to *CYC1*-like expression of *CYC7* both in a *CYP1* wild-type strain and in a strain carrying the semidominant mutation *CYP1-16* which reverses the oxygen-dependent expression of the two genes. Our results strongly support the hypothesis that the *CYP1*-binding sites in *CYC1* and *CYC7* are related forms of the same sequence and that the *CYP1-16* protein has altered specificity for the variant forms of the consensus sequences in both genes.

In *Saccharomyces cerevisiae*, the expression of the two nuclear genes, *CYC1* and *CYC7*, encoding the iso-1 and iso-2 cytochrome *c* proteins, respectively, is transcriptionally activated in the presence of oxygen (8, 13, 28). This activation occurs through the interaction of the *CYP1* (*HAP1*) protein with sequences upstream of each gene (12, 15, 16, 22, 23, 28). However, despite sharing a common regulatory factor, under aerobic conditions the transcription of *CYC1* is much higher than that of *CYC7* (20, 28). While part of this difference can be explained by the presence of other regulatory sequences which affect gene expression, such as upstream activation sequence 2 (UAS2) in *CYC1* (7) and the negative site in *CYC7* (24, 25), it is clear that the *CYP1* protein interacts differently with its target sites upstream from the two genes. This point is most dramatically demonstrated by the isolation of semidominant mutations in the *CYP1* gene, such as *CYP1-16* and *CYP1-18*, which reverse the levels of expression of the two genes (4, 5). In the mutant background, *CYC1* is expressed at low levels aerobically, while *CYC7* is expressed at high levels. We and others have proposed that this reciprocal change in gene expression is due to an altered DNA-binding specificity in the *CYP1* mutant proteins; the wild-type protein binds the *CYC1* target sequence well and the *CYC7* target sequence poorly, while the *CYP1-16* mutant protein binds *CYC7* well and *CYC1* poorly. This proposal assumes that the *CYC1*- and *CYC7*-binding sites share a similar sequence, such that a small change in the *CYP1* protein could cause a reciprocal change in its interaction with the two genes. Zitomer et al. have proposed such homology between the two sites and indicated how the *CYC7* site differed from that in *CYC1* (28). Recently, Pfeifer et al. (16) proposed that the *CYC1*- and *CYC7*-binding sites were so different that they could not be related forms of the same sequence, so that some novel mechanism must operate to enable the same protein to recognize these two sites.

In this report, we attempt to distinguish between these two hypotheses. We created single-base-pair changes in each copy of a repeated sequence in *CYC7* that we previously suggested would cause sufficient increased homology between the *CYC1* and *CYC7* sites as to result in *CYC7* expression mimicking that of *CYC1*: high aerobic expression in *CYP1* cells and low expression in *CYP1-16* cells. Our results clearly support the hypothesis that the two sites are variant forms of a single consensus sequence.

MATERIALS AND METHODS

Strains. The *Escherichia coli* strains used for the transformation and maintenance of plasmids were HB101 (2) and MC1061 (3). MC1061 is a *lacZ* mutant enabling *lacZ* expression of gene fusions to be screened. The *ung* mutant BW313 was used for in vitro mutagenesis (11). JM101 was used for the propagation of the M13um20-derived plasmids (26). Transformation of bacterial cells was done by the method of Hanahan (9).

The *S. cerevisiae* strains used in these studies, ZW13 and ZW10, were previously described and carry the *CYP1* and *CYP1-16* alleles, respectively (28). Transformations of these strains were done by the frozen-cell method (10), selecting in every case for the Trp^+ phenotype.

Media and cell growth. Yeast cells were grown under nonselective conditions in YPD (2% peptone, 1% yeast extract, 2% glucose). Tryptophan phenotypes were determined on complete plates minus tryptophan (27), and cytochrome *c* phenotypes were determined on glycerol plates (2% peptone, 1% yeast extract, 3% glycerol, 1.5% agar) or lactic acid plates (21). X—gal (5-bromo-4-chloro-3-indoyl-β-galactoside) plates (17) were used for screening *lacZ* expression of gene fusions.

For β-galactosidase assays, cells were diluted 100- to 500-fold from overnight cultures into 15 ml of YPD and grown either aerobically with vigorous shaking or anaerobically in flasks packed into sealed jars containing a GasPak

* Corresponding author.
† Permanent address: Departamento de Bioquimica y Biologia Molecular, Colegio Universitario de la Coruna, La Coruna, Spain.

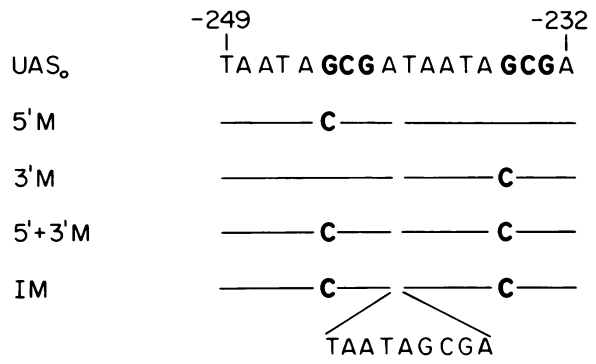

FIG. 1. Summary of the mutations at the UAS_o from *CYC7*. Boldface print represents the important residues implied in the proposed consensus sequence in the coding strand of UAS_o. In the other lines, only point mutations or integrated sequences are printed.

anaerobic system (BBL Microbiology Systems, Cockeysville, Md.). For anaerobic growth, the medium was supplemented with 20 μg of ergosterol per ml and 0.2% Tween 80 (required for sterol synthesis in the absence of oxygen). Cells were harvested at a mid-log density of A_{600} between 0.6 and 1.0.

Plasmids. The centromeric *TRP1-ARS1* plasmids YCp*CYC1*(2.4) and YCp*CYC7*(2)r containing the *CYC1* and *CYC7* genes, respectively, were described previously (13, 24). Δ41 is a derivative of YCp*CYC7*(2)r containing a 41-base-pair deletion in the *Bam*HI-*Xho*I fragment carrying the upstream regulatory region of *CYC7* (24). B1927Z is also a derivative of YCp*CYC7*(2)r which contains a deletion from −192 to −715 in the *CYC7* upstream region with a *Bam*HI site present at the site of the deletion and a *lacZ* gene inserted in frame into the coding sequence of *CYC7* (25). The bacteriophage vector M13um20, a derivative of M13mp18, was purchased from International Biotechnologies, Inc. (New Haven, Conn.).

Plasmid constructions. (i) Phage templates for mutagenesis. The *Xho*I-*Bam*HI fragment from YCp*CYC7*(2)r containing the upstream regulatory region of *CYC7* from −142 to −715 was subcloned into the polylinker region of M13um20 phage. After transfection of JM101 cells, clear plaques were picked and phage containing the right insert were identified by restriction analysis.

(ii) *CYC7* UAS mutations in YCp*CYC7*(2)r. After in vitro mutagenesis, the replicative forms of the mutant phage were digested with *Bam*HI and *Xho*I, and the fragment containing the mutation (−142 to −715) was excised from an agarose gel, purified with Gene-Clean (Bio 101), and ligated into the Δ41 vector, which had been digested with *Bam*HI and *Xho*I. After transformation of HB101 cells, the desired constructs were identified by restriction analysis. Δ41 was used because the *Xho*I-*Bam*HI upstream region of this plasmid was easily differentiated from that of the fragment-containing mutations.

(iii) *lacZ* fusions. The 4-kilobase *Xba*I-*Xho*I fragment of B1927Z containing the *CYC7-lacZ* fusion was gel purified and ligated to the 7-kilobase *Xba*I-*Xho*I fragment from each of the YCp*CYC7*(2)r derivatives containing the mutant upstream regulatory region of *CYC7*. MC1061 transformants were selected on ampicillin–X-gal plates, blue colonies were picked, and the desired plasmids were identified by restriction analysis.

In vitro mutagenesis. Site-specific mutagenesis was done with a uracil-containing template prepared by the method previously described by Kunkel (11). Two oligonucleotides were used. In the first, 5′-TATTATCGGTATTAGC-3′, the guanine in position 9 represented a mismatch at nucleotide −244 in the 5′ repeat. In the second, 5′-CCCTCGGTATTATCG-3′, the guanine in position 7 represented an identical mismatch with nucleotide −235 in the 3′ repeat. After phosphorylation by T4 polynucleotide kinase, the oligonucleotides were annealed to 0.5 μg of the uracil template and extended with T4 polymerase in the presence of 25 μg of gene 32 protein per ml and 2 U of T4 DNA ligase per ml. The newly synthesized double-stranded DNA was used to transform JM101 cells.

Differential plaque hybridization. Plaque hybridization was done as previously described (1) with the [γ-^{32}P]ATP-end-labeled synthetic oligonucleotides as specific probes for the mutants. Plaques giving a strong signal after the hybridization were selected for sequence analysis to confirm the presence of the mutation. For the construction of the double mutant, the 3′ mutant (at −235) was used as a template for mutagenesis with the 5′ synthetic oligonucleotide.

DNA sequence analysis. DNA sequence analyses were done by the dideoxy chain termination method of Sanger et al. (18). Universal primer was purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.).

β-Galactosidase assays. β-Galactosidase assays were performed on permeabilized yeast cells and units of enzyme activity were calculated as described previously (6).

Materials. Restriction enzymes were purchased from either Boehringer Mannheim Biochemicals or New England BioLabs, Inc. (Beverly, Mass.). T4 DNA polymerase, T4 DNA ligase, and the Klenow fragment of DNA polymerase were purchased from Boehringer Mannheim Biochemicals. Gene 32 protein was purchased from Bio-Rad Laboratories (Richmond, Calif.). All enzymes were used under the conditions recommended by the vendor. The oligonucleotides were purchased from Nadrian Seeman in this department.

Nomenclature. The base pairs of the *CYC1* and *CYC7* genes are numbered with the initiation of translation as the reference point. The A residue of the initiation codon in the coding strand is base 1, and bases 3′ to the coding sequence are numbered consecutively in negative integers.

We referred to the *CYP1-HAP1* locus as *CYP1* and its gene product as the *CYP1* protein because we used mutant alleles from the French group that uses the *CYP1* designation (4, 5).

We previously referred to the *CYC7* region that interacts with the *CYP1* protein as the positive site. Here we will refer to it as UAS_o to differentiate it from the negative site which has activation activity under anaerobic conditions (28).

RESULTS

The UAS_o region of *CYC7* contains a 9-base-pair direct repeat composed of the sequence TAATAGCGA. Mutations in the CG base pair at position 7 or in the GC base pair at position 8 in either repeat cause a decrease in *CYC7* expression (28). This region is also protected by the *CYP1* protein in crude extract binding assays (16). A comparison of this region to the *CYP1*-protected regions of the *CYC1* gene indicated a number of similarities with the striking difference being that there are CG residues at position 6 in the two copies in *CYC1*, whereas *CYC7* has a GC at the equivalent positions (see Fig. 1 for repeats and Fig. 2 for comparison). To test the importance of this GC in the *CYP1* protein-DNA interaction, we constructed a set of point mutations in the

CYC7 upstream region using site-directed mutagenesis. Two different synthetic oligonucleotides were used to introduce GC-to-CG transversions in position 3 of each repeat. These mutations were designated 3'M and 5'M for those in the 3' and 5' repeat, respectively. A double mutant, 5'+3'M, containing the two GC-to-CG changes in both repeats was also constructed with 3'M and the other oligonucleotide for the mutagenesis. In the course of this latter construction, presumably as a result of loop formation during the hybridization of the oligonucleotide to the template, an insertion mutant was obtained. Designated IM, this mutant contained three copies of the 9-base-pair repeat; two mutant copies of the repeat interrupted by a wild-type copy (Fig. 1).

Phenotypic expression in mutants and effect of the *CYP1-16* mutation. The mutagenized UAS_o regions were inserted upstream of the *CYC7* coding sequence, and the plasmids obtained were used to transform two strains of *S. cerevisiae*, ZW13 and ZW10. Both strains are cytochrome *c* deficient and so unable to grow on nonfermentable energy sources such as glycerol or lactate. ZW13 cells transformed with a wild-type *CYC7*-containing plasmid were capable of growth on glycerol but only very limited growth on lactate owing to the relatively low level of expression of *CYC7*. ZW10 cells carry the *CYP1-16* mutation which results in increased expression of the *CYC7* gene. Thus, ZW10 cells transformed with the wild-type plasmid grew well on either glycerol or lactic acid.

The mutations in UAS_o all caused increased *CYC7* expression in *CYP1* wild-type cells and decreased expression in *CYP1-16* cells. All the mutations increased the ability of ZW13 to grow on lactate compared with the same cells transformed with the wild-type *CYC7* plasmid (Table 1). This increase was greater for the double mutant than for the single mutants; the IM mutant behaved in a way similar to the single mutants. On the other hand, the mutations caused a decrease in the ability of ZW10 to grow on lactate, with the most dramatic effect caused by the double mutation. These results clearly demonstrated that the GC in position 6 of the 9-base-pair repeat plays an important role in the low levels of expression of *CYC7* in a wild-type *CYP1* background. When this GC base pair was substituted with a CG base pair, making the UAS_o region of *CYC7* more homologous to the UAS1 region of *CYC1*, the result was an increase in *CYC7* expression. These results also showed that the GC base pair

FIG. 2. DNA sequences that interact with *CYP1*. The sequences of the upstream regions of *CYC1* UAS1 A from −364 to −342 (the coding strand) and UAS1 B from −316 to −338 (the noncoding strand) and *CYC7* UAS_o from −251 to −229 (the coding strand) are compared. The arrows designate the direction toward the coding sequence, and the number in parentheses represents the number of the leftmost base. An asterisk above a residue signifies that a mutation was isolated at that residue which caused a decrease in gene expression (12, 28). Boxed areas represent DNA sequences protected from DNase I by a *CYP1* complex (15, 16). Those residues in boldface print represent the positions of apparent protein contacts as determined by methylation interference (15, 16). A cytosine or thymine in boldface print means that when the complementary guanine or adenine was methylated the protein complex did not form.

TABLE 1. Phenotypes of UAS_o mutations

CYC7 plasmid	Growth on lactate[a]	
	ZW13 (*CYP1*)	ZW10 (*CYP1-16*)
Wild type	−	+++
3'M	+	+
5'M	+	+
5'+3'M	++	−
IM	+	+

[a] −, No growth; +, ++, +++, varying colony size with the largest colony size represented by +++.

at this position is important for the recognition of this region by the *CYP1-16* protein.

Quantitation of gene expression in mutants and oxygen regulation. To quantitate the levels of *CYC7* expression from these mutants, we constructed a series of *CYC7-lacZ* fusions. The ZW13 and ZW10 strains were transformed with the plasmids YCp7Z and YCp1Z carrying the wild-type *CYC7-lacZ* and *CYC1-lacZ* fusion, respectively, as well as with fusion plasmids carrying the mutant UAS_o. The expression and regulation of the fusion genes in yeast cells transformed with these plasmids are summarized in Table 2 and generally confirm the results of phenotypic expression described in the previous section. Under aerobic conditions, in the *CYP1* wild-type background, the UAS mutations caused an increase of three- to fivefold in *CYC7* expression, indicating that the *CYP1* protein interacted better with the CG at position 6 versus the wild-type GC. In the *CYP1-16* background, the mutations in the UAS sequence of *CYC7* caused a great decrease in gene expression. The double mutant 5'+3'M resulted in a 20-fold decrease, making the level of expression of *CYC7* quite similar to that of *CYC1* in this strain. A five- to eightfold decrease was observed for the single mutants. For the insertion mutation, which contained a wild-type repeat flanked by two mutant repeats, the level of *CYC7* expression was slightly higher than that for the single mutants and nearly fivefold higher than in the double 5'+3'M mutant.

Under anaerobic conditions, there were no significant variations in the levels of *CYC7* expression in the mutants as was expected from the similar, low expression of *CYC7* and *CYC1* in both strains.

DISCUSSION

The two genes that encode the isoforms of cytochrome *c* in *S. cerevisiae* respond to the presence of oxygen in a regulatory process that has proven to be heme dependent (8,

TABLE 2. Quantitation of the effect of the UAS_o mutations

Plasmid	β-Galactosidase activity[a]			
	ZW13		ZW10	
	$+O_2$	$-O_2$	$+O_2$	$-O_2$
YCp7Z	1.1	0.32	24.0	0.22
YCp1Z	18.0	0.33	1.8	0.24
3'M	3.1	0.42	3.3	0.25
5'M	3.4	0.34	5.4	0.34
5'+3'M	4.8	0.41	1.4	0.30
IM	5.0	0.47	6.0	0.35

[a] Units of β-galactosidase activity were determined as described previously (6).

14) and is mediated by a *trans*-acting factor encoded by the *CYP1* gene (7, 22, 23, 28). It has been shown that *CYP1* protein is part of a complex that interacts with both the UAS1 region of *CYC1* and the UAS_0 region of *CYC7* (15, 16). There are two alternative hypotheses concerning the interaction of the protein with these two sites. In one, proposed by us and others, the binding sites in *CYC1* and *CYC7* share sequence or spatial homology or both, that is, the *CYP1*-binding sites represent variant forms of the same sequence. For the other, it has been suggested that the protein must recognize sites without apparent or extensive homology (16). We believe that the data reported here support the conclusion that these two sites are homologous and are viewed in a similar fashion by the protein.

We started these experiments with the premise that the major difference between the *CYC1* and *CYC7* UASs was the presence of GC base pairs at the same positions in a repeated sequence in *CYC7* compared with CG base pairs in *CYC1* (Fig. 2). A corollary to this hypothesis was that the mutation to *CYP1-16* led to an altered protein that either accommodated to the GC residues or actually used them as an important contact, thus causing increased *CYC7* and decreased *CYC1* expression. Our results support this hypothesis. The change of these GC residues to CG residues in *CYC7* led to increased expression in a *CYP1* background and decreased expression in a *CYP1-16* background. The effect, although qualitatively reciprocal, was not quantitatively reciprocal; the mutated *CYC7* gene was not expressed at a level identical to that of *CYC1* in a *CYP1-16* background. This was not surprising given the difference in other regulatory elements between the two genes, such as the presence of the negative site in *CYC7*.

In Fig. 2, the *CYC1*-UAS1 A and B sites are aligned with the *CYC7* UAS_0 with respect to the GC-CG residues. All the data relevant to *CYP1* binding or biological activity of these sequences are included. The overall pattern of residues sensitive to dimethyl sulfate inhibition of *CYP1* binding (bold letters) and mutable to loss of expression (asterisks) among the three sites is quite similar. Where they differ, as in the A+T-rich region of residues −331 to −226 in UAS1 B, −251 to −254 in UAS1 A, and −238 to −241 in the *CYC7* UAS_0, the dimethyl sulfate suggests that the protein binds to the minor groove where a distinction is not made between AT and TA base pairs (19). This functional and structural homology combined with the ability to convert *CYC7* into a *CYC1*-like gene by increasing its homology to *CYC1* with only a single-base-pair change in each copy of the repeat makes the invocation of novel DNA-protein interactions unnecessary to explain the binding to both *CYC1* and *CYC7*.

An additional feature of the protein-DNA interaction can be obtained from the data. The insertion mutant, IM, showed similar effects on gene expression as the single mutants. It can be inferred from these results that the protein binds to a dimer sequence cooperatively with spatial constraints resulting in the inability of the wild-type protein to take advantage of the presence of two mutant repeats when interrupted by the wild-type repeat.

The question remains as to whether the UAS1 A binds the *CYP1* protein. It is possible that this region binds *CYP1* more weakly than the other sites resulting in poor protection; the protein must be purified to resolve this question. Also, a discrepancy remains between our results suggesting that the *CYP1* protein binds less well to the *CYC7* UAS_0 and those of Pfeifer et al. (16) who, in competition experiments, found that *CYC1* and *CYC7* UASs competed equally well for *CYP1* binding. However, these results are inconclusive given that a crude extract was used and equilibrium conditions were not demonstrated.

In summary, we believe that the *CYP1* protein binds variant forms of the same sequence in the *CYC1* and *CYC7* genes and that the *CYP1-16* protein has altered specificity such that it recognizes or accommodates the GC residues present in the *CYC7* repeats.

ACKNOWLEDGMENTS

This study was supported by a Public Health Service grant from the National Institutes of Health. M.E.C. was supported by a fellowship from the Xunta de Galicia, Spain.

LITERATURE CITED

1. **Benton, W. D., and R. W. Davis.** 1977. Screening λgt recombinant clones by hybridization to single plaques in situ. Science **196**:180–182.
2. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **4**:459–472.
3. **Casadaban, M. J., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. **138**:179–207.
4. **Clavilier, L., G. Péré-Aubert, and P. P. Slonimski.** 1969. Mise en évidence de plusieurs loci indépendants impliqués dans la synthèse de l'iso-2-cytochrome c chez la levure. Mol. Gen. Genet. **104**:195–218.
5. **Clavalier, L., G. Péré-Aubert, M. Somlo, and P. P. Slonimski.** 1976. Réseau d'interactions entre des gènes non liés: régulation synergique ou antagoniste de la synthèse de l'iso-1-cytochrome c, de l'iso-2-cytochrome c et du cytochrome b_2. Biochimie **58**:155–172.
6. **Guarente, L.** 1983. Yeast promoters and *lacZ* fusion designed to study expression of cloned genes in yeast. Methods Enzymol. **101**:181–191.
7. **Guarente, L., B. Lalonde, P. Gifford, and E. Alani.** 1984. Distinctly regulated tandem upstream activation sites mediate catabolite repression of the *CYC1* gene of *S. cerevisiae*. Cell **36**:503–511.
8. **Guarente, L., and T. Mason.** 1983. Heme regulates the transcription of the *CYC1* gene of *S. cerevisiae* via an upstream activation site. Cell **32**:1279–1286.
9. **Hanahan, H.** 1983. Studies on the transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166**:557–580.
10. **Klebe, R. J., J. V. Harriss, Z. D. Sharp, and M. G. Douglas.** 1983. A general method for polyethylene-glycol-induced genetic transformation of bacteria and yeast. Gene **25**:333–341.
11. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82**:488–492.
12. **Lalonde, B., B. Arcangioli, and L. Guarente.** 1986. A single *Saccharomyces cerevisiae* upstream activation site (UAS1) has two distinct regions essential for its activity. Mol. Cell. Biol. **6**:4690–4696.
13. **Lowry, C. V., J. L. Weiss, D. A. Walthall, and R. S. Zitomer.** 1983. Modulator sequences mediate the oxygen regulation of *CYC1* and a neighboring gene in yeast. Proc. Natl. Acad. Sci. USA **80**:151–155.
14. **Lowry, C. V., and R. S. Zitomer.** 1984. Oxygen regulation of aerobic and anaerobic genes mediated by a common regulatory factor in yeast. Proc. Natl. Acad. Sci. USA **81**:6129–6133.
15. **Pfeifer, K., B. Arcangioli, and L. Guarente.** 1987. Yeast *HAP1* activator competes with the factor RC2 for binding to the upstream activation site UAS1 of the *CYC1* gene. Cell **49**:9–18.
16. **Pfeifer, K., T. Prezant, and L. Guarente.** 1987. Yeast *HAP1* activator binds to two upstream activation sites of different sequence. Cell **49**:19–27.
17. **Rose, M., and D. Botstein.** 1983. Construction and use of gene fusions to *lacZ* (β-galactosidase) that are expressed in yeast. Methods Enzymol. **101**:167–180.
18. **Sanger, F., S. Nicklen, and S. A. Coulson.** 1977. DNA sequenc-

ing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.

19. **Seeman, N. C., J. M. Rosenberg, and A. Rich.** 1976. Sequence-specific recognition of double helical nucleic acids by proteins. Proc. Natl. Acad. Sci. USA **73**:804–808.
20. **Sherman, F., and J. W. Stewart.** 1971. Genetics and biosynthesis of cytochrome c. Annu. Rev. Genet. **5**:257–296.
21. **Sherman, F., J. W. Stewart, E. Margoliash, J. Parker, and W. Campbell.** 1966. The structural gene for yeast cytochrome c. Proc. Natl. Acad. Sci. USA **55**:1498–1504.
22. **Verdiere, J., F. Creusot, and L. Guarente.** 1986. The overproducing *CYP1* mutation and the underproducing *hap1* mutations are alleles of the same gene which regulates in *trans* the expression of the structural genes encoding iso-cytochromes c. Curr. Genet. **10**:339–342.
23. **Verdiere, J., F. Creusot, and M. Guerineau.** 1985. Regulation of the expression of iso 2-cytochrome c gene in *S. cerevisiae*: cloning of the positive regulatory gene *CYP1* and identification of the region of its target sequence on the structural gene *CYP3*. Mol. Gen. Genet. **199**:524–533.
24. **Wright, C. F., and R. S. Zitomer.** 1984. A positive regulatory site and a negative regulatory site control the expression of the *Saccharomyces cerevisiae CYC7* gene. Mol. Cell. Biol. **4**:2023–2030.
25. **Wright, C. F., and R. S. Zitomer.** 1985. Point mutations implicate repeated sequences as essential elements of the *CYC7* negative upstream site in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **5**:2951–2958.
26. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. New M13 host strains and the complete sequences of M13mp and pUC vectors. Gene **33**:103–119.
27. **Zitomer, R. S., and B. D. Hall.** 1976. Yeast cytochrome c mRNA. J. Biol. Chem. **251**:6320–6326.
28. **Zitomer, R. S., J. W. Sellers, D. W. McCarter, G. A. Hastings, P. Wick, and C. V. Lowry.** 1987. Elements involved in oxygen regulation of the *Saccharomyces cerevisiae CYC7* gene. Mol. Cell. Biol. **7**:2212–2220.