SUC Genes of Yeast: A Dispersed Gene Family

M. CARLSON, B. C. OSMOND, AND D. BOTSTEIN
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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<sup>−</sup> 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<sup>−</sup> alleles, as illustrated in Figure 1. A SUC locus containing a suc<sup>−</sup> allele could contain no DNA related to an active SUC gene (Fig. 1c) or it could contain a "silent" SUC gene, one that is not expressed or produces a defective product (Fig. 1b).

Genetic Analysis of suc<sup>−</sup> Alleles

To investigate the nature of suc<sup>−</sup> alleles, a yeast strain was constructed carrying suc<sup>−</sup> alleles at all SUC loci (i.e., its genotype is suc<sup>−</sup> suc<sup>−</sup> suc<sup>−</sup> suc<sup>−</sup> suc<sup>−</sup>). 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 SUC<sup>−</sup> suc<sup>−</sup>; Lacroute 1968], and DBY473, a strain derived by mutation from S288C [the relevant genotype is SUC<sup>−</sup> suc<sup>−</sup>]) were crossed, and a sucrose nonfermenting recombinant was recovered. This procedure resulted in a hybrid strain of the desired genotype (suc<sup>−</sup> 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 covering 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 and the suc<sup>−</sup> allele from its FL100 ancestor and S288C carries SUC2.

Reversion of suc<sup>−</sup> alleles. The "S288Cure<sup>−</sup>" 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<sup>−</sup> 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 x 10<sup>−5</sup>) by plating the S288Cure<sup>−</sup> strain on a medium requiring sucrose fermentation for growth. Is each of nine independent Suc<sup>−</sup> revertants, the Suc<sup>−</sup> character was linked to the SUC2 locus. These data suggest that the suc<sup>−</sup> 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 suc<sup>−</sup> allele. An experiment was designed to detect SUC-gene information at the SUC2 locus in the S288Cure<sup>−</sup> strain. It was based on the idea that a silent suc<sup>−</sup> allele might be able to recombine with a suc<sup>+</sup> 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 suc<sup>−</sup> gene contains a single lesion accounting for its failure to function, then it might be expected to have the functional alleles of the suc<sup>−</sup> mutations intact; therefore, recombination between the suc<sup>−</sup> and the suc<sup>+</sup> alleles (producing a SUC<sup>−</sup> recombinant) would be possible.
To test for recombination, diploid strains heteroallelic at the SUC2 locus for suc\(^{-}\) and each of three suc2am 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-hydrolyzing 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 homokaryotic 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 suc2am\(^{2}\) is a silent SUC gene ca-

![Figure 1: Models for the structure of suc\(^{-}\) alleles. Shown are schematic representations (not to scale) of a chromosome with a SUC locus. (a) The centromere. (a) A SUC\(^{+}\) allele at the locus (C3). (b) A suc\(^{-}\) allele is depicted as a si-
tent gene, a copy of a SUC gene (C3) containing a le-sion(s) (B). The defect(s) could be a point mutation, inser-
tion, 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 implica-
tions are intended regarding the presence or absence of se-
quences normally adjacent to the SUC gene.](image1)

![Figure 2: Construction of the suc\(^{-}\) strain congenic to S288C. Illustrated is the procedure for constructing a strain (called DBY938 or S288Cauc\(^{-}\)) carrying suc\(^{-}\) alleles at all SUC loci and congenic to S288C at all loci except SUC2. A derivative of FL100, strain DBY631 (a SUC\(^{7}\) suc\(^{-}\) strain), was crossed with a derivative of S288C, strain DBY473 to SUC\(^{7}\) suc\(^{-}\) his3. For simplicity, only the two chromo-
somes carrying the SUC2 and SUC7 loci are shown, with their centromeres represented by open and filled circles, re-
spectively, both strains have suc\(^{-}\) alleles at all other SUC loci. A Suc\(^{-}\) recombinant recovered from this cross was suc-
ccessively 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).](image2)

![Figure 3: Recombination tests with the suc\(^{-}\) allele. Diploids heteroallelic for suc\(^{-}\) each of three mutant alleles, suc2-20, suc2-273am, and suc2-231am, were constructed. The suc\(^{-}\) parent of each diploid an S288Cauc\(^{-}\) strain derived from the strain backcrossed described in the legend to Fig. The three suc2am mutations were isolated in the S288C genetic background and mapped by mitotic recombination with respect to each other (M. Carlson et al. in prep.). The yield of Suc\(^{-}\) recombinants selected by sunlamp radiation was determined according to the method of Lawrence and Christensen (1974), with selection for Suc\(^{-}\) colonies on rich medium (YPF; She et al. 1974) containing 2% sucrose under aerobic conditions. Diploids homozygous for each of the suc2am mutations yielded no Suc\(^{-}\) recombinants (data not shown). Diploids heteroallelic for all three pairs of suc2am alleles gave rise to Suc\(^{-}\) recombinants; data shown for one of the pairs.](image3)
SUC GENES OF YEAST

801

Figure 3. Recombination tests with the nuc2* allele. Diploids heteroallelic for nuc2* and each of three mutant alleles, nuc2-202am, nuc2-203am, and nuc2-213am, were constructed. The nuc2* parent of each diploid was an S288C nuc2* strain derived from the same strain crosses described in the legend to Fig. 2. The three nuc2* 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 ultraviolet radiation was determined according to the method of Lawrence and Christensen (1974), with selection for Suc* recombinants on rich medium (YE, Sauer et al. 1974) containing 2% sucrose under aerobic conditions. Diploids homozygous for each of the nuc2* mutations yielded no Suc* recombinants (data not shown). Diploids heteroallelic for all three pairs of nuc2* mutations gave rise to Suc* recombinants; data are shown for one of the pairs.

Figure 4. Restriction maps of cloned SUC2 DNA segments. Recombinant plasmids carrying the suc2* 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 HindIII (H) are also indicated. Cleavage of this DNA with both BamHI and HindIII 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 number 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 SUC3, where the active gene is, and SUC2, where the nuc2* 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 nuc2* allele at the SUC2 locus of strain FL100 contains SUC DNA. These studies also imply that the nuc2* allele 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 BamHI
Figure 5. Blot hybridization analysis of SUC genes. (a) Total DNAs from the following strains were digested with BamH1 and electrophoresed in a 0.5% agarose gel; a strain derived by mutation from S288C (DBY393; a suc2Δ2:215am 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 (a suc2Δ2:215am lys2 his4, derivative of S288C). The DNA fragments were transferred to nitrocellulose (Southern 1975) and hybridized to 32P-labeled DNA probe prepared from the subclone of fragment L (see Fig. 4) by nick translation (Rigby et al. 1977). Hybridization was carried out in 5x 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 5x SSC at 65°C. An autoradiograph is shown. The bands represent suc2+, SUC2 (suc2A), and SUC7 DNA were identified by this and other blot hybridization experiments in conjunction with genetic analysis. As expected for 2, segregation, two spores of the tetrad (C, D) have the suc2Δ2 band and the other two spores (A, B) have the SUC2 band; two spore (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 BamH1, hybridization with fragment-1 probe) were the only conditions found in which the suc2Δ2 and SUC2 fragments failed to comigrate. (b) DNAs from the following strains carrying one active SUC gene were digested with BamH1 and analyzed by blot hybridization exactly as described in a: R251-4A (a SUC1::ura3 ade2); 1412-4D (a SUC3::MELI::MELI::MGL2::MGL2::GAL::ade2); SS-12A (a SUC4::his4); and 2880-8C (a SUC5::ade2). 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 suc2Δ2 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 suc2Δ2 allele of FL100 (data not shown), which suggests that each of these strains contains the suc2Δ2 allele in addition to its active SUC gene. The other fragments in the SUC1, 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 suc2Δ2 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 Suc2+ 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 suc2Δ2 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 suc2Δ2 form.

Possibly the simplest interpretation of these data is that the suc2Δ2 allele is a naturally occurring mutant allele that arose by mutation of a SUC2+ gene. However, the apparent ubiquity of the suc2Δ2 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 an-ecracy 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 a progenitor to the dispersed active SUC genes, will have to be explored.

The existence of silent copies of active genes (some- times called pseudogenes) is not rare in eukaryotes. Apparent 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 (Frisch et al. 1980; Lauer et al. 1980), rabbit (Hardison et al. 1979), and mouse (Vanin et al. 1980).

Unlike suc2+, the suc2Δ2 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 suc2Δ2 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.

ACKNOWLEDGMENTS

This research was supported by American Cancer Society (VC245) and Stittites of Health (GM-21253). M. C. Jane Coffin Children's Memorial Fund.

REFERENCES


Lauer, J., C. J. Shen, and T. Maizel. 1979. Chromosomal arrangement of human...
ACKNOWLEDGMENTS

This research was supported by grants from the American Cancer Society (VC-245) and the National Institutes of Health (GM-21253). M. C. was a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

REFERENCES


SUC GENES OF YEAST

803