GENETIC EVIDENCE FOR A SILENT *SUC* GENE IN YEAST

MARIAN CARLSON, BARBARA C. OSMOND AND DAVID BOTSTEIN

Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass. 02139

ABSTRACT

The *SUC* genes (*SUC1–SUC7*) of Saccharomyces are a family of genes that are dispersed in the yeast genome. A SUC^+ allele at any locus confers the ability to produce the enzyme invertase and, thus, to ferment sucrose. Most yeast strains do not carry SUC^+ alleles at all possible *SUC* loci. We have investigated the naturally occurring negative (suc^o) alleles present at *SUC* loci with the aim of distinguishing between two possible models for the structure of suc^o alleles: (1) suc^o alleles correspond to a simple absence of *SUC* genetic information; (2) suc^o alleles are "silent" *SUC* genes that either produce a defective product or are not expressed. To facilitate these studies, sucrose-nonfermenting strains were constructed that are congenic to *S. cerevisiae* strain S288C ($SUC2^+$), but carry at the *SUC2* locus the naturally occurring negative allele, $suc2^o$, of strain FL100 (LACROUTE 1968). These strains were used to study the genetic properties of the $suc2^o$ allele of FL100 and the suc^o alleles ($suc1^o$, $suc3^o$, etc.) of S288C. The $suc2^o$ allele was shown to revert to an active Suc^+ state and to provide functional information at three points in the *SUC2* gene in recombination experiments; this $suc2^o$ gene thus appears to be a "silent" gene. Similar tests for silent *SUC* genes in S288C (corresponding to loci other than *SUC2*) failed to reveal any additional silent genes.

THE *SUC* genes of yeast (Saccharomyces) are a family of nonallelic genes responsible for sucrose utilization by yeast (reviewed by MORTIMER and HAWTHORNE 1969). Each SUC^+ gene confers upon strains carrying it the ability to produce the enzyme invertase, which cleaves sucrose extracellularly (DE LA FUENTES and SOLS 1962) to yield glucose and fructose. Two forms of invertase are made: a glycosylated form, which is secreted into the periplasmic space, and an apparently nonglycosylated form, which remains within the cell (NEUMANN and LAMPEN 1967; GASCON and LAMPEN 1968; GASCON, NEUMANN and LAMPEN 1968; OTTOLENGHI 1971). Six nonallelic *SUC* loci (*SUC1–SUC6*) were previously identified by segregational analysis of different Saccharomyces strains (Table 1) (GILLILAND 1949; WINGE and ROBERTS 1952; MORTIMER and HAWTHORNE 1966). Any individual haploid strain of yeast may have zero, one, or several SUC^+ alleles; for example, the early studies of GILLILAND (1949) and WINGE and ROBERTS (1952) showed that *S. chevalieri* possesses three SUC^+ genes, but *S. italicus* has none. Thus, both the number of SUC^+ alleles in the genome and their chromosomal locations vary among different strains.

Such variability is unusual; most known genes specifying metabolic functions in yeast appear to occupy constant positions on the genetic map. Other notable

TABLE 1

SUC loci in yeast

Locus	Chromosome
SUC1	VII
SUC2	IX
SUC3	II
SUC4	Not mapped
SUC5	IV
SUC6	Not mapped
SUC7*	Not mapped

Map positions have been determined for *SUC1* (MORTIMER and HAWTHORNE 1966), *SUC2* (ONO, STEWART and SHERMAN 1979), and *SUC3* (KAWASAKI 1979). KAWASAKI (1979) mapped an unidentified *SUC* locus on chromosome *IV*; we crossed his *SUC⁺* strain N422–8C to *SUC1–SUC5* and *SUC7* testers (see MATERIALS AND METHODS) and, from the cross to a *SUC5* tester, we recovered no sucrose-nonfermenting segregants among 52 spores, including 7 complete tetrads.
* The identification of *SUC7* is described in MATERIALS AND METHODS.

exceptions besides the *SUC* genes are the *MAL* and *MGL* genes, which are responsible for fermentation of maltose and α-methylglucoside, respectively, and which constitute gene families exhibiting variability in the number and location of active alleles in different strains (MORTIMER and HAWTHORNE 1969). These families are apparently analogous in organization to the *SUC* gene family.

Most yeast strains do not carry *SUC⁺* alleles at all known *SUC* loci, but rather carry naturally occurring negative alleles at some or all *SUC* loci in their genomes. We have investigated the genetic properties of these naturally occurring negative alleles, which are here denoted suc^o ($suc1^o$, $suc2^o$, etc.) to distinguish them from negative mutations derived from active *SUC⁺* genes in the laboratory. Two contrasting models for the structure of suc^o alleles are illustrated in Figure 1. A *SUC* locus bearing a suc^o allele could contain no genetic information related to an active *SUC⁺* gene (Figure 1c); alternatively, it could contain a "silent" *SUC* gene that, due to some lesion(s), either is not expressed or produces a defective product (Figure 1b). In this paper, we present genetic evidence that the $suc2^o$ allele of *S. cerevisiae* strain FL100 is a silent *SUC* gene able to revert to an active Suc⁺ state and to provide functional information at three points in the gene by recombination.

MATERIALS AND METHODS

Yeast strains: S288C (a *SUC2⁺ mal° gal2*) was obtained from G. FINK. Strains derived from S288C by mutation carry *suc2, ade2, his4* and *lys2* mutant alleles, which are described in the accompanying paper (CARLSON, OSMOND and BOTSTEIN 1981), and also can^r, a spontaneous mutation conferring resistance to canavanine, isolated by D. MOIR. Strain 7502–10D (a *rad52–1*) was obtained from G. FINK; the *rad52* allele had been crossed into the S288C genetic background twice successively. 7502–10D was crossed to DBY938 (a *suc° ade2*), a sucrose-nonfermenting "S288C $suc2^o$" strain (see RESULTS for description), and 2:2 segregations for sucrose utilization were observed in tetrads. Sucrose-nonfermenting segregants DBY896 (a *rad52–1 suc2° ade2*) and DBY895 (α *rad52–1 suc2° ade2*) were recovered. 7502–10D was also crossed to an S288C derivative carrying the *suc2–215*am allele, and DBY897 (a *rad52–1 suc2–215*am *lys2–801*am) was
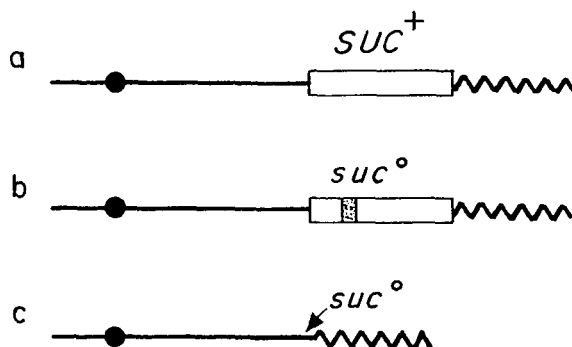
FIGURE 1.—Models for the structure of *suc⁰* alleles. The diagrams show schematic representations (not to scale) of a chromosome carrying a *SUC* locus; the filled circle represents the centromere. (a) A *SUC⁺* allele at the *SUC* locus is represented by an open bar. (b) A *suc⁰* allele is depicted as a "silent" gene, a copy of a *SUC⁺* gene (the open bar) containing a lesion(s) (the shaded region); the defect(s) could be a point mutation, insertion, deletion, inversion, *etc.*, and need not be in the center of the gene. (c) A *suc⁰* 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.

recovered; the presence of the *suc2–215*am allele in this segregant was confirmed by its simultaneous reversion with the *lys2–801*am marker.

Strains used in allelism tests for *SUC1⁺*, *SUC2⁺*, *SUC3⁺*, *SUC4⁺* and *SUC5⁺* were obtained and analyzed as follows. S288C derivatives served as *SUC2⁺* tester strains. Other testers, obtained from the Yeast Genetic Stock Center (Berkeley, CA), were: R251–4A (*α SUC1 mal⁰ ura1 ade2*); 1412–4D (**a** *SUC3 MAL3 MEL1 MGL2 MGL3 ade2 GAL*), SS–4A (**a** *SUC4 mal⁰ ade1*) and 2080–8C (**a** *SUC5 mal⁰ ade6*). To confirm the *SUC* genotypes of these tester strains, each was crossed to a sucrose-nonfermenting S288C *suc2⁰* strain (see RESULTS for description); tetrad analysis showed 2:2 (Suc⁺:Suc⁻) segregations in all cases except that of 1412–4D, for which insufficient data were obtained due to poor spore viability. Crosses involving all possible pairs of *SUC⁺* genes then were undertaken, using these tester strains and *SUC⁺* derivatives recovered from the crosses to S288C *suc2⁰* strains. Frequent segregation of sucrose-nonfermenting spores was observed from crosses involving each pair of *SUC⁺* genes. These data confirm that the 5 tester strains each carry a *SUC⁺* gene at a different locus.

FL100 (**a** *suc2⁰ SUC7⁺*) (LACROUTE 1968) and strains derived from FL100 by mutation were obtained from F. LACROUTE. The *SUC* genotype of FL100 was determined by segregational analysis of crosses between FL100 derivatives and tester strains. Tetrads from a cross to a sucrose-nonfermenting "S288C *suc2⁰*" strain gave 2:2 (Suc⁺:Suc⁻) segregations, indicating that FL100 carries a single *SUC⁺* allele. Crosses to *SUC1⁺*, *SUC2⁺*, *SUC3⁺*, *SUC4⁺* and *SUC5⁺* tester strains yielded frequent sucrose-nonfermenting segregants, showing that the FL100 *SUC⁺* gene is not allelic to these *SUC* genes. Because the *SUC6⁺* allele is no longer available from the Yeast Genetic Stock Center to test allelism, the FL100 *SUC⁺* locus has been designated *SUC7*. FL100 produces both glycosylated and nonglycosylated forms of invertase, as judged from the gel assay described earlier (data not shown; CARLSON, OSMOND and BOTSTEIN 1981). FL100 does not ferment raffinose, a substrate for many invertases; this failure appears to be a property of the *SUC7⁺* gene because *SUC7⁺* strains congenic to S288C (CARLSON, OSMOND and BOTSTEIN 1981) also are unable to ferment raffinose (data not shown).

Genetic methods and media: General genetic methods, media and methods for scoring ability to ferment sugars have been described (CARLSON, OSMOND and BOTSTEIN 1981). YEP-α-methylglucoside contained 2% of the sugar (Pfanstiehl Laboratories), sterilized by filtration. As before, all scoring for sugar utilization was carried out under anaerobic conditions. For tetrad analysis in which segregation of *SUC* markers was scored, diploids were purified by single colony isolation

prior to sporulation. The *rad52-1* mutation was scored by inability to grow on YEP-glucose medium containing 0.008% methyl methanesulfonate (MALONE and ESPOSITO 1980). Random spore analysis was carried out essentially as described by BRANDRISS, SOLL and BOTSTEIN (1975).

Isolation of sucrose-fermenting revertants: Single colonies of the sucrose-nonfermenting strain were grown in YEP-glucose liquid medium. Cells were usually washed with water and approximately 6×10^7 cells were plated on YEP-sucrose medium. To obtain spontaneous revertants, plates were incubated under anaerobic conditions in a GasPak Disposable Anaerobic System (BBL) at 30° for 5–12 days. To induce reversion with ethyl methansulfonate (EMS), a sterile Bacto-Concentration Disk (Difco Laboratories) was placed in the center of the plate and a drop of the mutagen was allowed to soak into the disk; plates were then incubated as above. UV-induced revertants were obtained by exposing the plates to 23 Jm^{-2} of ultraviolet radiation just prior to incubation. Revertant colonies were purified and tested for ability to utilize sucrose. Revertants were considered to be independent only if derived from different single colonies of the parent strain.

RESULTS

Construction of strains carrying only suc^o *alleles:* A sucrose-nonfermenting strain carrying naturally occurring negative alleles at all *SUC* loci was essential for genetic studies of the *suc^o* alleles. Because different strains might differ in the nature of their *suc^o* alleles or with respect to other genes affecting sucrose utilization, studies were carried out with strains having the genetic background of the *S. cerevisiae* laboratory strain S288C. S288C bears a single *SUC$^+$* allele, at the *SUC2* locus, and has *suc^o* alleles at all other SUC loci. A sucrose nonfermenting strain congenic to S288C but lacking the *SUC2$^+$* allele, and carrying instead a *suc2^o* allele, was constructed by the following procedure (illustrated in Figure 2). DBY473, an isogenic derivative of S288C (*SUC2$^+$*) was crossed to DBY631, an isogenic derivative of FL100 (*SUC7$^+$*; see MATERIALS AND METHODS) and a sucrose-nonfermenting recombinant was recovered from the cross. This recombinant carries a naturally occurring negative allele at the *SUC2* locus (*suc2^o*) derived from its FL100 ancestor, and a naturally occurring negative allele at the *SUC7* locus (*suc7^o*) derived from its S288C ancestor. In addition it carries *suc^o* alleles derived from either FL100 or S288C at all other *SUC* loci. To obtain a strain carrying the *suc2^o* allele but otherwise congenic with S288C, this recombinant was serially backcrossed to derivatives of S288C ten times, each time recovering a haploid spore clone unable to ferment sucrose. The resulting sucrose-nonfermenting congenic strains (from the ninth and tenth backcrosses) are referred to as "S288C *suc2^o*" strains. These strains contain naturally occurring negative alleles at all *SUC* loci: the *suc2^o* allele from FL100 and all other *suc^o* alleles (*suc1^o*, *suc3^o*, *etc.*) from S288C.

"Reversion" of suc^o *alleles:* The S288C *suc2^o* strains are suitable strains for investigating the genetic properties of *suc^o* alleles because, lacking a *SUC$^+$* gene, they are unable to produce invertase or ferment sucrose. If any one of the *suc^o* alleles was a silent *SUC* gene, as illustrated in Figure 1b, that allele might be able to mutate, or "revert," to an active Suc$^+$ state, and thereby enable the strain to produce invertase and utilize sucrose. The ability of *suc^o* alleles to revert was tested by plating S288C *suc2^o* strain DBY938 on medium selective for sucrose-fermenting revertants. Revertant colonies arose at a spontaneous frequency of
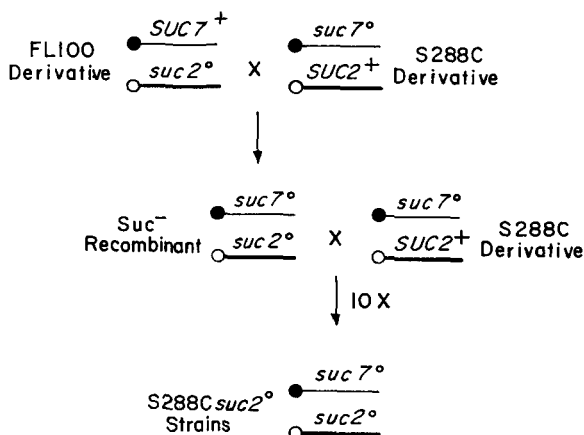
FIGURE 2.—Construction of *suc⁰* strains congenic to S288C. The diagram illustrates the procedure followed to construct the "S288C *suc2⁰*" strains carrying *suc⁰* alleles at all *SUC* loci and congenic to S288C at all loci except *SUC2* (see text for explanation). A derivative of FL100, strain DBY631 (a *SUC7⁺ suc2⁰ ura3*), was crossed with a derivative of S288C, DBY473 (α *SUC2⁺ suc7⁰ 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 *suc⁰* alleles at all other *SUC* loci. A Suc⁻ recombinant recovered from this cross was backcrossed 10 times successively to strains derived by mutation from S288C. Sucrose-nonfermenting strains recovered from the backcrosses are congenic to S288C and are called "S288C *suc2⁰*" strains.

approximately 2×10^{-8} revertants per cell plated. The colonies were large and resembled wild-type *SUC⁺* colonies in being surrounded by a "halo," apparently produced by growth of sucrose-nonfermenting cells fed by the revertant colony. Nine independent revertants, each derived from a different single colony isolate of DBY938, were purified and analyzed. All nine grew on both YEP-sucrose and YEP-raffinose, and none were temperature sensitive; *SUC2⁺* strains utilize raffinose, which is a poorer substrate for invertase than sucrose (GASCON, NEUMANN and LAMPEN 1968). The loci containing the revertant Suc⁺ characters were mapped genetically. In all nine, the reversion site is tightly linked to the *SUC2* locus, as judged by tetrad analysis of crosses between the revertants and a *SUC2⁺* tester strain. Segregations of 4:0 (Suc⁺:Suc⁻) were observed in all complete tetrads, and no sucrose-nonfermenting segregants were recovered among more than 20 spores tested from each cross (Table 2).

Similar results were obtained in other experiments. Spontaneous sucrose-utilizing revertants were selected from sucrose-nonfermenting segregants recovered from the fifth, sixth and ninth backcrosses in the construction of the S288C *suc2⁰* strains. Six independent revertants were crossed to *SUC2⁺* tester strains; 4:0 (Suc⁺:Suc⁻) segregations were observed and no sucrose-nonfermenting segregants were recovered among a total of 103 spores.

The sucrose-fermenting revertants were then examined to determine whether they resemble wild-type *SUC2⁺* strains with respect to production of invertase. Wild-type *SUC2⁺* strains produce both forms of the enzyme under derepressing

TABLE 2

Phenotypic segregation in crosses of Suc⁺ revertants to SUC2⁺ tester strains

Revertant	Number of spores		Number of complete tetrads
	Suc⁺	Suc⁻	
R1	21	0	3
R3	26	0	5
R5	27	0	6
R6	28	0	7
R7	28	0	7
R8	27	0	6
R11	28	0	7
R12	24	0	4

Sucrose-fermenting revertants of DBY938 (**a** *ade2 suc°*) were crossed to DBY783 (*α his4 canʳ SUC2⁺*), and tetrad analysis was carried out.

conditions (low glucose concentration) and produce only the nonglycosylated form under repressing conditions (high glucose) (GASCON and LAMPEN 1968; OTTOLENGHI 1971). Revertant R1 (see Table 2) was assayed for the presence of the nonglycosylated and glycosylated forms of invertase, as described in the legend to Figure 3. Like the wild type, revertant R1 produced both forms of invertase during growth under derepressing conditions and produced only nonglycosylated invertase under repressing conditions; thus, revertant R1 appeared to be normally regulated by glucose repression. However, very little nonglycosylated activity was detected, and further experiments have suggested that the nonglycosylated invertase of revertant R1 migrates somewhat differently on these gels from that of wild type (data not shown). Revertants R3, R5 and R7 were also found to produce both invertases under conditions of glucose derepression (data not shown).

These findings that the *suc2°* allele can revert to an active Suc⁺ state strongly suggest that the *suc2°* allele is a silent *SUC* gene, as illustrated in Figure 1b. However, these data do not exclude alternative explanations consistent with the model shown in Figure 1c; for example, the reversion event could have involved transposition of *SUC* DNA from a silent "library" locus to a special site at the *SUC2* locus.

Recombination analysis: If, as the reversion studies suggest, the *suc2°* allele is a silent gene with a single revertible lesion accounting for its failure to function, then the *suc2°* gene might have functional alleles of one or more *suc2* amber mutations (CARLSON, OSMOND and BOTSTEIN 1981). The *suc2°* allele would then be able to recombine with a *suc2*-am allele to produce sucrose-fermenting recombinants. Detecting such recombination would provide strong evidence for the presence of *SUC* gene information in the *suc2°* allele.

To test for recombination, diploids heteroallelic for the *suc2°* allele and each of three *suc2*-am mutations (*suc2–202, suc2–215* and *suc2–231;* CARLSON, OSMOND and BOTSTEIN 1981) were constructed, as were homoallelic control strains. The frequency of sunlamp-radiation-stimulated mitotic recombination was de-
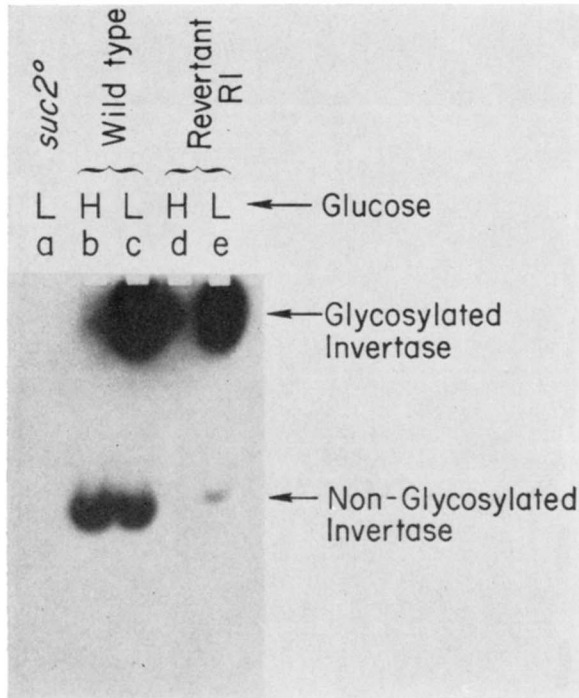
FIGURE 3.—Production of invertase by revertant R1. Glucose-repressed and derepressed strains were assayed for the presence of glycosylated and nonglycosylated invertase by electrophoresis of cellular extracts in a polyacrylamide gel, followed by detection of activity *in situ* (GABRIEL and WANG 1969) as described by CARLSON, OSMOND and BOTSTEIN (1981). (a) S288C *suc2°* strain DBY938 (**a** *suc°* *ade2*), grown in low glucose (L); (b) *SUC2+* strain DBY963 (**a** *SUC+* *his4* *lys2*; S288C derivative), grown in high glucose (H); (c) DBY963, low glucose; (d) revertant R1, high glucose; (e) revertant R1, low glucose. The levels of nonglycosylated invertase activity detected were reproducibly lower in revertant R1 than in DBY963. The activity present in repressed cells of revertant R1 was barely detectable.

termined for each diploid (LAWRENCE and CHRISTENSEN 1974). Figure 4 shows that sucrose-fermenting recombinants were produced from diploids heteroallelic for *suc2°* and each *suc2*-am allele, and that the yield of recombinants increased linearly with an increasing dose of sunlamp radiation. For comparison, the rate of recombination in a *suc2–202/suc2–231* diploid was also determined in the same experiment. The rates observed for *suc2°/suc2*-am diploids were low, relative to the rate for the *suc2–202/ suc2–231* diploid, a discrepancy that was reproduced in other experiments (data not shown) and that may be attributable to the different origins of the *suc2°* and *suc2*-am alleles.

Control experiments with diploids homoallelic for each *suc2* allele showed that the sucrose-fermenting progeny detected in the heteroallelic diploids indeed resulted from recombination between *suc2* alleles, not from other events such as reversion: no or few sucrose-fermenting progeny were produced from the homoallelic control strains. An additional control experiment was suggested by the
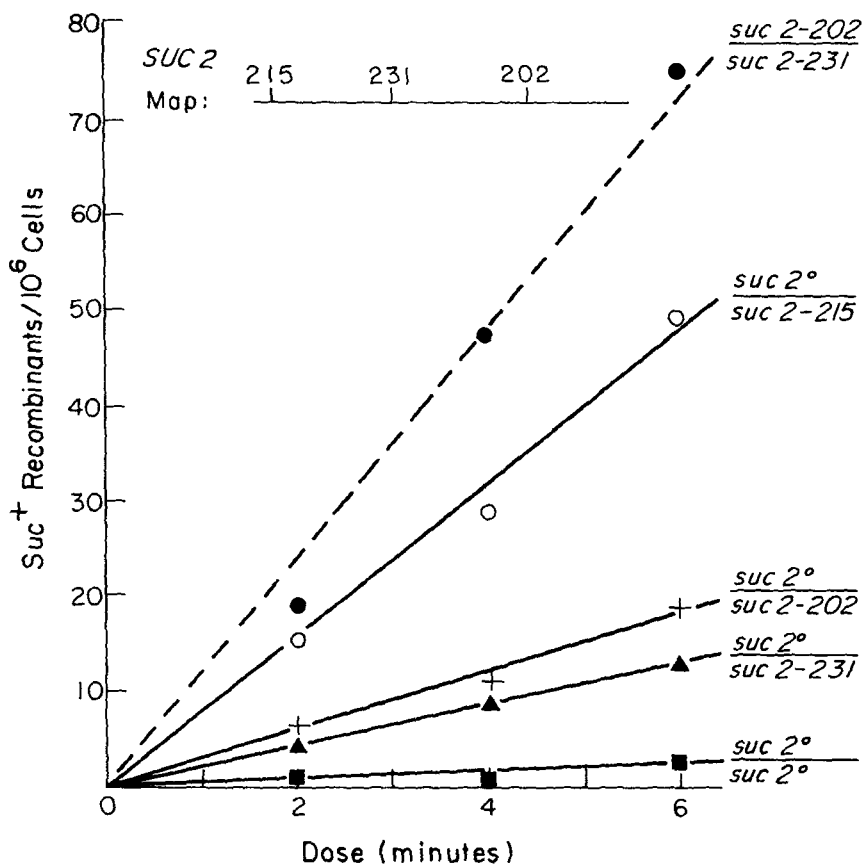
FIGURE 4.—Sunlamp-radiation-induced recombination in $suc2^o/suc2$-am diploids. The $suc2^o$ parents of the diploids were S288C $suc2^o$ strains from the 9th backcross (see RESULTS). Amber alleles $suc2$-202, $suc2$-231, and $suc2$-215 were mapped previously (CARLSON, OSMOND and BOTSTEIN 1981). The rate of sunlamp-radiation-induced mitotic recombination was determined by the method of LAWRENCE and CHRISTENSEN (1974), with selection for sucrose-fermenting recombinants on YEP-sucrose medium under anaerobic conditions, as described by CARLSON, OSMOND and BOTSTEIN (1981). The rates (in units of recombinants per 10^6 survivors per minute sunlamp irradiation) were 7.7 for $suc2^o/suc2$-215, 3.0 for $suc2^o/suc2$-202, 2.2 for $suc2^o/suc2$-231, 0.18 for $suc2^o/suc2^o$, and 12.2 for $suc2$-202$/suc2$-231 (previous value was 11.9; CARLSON, OSMOND and BOTSTEIN 1981). Diploids homozygous for each of the $suc2$-am mutations yielded no Suc+ progeny (data not shown).

observation that the $rad52$-1 mutation reduces spontaneous mitotic recombination (MALONE and ESPOSITO 1980; PRAKASH et al. 1980); it seemed likely that sunlamp-induced recombination also would be affected. A $rad52$-1/$rad52$-1 $suc2^o/suc2$-215 am diploid was constructed, and, as expected, the yield of sucrose-fermenting progeny from this strain was found not to be stimulated by sunlamp radiation (data not shown).

Genetic analysis of two sunlamp-induced recombinants provided further evidence that the sucrose-fermenting progeny resulted from recombination at the

SUC2 locus. As part of an experiment similar to that shown in Figure 4, sucrose-fermenting progeny were recovered from a *suc2°/suc2–231*am diploid exposed to 4 minutes of sunlamp radiation. Two such sucrose-fermenting diploids were purified and sporulated. The Suc+ character from each diploid showed 2:2 segregation in several tetrads. A sucrose-fermenting haploid segregant was recovered from each diploid and was crossed to a *SUC2+* tester strain; tetrad analysis showed 4+:0– segregations for sucrose utilization (6 tetrads and 4 tetrads in the two cases). These data indicate that each sucrose-fermenting diploid carries a *SUC2+* allele, as expected if the Suc+ character resulted from a recombination event at the *SUC2* locus.

Reversion at other loci: The reversion experiments described above yielded sucrose-fermenting revertants of S288C *suc2°* strains by mutation of the *suc2°* allele. The failure to recover revertants at other *SUC* loci has two possible explanations: (1) the *suc°* alleles at loci other than *SUC2* are unable to revert to an active Suc+ state, perhaps because they correspond to an absence of *SUC* information, as illustrated in Figure 1c; or (2) the other *suc°* alleles revert at a much lower frequency than *suc2°* and our sample was too small to include such a revertant.

A more extensive search for revertants was undertaken. Sucrose-fermenting revertants were selected from *suc2* mutant strains, many of which reverted at lower spontaneous frequency than did strains bearing the *suc2°* allele derived from FL100 (M. CARLSON, unpublished data). In most cases, reversion was induced by treatment with ethyl methanesulfonate or UV. Revertant colonies in a a range of sizes with and without halos were recovered; a variety of colonies was picked, purified and characterized. Revertants of *suc2*-am mutants were tested for simultaneous reversion of another amber marker (*lys2–801*) present in the strains; co-revertants were eliminated from further consideration on the assumption that they carried amber suppressors.

Seventeen independent revertants that carried no suppressor of *lys2–801* were crossed to a *SUC2+* strain to test for linkage of the Suc+ element to *SUC2*. Nine revertant loci are tightly linked to *SUC2*, as judged by 4+:0– segregation for sucrose utilization in tetrads and failure to recover sucrose-nonfermenting segregants; the sites of reversion in the other eight strains are not at *SUC2*, as judged by frequent recovery of sucrose-nonfermenting segregants. The properties of these 17 revertants are listed in Table 3.

The strains in which the site of reversion is linked to *SUC2* utilize raffinose and produce invertase; they had formed large colonies with halos on the original selection plates. Eight additional revertants (see legend to Table 3) that had shown similar colony morphology were crossed to a *SUC2+* tester bearing a *can^r* allele; in each case, analysis of random spores revealed no sucrose-nonfermenting segregants. Thus, all the analyzed revertants with this characteristic colony morphology apparently had reverted at the *SUC2* locus. One exceptional revertant of the *suc2–231* strain had this same colony morphology, utilized raffinose, and produced invertase. However, 4:0, 3:1, and 2:2 (Suc+:Suc-) segregations were observed in a cross to a *SUC2+* tester. Extensive genetic and physical analy-

TABLE 3

Properties of Suc⁺ revertants

Revertant strain	Mutant allele	Inducing mutagen	Linkage to SUC2	Growth on YEP-raffinose	Halo	Invertase
DBY972	suc2–533	EMS	+	+	+	+
DBY973	suc2–418	EMS	+	+	+	+
DBY974	suc2–428	EMS	+	+	+	+
DBY964	suc2–215am	None	+	+	+	+
DBY965	suc2–215am	UV	+	+	+	+
DBY966	suc2–215am	UV	+	+	+	+
DBY989	suc2–231am	None	+	+	+	ND
DBY990	suc2–231am	UV	+	+	+	ND
DBY991	suc2–231am	UV	+	+	+	ND
DBY975	suc2–432	EMS	—	—	—	—
DBY976	suc2–418	EMS	—	—	—	—
DBY977	suc2–171	EMS	—	—	—	—
DBY967	suc2–215am	None	—	—	—	—
DBY968	suc2–215am	EMS	—	—	—	—
DBY969	suc2–215am	EMS	—	—	—	—
DBY970	suc2–215am	UV	—	—	—	—
DBY971	suc2–215am	UV	—	—	—	—

The site of reversion was classified as tightly linked to *SUC2* on the basis of at least five complete tetrads, except in the case of DBY989, for which only 19 spores, including four complete tetrads, were recovered. Among the revertants that failed to grow on YEP-raffinose (and grew weakly on YEP-sucrose), some appeared temperature sensitive when growth on YEP-sucrose at 26° and 35° was compared, so that all crosses for this group were scored at 26°.

Invertase was assayed using the gel assay described in the legend to Figure 3. Both the glycosylated and nonglycosylated forms of the enzyme were detected in strains scored "+" following growth under derepressing conditions. Neither form was detected in strains scored "—"; for these strains, invertase was assayed at 26° under conditions in which invertase was easily detected in "+" strains.

Not included in this Table are eight additional revertants carrying a Suc⁺ determinant closely linked to *SUC2* (see RESULTS): two EMS-induced revertants and one UV-induced revertant of the *suc2–215*am strain, and five UV-induced revertants of the *suc2–231*am strain. The UV-induced revertants were derived from some of the same single colonies as the revertants shown above; however, since UV irradiation stimulated reversion at least 10-fold, it is likely that these were independent revertants.

"N.D." means not determined.

sis revealed this strain to have undergone two genetic events: a duplication of chromosome *IX* and a reversion event at the site of the *suc2*–am mutation on one of the ninth chromosomes (data not shown).

The strains in which the reversion event had occurred at a locus other than *SUC2* shared a number of properties. First, these revertants all had formed colonies without halos on the original selection plates. Second, when tested by spotting cell suspensions, they grew slowly on YEP-sucrose and failed to grow on YEP-raffinose. Third, no invertase activity was detected (see legend to Table 3). Because maltases and α-methylglucosidases cleave sucrose (KHAN and EATON 1967; KHAN and HAYNES 1972; KHAN, ZIMMERMAN and EATON 1973), the capacity of the revertants for growth on YEP-maltose and YEP-α methylglucoside was tested; like the parental strains, they failed to grow. In addition, the revertants showed no enhanced growth relative to parental strains on plates containing low concentrations (0.2%) of glucose or fructose.

Further genetic analysis was carried out on some of these unusual revertants. To determine whether the dominant Suc$^+$ mutations carried by DBY975, DBY976, DBY977 and DBY969 are allele-specific suppressors, each was crossed to a *suc2-215*am strain and/or a S288C *suc2°* strain; the observed 2:2 segregations for sucrose fermentation ruled out this possibility. Sucrose-fermenting strains of both mating types were recovered from these crosses. To test allelism of the Suc$^+$ mutations, DBY975 and a derivative of DBY977 were crossed to one another and 4:0 (Suc$^+$:Suc$^-$) segregations were found (7 tetrads), indicating that the new Suc$^+$ genes in these strains are allelic or tightly linked. Similar analysis (7 tetrads) showed that the sites of reversion in DBY968 and DBY969 are tightly linked, but at a different locus from that in DBY975. Neither revertant Suc$^+$ locus shows tight linkage to *SUC1, SUC3, SUC4, SUC5* or *SUC7*, as judged by the frequent recovery of sucrose-nonfermenting segregants from crosses to tester strains.

Thus, these revertants provided no evidence that the *SUC* loci of S288C (other than *SUC2*) carry silent genes similar to the *suc2°* gene of FL100. No reversion events at known *SUC* loci were identified, and no invertase was detected in the revertants.

A revertible suc2° *allele in another strain*: The above data suggest that S288C does not contain easily revertible silent *suc°* genes like the *suc2°* allele derived from FL100. FL100 is not, however, unusual; the following experiment showed that strain R251–4A also carries a revertible *suc2°* allele. Strain R251–4A (*SUC1$^+$*) was crossed to an S288C derivative (*SUC2$^+$*), and a sucrose-nonfermenting segregant was recovered DBY994 (*suc°*). This strain contains a *suc2°* allele, and probably other *suc°* alleles, derived from R251–4A. Spontaneous Suc$^+$ revertants of DBY994 were selected, and one was crossed to a *SUC2$^+$* strain. Segregations of 4+:0– for sucrose fermentation in 7 tetrads indicated linkage of the new Suc$^+$ determinant to *SUC2*. Thus, both strains tested, FL100 and R251–4A, carry a *suc2°* allele capable of reverting to an active Suc$^+$ state.

DISCUSSION

The *suc2°* allele derived from FL100 reverts to an active Suc$^+$ state, conferring ability to produce invertase and ferment sucrose. Recombination experiments confirmed the presence of *SUC* genetic information in the *suc2°* allele; it is able to provide functional information at three points in the *SUC2* gene. These data indicate that this *suc2°* allele is a silent gene, as illustrated in Figure 1b, that does not confer ability to utilize sucrose either because it is not expressed, or because it produces a defective product. The reversion and recombination experiments also suggest that the *suc2°* defect is not a gross rearrangement or deletion. A second strain, R251–4A, was shown also to carry a revertible *suc2°* allele.

These conclusions have been confirmed by physical studies of these *suc°* genes (CARLSON, OSMOND and BOTSTEIN 1980). Recombinant plasmids containing cloned *SUC2* DNA were used as hybridization probes to detect homologous sequences in genomic DNA from strains of known *SUC* genotype. DNA sequences homologous to *SUC2* DNA and corresponding by genetic criteria to the *suc2°*

allele were detected in FL100 and S288C *suc2°* strains, thus confirming that *suc2°* is a silent gene. The genome of strain R251–4A was also shown to contain a silent *SUC* gene.

The simplest explanation for the presence of a silent *suc2°* allele in the genome of FL100 (and R251–4A) is that it arose by mutation of a *SUC2+* gene and represents a naturally occurring mutant allele. Revertant Suc+ alleles would then result from intragenic mutation. Other more complicated interpretations can also be devised, and we cannot exclude the possibility that this silent gene has an unrecognized function in cellular metabolism. Alternatively, the *suc2°* gene may serve some long-term evolutionary purpose; for example, KOCH (1972) and RIGBY, BURLEIGH and HARTLEY (1974) have proposed that silent gene copies of active genes are important intermediates in the evolution of new proteins.

Other examples of silent genes, or pseudogenes, in eukaryotes have been reported. Apparently defective or inactive copies of active genes have been detected by physical methods in the 5S ribosomal genes in Xenopus (MILLER *et al.* 1978) and in the globin families of man (FRITSCH, LAWN and MANIATIS 1980; PROUD-FOOT and MANIATIS 1980), monkey (MARTIN *et al.* 1980), rabbit (LACY and MANIATIS 1980), and mouse (NISHIOKA, LEDER and LEDER 1980; VANIN *et al.* 1980; JAHN *et al.* 1980).

The nature of the *suc°* alleles (*suc1°*, *suc3°*, etc.) normally present in S288C remains unclear from these studies. All reversion events in sucrose-nonfermenting derivatives of S288C that generated fully active Suc+ elements occurred at the *SUC2* locus. Although revertants carrying Suc+ determinants at loci other than *SUC2* were recovered, they grew only slowly on YEP-sucrose and not at all on YEP-raffinose; moreover, no invertase activity was detected in these strains. A possible explanation of these data is that the revertants produce weakly active or unstable invertase. The new Suc+ determinants tested are apparently not allelic to *SUC1–SUC5* or *SUC7*; however, *SUC6* was not available for testing, and other *SUC* loci may as yet remain unidentified. Thus, these genetic results cannot exclude the possibility that silent *suc°* genes are present in S288C; however, no such silent genes were detected by physical techniques. Using cloned *SUC2* DNA as a probe, we detected no homologous sequences corresponding to *suc°* alleles in S288C (CARLSON, OSMOND and BOTSTEIN 1980).

The ability of these revertants to utilize sucrose may be due to the presence of an enzyme other than invertase able to cleave sucrose, and/or a sucrose uptake system. For example, maltases and α-methylglucosidases cleave sucrose *in vitro* (KAHN and EATON 1967; KAHN and HAYNES 1972; KHAN, ZIMMERMANN and EATON 1973), and *MAL4 suc°* strains, which produce maltase constitutively but make no invertase, can grow on sucrose (KHAN, ZIMMERMANN and EATON 1973). Although these revertants are unable to grow on YEP-maltose or YEP-α-methylglucoside, this finding does not exclude involvement of the *MAL* or *MGL* genes. In bacterial systems, a variety of mechanisms have been reported by which a strain evolved to utilize an unusual substrate, including constitutive or otherwise altered regulation of expression of the structural gene for an enzyme or permease possessing some activity toward the new substrate; duplication of

such a gene; improvement in catalytic efficiency or substrate affinity of such an enzyme or permease; and evolution of new substrate specificities (reviewed by HEGEMAN and ROSENBERG 1970). For example, growth on lactose of *E. coli* strains carrying a deletion in *lacZ* was attributed to evolution of a novel lactase encoded by a different gene (CAMPBELL, LENGYEL and LANGRIDGE 1973; HALL and HARTL 1974).

LITERATURE CITED

BRANDRISS, M. C., L. SOLL and D. BOTSTEIN, 1975   Recessive lethal amber suppressors in yeast. Genetics **79**: 551–560.

CAMPBELL, J. H., J. A. LENGYEL and J. LANGRIDGE, 1973   Evolution of a second gene for β-galactosidase in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **70**: 1841–1845.

CARLSON, M., B. C. OSMOND and D. BOTSTEIN, 1980   *SUC* genes of yeast: a dispersed gene family. Cold Spring Harbor Symp. Quant. Biol. **45**: 799–803. ——, 1981   Mutants of yeast defective in sucrose utilization. Genetics **97**: 25–40.

DE LA FUENTE, G. and A. SOLS, 1962   Transport of sugars in yeasts. II. Mechanisms of utilization of disaccharides and related glycosides. Biochim. Biophys. Acta **56**: 49–62.

FRITSCH, E. F., R. M. LAWN and T. MANIATIS, 1980   Molecular cloning and characterization of the human β-like globin gene cluster. Gene **19**: 959–972.

GABRIEL, O. and S.-F. WANG, 1969   Determination of enzymatic activity in polyacrylamide gels. I. Enzymes catalyzing the conversion of nonreducing substrates to reducing products. Anal. Biochem. **27**: 545–554.

GASCON, S. and J. O. LAMPEN, 1968   Purification of the internal invertase of yeast. J. Biol. Chem. **243**: 1567–1572.

GASCON, S., N. P. NEUMANN and J. O. LAMPEN, 1968   Comparative study of the properties of the purified internal and external invertases from yeast. J. Biol. Chem. **243**: 1573–1577.

GILLILAND, R. B., 1949   A yeast hybrid heterozygotic in four fermentation characters. Compt. Rend. Trav. Lab. Carlsberg, Ser. Physiol. **24**: 347–356.

HALL, B. G. and D. L. HARTL, 1974   Regulation of newly evolved enzymes. I. Selection of a novel lactase regulated by lactose in *Escherichia coli*. Genetics **76**: 391–400.

HEGEMAN, G. D. and S. L. ROSENBERG, 1970   The evolution of bacterial enzyme systems. Ann. Rev. Microbiol. **24**: 429–462.

JAHN, C. L., C. A. HUTCHINSON, III, S. J. PHILLIPS, S. WEAVER, N. L. HAIGWOOD, C. F. VOLIVA and M. H. EDGELL, 1980   DNA sequence organization of the β-globin complex in the BALB/c mouse. Cell **21**: 159–168.

KAWASAKI, G., 1979   Karyotypic instability and carbon source effects in cell cycle mutants of *Saccharomyces cerevisiae*. Ph.D. Dissertation, Univ. of Washington, Seattle, WA.

KHAN, N. A. and N. R. EATON, 1967   Purification and characterization of maltase and α-methyl-glucosidase from yeast. Biochim. Biophys. Acta **146**: 173–180.

KHAN, N. A. and R. H. HAYNES, 1972   Genetic redundancy in yeast: Non-identical products in a polymeric gene system. Molec. Gen. Genet. **118**: 279–285.

KHAN, N. A., F. K. ZIMMERMANN and N. R. EATON, 1973   Genetic and biochemical evidence of sucrose fermentation by maltase in yeast. Molec. Gen. Genet. **123**: 43–50.

KOCH, A. L., 1972 Enzyme evolution: I. The importance of untranslatable intermediates. Genetics **72**: 297–316.

LACROUTE, F., 1968 Regulation of pyrimidine biosynthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **95**: 824–832.

LACY, E. and T. MANIATIS, 1980 The nucleotide sequence of a rabbit β-globin pseudogene. Cell **21**:545–553.

LAWRENCE, C. W. and R. CHRISTENSEN, 1974 Fine structure mapping in yeast with sunlamp radiation. Genetics **76**: 723–733.

MALONE, R. E. and R. E. ESPOSITO, 1980 The *RAD52* gene is required for homothallic interconversion of mating types and spontaneous mitotic recombination in yeast. Proc. Natl. Acad. Sci. U.S. **77**: 503–507.

MARTIN, S. L., E. A. ZIMMER, Y. W. KAN and A. C. WILSON, 1980 Silent δ-globin gene in Old World monkeys. Proc. Natl. Acad. Sci. U.S. **77**: 3563–3566.

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**: 717–725.

MORTIMER, R. K. and D. C. HAWTHORNE, 1966 Genetic mapping in Saccharomyces. Genetics **53**: 165–173. ——, 1969 Yeast genetics. pp. 385–460. In: *The Yeasts*, Vol. 1. Edited by A. H. ROSE and J. S. HARRISON. Academic Press, New York.

NEUMANN, N. P. and J. O. LAMPEN, 1967 Purification and properties of yeast invertase. Biochemistry **6**: 468–475.

NISHIOKA, Y., A. LEDER and P. LEDER, 1980 An unusual alpha globin-like gene that has cleanly lost both globin intervening sequences. Proc. Natl. Acad. Sci. U.S. **77**: 2806–2809.

ONO, B.-I., J. W. STEWART and F. SHERMAN, 1979 Yeast UAA suppressors effective in Ψ+ strains: serine-inserting suppressors. J. Mol. Biol. **128**: 81–100.

OTTOLENGHI, P., 1971 Some properties of five non-allelic β-D-fructofuranosidases (invertases) of *Saccharomyces*. Compt. Rend. Trav. Lab Carlsberg **38**: 213–221.

PRAKASH, S., L. PRAKASH, W. BURKE and B. A. MONTELONE, 1980 Effects of the *RAD52* gene on recombination in *Saccharomyces cerevisiae*. Genetics **94**: 31–50.

PROUDFOOT, N. J. and T. MANIATIS, 1980 The structure of a human α-globin pseudogene and its relationship to α-globin gene duplication. Cell **21**: 537–544.

RIGBY, P. W. J., B. D. BURLEIGH, JR. and B. S. HARTLEY, 1974 Gene duplication in experimental enzyme evolution. Nature **251**: 200–204.

VANIN, E. F., G. I. GOLDBERG, P. W. TUCKER and O. SMITHIES, 1980 A mouse α-globin-related pseudogene lacking intervening sequences. Nature **286**: 222–226.

WINGE, O. and C. ROBERTS, 1952 The relation between the polymeric genes for maltose, raffinose and sucrose fermentation in yeasts. Compt. Rend. Trav. Lab. Carlsberg, Ser. Physiol. **25**: 141–171.

Corresponding editor: E. JONES