SUC Genes of Yeast: A Dispersed Gene Family

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The *SUC* genes of yeast (*Saccharomyces*) genetically appear to constitute a family of repeated genes that are dispersed in the yeast genome. Each SUC^+ gene confers upon the strains carrying it the ability to produce invertase, a primarily extracellular and glycosylated enzyme that cleaves sucrose to yield fructose and glucose. Thus, strains carrying a SUC^+ allele can ferment sucrose. An unusual feature of this dispersed gene family is that different *Saccharomyces* strains (or species) have SUC^+ alleles at different chromosomal loci; to date, six (possibly seven) unlinked *SUC* loci have been identified (Table 1) (reviewed by Mortimer and Hawthorne 1969). Any individual haploid strain of yeast may have zero, one, or several SUC^+ alleles. Thus, the number and location of SUC^+ genes is variable. Although the *MAL* genes and *MGL* genes of yeast (responsible for fermentation of maltose and alpha-methylglucoside, respectively) show similar variability (Mortimer and Hawthorne 1969), most known genes specifying metabolic functions in yeast appear to occupy a constant position on the yeast genetic map, and most such genes appear to occupy the same relative map positions in different *Saccharomyces* strains.

Since a given strain usually does not have SUC^+ genes at all six (or seven) loci at which SUC^+ genes have been found, we set out to investigate the naturally occurring negative alleles at *SUC* loci not containing an active SUC^+ gene. The notation *suc*° is used to denote such naturally occurring negative alleles in order to distinguish them from negative mutations (mutagen-induced or spontaneous) derived from an active SUC^+ gene in the laboratory.

Two models representing opposite extremes can be envisioned for the structure of *suc*° alleles, as illustrated in Figure 1. A *SUC* locus containing a *suc*° allele could contain no DNA related to an active SUC^+ gene (Fig. 1c) or it could contain a "silent" *SUC* gene, one that either is not expressed or produces a defective product (Fig. 1b).

Genetic Analysis of *suc*° Alleles

To investigate the nature of *suc*° alleles, a yeast strain was constructed carrying *suc*° alleles at all *SUC* loci (i.e., its genotype is *suc1*° *suc2*° *suc3*° . . . *sucN*°). As illustrated in Figure 2, two SUC^+ strains that carried active SUC^+ genes at different loci (namely, DBY631, a strain derived by mutation from FL100 [the relevant genotype is $SUC7^+$ *suc2*°; Lacroute 1968], and DBY473, a strain derived by mutation from S288C [the relevant genotype is $SUC2^+$ *suc7*°]) were crossed, and a sucrose nonfermenting recombinant was recovered. This procedure resulted in a hybrid strain of the desired genotype (*suc*° at all *SUC* loci). For purposes of further genetic analysis, it was desirable to make this strain otherwise congenic with the standard strain (S288C). This was accomplished by backcrossing the Suc^- recombinant to a derivative of S288C ten times in succession, each time recovering a haploid spore unable to ferment sucrose (Fig. 2). The resulting sucrose nonfermenting strain (DBY938) should be essentially identical to S288C except at the *SUC2* locus, where the nonfermenting strain carries the *suc2*° allele from its FL100 ancestor and S288C carries $SUC2^+$.

Reversion of* suc° *alleles. The "S288C*suc2*°" strain DBY938 contains negative alleles at all of its *SUC* loci. It produces no invertase and therefore fails to ferment sucrose. If any of the *suc*° alleles of this strain were a silent copy of a SUC^+ gene, then that allele might revert to an active SUC^+ state. The particular *SUC* locus containing the regenerated gene could then be determined by mapping genetically the new SUC^+ character.

We obtained revertants (at a frequency of about 2×10^{-8}) by plating the S288C*suc2*° strain on a medium requiring sucrose fermentation for growth. In each of nine independent Suc^+ revertants, the Suc^+ character was linked to the *SUC2* locus. These data suggest that the *suc2*° allele derived from FL100 is a silent *SUC* gene, as illustrated in Figure 1b. An alternative interpretation more consistent with the structure shown in Figure 1c cannot, however, be excluded by these data. The reversion event could have involved the transposition of *SUC* DNA from a silent "library" locus to a special site (containing no *SUC*-gene information) at the *SUC2* locus.

Recombination rescue of* SUC-*gene information from the* suc2° *allele. An experiment was designed to detect *SUC*-gene information at the *SUC2* locus in the S288C*suc2*° strain. It was based on the idea that a silent *suc2*° gene might be able to recombine with a $suc2^-$ allele derived by mutagenesis of the active $SUC2^+$ gene. A set of characterized nonsense (amber) mutations of the active $SUC2^+$ gene of S288C was isolated previously (M. Carlson et al., in prep.). These mutants fail (in the absence of an amber suppressor) to ferment sucrose or to make invertase. If, as suggested by the reversion studies, the *suc2*° gene contains a single lesion accounting for its failure to function, then it might be expected to have the functional alleles of the *suc2*am mutations intact; therefore, recombination between the *suc2*am and the *suc2*° alleles (producing a SUC^+ recombinant) would be possible.

Table 1. *SUC* Loci in Yeast

Locus	Chromosome
SUC1	VII
SUC2	IX
SUC3	II
SUC4	not mapped
SUC5	not mapped
SUC6	not mapped
SUC7[a]	not mapped

Six unlinked *SUC* loci (*SUC1–SUC6*) were identified by genetic analysis (reviewed by Mortimer and Hawthorne 1969). The map positions of *SUC1* (Mortimer and Hawthorne 1966), *SUC2* (Ono et al. 1979), and *SUC3* (Kawasaki 1979) have been determined.

[a] A new SUC^+ allele in strain FL100 (from F. Lacroute, Université Louis Pasteur, Strasbourg, France) was identified that is not linked to the $SUC1^+$–$SUC5^+$ alleles obtained from the Yeast Genetic Stock Center (Berkeley, California). Since the $SUC6^+$ allele is no longer available and could not be tested, the new *SUC* locus in FL100 has been denoted *SUC7*.

To test for recombination, diploid strains heteroallelic at the *SUC2* locus for *suc2°* and each of three *suc2*am alleles were constructed, along with strains heteroallelic for different amber alleles and homoallelic control strains. All of these diploid strains were phenotypically Suc^-, so mitotic recombination could be detected readily by the appearance of sucrose-fermenting progeny. Mitotic recombination was stimulated by increasing doses of sunlamp radiation (Lawrence and Christensen 1974). The results of this experiment (Fig. 3) clearly show that all of the heteroallelic strains gave Suc^+ recombinants and that the yield increased linearly with increasing doses of sunlamp radiation. The homoallelic control strains produced no or few Suc^+ recombinants. These results confirm that *suc2°* is a silent *SUC* gene capable both of reverting to SUC^+ and of donating good *SUC*-gene information at three different points in the *SUC2* gene in recombination experiments.

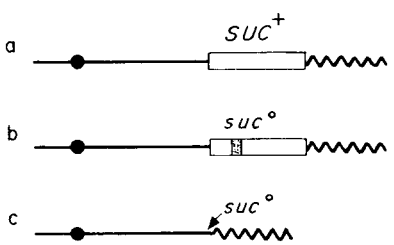

Figure 1. Models for the structure of *suc°* alleles. Shown are schematic representations (not to scale) of a chromosome with a *SUC* locus. (●) The centromere. (*a*) A SUC^+ allele at the locus (▭). (*b*) A *suc°* allele is depicted as a silent gene, a copy of a SUC^+ gene (▭) containing a lesion(s) (▩). The defect(s) could be a point mutation, insertion, deletion, inversion, etc., and need not be in the center of the gene. (*c*) A *suc°* allele is shown as a *SUC* locus that contains no DNA related to the SUC^+ gene. No implications are intended regarding the presence or absence of sequences normally adjacent to the SUC^+ gene.

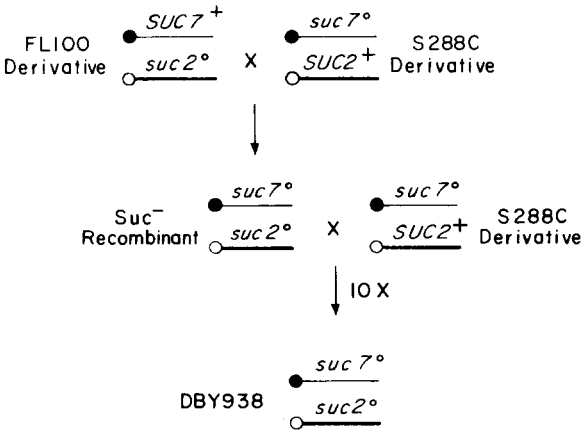

Figure 2. Construction of the *suc°* strain congenic to S288C. Illustrated is the procedure for constructing a strain (called DBY938 or S288C*suc2°*) carrying *suc°* alleles at all *SUC* loci and congenic to S288C at all loci except *SUC2*. A derivative of FL100, strain DBY631 (**a** $SUC7^+$ *suc2° ura3*), was crossed with a derivative of S288C, strain DBY473 (*α* $SUC2^+$ *suc7° his4*). For simplicity, only the two chromosomes carrying the *SUC2* and *SUC7* loci are shown, with their centromeres represented by open and filled circles, respectively; both strains have *suc°* alleles at all other *SUC* loci. A Suc^- recombinant recovered from this cross was successively backcrossed ten times to strains derived by mutation from S288C. One of the Suc^- strains recovered from the tenth backcross was DBY938 (**a** *suc° ade2*).

Physical Analysis of *SUC* Genes

The genetic analysis summarized above gave information about only one of the *suc°* genes, *suc2°*. To examine the nature of the other *suc°* alleles, a hybridization probe specific for *SUC* DNA was needed. To this end, a *suc2*am gene was cloned by complementation of a $suc2^-$ mutation in a yeast strain carrying an amber suppressor. A library of recombinant plasmids was constructed by preparing a partial digest of DNA from a *suc2*am yeast strain and ligating the fragments to a plasmid vector that could be maintained and selected for in both *Escherichia coli* and yeast. Details of these experiments will be published elsewhere (M. Carlson and D. Botstein, in prep.). From this library, we recovered a series of recombinant plasmids containing overlapping cloned fragments, each of which complemented a $suc2^-$ defect in yeast only when an amber suppressor was present in the strain. From this amber phenotype, it was known that the DNA common to the overlapping set must contain *SUC2* information (and not some other *SUC* gene or some unrelated gene). Figure 4 shows restriction maps of the *SUC2* clones; a 4-kb region is common to all of them. Two fragments of the common region were subcloned into the plasmid vector pBR322 (Bolivar et al. 1977). These subclones were used in blot hybridization experiments (Southern 1975) to probe total yeast genomic DNA digested with restriction en-

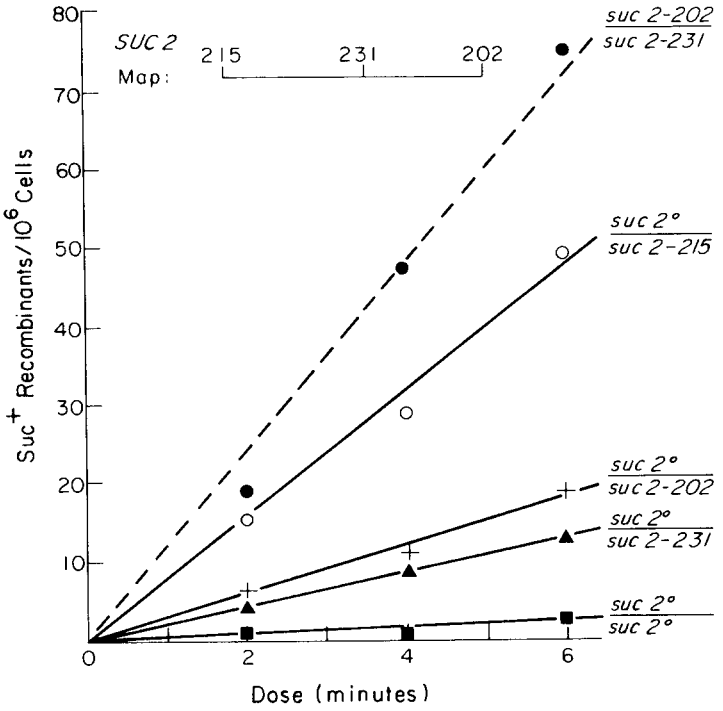

Figure 3. Recombination tests with the *suc2°* allele. Diploids heteroallelic for *suc2°* and each of three mutant alleles, *suc2-202*am, *suc2-215*am, and *suc2-231*am, were constructed. The *suc2°* parent of each diploid was an S288C*suc2°* strain derived from the ninth backcross described in the legend to Fig. 2. The three *suc2*am mutations were isolated in the S288C genetic background and were mapped by mitotic recombination with respect to each other (M. Carlson et al., in prep.). The yield of Suc^+ recombinants induced by sunlamp radiation was determined according to the method of Lawrence and Christensen (1974), with selection for Suc^+ recombinants on rich medium (YEP; Sherman et al. 1974) containing 2% sucrose under anaerobic conditions. Diploids homozygous for each of the *suc2*am mutations yielded no Suc^+ recombinants (data not shown). Diploids heteroallelic for all three pairs of *suc2*am mutations gave rise to Suc^+ recombinants; data are shown for one of the pairs.

zymes for the presence of fragments homologous to *SUC2* DNA. Key results were checked by using as probes purified restriction fragments covering the entire common region.

When total genomic DNAs from strains derived directly from S288C (*SUC2*$^+$) or FL100 (*SUC7*$^+$*suc2°*) were digested with *Bam*HI and analyzed by blot hybridization, one fragment homologous to the *SUC2* probe was detected in the digest of S288C-related DNA, and

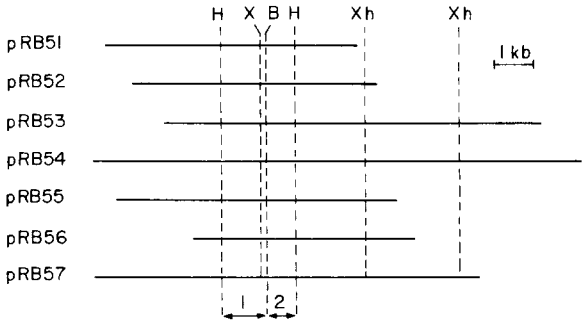

Figure 4. Restriction maps of cloned *SUC2* DNA segments. Recombinant plasmids carrying the *suc2*am gene were mapped by digesting plasmid DNA with restriction enzymes and electrophoresing in agarose gels. Shown are the overlapping yeast DNA segments from seven plasmids; both possible orientations of yeast DNA sequences with respect to vector DNA were included among the seven plasmids (data not shown). The restriction sites for endonucleases *Bam*HI (B), *Xba*I (X), and *Xho*I (Xh) are shown. Two of the several sites for *Hin*dIII (H) are also indicated. Cleavage of this DNA with both *Bam*HI and *Hin*dIII produced two fragments (1, 2) that were subcloned in pBR322 (Bolivar et al. 1977) and used as hybridization probes in subsequent experiments.

two homologous fragments were detected in FL100 DNA (Fig. 5a). The same numbers of fragments were detected with a variety of other restriction enzymes and *SUC2* DNA probes. These results suggest that in S288C, only one of the *SUC* loci (*SUC2*) contains *SUC* DNA, and in FL100, two loci (presumably *SUC7*, where the active gene is, and *SUC2*, where the *suc2°* allele is) contain *SUC* DNA. These experiments do not rule out, however, the possibility that each band detected by blot hybridization was composed of fragments derived from several identical *SUC* genes at different chromosomal loci. To eliminate this possibility, a diploid was made by crossing an isogenic derivative of FL100 with a derivative of S288C, and the four products (spores) from the meiosis of a single diploid cell were grown up and analyzed. If, indeed, each band in the blot hybridization represented a *SUC* gene at a single locus, then the pattern of bands observed in the spores should follow the Mendelian segregation of the alleles at each locus. As shown in Figure 5a, the hybridization pattern showed the expected 2:2 segregation of the bands, confirming that only one locus contains *SUC* DNA in S288C, and two loci contain *SUC* DNA in FL100.

These physical experiments confirm the inference from the genetic experiments described above that the *suc2°* allele at the *SUC2* locus of strain FL100 contains *SUC* DNA. These studies also imply that the *suc°* alleles at the other *SUC* loci correspond not to silent copies of a *SUC* gene but to the absence of *SUC* information, as illustrated in Figure 1c.

The *SUC* genes in four other laboratory strains, each carrying one active *SUC*$^+$ allele at the *SUC1, SUC3, SUC4,* or *SUC5* locus, were also analyzed by blot hybridization. Figure 5b shows that digestion with *Bam*HI

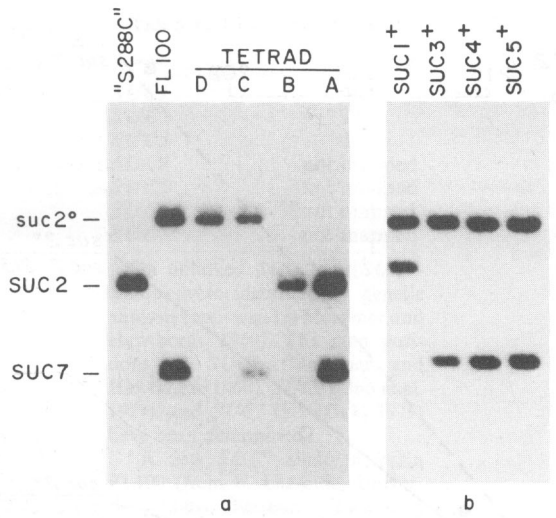

Figure 5. Blot hybridization analysis of *SUC* genes. (*a*) Total DNAs from the following strains were digested with *Bam*HI and electrophoresed in a 0.5% agarose gel: a strain derived by mutation from S288C (DBY939 α *suc2-215*am *ade2*, labeled "S288C"); FL100; the four spores (*A, B, C, D*) of a tetrad from the diploid made by crossing DBY615 (**a** *SUC7*$^+$ *ura3*, derivative of FL100) with DBY940 (α *suc2-215*am *lys2 his4*, derivative of S288C). The DNA fragments were transferred to nitrocellulose (Southern 1975) and hybridized to ^{32}P-labeled DNA probe prepared from the subclone of fragment 1 (see Fig. 4) by nick translation (Rigby et al. 1977). Hybridization was carried out in 5× SSCP (0.6 M NaCl, 0.075 M sodium citrate, 0.1 M sodium phosphate at pH 7) containing 0.5% SDS at 65 °C. Filters were washed in 5× SSCP at 65 °C. An autoradiograph is shown. The bands representing *suc2°*, *SUC2* (*suc2*am), and *SUC7* DNA were identified by this and other blot hybridization experiments in conjunction with genetic analysis. As expected for 2:2 segregation, two spores of the tetrad (*C, D*) have the *suc2°* band and the other two spores (*A, B*) have the *SUC2* band; two spores (*A, C*) have the *SUC7* band (and exhibited the *SUC7*$^+$ phenotype) and the other two spores (*B, D*) have no additional band. The conditions used in this experiment (digestion with *Bam*HI; hybridization with fragment-1 probe) were the only conditions found in which the *suc2°* and *SUC2* fragments failed to comigrate. (*b*) DNAs from the following strains carrying one active *SUC*$^+$ gene were digested with *Bam*HI and analyzed by blot hybridization exactly as described in *a*: R251-4A (α *SUC1*$^+$ *ura1 ade2*); 1412-4D (**a** *SUC3*$^+$ *MAL3*$^+$ *MEL1*$^+$ *MGL2*$^+$ *MGL3*$^+$ *GAL*$^+$ *ade2*); SS-12A (α *SUC4*$^+$ *his4*); and 2080-8C (**a** *SUC5*$^+$ *ade6*). These strains were obtained from the Yeast Genetic Stock Center. An autoradiograph is shown, and the lanes are labeled with the *SUC* genotypes of the strains. The upper fragment in each lane comigrated with the fragment corresponding to the *suc2°* allele (data not shown).

generated two fragments homologous to the *SUC* DNA probe from the DNA of each strain. A variety of other restriction enzymes were used (not shown) with similar results. In all cases, one of the two homologous fragments comigrated with the fragment associated above with the *suc2°* allele of FL100 (data not shown), which suggests that each of these strains contains the *suc2°* allele in addition to its active *SUC*$^+$ gene. The other fragments in the *SUC3*$^+$, *SUC4*$^+$, and *SUC5*$^+$ strains (presumably corresponding to the active *SUC*$^+$ alleles) comigrated with one another in this experiment but were distinguishable in experiments using other restriction enzymes. Again, these data support the idea that, with the exception of *SUC2*, the negative alleles at the *SUC* loci correspond to the absence of *SUC* information.

DISCUSSION

The *suc2°* allele present at the *SUC2* locus of strain FL100 (and possibly other laboratory strains of yeast) appears to be a naturally occurring silent gene somehow unable to confer the Suc$^+$ phenotype upon strains carrying it. This failure could be due to a failure in expression of the gene or to a defect in the product of the gene. The *suc2°* defect(s) cannot be gross, since *suc2°* reverts to functionality at a reasonable frequency and provides correct information in recombination tests at three points in the gene. The blot hybridization data revealed some restriction-site polymorphism in the neighborhood of the *SUC* gene (see Fig. 5) but showed no evidence of a major rearrangement of the *SUC* DNA in the *suc2°* form.

Possibly the simplest interpretation of these data is that the *suc2°* allele is a naturally occurring mutant allele that arose by mutation of a *SUC2*$^+$ gene. However, the apparent ubiquity of the *suc2°* gene among *SUC*$^+$ strains with active alleles at loci other than *SUC2*, as judged by the presence of restriction fragments of common size, remains unexplained. A trivial explanation—that the ubiquity of the silent gene is due to common ancestry in the laboratory—cannot easily be excluded because the histories of the standard strains are not known in sufficient detail. Analysis of *Saccharomyces* strains obtained directly from nature will be required to determine whether silent *SUC* alleles are a general feature in yeast genomes. If so, the possibility that the silent gene(s) serves an important function, perhaps as progenitor to the dispersed active *SUC*$^+$ genes, will have to be explored.

The existence of silent copies of active genes (sometimes called pseudogenes) is not rare in eukaryotes. Apparently defective or inactive copies of active genes have been detected in the 5S ribosomal genes in *Xenopus* (Miller et al. 1978) and in the globin families of man (Fritsch et al. 1980; Lauer et al. 1980), rabbit (Hardison et al. 1979), and mouse (Vanin et al. 1980).

Unlike *suc2°*, the *suc°* alleles at most of the *SUC* loci in the strains examined do not contain *SUC*-gene information. This finding suggests that the presence of active *SUC* genes at these loci in some strains results from movement of *SUC* information during the evolution of yeast strains. Such movement could have occurred either by a series of gross chromosomal rearrangements or, perhaps, by the transposition of a specific element containing an active *SUC*$^+$ gene. The *suc°* alleles would then represent either the complete absence of any special information (i.e., just random sequences into which *SUC* DNA became inserted) or some kind of specific preferred integration site for the postulated specific element. We cannot distinguish between these possibilities at present.

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REFERENCES

BOLIVAR, F., R. L. RODRIGUEZ, P. J. GREENE, M. C. BETLACH, H. L. HEYNEKER, H. W. BOYER, J. H. CROSA, and S. FALKOW. 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene* **2:** 95.

FRITSCH, E. F., R. M. LAWN, and T. MANIATIS. 1980. Molecular cloning and characterization of the human beta-like globin gene cluster. *Cell* **19:** 959.

HARDISON, R. C., E. T. BUTLER III, E. LACY, T. MANIATIS, N. ROSENTHAL, and A. EFSTRATIADIS. 1979. The structure and transcription of four linked rabbit beta-like globin genes. *Cell* **18:** 1285.

KAWASAKI, G. 1979. "Karyotypic instability and carbon source effects in cell-cycle mutants of *Saccharomyces cerevisiae.*" Ph.D. thesis, University of Washington, Seattle.

LACROUTE, F. 1968. Regulation of pyrimidine biosynthesis in *Saccharomyces cerevisiae. J. Bacteriol.* **95:** 824.

LAUER, J., C.-K. J. SHEN, and T. MANIATIS. 1980. The chromosomal arrangement of human alpha-like globin genes: Sequence homology and alpha-globin gene deletions. *Cell* **20:** 119.

LAWRENCE, C. W. and R. CHRISTENSEN. 1974. Fine structure mapping in yeast with sunlamp radiation. *Genetics* **76:** 723.

MILLER, J. R., E. M. CARTWRIGHT, G. G. BROWNLEE, N. V. FEDEROFF, and D. D. BROWN. 1978. The nucleotide sequence of oocyte 5S DNA in *Xenopus laevis.* II. The GC-rich region. *Cell* **13:** 717.

MORTIMER, R. K. and D. C. HAWTHORNE. 1966. Genetic mapping in *Saccharomyces. Genetics* **53:** 165.

———. 1969. Yeast genetics. In *The yeasts* (ed. A. H. Rose and J. S. Harrison), vol. 1, p. 385. Academic Press, New York.

ONO, B.-I., J. W. STEWART, and F. SHERMAN. 1979. Yeast UAA suppressors effective in ψ^+ strains: Serine-inserting suppressors. *J. Mol. Biol.* **128:** 81.

RIGBY, P. W. J., M. DIECKMANN, C. RHODES, and P. BERG. 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* **113:** 237.

SHERMAN, F., G. R. FINK, and C. W. LAWRENCE. 1974. *Methods in yeast genetics,* p. 61. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

SOUTHERN, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98:** 503.

VANIN, E. F., G. I. GOLDBERG, P. W. TUCKER, and O. SMITHIES. 1980. A mouse α-globin-related pseudogene lacking intervening sequences. *Nature* **286:** 222.