MUTANTS OF YEAST DEFECTIVE IN SUCROSE UTILIZATION

MARIAN CARLSON, BARBARA C. OSMOND AND DAVID BOTSTEIN

Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Manuscript received December 17, 1980
Revised copy received March 6, 1981

ABSTRACT

Utilization of sucrose as a source of carbon and energy in yeast (Saccharomyces) is controlled by the classical *SUC* genes, which confer the ability to produce the sucrose-degrading enzyme invertase (MORTIMER and HAWTHORNE 1969). Mutants of *S. cerevisiae* strain S288C (*SUC2*+) unable to grow anaerobically on sucrose, but still able to use glucose, were isolated. Two major complementation groups were identified: twenty-four recessive mutations at the *SUC2* locus (*suc2*$^-$); and five recessive mutations defining a new locus, *SNF1* (for *s*ucrose *n*onfermenting), essential for sucrose utilization. Two minor complementation groups, each comprising a single member with a leaky sucrose-nonfermenting phenotype, were also identified. The *suc2* mutations isolated include four suppressible amber mutations and five mutations apparently exhibiting intragenic complementation; complementation analysis and mitotic mapping studies indicated that all of the *suc2* mutations are alleles of a single gene. These results suggest that *SUC2* encodes a protein, probably a dimer or multimer. No invertase activity was detected in *suc2* mutants.—The *SNF1* locus is not tightly linked to *SUC2*. The *snf1* mutations were found to be pleiotropic, preventing sucrose utilization by *SUC2*+ and *SUC7*+ strains, and also preventing utilization of galactose, maltose and several nonfermentable carbon sources. Although *snf1* mutants thus display a petite phenotype, classic petite mutations do not interfere with utilization of sucrose, galactose or maltose. A common feature of all the carbon utilization systems affected by *SNF1* is that all are regulated by glucose repression. The *snf1* mutants were found to produce the constitutive nonglycosylated form of invertase, but failed to produce the glucose-repressible, glycosylated, secreted invertase. This failure cannot be attributed to a general defect in production of glycosylated and secreted proteins because synthesis of acid phosphatase, a glycosylated secreted protein not subject to glucose repression, was not affected by *snf1* mutations. These findings suggest that the *SNF1* locus is involved in the regulation of gene expression by glucose repression.

YEASTS are able to utilize sucrose as a source of carbon and energy by producing the enzyme invertase, which cleaves sucrose to yield glucose and fructose. Invertase also hydrolyzes related glycosides such as raffinose (reviewed by LAMPEN 1971). Yeast cells produce two forms of the enzyme: a heavily glycosylated enzyme secreted into the periplasmic space ("external" invertase) and an apparently nonglycosylated form retained within the cell ("internal" invertase; NEUMANN and LAMPEN 1967; GASCON and LAMPEN 1968; GASCON, NEUMANN and LAMPEN 1968; OTTOLENGHI 1971). The secreted enzyme is responsible for the first step in sucrose fermentation, the extracellular hydroly-

sis of sucrose (DE LA FUENTE and SOLS 1962); the role of the internal enzyme is not understood. The level of invertase activity is regulated by glucose repression; 100- to 1000-fold changes in activity have been reported in various strains (GASCON and LAMPEN 1968; OTTOLENGHI 1971). Changes in the amount of the glycosylated enzyme are largely responsible for the increased activity under conditions of glucose derepression; the level of the internal nonglycosylated enzyme changes relatively little with changes in glucose concentration (GASCON and LAMPEN 1968; OTTOLENGHI 1971).

The relationship between the two forms of invertase is not clear. The molecular weight of the internal enzyme is 135,000 (GASCON and LAMPEN 1968), and the external enzyme has a protein moiety composed of two apparently identical 60,000 dalton subunits (TRIMBLE and MALEY 1977). The two forms have similar kinetic properties and are serologically cross-reactive (GASCON, NEUMANN and LAMPEN 1968); however, GASCON, NEUMANN and LAMPEN (1968) also have reported that their amino acid compositions differ markedly. Attempts to demonstrate a precursor-product relationship have been unsuccessful (LAMPEN *et al.* 1972; GALLILI and LAMPEN 1977).

Sucrose utilization and production of invertase are controlled by a family of genes called *SUC* genes (reviewed by MORTIMER and HAWTHORNE 1969). Six different *SUC* loci (*SUC1–SUC6*) have been identified by segregational analysis of crosses between different Saccharomyces strains (GILLILAND 1949; WINGE and ROBERTS 1952; MORTIMER and HAWTHORNE 1966). A SUC^+ allele at any one of these loci confers the ability to produce invertase and, thus, to ferment sucrose. An individual haploid yeast strain may contain zero, one or several SUC^+ alleles in its genome. Other genes necessary for sucrose fermentation, but common to different Saccharomyces strains, would not have been identified by these studies. Whether a *SUC* locus contains structural gene(s) for one or both invertases and/or regulatory genes is not entirely clear.[1] HACKEL (1975) isolated nine mutants of a $SUC3^+$ strain that failed to produce either form of invertase; in all cases the mutation was tightly linked to the $SUC3^+$ locus, suggesting the presence of either a regulatory or structural gene needed for production of both enzymes. He also studied other SUC^+ strains with similar results (HACKEL 1977). GROSSMANN and ZIMMERMANN (1979) reported that the structural genes for the nonglycosylated forms of invertase are linked to *SUC* loci on the basis of detecting heterodimeric forms of the internal enzyme in strains carrying two different SUC^+ genes.

We have undertaken a mutational analysis of sucrose utilization with the aim of characterizing the genetic properties of a SUC^+ locus and perhaps identifying other genes, besides *SUC* genes, required for sucrose utilization. Because yeast strains differ with respect to the SUC^+ genes they carry, and perhaps also with respect to other, as yet unidentified, genes involved in sucrose fermentation, our genetic analysis concentrated on strains isogenic to a common laboratory strain of *S. cerevisiae*, S288C. The S288C genome contains a single SUC^+ allele, at the *SUC2* locus. We have isolated and characterized many mutations at the *SUC2* locus of S288C, all of which appear to be alleles of a single gene. We have also

identified a new locus (*SNF1*) involved in the utilization of sucrose and other carbon sources that are regulated by glucose repression.

MATERIALS AND METHODS

Yeast strains: Except for the tester strains described below, all the strains used in this study were derived from S288C (*α SUC2+ gal2 mal^o*) by mutation. S288C and strains carrying the following alleles isolated in the S288C genetic background were obtained from G. FINK: a mating type, *ede2–101*oc, *his4–619*, *his4–86* (ABC oc), *his4–539* (ABC am). A spontaneous *GAL+* revertant (DBY993) was isolated by selection for anaerobic growth on YEP-galactose. Spontaneous *lys2* mutants were isolated from strains carrying either *his4–86*oc or *his4–539*am by selecting for growth on α-aminoadipate medium, as described by CHATTOO *et al.* (1979). To identify *lys2* nonsense mutations, mutants were tested for the frequency of simultaneous reversion to lysine and histidine prototrophy. Frequent co-revertants were obtained from *lys2–801 his4–539*am and *lys2–802 his 4–86*oc strains, and a cross of a co-revertant from each strain to a LYS+ HIS+ tester yielded histidine- and lysine-requiring segregants, as expected if co-reversion occurred by acquisition of a nonsense suppressor. On the basis of these data, *lys2–801* and *lys2–802* were considered to be amber and ochre mutations, respectively. Both were identified as *lys2* alleles by complementation tests with strains DBY766 (**a** *lys2 trp1*; originally from the Cold Spring Harbor Yeast Genetics Course) and DBY731 (**a** *lys1–1 leu2–1*).

The construction of "S288C *suc2^o*" strains by serial backcrosses to S288C is described in the accompanying paper (CARLSON, OSMOND and BOTSTEIN 1981). These strains are congenic to S288C, but differ at the *SUC2* locus; instead of the *SUC2+* gene of S288C, the S288C *suc2^o* strains carry a naturally occurring negative allele (*suc2^o*) derived from strain FL100 (LACROUTE 1968; **a** *suc2^o SUC7+*). Strain DBY987 (**a** *snf1–28 suc2^o ade2–101*) is a segregant from a cross of a *snf1–28* mutant (see RESULTS) by an "S288C *suc2^o*" strain. Strain DBY988 (*α suc2^o SUC7+ his4–619*) is congenic to S288C and carries the *suc2^o* and *SUC7+* alleles of FL100; it was constructed by a procedure analogous to that used in construction of "S288C *suc2^o*" strains.

Cytoplasmic petite strains presumed to lack mitochondrial DNA (*ρ^o*) were derived by growing cells in minimal medium containing 10 μg/ml ethidium bromide (SHERMAN, FINK and LAWRENCE 1978).

Genetic symbols are those proposed by PLISCHKE *et al.* (1976).

General genetic methods: Standard yeast genetic procedures of crossing, sporulation and tetrad analysis were followed, as described by MORTIMER and HAWTHORNE (1969) and SHERMAN, FINK and LAWRENCE (1978).

Media: Rich medium (YEP) was 1% Bacto-yeast extract and 2% Bacto-peptone, solidified with 2% Bacto-agar for plates. YEP-glucose contained 1% glucose when used in testing for ability to ferment sucrose, and 2% glucose otherwise. YEP-sucrose, YEP-raffinose, YEP-maltose and YEP-galactose each contained 2% of the indicated sugar. Sucrose, raffinose and maltose (Pfanstiehl Laboratories) were sterilized by filtration. YEP-glycerol, YEP-ethanol and YEP-lactate each contained 3% (v/v) of glycerol, ethanol or sodium lactate, respectively. Minimal media were prepared and supplemented with nutrients as described by SHERMAN, FINK and LAWRENCE (1978). Sporulation medium contained 1% potassium acetate, 0.1% Bacto-yeast extract, 0.05% glucose, 0.02% raffinose, 2% Bacto-agar and nutritional requirements.

Scoring for ability to ferment sugars: Ability to ferment sucrose (Suc phenotype) was scored by comparing relative growth on YEP-1% glucose and YEP-sucrose plates under anaerobic conditions in a GasPak Disposable Anaerobic System (BBL). Anaerobiosis was required to reduce background growth of respiratory-proficient strains lacking invertase. Raffinose, maltose and galactose utilization were scored similarly, using YEP-raffinose, YEP-maltose and YEP-galactose plates. Cell suspensions were spotted on plates using a metal head with inoculating rods, as described by SHERMAN *et al.* (1974). Conventional replica-plating procedures with velveteens were occasionally used.

Mutagenesis and isolation of mutants: Strains were treated with 3% ethyl methanesulfonate (Eastman Organic Chemicals), as described by SHERMAN, FINK and LAWRENCE (1978). Aliquots

were transferred into sterile 5% sodium thiosulfate after treatment for 30 min, 45 min and 60 min. Cells were then diluted into sterile water and stored at 4° for 2 days while cell survival was determined; cells were stored under conditions nonpermissive for growth between the time of mutagenesis and plating, so that the mutants recovered would be independent. In a typical experiment, 30% of the cells survived following treatment for 45 min. Survivors were plated for single colonies on YEP-glucose medium at 30° and screened for sucrose-nonfermenting mutants at 30° or 35°, as described above, by replica-plating with velveteens. Putative mutants were tested again for inability to ferment sucrose by spotting cell suspensions. Some mutants were originally identified by comparing aerobic growth of colonies with glucose or sucrose as carbon source in minimal medium or in YEP medium containing ethidium bromide.

Complementation analysis: To test pairs of mutations for complementation, heterozygous diploids were constructed and, in most cases, isolated by prototrophic selection. In the few cases where selection against both haploid parents was not possible, diploids were identified following single colony isolation by testing for ability to sporulate. The ability of the diploid to utilize the indicated sugar was then determined.

Fine-structure mapping: Mapping analysis was based on the method of LAWRENCE and CHRISTENSEN (1974) for determining the rate of sunlamp-radiation-induced mitotic recombination. Diploids heteroallelic for noncomplementing pairs of mutations and homoallelic control diploids were constructed as above and purified by single colony isolation. For each diploid, a culture of YEP-glucose was inoculated and grown, with shaking, for 36 hr. Cells were washed and sonicated briefly to disperse cell clumps. The number of viable cells was determined by plating a suitable dilution on YEP-glucose medium. Approximately 3×10^6 viable cells were plated on YEP-sucrose medium and exposed in closed plastic petri dishes to 0, 2, 4 or 6 minutes of sunlamp radiation under an array of four FS20T12 fluorescent sunlamps (Westinghouse). Plates were incubated in the dark under anaerobic conditions at 30° for 3 days before scoring. The dose-response curves were approximately linear; slopes were approximated by the method of least squares.

Gel assay for invertase: To obtain cells repressed and derepressed for invertase, 5 ml of cells growing exponentially (Klett ≃50, green filter) in YEP-2% glucose liquid medium at 30° were collected by centrifugation, washed with YEP and resuspended in an equal volume of either YEP-2% glucose (repressing conditions) or YEP-0.05% glucose (derepressing conditions). Cultures were incubated for another 2.5 to 3 hr with aeration, then harvested by centrifugation and chilled on ice. Cells were washed with cold 25 mM Tris-phosphate buffer (pH 6.7) and collected by centrifugation in 6 × 50 mm glass culture tubes. Cell pellets were frozen at —20° and thawed on ice by addition of 20 μl of cold lysis buffer [25 mM Tris-phosphate (pH 6.7), 10% glycerol, 0.1 mM dithiothreitol, 1 mM EDTA and 2 mM phenylmethyl sulfonyl fluoride]. Glass beads (0.45–0.50 mm diameter) were added until the level of beads reached the meniscus, and cells were broken by vortexing 5 × 15 sec at 4° (HOPPER, BANKS and EVANGELIDIS 1978). An additional 20 μl of lysis buffer was added, and cell debris and glass beads were removed by centrifugation for 1 min at 4° in an Eppendorf microfuge. Supernatant (25 μl) was removed, bromphenol blue was added, and proteins were immediately fractionated by electrophoresis in a 5.5% polyacrylamide slab gel containing 0.1 M Tris -phosphate buffer (pH 6.7) The gel was prepared from a stock of 20% acrylamide, 1% N,N′-methylene bisacrylamide and was polymerized by addition of 0.1% TEMED and 0.03% ammonium persulfate. The tray buffers were 0.1 M Tris-phosphate (pH 6.7). Electrophoresis was carried out at 5V/cm for 5 hr at 4°.

Invertase activity was detected by incubating the gel in 0.1 M sucrose, 0.1 M sodium acetate (pH 4.6) at 30° for 15 min and then staining with 0.1% 2,3,5-triphenyl tetrazolium chloride in 0.5 N NaOH at 100° (GABRIEL and WANG 1969). Gels were sometimes subsequently stained with Coomassie Brilliant Blue to detect protein.

Assay for acid phosphatase: Cells were grown with shaking in SMD medium (BOSTIAN *et al.* 1980) containing low or high phosphate, generously provided by K. BOSTIAN. Cells were harvested in exponential phase by centrifugation, washed twice with water and assayed for acid phosphatase (VAN RIJN, BOER and STEYN-PARVÉ 1972). Cell densities were determined with a Klett-Summerson photoelectric colorimeter. Control strains H42 (**a** *gal4 PHOC PHOE*) and P144-2D (*α phoC*

arg⁻ phoD) were obtained from K. BOSTIAN; H42 produces derepressible and constitutive acid phosphatases, and P144–2D produces virtually no acid phosphatase.

RESULTS

Isolation of mutants: Mutants able to utilize glucose, but not sucrose, for growth were isolated from S288C and two isogenic strains carrying nonsense markers, DBY916 (**a** *his4–86*oc *lys2–802*oc) and DBY963 (**a** *his4–539*am *lys2–801*am), as described in MATERIALS AND METHODS. Because sucrose is hydrolyzed by external invertase to yield glucose and fructose, these sucrose-nonfermenting mutants were expected to lack external invertase activity. Mutants were recovered from six separate experiments, and precautions were taken to ensure the independence of mutants recovered in a single experiment (see MATERIALS AND METHODS).

Each mutant was crossed to a *SUC2*$^+$ derivative of S288C to determine whether the deficiency in sucrose utilization segregated 2:2 as expected for a mutation in a single nuclear gene. Only those mutants in which the sucrose-nonfermenting phenotype could be attributed to a single nuclear mutation were characterized further. Thirty-one mutants were recovered among 22,000 colonies screened.

Identification of nonsense mutations: Those mutations isolated in strains DBY 963 (*his4*–am *lys2*–am) and DBY916 (*his4*–oc *lys2*–oc) were tested for co-reversion with the nonsense markers by first plating each mutant strain on medium selective for simultaneous reversion to histidine and lysine prototrophy, and then testing co-revertants for growth on sucrose. Four Suc$^-$ mutations isolated in DBY-963 (*suc2–202*, *suc2–215*, *suc2–231* and *suc2–748*; see below) reverted simultaneously with the amber markers and were presumed to be amber mutations. To confirm this conclusion, a co-revertant of each of the four mutant strains was crossed to DBY782 (*α ade2 SUC2*$^+$; S288C derivative) for tetrad analysis; as expected, histidine-requiring, lysine-requiring and sucrose-nonfermenting segregants were recovered, and the phenotypic segregations observed were consistent with the segregation of an amber suppressor able to suppress the *his4*–am, *lys2*–am and Suc$^-$ mutations.

Complementation analysis: Mutations were analyzed for their ability to complement one another for anaerobic growth on YEP-sucrose at 30°. Four complementation groups were identified, as shown in Table 1. The major group comprises 24 recessive mutations, including the four amber mutations, that failed to complement amber mutation *suc2–215*. These mutations were identified as *suc2* mutations by their inability to complement the naturally occurring *suc°* alleles in one of the S288C *suc2°* strains, DBY936 (*α suc2° ade2*) (see MATERIALS AND METHODS and CARLSON, OSMOND and BOTSTEIN 1981). By its construction, DBY936 differs from S288C only at the *SUC2* locus (and in nutritional markers); thus, mutations failing to complement its sucrose utilization deficiency are, by definition, *suc2*$^-$ mutations.

All pairwise combinations of *suc2* mutations were then tested for complementation. No complementation was observed, with four exceptions: *suc2–437* com-

TABLE 1

Complementation analysis of Suc⁻ mutations

Mutant allele	Parent strain	Complementation						
		suc2°	*suc2–215*am	Group 1A alleles*	Group 1B alleles*	Group 1C *suc2–437*	*snf1–31*	Group 2 alleles*
Group 1A								
suc2–1	S288C	—	—	—	—	—	+	ND
suc2–2	DBY916	—	—	—	—	—	+	ND
suc2–171	DBY916	—	—	—	—	—	+	ND
suc2–172	DBY916	—	—	—	—	—	+	ND
*suc2–202*am	DBY963	—	—	—	—	—	+	ND
suc2–206	DBY963	—	—	—	—	—	+	ND
*suc2–215*am	DBY963	—	—	—	—	—	+	ND
*suc2–231*am	DBY963	—	—	—	—	—	+	ND
suc2–236	DBY963	—	—	—	—	—	+	ND
suc2–418	DBY916	—	—	—	—	—	+	ND
suc2–428	DBY916	—	—	—	—	—	+	ND
suc2–432	DBY916	—	—	—	—	—	+	ND
suc2–533	DBY916	—	—	—	—	—	+	ND
suc2–538	DBY916	—	—	—	—	—	+	ND
suc2–686	DBY916	—	—	—	—	—	+	ND
suc2–693	DBY916	—	—	—	—	—	+	ND
suc2–725	DBY963	—	—	—	—	—	+	ND
*suc2–748*am	DBY963	—	—	—	—	—	+	ND
suc2–760	DBY963	—	—	—	—	—	+	ND
Group 1B								
suc2–8	S288C	—	—	—	—	+	+	ND
suc2–191	DBY963	—	—	—	—	+	+	ND
suc2–684	DBY916	—	—	—	—	+	+	ND
suc2–739	DBY963	—	—	—	—	+	+	ND
Group 1C								
suc2–437	DBY916	—	—	—	+	—	+	+
Group 2								
snf1–20	S288C	+	+	ND	ND	+	—	—
snf1–25	S288C	+	+	ND	ND	+	—	—
snf1–28	S288C	+	+	ND	ND	+	—	—
snf1–30	DBY916	+	+	ND	ND	+	—	—
snf1–31	DBY916	+	+	ND	ND	+	—	—
Group 3†								
C9	DBY916	+	+	ND	ND	+	+	+
Group 4†								
*ts*C25‡	DBY916	+	+	ND	ND	+	+	ND

* All alleles in each Group were tested for complementation.
† C9 and *ts*C25 complemented one another.
‡ Complementation tests with *ts*C25 were carried out at 35°.

plemented *suc2–8*, *suc2–191*, *suc2–684* and *suc2–739* for growth on YEP-sucrose at 30° and 35°, but not for growth on YEP-raffinose. *SUC2*$^+$ strains grow slowly on raffinose, which is a poorer substrate than sucrose for invertase (GASCON, NEUMANN and LAMPEN 1968).

A second complementation group includes five mutations that complemented *suc2–215*am and *suc2*^{o}, but failed to complement one another. These mutations define a new locus involved in sucrose utilization, designated *SNF1* (*s*ucrose *n*on*f*ermenting).

Two additional recessive mutations were recovered, both leaky and each apparently representing a separate complementation group. One of these is temperature sensitive; mutation *ts*C25 had no effect at 30°, but conferred a reduced ability to grow on sucrose, as compared to glucose, at 35°. Neither mutation was characterized further, due to the difficulties inherent in working with their leaky phenotypes.

SNF1 *is not linked to* SUC2: To determine whether the *SNF1* and *SUC2* loci are linked, a *snf1* mutant (*SUC2*$^+$ *snf1–28*) was crossed to DBY936 (*suc2*^{o} *SNF*$^+$). Tetrad analysis showed at least one sucrose-fermenting recombinant in each of five tetrads, showing that *SNF1* and *SUC2* are not tightly linked. The *snf1* mutations will be discussed in detail below.

Properties of the suc2 *mutants*: The *suc2* mutants were tested for anaerobic growth on YEP-sucrose and YEP-raffinose at 26°, 30° and 35°. All *suc2* mutants failed to grow on YEP-sucrose or YEP-raffinose at any temperature, with the following exceptions. The *suc2–2* mutation allowed slow growth on YEP-sucrose, but not on YEP-raffinose, at all temperatures. Both *suc2–686* and *suc2–693* showed temperature-sensitive phenotypes, conferring a nearly normal capacity to grow on sucrose at 26° and 30° and a somewhat reduced capacity at 35°; however, neither conferred the ability to grow on YEP-raffinose at any temperature. Moreover, the Suc$^+$ phenotypes associated with these alleles were recessive in complementation tests; diploids heteroallelic for *suc2–686* or *suc2–693* and other *suc2* mutations failed to grow on YEP-sucrose at 30° or, in a few cases, grew weakly. Interestingly, the homoallelic diploids did not grow nearly as well on YEP-sucrose at 30° as did the mutant haploids, in comparison to wild-type controls. These two mutant alleles were recovered from the same mutagenesis and their similarity in phenotype suggests that they may not, despite the precautions taken, be independent.

Fine-structure mapping of the SUC2 *locus*: A fine-structure map, including the *suc2*–am mutations and the complementing *suc2* mutations, was constructed from the frequencies of sunlamp-radiation-induced mitotic recombination between pairs of alleles (LAWRENCE and CHRISTENSEN 1974). Diploids heteroallelic for noncomplementing pairs of *suc2* alleles and diploids homoallelic for each of the *suc2* alleles were constructed, and mitotic recombinants were detected as sucrose-fermenting progeny. Mitotic recombination was stimulated by increasing doses of sunlamp radiation, and an approximate map, shown in Figure 1, was derived from the slopes of the linear dose-response curves. Amber mutations *suc2–231* and *suc2–748* are very tightly linked and were isolated from the same

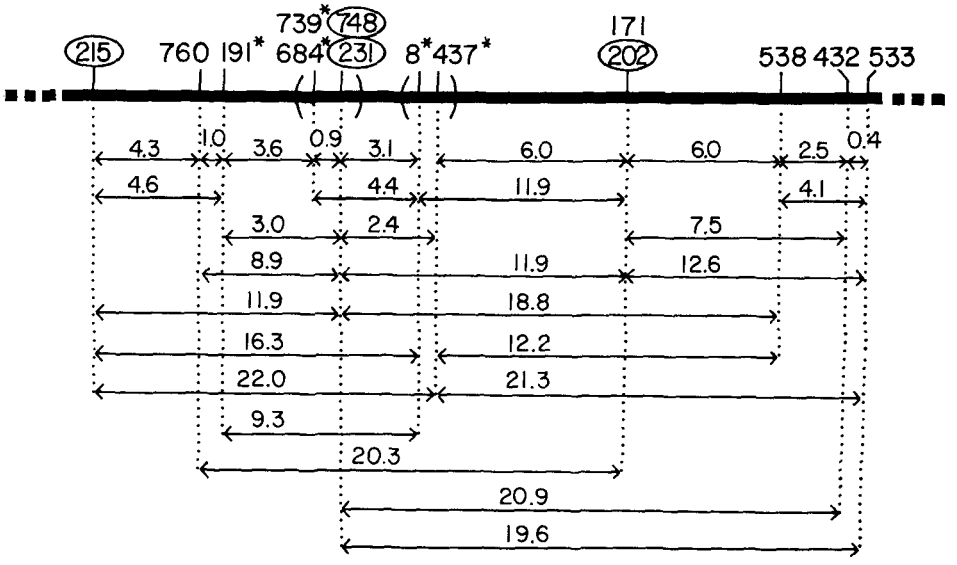

FIGURE 1.—Genetic map of the *SUC2* locus, based on sunlamp-radiation-induced recombination frequencies in heteroallelic diploids. Amber alleles are circled, and alleles involved in intragenic complementation are starred; *suc2–191*, *suc2–684*, *suc2–739* and *suc2–8* complement *suc2–437*. No recombination was detected in *suc2–684/suc2–739*, *suc2–231/suc2–748* or *suc2–171/suc2–202* diploids. The data shown are based on experiments with *suc2–684*, *suc2–231* and *suc2–202*, but data from experiments with the other allele of each pair were consistent with the map order shown. Rates of recombination are shown in units of recombinants per 10^6 survivors per minute sunlamp radiation. No recombination was detected in any of the 14 homoallelic diploids.

mutagenesis of DBY963. Mutations *suc2–684* and *suc2–739* are also tightly linked and both complemented *suc2–437*; however, they are unquestionably independent as they were isolated from different strains. Two other tightly linked mutations, *suc2–171* and *suc2–202*, were also isolated from different strains.

Both forms of invertase are lacking in suc2 *mutants*: A representative set of 12 mutants was analyzed for the presence of both the glycosylated and nonglycosylated forms of invertase, using a gel assay. A crude cellular lysate was subjected to electrophoresis in a polyacrylamide gel to separate the two forms of invertase, and enzymatic activity was detected *in situ* (GABRIEL and WANG 1969), as described in MATERIALS AND METHODS. As shown in Figure 2, both enzymes were detected in wild-type S288C cells grown under derepressing conditions (low glucose concentration), but only the nonglycosylated form was detected in S288C cells grown under repressing conditions (high glucose). No invertase activity was detected in *suc2* mutants (except *suc2–686*; *suc2–693* was not tested) or S288C *suc2°* strains grown in low glucose.

Properties of the snf1 *mutants*: The five *snf1* mutants were isolated as sucrose nonfermenters; however, it was immediately evident that they also grow somewhat poorly on YEP-glucose. They formed small colonies on YEP-2% glucose plates aerobically; in tests carried out by spotting cell suspensions, they grew under anaerobic conditions somewhat more slowly than wild type on YEP-2% glucose and much more slowly on YEP-0.2% glucose. They showed a marked

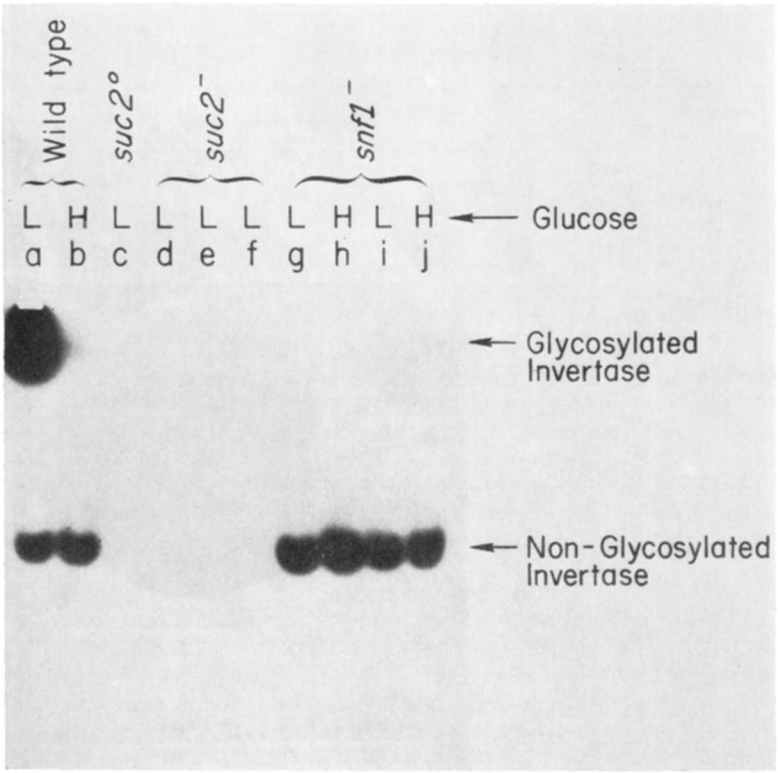

FIGURE 2.—Assays of sucrose-nonfermenting mutants for invertase. Strains were grown under glucose-repressing or derepressing conditions, as indicated, and were assayed for the presence of glycosylated and nonglycosylated invertase by the gel assay described in MATERIALS AND METHODS. (a) *SUC2*+ strain DBY963, grown in low glucose (L); (b) DBY963, grown in high glucose (H); (c) S288C *suc2°* strain DBY938 (**a** *suc°* *ade2*), low glucose; (d) *suc2–231*am mutant, low glucose; (e) *suc2–533* mutant, low glucose; (f) *suc2–760* mutant, low glucose; (g) *snf1–28* mutant, low glucose; (h) *snf1–28* mutant, high glucose; (i) *snf1–31* mutant, low glucose; (j) *snf1–31* mutant, high glucose.

deficiency in aerobic growth on YEP-glycerol, YEP-ethanol and YEP-lactate (the classic petite phenotype), although the deficiency was not as complete as that of a petite (ρ^o) control strain (see MATERIALS AND METHODS). The petite phenotype of the *snf1* mutants co-segregated in tetrads with inability to ferment sucrose, and the five *snf1* mutants failed to complement one another for growth on YEP-ethanol. To ascertain whether the reduced capacity for growth on glucose exhibited by the *snf1* mutants relative to wild-type strains could be remedied by sufficiently high glucose concentrations, growth rates of *snf1* mutants and the wild type were determined in liquid YEP medium containing 7.5% glucose. The two *snf1* mutants tested (*snf1–20* and *snf1–28*) grew more slowly than wild type in 0.1% glucose, but grew at nearly the same rate as wild type in 7.5% glucose (Table 2). Also, as has been found for other petite strains, a *snf1–28/snf1–28* diploid was defective in sporulation; less than 1% of the diploids sporulated under conditions that normally induced extensive sporulation of S288C-

TABLE 2

Growth rates of snf1 *mutants*

SNF1 genotype	Doubling time (hrs) 0.1% glucose	7.5% glucose
SNF1+	3.1	2.0
snf1–20	> 10	2.4
snf1–28	> 10	2.1

A fresh culture was grown from a single colony in YEP-2% glucose liquid medium. Cells were diluted at least 50-fold into YEP-7.5% glucose in a Klett flask and incubated at 26° with rotatory shaking. Growth was monitored, using the Klett-Summerson photoelectric colorimeter equipped with a no. 54 green filter. When the culture was growing exponentially, cells were collected by filtration, washed with YEP and resuspended in an equal volume of YEP-7.5% glucose or YEP-0.1% glucose. Growth rates were again monitored, and doubling times were estimated.

derived strains. The *snf1* mutants are known to carry functional mitochondrial genomes because diploids constructed by mating *snf1–20*, *snf1–28* and *snf1–31* mutants to a cytoplasmic petite (ρ^o) mutant were able to grow on YEP-glycerol.

The deficiency in sucrose utilization of the *snf1* mutants is not due directly to their petiteness. Petite, presumably ρ^o, derivatives of a *SUC2+ GAL+* strain (see MATERIALS AND METHODS) were able to ferment sucrose and galactose. Nor is the deficiency dependent on anaerobiosis. Because the *snf1* mutants are petite, their ability to utilize sucrose could be scored clearly under aerobic as well as anaerobic conditions, and they were sucrose nonfermenting under both conditions. This observation ruled out the possibility that anaerobiosis affects their sucrose utilization phenotype.

snf1 *mutations prevent the expression of* SUC7+: The *snf1* mutations prevent utilization of sucrose in strains carrying a *SUC2+* allele. To determine whether the *snf1* mutations also affected the function of other *SUC* loci, we crossed DBY987 (*snf1–28 suc2°*) with DBY988 (*SNF+ SUC7+ suc2°*; see MATERIALS AND METHODS for strain descriptions). The observed phenotypic segregations in tetrads for ability to ferment sucrose [1(0+:4−); 4(1+:3−); 1(2+:2−)] were those expected from independent segregation of *SUC7+* and *snf1*, if *snf1 SUC7* haploids have a sucrose-nonfermenting phenotype. To confirm that a strain of genotype *snf1–28 SUC7+* is unable to ferment sucrose, each of the four spore clones of a tetratype ascus (1+:3−) was mated to an S288C *suc2°* strain (*SNF1+ suc°*) to test for the presence of a *SUC7+* gene by complementation. The genotypes inferred for each spore clone are shown in Table 3. As predicted, one of the sucrose nonfermenting spores complemented the tester strain for growth on sucrose and presumably had the genotype *snf1–28 SUC7+*.

snf1 *mutations prevent utilization of other sugars*: A common feature of the systems for utilizing sucrose and nonfermentable carbon sources (glycerol, ethanol and lactate) in yeast is that both are regulated by glucose repression (POLAKIS and BARTLEY 1965; POLAKIS, BARTLEY and MEEK 1965; GASCON and LAMPEN 1968; OTTOLENGHI 1971; PERLMAN and MAHLER 1974). Because induc-

TABLE 3

Effect of snf1 *on the phenotype of* SUC7+: *tetratype ascus from* SNF1+ SUC7+ × snf1–28 suc^0

Spore clone	Relevant genotype	Phenotype
4A	*SUC7+ snf1–28*	Suc⁻
4B	*SUC7+ SNF1+*	Suc+
4C	suc^0 *SNF1+*	Suc⁻
4D	suc^0 *snf1–28*	Suc⁻

Genotypes were assigned to the spore clones of this tetratype ascus from DBY987 × DBY988, as explained in the text.

tion of enzymes involved in maltose and galactose fermentation is also subject to glucose repression (VAN WIJK *et al.* 1969; ADAMS 1972), we carried out experiments to test the effects of *snf1* on utilization of these sugars. A direct test of the ability of *snf1* mutants to ferment maltose and galactose was not feasible, because the S288C genetic background in which the *snf1* mutations were isolated was mal^0 *gal2*. Therefore, the effect of *snf1* alleles on maltose and galactose fermentation was determined by tetrad analysis of crosses between *snf1* mutants and *SNF+* strains carrying *MAL2+*, *MAL3+*, *MAL4+* or *GAL+* alleles in experiments analogous to those described above with *SUC7+*. Segregations of the *snf1* muta-

TABLE 4

Phenotypic segregation in crosses of snf1 *mutants to* MAL+ *and* GAL+ *strains*

Cross	Relevant diploid genotype	Phenotype scored	Number of tetrads 0+:4−	1+:3−	2+:2−
DBY934 × 1453–3A	$\frac{snf1\text{–}28}{SNF+}$ $\frac{mal^0}{MAL2+}$ $\frac{gal2}{GAL+}$	Mal	0	5	2
		Gal	4	3	0
DBY934 × DBY1000	$\frac{snf1\text{–}28}{SNF+}$ $\frac{mal^0}{MAL3+}$	Mal	1	3	2
DBY992 × 1403–7A	$\frac{snf1\text{–}28}{SNF+}$ $\frac{mal^0}{MAL4+}$	Mal	1	5	0
DBY992 × DBY993	$\frac{snf1\text{–}28}{SNF+}$ $\frac{gal2}{GAL+}$	Gal	0	3	2

Strains DBY934 (**a** *snf1–28 SUC2+ gal2 his4–619*), DBY992 (**a** *snf1–28 SUC2+ gal2 ade2–101*) and DBY993 (**a** *suc2–202 GAL+ his4–539 lys2–801*) were derivatives of S288C. Strains 1453–3A (**a** suc^0 *MAL2 MEL1 his4 leu2*), 1412–4D (**a** *SUC3 MAL3 MEL1 MGL2 MGL3 ade2*) and 1403–7A [**a** suc^0 *MAL4 MGL3 gal3 gal4 trp1 ura3* (*MEL1*?)] were obtained from the Yeast Genetic Stock Center (Berkeley, CA). Strain DBY1000 (**a** *SUC3 MAL3 his4 ade2*) was a segregant from a cross between 1412–4D and an S288C $suc2^0$ strain.

MAL and *GAL* genotypes of selected spore clones were inferred from complementation tests with mal^0 *gal2 SNF+* tester strains (see RESULTS). Phenotypically maltose- and galactose-nonfermenting spores were assigned the following genotypes: *snf1–28 MAL3+* (5 spores); *snf1–28 MAL2+ GAL+* (1 spore); *snf1–28 GAL+* (1 spore from DBY934 × 1453–3A; 3 spores from DBY992 × DBY993); *snf1–28 MAL4+* (1 spore). Segregation of nutritional markers (2:2) was also followed in these crosses.

tion, scored by inability to grow on YEP-glycerol or YEP-ethanol, were 2:2 in all crosses. As shown in Table 4, phenotypic ratios of 0+:4–, 1+:3–, and 2+:2– for maltose and galactose utilization were observed in tetrads. All *snf1* spore clones were nonfermenters. The *MAL* and *GAL* genotypes of several such spore clones from each cross were inferred from their ability to complement *SNF*$^+$ *mal*^{o} *gal2* tester strains for growth on maltose and galactose; for each cross, one or more phenotypically nonfermenting spore clones of genotype *snf1 MAL*$^+$ or *snf1 GAL*$^+$ were identified (see legend to Table 4). The *GAL* genotype was tested for all spores of the five tetrads from the cross of DBY992 by DBY993, and *GAL*$^+$ segregated 2:2 in this cross, as did *snf1*; the phenotypic and inferred genotypic segregations in a representative tetratype ascus are shown in Table 5.

snf1 *mutants produce only nonglycosylated invertase*: Because the *snf1* mutants failed to ferment sucrose, it was unlikely that they secreted invertase (DE LA FUENTE and SOLS 1962). To determine whether they produced either form of invertase, the *snf1* mutants were analyzed by the gel assay described above. During growth in glucose-repressing conditions, the *snf1* mutants produced the nonglycosylated form of invertase, as did wild-type strains (Figure 2). During growth in derepressing conditions, however, the *snf1* mutants produced the nonglycosylated enzyme, but failed to produce activity corresponding to the glycosylated form. Although this gel assay is not quantitative, no disproportionately large amount of activity was detected in derepressed *snf1* mutants at the position of the nonglycosylated enzyme, as might have been expected were activity equivalent to that of the glycosylated secreted enzyme accumulating in a nonglycosylated state within the cell.

Assay of acid phosphatase in snf1 *mutants*: To determine whether the *snf1* phenotype includes a defect in production of a glycosylated secreted enzyme not regulated by glucose repression, acid phosphatase was assayed (VAN RIJN, BOER and STEYN-PARVÉ 1972). Acid phosphatase activity in cells grown under derepressing and repressing conditions (low and high phosphate concentrations, respectively) was compared. Table 6 shows that several *snf1* mutants and their wild-type (*SNF*$^+$) parent derepressed acid phosphatase when grown in low phosphate.

TABLE 5

Effect of snf1 *on galactose utilization: tetratype ascus from* GAL+ SNF+ × gal2 snf1–28

Spore clone	Relevant genotype	Phenotype
2A	*gal2 SNF+*	Gal–
2B	*GAL+ snf1–28*	Gal–
2C	*gal2 snf1–28*	Gal–
2D	*GAL+ SNF+*	Gal+

The spore clones of this tetratype ascus from DBY992 × DBY993 were assigned *GAL* genotypes on the basis of their ability to complement *SNF+ mal*^{o} *gal2* strains for growth on galactose; *SNF* genotypes were determined by ability to utilize glycerol (see text).

TABLE 6

Derepression of acid phosphatase in snf1 *mutants*

Experiment	Strain	Relevant genotype	Acid phosphatase activity* (units/10^6 cells)	
			Low phosphate	High phosphate
1	S288C	*SNF+ PHO+*	2.7	0.4
	DBY1036	*snf1-28 PHO+*	3.7	0.5
	H42	*PHO+*	7.0	0.7
	P144-2D	*phoC phoD*	<0.1	<0.1
2	S288C	*SNF+ PHO+*	3.6	<0.1
	DBY1037	*snf1-20 PHO+*	1.4	0.1
	DBY1038	*snf1-30 PHO+*	2.6	0.1

* External acid phosphatase was assayed in whole cells grown in low or high concentrations of phosphate, as described in MATERIALS AND METHODS. Units are nmoles of p-nitrophenol released per minute.

DISCUSSION

We isolated and characterized 31 mutants of S288C (*SUC2+*) unable to ferment sucrose. Each of these mutants carries a single nuclear mutation responsible for its sucrose-nonfermenting phenotype. The 31 mutations define two major complementation groups essential for utilization of sucrose. One group was identified with the *SUC2* locus by complementation tests with an S288C *suc2°* strain congenic to S288C but carrying a *suc2°* allele, rather than *SUC2+*. The recovery of *suc2* mutations was anticipated on the basis of previous work (HACKEL 1977). A second major group, consisting of the five *snf1* mutations, was not anticipated; these mutations proved to have pleiotropic effects on utilization of sugars other than sucrose.

The 24 *suc2* mutations appear to define a single gene at the *SUC2* locus. All *suc2* mutations fail to complement one another, with the exception of four pairs: *suc2-8*, *-191*, *-684* and *-739* complement *suc2-437* for growth on sucrose. These four cases are most likely examples of intragenic complementation because no complementation was observed for growth on raffinose (indicating incomplete restoration of the wild-type phenotype) and because each of these five mutations fail to complement every other *suc2* mutation. Mitotic mapping studies also support this interpretation; the five complementing mutations all map at positions flanked by sites of noncomplementing *suc2* mutations. Thus, the map order of 14 mutations is most consistent with the interpretation that the complementing, as well as the noncomplementing, alleles are all alleles of a single gene. It should be noted, however, that MOORE and SHERMAN (1975) have shown that allele orders determined by five commonly used mapping methods, including the sunlamp-radiation method used here, are occasionally incorrect.

Among the *suc2* mutations recovered are four amber mutations, identified by co-reversion with other amber markers carried by the mutant strain. Genetic analysis confirmed that the sucrose fermenting phenotype of co-revertants is due to suppression. Isolation of suppressible amber mutations in the *SUC2* gene indicates that this gene encodes a protein, and the intragenic complementation ob-

served suggests that this protein is dimeric or multimeric. Also recovered were two unusual mutations, *suc2–686* and *–693*, that show a deficiency in growth on raffinose, but nearly normal, although somewhat temperature-sensitive, ability to utilize sucrose; the sucrose-fermenting phenotype of these alleles was recessive in most heteroallelic diploids and weaker in homoallelic diploids than in haploids, as compared to wild-type controls. These latter observations remain unexplained.

Invertase activity was not detected in the *suc2* mutants tested, with the exception of the *suc2–686* mutant (*suc2–693* was not tested). The absence of either form of invertase is in accord with previous studies of *suc*$^-$ mutants (HACKEL 1975, 1977). These results are also consistent with the evidence of GROSSMANN and ZIMMERMANN (1979) that each *SUC* locus contains a structural gene for the internal invertase (and probably also for the glycosylated secreted invertase).

Our mutational analysis provides evidence for only one gene at the *SUC2* locus. The possibility remains, however, that the locus includes another gene or genes essential for sucrose utilization, but in which mutations are not easily recovered. For example, mutations in a gene that is duplicated in the genome would have no phenotype. The *MAL*$^+$ loci of yeast, a gene family responsible for maltose utilization, appear to be a case in point. NAUMOV (1976) has suggested on the basis of complementation analysis that each *MAL*$^+$ locus comprises two closely linked complementary genes, one of which was not identified by segregational or mutational analysis due to the presence of more than one active copy in the genomes of the strains investigated.

We have identified a novel locus essential for sucrose fermentation, the *SNF1* locus. This locus is not tightly linked to *SUC2* and affects utilization of not only sucrose (*via SUC2*$^+$ and *SUC7*$^+$ genes), but also galactose, maltose (*via MAL2*$^+$, *MAL3*$^+$ and *MAL4*$^+$ genes) and nonfermentable carbon sources. Failure to utilize nonfermentable carbon sources is one aspect of the petite phenotype exhibited by *snf1* mutants; however, the petite phenotype *per se* does not include the inability to ferment sucrose and galactose, because petite (ρ^o) derivatives of S288C are able to ferment both sugars. Utilization of all these sugars is repressible by glucose. KHAN and EATON (1971) have reported that the maltase activity controlled by the *MAL4*$^+$ allele used here (from strain 1403–7A) is constitutive and resistant to glucose repression; however, the regulation of maltose uptake was not investigated and may remain subject to glucose repression. These observations suggest that the *snf1* mutants are defective in derepressing expression of glucose-repressible genes in response to low glucose concentrations. Biochemical evidence also supports this view; under derepressing conditions, *snf1* mutants produce the essentially constitutive internal invertase, but not the glucose-repressible glycosylated enzyme. Assays of secreted acid phosphatase confirmed that the *snf1* mutants are capable of producing a secreted glycosylated protein not subject to glucose repression and, in addition, clearly distinguished *snf1* mutants from the secretory (*sec*) mutants of NOVICK, FIELD and SCHEKMAN (1980). In summary, the *SNF1* locus appears to be involved in the regulation of gene expression by glucose repression.

This work was supported by grants from the American Cancer Society (MV-90A) and the Public Health Service (GM21253). M. CARLSON was a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research. We thank MARJORIE BRANDRISS and DEBORAH DARAGO for use of their sunlamp equipment and RUSSELL MAURER for a critical reading of the manuscript.

[1] Direct confirmation that *SUC2* is a structural gene encoding invertase has been obtained by comparison of a partial invertase amino acid sequence (D. PERLMAN, H. O. HALVORSON and L. E. CANNON, personal communication) with a partial nucleotide sequence of cloned *SUC2* DNA (M. CARLSON, P. GRISAFI and D. BOTSTEIN, unpublished). Also, L. RODRIGUEZ, J. O. LAMPEN and V. L. MACKAY recently published (Mol. Cell. Biol. **1**: 469–474, 1981) genetic evidence showing that *SUC1* is also a structural gene encoding invertase.

LITERATURE CITED

ADAMS, B. G., 1972 Induction of galactokinase in *Saccharoymces cerevisiae*: kinetics of induction and glucose effects. J. Bacteriol. **111**: 308–315.

BOSTIAN, K. A., J. M. LEMIRE, L. E. CANNON and H. O. HALVERSON, 1980 *In vitro* synthesis of repressible yeast acid phosphatase: Identification of multiple mRNAs and products. Proc. Natl. Acad. Sci. U.S. **77**: 4504–4508.

CARLSON, M., B. C. OSMOND and D. BOTSTEIN, 1981 Genetic evidence for a silent *SUC* gene in yeast. Genetics **97**: 41–54.

CHATTOO, B. B., F. SHERMAN, D. A. AZUBALIS, T. A. FJELLSTEDT, D. MEHNERT and M. OGUR, 1979 Selection of *lys2* mutants of the yeast *Saccharomyces cerevisiae* by the utilization of α-aminoadipate. Genetics **93**: 51–65.

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.

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.

GALLILI, G. and J. O. LAMPEN, 1977 Large and small invertases and the yeast cell cycle. Pattern of synthesis and sensitivity to tunicamycin. Biochim. Biophys. Acta **475**: 113–122.

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.

GROSSMANN, M. K. and F. K. ZIMMERMANN, 1979 The structural genes of internal invertases in *Saccharomyces cerevisiae*. Molec. Gen. Genet. **175**: 223–229.

HACKEL, R. A., 1975 Genetic control of invertase formation in *Saccharomyces cerevisiae*. I. Isolation and characterization of mutants affecting sucrose utilization. Molec. Gen. Genet. **140**: 361–370. ——, 1977 Genetic and biochemical studies of sucrose fermentation in the yeast *Saccharomyces cerevisiae*. Ph.D. Dissertation, City University of New York, N.Y.

HOPPER, A. K., F. BANKS and V. EVANGELIDIS, 1978 A yeast mutant which accumulates precursor tRNAs. Cell **14**: 211–219.

KHAN, N. A. and N. R. EATON, 1971 Genetic control of maltase formation in yeast. I. Strains producing high and low basal levels of enzyme. Molec. Gen. Genet. **112**: 317–322.

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

LAMPEN, J. O., 1971 Yeast and neurospora invertases. pp. 291–305. In: *The Enzymes*, 3rd Edition, Vol. 5. Edited by P. D. BOYER. Academic Press, New York.

LAMPEN, J. O., S.-C. KUO, F. R. CANO and J. S. TKACZ, 1972 Structural features in synthesis of external enzymes by yeast. pp. 819–824. In: *Fermentation Technology Today*, Proc. of the IVth Intl. Fermentation Symposium. Edited by G. TERUI. Society of Fermentation Technology, Osaka, Japan.

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

MOORE, C. and F. SHERMAN, 1975 Role of DNA sequences in genetic recombination in the iso-1-cytochrome *c* gene of yeast. I. Discrepancies between physical distances and genetic distances determined by five mapping procedures. Genetics **79:** 397–418.

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.

NAUMOV, G. I., 1976 Comparative genetics of yeasts. XVI. Genes for maltose fermentation in *Saccharomyces carlsbergensis* N.C.Y.C. 74. Genetika **12:** 87–100.

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

NOVICK, P., C. FIELD and R. SCHEKMAN, 1980 Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell **21:** 205–215.

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

PERLMAN, P. S. and H. R. MAHLER, 1974 Derepression of mitochondria and their enzymes in yeast: Regulatory aspects. Arch. Biochem. Biophys. **162:** 248–271.

PLISCHKE, M. E., R. C. VON BORSTEL, R. K. MORTIMER and W. E. COHN, 1976 Genetic markers and associated gene products in *Saccharomyces cerevisiae*. pp. 767–832. In: *Handbook of Biochemistry and Molecular Biology, Nucleic Acids*, Vol. II, 3rd Edition. Edited by G. D. FASMAN. CRC Press, Cleveland, Ohio.

POLAKIS, E. S. and W. BARTLEY, 1965 Changes in the enzyme activities of *Saccharomyces cerevisiae* during aerobic growth on different carbon sources. Biochem. J. **97:** 284–297.

POLAKIS, E. S., W. BARTLEY and G. A. MEEK, 1965 Changes in the activities of respiratory enzymes during the aerobic growth of yeast on different carbon sources. Biochem. J. **97:** 298–302.

SHERMAN, F., G. R. FINK and C. W. LAWRENCE, 1978 *Cold Spring Harbor Laboratory Manual for a Course, Methods in Yeast Genetics*, revised edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

SHERMAN, F., J. W. STEWART, M. JACKSON, R. A. GILMORE and J. H. PARKER, 1974 Mutants of of yeast defective in iso-1-cytochrome *c*. Genetics **77:** 255–284.

TRIMBLE, R. B. and F. MALEY, 1977 Subunit structure of external invertase from *Saccharomyces cerevisiae*. J. Biol. Chem. **252:** 4409–4412.

VAN RIJN, H. J. M., P. BOER and E. P. STEYN-PARVÉ, 1972 Biosynthesis of acid phosphatase of baker's yeast. Factors influencing its production by protoplasts and characterization of the secreted enzyme. Biochim. Biophys. Acta **268:** 431–441.

VAN WIJK, R., J. OUWEHAND, T. VAN DE BOS and V. V. KONINGSBERGER, 1969 Induction and catabolite repression of α-glucosidase synthesis in protoplasts of *Saccharomyces carlsbergensis* Biochim. Biophy. Acta **186:** 178–191.

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