SUC1 Gene of *Saccharomyces*: a Structural Gene for the Large (Glycoprotein) and Small (Carbohydrate-Free) Forms of Invertase

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Saccharomyces cerevisiae revertant strain D10-ER1 has been shown to contain thermosensitive forms of the large (glycoprotein) and small (carbohydrate-free) invertases and a very low level of the small enzyme, along with a wild-type level of the large form (T. Mizunaga et al., Mol. Cell. Biol. 1:460-468, 1981). These characteristics cosegregated in crosses of the revertant strain with wild-type sucrose-fermenting (*SUC1*) or nonfermenting (*suc0*) strains. In addition, there is tight linkage between sucrose and maltose fermentation in revertant D10-ER1 (characteristic of the *SUC1* and *MAL1* genes). From this we infer that a single reversion event is responsible for the several changes observed in D10-ER1, and that this mutation maps within or very close to the *SUC1* gene present in the ancestor strain 4059-358D. The revertant *SUC1* allele in D10-ER1 (termed *SUC1-R1*) was expressed independently of the wild-type *SUC1* gene when both were present in diploid cells. Diploids carrying only the wild-type or the mutant genes synthesized invertases with the characteristics of the parental *Suc*⁺ haploids. The possibility that a modifier gene was responsible for the alterations in the invertases of revertant D10-ER1 was ruled out by appropriate crosses. We conclude that *SUC1* is a structural gene that codes for both the large and the small forms of invertase and suggest that *SUC2* through *SUC5* are structural genes as well.

Saccharomyces cerevisiae contains both a large (glycoprotein) invertase and a small (carbohydrate-free) form (7, 8). The production of these enzymes is dependent on the presence of any one of the nonallelic *SUC* genes whose functions have been obscure. Ethyl methane sulfonate mutagenesis of *S. cerevisiae* 4059-358D (*SUC1*) yielded an invertase-negative mutant (D10). Crosses of this mutant with a *suc0* strain or with the *SUC1* parent 4059-358D gave results consistent with the presence in D10 of a mutant allele of the *SUC1* gene. On this basis, mutant D10 was provisionally termed *suc1-1* (5).

Treatment of mutant D10 with ethyl methane sulfonate led to the isolation of a *Suc*⁺ revertant (D10-ER1) that produces as much large invertase as strain 4059-358D but only trace amounts of the small enzyme (1; R. Hackel, Ph.D. thesis, City University of New York, New York, 1977). In addition, both the large and small invertases of the revertant are thermosensitive (5). Preliminary studies indicated that the thermosensitivity of the two invertases and the low level of the small form cosegregated in crosses of D10-ER1 with *SUC1* or *suc0* strains and that the second mutation in the revertant mapped close to or

within the *SUC1* gene (5). In this communication further genetic experiments are presented which provide strong evidence that the revertant *SUC* gene in D10-ER1 is an allele of *SUC1* and that *SUC1* is a structural gene for both the large and small forms of invertase.

MATERIALS AND METHODS

Yeast strains. Genotypes of the strains used in this study are presented in Table 1.

Culture media. Media for maintenance of the strains, for maximal invertase production, and for genetic analyses have been described previously (1, 4). Fermentation of sucrose and maltose was tested on solid YEP medium (0.3% yeast extract, 0.5% peptone, 2% agar) containing 0.0055% bromocresol purple. Sucrose or maltose was added (2% final concentration) after the medium was autoclaved.

Invertase assay. Invertase was assayed at 30°C as described by Goldstein and Lampen (2).

Genetic analysis. Crosses between a and a haploid strains were carried out by mixing cells on solid YEPD medium (1% yeast extract, 2% peptone, 2% glucose, 2% agar), and zygotes were selected by micromanipulation. Ascus dissection, tetrad analysis, and mitotic recombination analysis were performed by standard methods (6).

Preparation of crude extracts. Yeast cells were grown in the indicated medium and harvested by centrifugation. The cells were washed twice with

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TABLE 1. Genotypes of yeast strains

Strain	Known genetic markers ^a	Source or reference
4059-358D	α <i>SUC1 MAL1 MGL ade1 ade2 leu1 CUP</i>	(1)
D10-ER1 ^b	α <i>SUC1-R1 MAL1 ade1 ade2 ade leu1</i>	(1)
D10-ER1-3A	a <i>SUC1-R1 MAL1</i>	(1)
dgr3	a <i>SUC3 MAL3</i>	(1)
Z1-2D	α <i>suc0 mal0 leu1 lys1</i>	(1)
A3	a <i>SUC mal0 leu2-2 leu2-112 his3-11 his3-15 can1</i>	B. Tye
M415	α <i>suc0 ade3 tyr1</i>	M. Leibowitz
XV570-1-1A	a <i>SUC1-R1 leu1 ade</i>	This paper
XV570-1-1B	α <i>SUC1-R1 ade</i>	This paper
XV570-1-1C	a <i>SUC1 leu1 ade</i>	This paper
XV570-1-1D	α <i>SUC1 ade</i>	This paper
XV570-1-5A	α <i>SUC1 ade</i>	This paper
XV570-1-5B	a <i>SUC1-R1 leu1 ade</i>	This paper
XV570-1-5C	a <i>SUC1 leu1 ade</i>	This paper
XV570-1-5D	α <i>SUC1-R1 ade</i>	This paper
XV571-1-10A	α <i>suc0 leu1</i>	This paper
XV571-1-10B	α <i>SUC1-R1 leu1</i>	This paper
XV571-1-10C	a <i>SUC1-R1 lys1</i>	This paper
XV571-1-10D	a <i>suc0 lys1</i>	This paper
XV571-1-35A	α <i>SUC1-R1 lys1</i>	This paper
XV571-1-35B	a <i>SUC1-R1 leu1</i>	This paper
XV571-1-35C	a <i>suc0 leu1</i>	This paper
XV571-1-35D	α <i>suc0 lys1</i>	This paper
XV572-1-4B	α <i>suc0 his3 leu1 lys1</i>	This paper
XV572-1-7B	α <i>suc0 leu1 leu2 lys1</i>	This paper
XV598-3-4B	a <i>SUC1-R1</i>	This paper
XV598-3-32A	α <i>suc0 ade3 tyr1</i>	This paper

^a *ade*, *leu*, *lys*, *his*, and *tyr* refer to requirements for adenine, leucine, lysine, histidine, and tyrosine, respectively; *can* refers to a mutation conferring resistance to canavanine. *CUP* indicates copper resistance. *MGL* indicates fermentation of α -methyl glucoside. *MAL* refers to a maltose gene, *SUC* refers to a sucrose gene, and *mal* and *suc* indicate the lack of these genes. The numbers following the gene symbol indicate the locus; numbers following the hyphen indicate the allele of the respective locus (if known). **a** and α indicate the mating type.

^b Strain D10-ER1, and presumably 4059-358D as well, carries an *ade* mutation which is not *ade1*, 2, 3, or 8. When applied to other strains, *ade* simply means that at least one *ade* mutation is present.

distilled water and once with 50 mM tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 7.5) containing 10 mM CaCl₂, 1 mM MgCl₂, and 0.1 M NaCl and were then suspended in this buffer (1 ml/g of cell paste). Cell suspensions were shaken in a Braun MSK homogenizer (Melsungen, Germany) with 0.45-mm glass beads (1 g/g of cell paste) for 2 min and centrifuged for 10 min at 10,000 $\times$ *g*. After a second centrifugation for 20 min at 40,000 $\times$ *g*, the supernatants were collected and used immediately for determination of the thermosensitivity of the two invertase forms and the percentage of the total activity present as small invertase.

Concanavalin A-Sepharose chromatography. Concanavalin A-Sepharose columns (0.5 by 6 cm) were used to separate large invertase, which binds to the lectin, from the small form of the enzyme, which flows through the column. The procedure was that described by Abrams et al. (1).

Materials. Media components were from Difco Laboratories, Detroit, Mich. Nutritional supplements for synthetic media were purchased from Calbiochem (La Jolla, Calif.). Bromocresol purple was from Sigma Chemical Co., St. Louis, Mo. All other reagents were analytical grade.

RESULTS

Segregation of sucrose fermentation in crosses of D10-ER1-3A with sucrose-fermenting and -nonfermenting strains. *S. cerevisiae* D10-ER1 and D10-ER1-3A are mutant strains which are characterized by alterations in the level of small invertase as well as in the thermostability of both the large and the small forms of the enzyme (5). D10-ER1 is an ethyl methane sulfonate-induced Suc⁺ revertant of an invertase-negative mutant, D10 (*suc1-1 MAL1*), which was itself derived from strain 4059-358D (*SUC1 MAL1*). D10-ER1-3A was obtained by crossing D10-ER1 with the segregational sucrose-nonfermenting strain EZ-5B (*a suc0 mal0*) (1).

As a working hypothesis, we considered that D10-ER1 and D10-ER1-3A may contain a revertant allele of the *suc1-1* mutation in strain D10. In our preliminary genetic studies, both D10-ER1 and the meiotic segregant D10-ER1-3A were found to be capable of fermenting both

maltose and sucrose (as does 4059-358D); there is known to be tight linkage between the *SUC1* and *MAL1* genes of *Saccharomyces* (6). Consequently, we tentatively termed the revertant gene *SUC1-R1*. To determine whether this classification is correct, D10-ER1-3A was crossed with the ancestral strain 4059-358D and with three sucrose-nonfermenting strains. The segregation of sucrose fermentation in those crosses is presented in Table 2. The 4:0 segregation in all of the 34 tetrads derived from cross XV570 gives support to the hypothesis that *SUC1* is the gene responsible for sucrose fermentation in D. 0-ER1-3A.

In crosses XV571, XV591 and XV592, there were relatively few four-spored asci for dissection, a condition which leads to a high frequency of "false asci." In the worst case, cross XV571, there were 21 false asci and 10 incomplete asci out of 65 tetrads dissected. Although many of the false tetrads can be detected by the non-2:2 segregation of markers, the few markers present in these crosses (four or five in addition to *SUC1-R1*) prevent the complete elimination of such asci from the data. Thus, the six asci with 3 Suc^+ :1 Suc^- (as compared to the 66 that scored 2 Suc^+ :2 Suc^-) are probably false asci or possibly gene conversions. The data from all four crosses taken together are not consistent with the presence of more than one *SUC* gene in the crosses unless these are closely linked.

Cosegregation of sucrose and maltose fermentation. The known tight linkage between *SUC1* and *MAL1* genes (6) provided an opportunity to obtain additional information about the possible allelism of *SUC1* and the revertant *SUC* gene.

Tetrads from crosses XV570 and XV571 were plated on YEP-maltose-bromocresol purple medium to test for maltose fermentation. The segregation pattern clearly corresponded to that for sucrose fermentation (Table 2). In cross XV570 all of the sucrose-positive spores were also maltose fermenters, and in cross XV571 only 3 out of 34 tetrads analyzed contained spores which were $Suc^+ Mal^-$.

Cosegregation of the low level of small invertase and the thermosensitivity of

large invertase in crosses XV570 and XV571. The known modifications affecting the invertases of revertants D10-ER1 and D10-ER1-3A (low content of small invertase and thermosensitivity of both small and large forms of the enzyme) should cosegregate in crosses if they are the result of a single mutation. The segregation of those characters in one tetrad from diploid HZ400 was in agreement with that prediction (5). To test this assumption more fully, two tetrads from cross XV570 and two from cross XV571 were randomly selected, and their level of small invertase and the thermostability of the large enzyme were measured. As shown in Table 3, in the tetrads from cross XV570 two of the four Suc^+ spores had a thermosensitive large invertase, and the same spores were deficient in small invertase. The tetrads from cross XV571 exhibited a 2:2 segregation for sucrose fermentation, and the sucrose-positive spores had a thermosensitive large invertase and a low content of small enzyme.

Expression of wild-type and mutant genes in diploids. If the modifications of the invertases in mutant D10-ER1 and derived strains are the results of a single mutation in a structural gene, one would expect the mutant gene to be expressed independently of the wild-type gene when both are present in a cell. Invertase-producing diploids were constructed by crossing mutant and wild-type sucrose-fermenting haploids as well as a sucrose-nonfermenting strain in all possible combinations. The level of small invertase and the thermostability of the large enzyme in the diploids were compared with the values for the original haploids (Table 4).

The characteristics of the diploids homozygous for the *SUC1* gene, either two wild-type *SUC1* genes (XR-1) or two revertant *SUC1-R1* genes (XR-2), were similar to those of the corresponding parental haploids. In the *SUC1/SUC1* diploid, the percentage of small invertase was approximately the same as in the wild-type parental haploid strains, and the large invertase was almost completely thermostable. In the *SUC1-R1/SUC1-R1* diploid, the percentage of small invertase was as low as in the mutant parental strains, and the large invertase was

TABLE 2. Segregation of sucrose and maltose fermentation in crosses of D10-ER1-3A with other strains

Cross	Strain crossed with D10-ER1-3A	Segregation of $Suc^+ : Suc^-$					Segregation of $Mal^+ : Mal^-$				
		4:0	3:1	2:2	1:3	0:4	4:0	3:1	2:2	1:3	0:4
XV570	4059-358D (<i>SUC1</i>)	34 ^a	0	0	0	0	34	0	0	0	0
XV571	Z1-2D (<i>suc0</i>)	0	3	31	0	0	0	1	32	1	0
XV591	XV572-1-4B (<i>suc0</i>) ^b	0	1	22	0	0	— ^c	—	—	—	—
XV592	XV572-1-7B (<i>suc0</i>) ^b	0	2	13	0	0	—	—	—	—	—

^a Number of tetrads.

^b Spores derived from the cross XV572 between strains Z1-2D and A3.

^c —, Not done.

TABLE 3. *Cosegregation of a low level of small invertase and thermosensitivity of large invertase in crosses XV570 and XV571*

Spore	Sucrose fermentation ^a	Small invertase (% of total invertase) ^b	Thermosensitivity of large invertase ^c
XV570-1-1A	+	0.2	ts
1B	+	0.1	ts
1C	+	6.4	N
1D	+	3.1	N
XV570-1-5A	+	4.2	N
5B	+	0.3	ts
5C	+	6.1	N
5D	+	0.5	ts
XV571-1-10A	—	— ^d	
10B	+	0.5	ts
10C	+	0.2	ts
10D	—	—	
XV571-1-35A	+	0.3	ts
35B	+	0.4	ts
35C	—	—	
35D	—	—	

^a Determined on YEP-sucrose-bromocresol purple plates as described in the text.

^b Cell extracts, prepared as described in the text, were fractionated on columns of concanavalin A-Sepharose. Invertase activity not retained by the column was considered to be the small form of the enzyme.

^c Invertase samples (crude extracts) containing 0.010 to 0.015 U of enzyme were incubated for 10 min in 0.2 N sodium acetate buffer (pH 4.9) at 60°C. The samples were quickly cooled on ice and assayed for residual activity. N (normal), More than 90% of original activity retained; ts (thermosensitive), less than 10% retained.

^d Cells were invertase negative (<0.2 mU of invertase per mg of cells). The wild-type strain 4059-358D produces 5 to 8 U/mg of cells under similar conditions (1).

inactivated as readily as the enzyme of the mutant haploids.

When a haploid carrying the wild-type invertases was crossed with a mutant strain, the resulting diploid (XV570-1) had approximately half the level of small invertase present in the wild-type parent, and about 50% of the large invertase was thermostable. This is the result expected if both wild-type and mutant genes are expressed to the same extent.

Crossing a sucrose nonfermenter with a wild-type *SUC1* strain or a *SUC1-R1* strain yielded diploids, XR-3 and XV571, respectively, whose invertases exhibited the characteristics of the parental *SUC1* or *SUC1-R1* haploid.

Wild-type and mutant *SUC1* haploids were also crossed with a *SUC3* strain to produce

diploids containing two different *SUC* genes. The characteristics of the invertases synthesized by the *SUC1/SUC3* diploid (XR-5) or the *SUC1-R1/SUC3* diploid (XR-4) were similar to those of the corresponding *SUC1/SUC1* or *SUC1-R1/SUC1* diploids. From these results it is evident that there is independent expression of the normal and the mutant genes when they are present in the same diploid cell.

Does a modifier exist which causes the thermosensitivity of the invertases? The correlation between small invertase content and thermostability of large invertase gives support to the assumption that both characters, and consequently both enzymes, are coded by a single structural *SUC1* gene. Nevertheless, the possible existence of a separate modifier gene which could alter both the level of small invertase and the thermostability of the two enzymes was considered. If such a gene were to exist, the parental strain 4059-358D should be Mod⁻ (modifier absent or inactive), whereas the mutant strains D10-ER1 and D10-ER1-3A should be Mod⁺ (modifier present), since 4059-358D has a normal invertase whereas the two other strains have thermosensitive enzymes (5). According to this hypothesis, the segregation in cross XV570 should be 2 Mod⁺:2 Mod⁻, that is, two spores with normal invertase and two with sensitive enzymes per tetrad. This prediction is in agreement with the results obtained (Table 3). Similarly, in cross XV571 the parental strain Z1-2D should be Mod⁺ because all the sucrose-fermenting strains obtained in this cross have thermosensitive invertase. To test this possibility, Z1-2D was crossed with XV570-1-1C which, according to the hypothesis, should be a Mod⁻ strain. From this cross nine tetrads with a 2:2 segregation for sucrose fermentation were analyzed. Crude extracts were prepared from cells derived from all of the sucrose-fermenting spores, and the thermosensitivity of the invertase was assayed as described in Table 3. All of the 18 spores tested had stable invertase. From this result we conclude that Z1-2D is not a Mod⁺ strain. Consequently, there is no evidence for the existence of a modifier whose activity is responsible for the temperature sensitivity of the large and small invertases, unless such a modifier is closely linked to or allelic with *SUC1*. We should point out, however, that we cannot entirely eliminate the possibility that the wild-type strain 4059-358D itself contains a modifier whose action is essential for the production of active thermostable invertase and which is altered in the mutants. It is difficult to propose a biochemical basis for such a modification. In addition, the process would have to be highly specific because, for example, the analogous yeast en-

TABLE 4. Small invertase level and thermostability of large invertase in diploids and in the parental haploid strains: independent expression of wild-type and mutant phenotypes

Strain	SUC gene(s)	Small invertase ^a (% of total invertase)	Residual activity of large invertase (%) after heating at 60°C for ^b :	
			5 min	30 min
XR-1 (4059-358D × XV570-1-5C)	<i>SUC1/SUC1</i>	4.6	94.5	86
XV570-1 (4059-358D × D10-ER1-3A)	<i>SUC1/SUC1-R1</i>	2.3	63.5	47
XR-2 (D10-ER1 × D10-ER1-3A)	<i>SUC1-R1/SUC1-R1</i>	0.2	3.5	0
XR-3 (Z1-2D × XV570-1-5C)	<i>suc0/SUC1</i>	5.6	94.5	91
XV571-1 (Z1-2D × D10-ER1-3A)	<i>suc0/SUC1-R1</i>	0.2	6	0
XR-5 (4059-358D × dgr3)	<i>SUC1/SUC3</i>	5.7	91	84.5
XR-4 (D10-ER1 × dgr3)	<i>SUC1-R1/SUC3</i>	2.3	47	35
4059-358D	<i>SUC1</i>	5.3	98.5	88.5
XV570-1-5C	<i>SUC1</i>	6.1	94	88
D10-ER1	<i>SUC1-R1</i>	0.2	7	0
D10-ER1-3A	<i>SUC1-R1</i>	0.1	2.5	0
dgr3	<i>SUC3</i>	5	97	80

^a See footnote b, Table 3.^b See footnote c, Table 3.

zyme acid phosphatase is not affected in the mutants.

DISCUSSION

The major conclusion to be reached from the work presented here and in the accompanying paper (5) is that the *SUC1* gene of yeast is a structural gene for both the large glycoprotein invertase and the small carbohydrate-free form. The basis for this conclusion is summarized below.

The *SUC1* gene in revertant D10-ER1 (and the derived strains) appears to be an allele of *SUC1*. (i) The original ancestor (strain 4059-358D) carries the *SUC1* gene tightly linked to *MAL1*. (ii) The invertase-negative mutant D10 appears to carry a mutant allele (termed *suc1-1*) of the *SUC1* gene (5). (iii) The segregation of sucrose fermentation in a cross between the Suc⁺ revertant and a *SUC1* strain was 4:0. (iv) Sucrose and maltose fermentation cosegregated in crosses of the mutant with either a *SUC1* or a *suc0* strain. Thus the *SUC1-R1* gene of revertant D10-ER1 appears to map within or very close to the *SUC1* gene of strain 4059-358D.

The second major finding is that the low level of small invertase and the thermosensitivity of the large and the small enzymes in the revertant (*SUC1-R1*) strain D10-ER1 are both consequences of a single mutation in or very close to the *SUC1* gene. These characteristics cosegregated with the *SUC1* gene in the HZ400 series of spores (5) and in crosses XV570 and XV571 (Table 3). Furthermore, there was no evidence for the existence of a modifier gene that might be responsible for the changes in the invertases.

The purified large invertases from strain 4059-358D and the revertant D10-ER1 are antigenically and catalytically similar and showed parallel behavior during purification (5). These findings are consistent with the interpretation that the D10-ER1 enzyme is an altered form of the 4059-358D enzyme.

Lastly, the fact that the mutant gene is expressed independently of the wild-type gene in diploids is most readily explained if one assumes that *SUC1* is a structural gene and that it codes for both the large and small forms of invertase.

There is considerable evidence that *SUC2* to *SUC5* are also structural genes. Ottolenghi (7, 8) has shown that strains carrying any one of the genes *SUC1* to *SUC5* produce both large and small invertases, and that these forms are comparable in size to those in *SUC1* strains. The kinetic constants for cleavage of sucrose by the partially purified enzymes are similar but not identical (7). This behavior would be expected for a series of closely related genes, probably derived from a common progenitor, that had been inherited independently for a substantial period of time. In addition, we have demonstrated that the protein portions of the large glycoprotein invertases of the *SUC1* strain 4059-358D, the *SUC1-R1* mutant D10-ER1, and the *SUC2* hyperproducing mutant FH4C are of approximately the same size and cross-react antigenically.

Grossmann and Zimmermann (3) reported that the electrophoretic mobility of the native small invertases was characteristic of the *SUC* gene responsible for their production and that the enzyme from their *SUC1* strain moved more slowly than the others under these conditions.

Cells containing *SUC1* and one of the other *SUC* genes formed invertase bands characteristic of the two individual genes and an intermediate band of invertase which they considered to be a hybrid. This result is consistent with our findings that the purified small invertase from mutant FH4C (*SUC2*) is a dimer with a native molecular weight of approximately 120,000 and a subunit weight of about 60,000 as measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (L. Rodriguez and J. McCullough, unpublished data).

We consider it likely that genes *SUC2* to *SUC5* are like *SUC1* in that they are structural genes for both the large and small invertases. To our knowledge, mutations in the *SUC* genes have been identified with modified invertase proteins only in the *SUC1-R1* revertant strain D10-ER1 (5; see above). Nevertheless, the catalytic and biochemical similarities among the products of the several *SUC* genes and the available genetic data all support the designation of *SUC1* to *SUC5* as structural genes.

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