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A Zinc Finger Protein from *Candida albicans* Is Involved in Sucrose Utilization

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A sucrose-inducible α-glucosidase activity that hydrolyzes sucrose in *Candida albicans* has been demonstrated previously. The enzyme is assayable in whole cells and was inhibited by both sucrose and maltose. A *C. albicans* gene (*CASUC1*) that affects sucrose utilization and α-glucosidase activity was cloned by expression in a *Saccharomyces cerevisiae suc2* mutant (2102) devoid of invertase genes. *CASUC1* enabled the *S. cerevisiae* mutant to utilize both sucrose and maltose. DNA sequence analysis revealed that *CASUC1* encodes a putative zinc finger-containing protein with 28% identity to a maltose-regulatory gene (*MAL63*) of *S. cerevisiae*. The gene products of *CASUC1* and *MAL63* are approximately the same size (501 and 470 amino acids, respectively), and each contains a single zinc finger located at the N terminus. The zinc fingers of *CASUC1* and *MAL63* comprise six conserved cysteines (C_6 zinc finger) and are of the general form Cys-Xaa_2-Cys-Xaa_6-Cys-$Xaa_{variable}$-Cys-Xaa_2-Cys-Xaa_6-Cys (where Xaa_n indicates a stretch of the indicated number of any amino acids). Both contain five amino acids in the variable region. *CASUC1* also complemented the maltose utilization defect of an *S. cerevisiae* mutant (TCY-137) containing a defined mutation in a maltose-regulatory gene. The sucrose utilization defect of type II *Candida stellatoidea*, a sucrase-negative mutant of *C. albicans*, was corrected by *CASUC1*. Determinations of α-glucosidase activity in whole cells revealed that activity was restored in transformants cultivated on either sucrose or maltose. To our knowledge, this is the first zinc finger-encoding gene, as well as the first putative regulatory gene, to be identified in *C. albicans*.

Candida albicans, the major opportunistic fungal pathogen of humans, causes serious systemic disease in immunocompromised patients and topical infections in healthy individuals (22). Recent studies of the molecular genetics of *C. albicans* have been facilitated by the development of a DNA-mediated transformation system for this diploid imperfect fungus (35). *Candida stellatoidea* is a closely related yeast that is considered a sucrose-negative variant of *C. albicans* (43). Following the discovery of high DNA identity between these two yeasts, the differential ability to assimilate sucrose was thought to be insufficient to warrant separate species classification.

Recently, isolates of *C. stellatoidea* have been classified into two genetically distinct types, called I and II (39). Isolates of type I are clearly distinguishable from those of type II and *C. albicans* by their unique electrophoretic karyotype and the fact that they are avirulent in mouse models. In addition, type I differs from type II and *C. albicans* in mitochondrial DNA restriction patterns, midrepeat sequence of nuclear DNA, resistance to UV irradiation, growth rate in vitro, and proteinase activity on bovine serum albumin agar at pH 3.8 (38, 39). Type II *C. stellatoidea* is identical to a reference strain of *C. albicans* in all of these properties and has been shown to be simply a sucrase-negative mutant of *C. albicans* (37). The sucrose-negative phenotypes of type I and type II *C. stellatoidea* are due to different mutations, as hybrids produced by protoplast fusion of the two types were capable of assimilating sucrose (38). Suc^+ prototrophs have been obtained from both types of *C. stellatoidea* by selection on sucrose-containing medium (37, 57). The Suc^+ derivatives of type II have characteristics of typical revertants (37); however, the Suc^+ isolates of type I have several interesting properties atypical of revertants. In contrast to the parent strains, the Suc^+ derivatives of type I are virulent in mouse models and exhibit a different electrophoretic karyotype, an indication that a chromosomal rearrangement has taken place (57).

Very little is known biochemically about sucrose utilization in *C. albicans*. Recently, a sucrose-inducible enzyme activity that hydrolyzes sucrose has been demonstrated in whole-cell assays (37). The enzyme appears to be an extracellular α-glucosidase rather than invertase (β-fructofuranosidase). This was not a surprising finding, as *C. albicans* does not grow on raffinose, a substrate of invertase. The α-glucosidase activity was inhibited by sucrose and, to a lesser extent, by maltose.

The purpose of this study was to clone the gene(s) involved in sucrose utilization from *C. albicans*, with the long-term goal being to understand their organization and regulation of expression in the Suc^+ derivatives of *C. stellatoidea* as well as in *C. albicans*. The natural occurrence of two different mutations affecting the same process provides a unique opportunity to dissect the steps involved in sucrose utilization genetically and biochemically. Such studies have not as yet been carried out in *C. albicans*, owing largely to the inherent difficulties of performing genetic studies on an asexual diploid. At present, very little is known about gene regulation in *C. albicans*; neither transcription factors nor regulatory sequences have been identified. DNA sequence analysis of a limited number of structural genes from *C. albicans* has revealed that the 5′ and 3′ noncoding regions of these genes contain features characteristic of *Saccharomyces cerevisiae* genes, but they have not been studied extensively (reviewed by Kurtz et al. [36]).

Here we report the isolation, by selection in *S. cerevisiae*,

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TABLE 1. Yeast strains used in this study

Strain	Genotype	Relevant phenotypes	Source or reference
C. albicans			
SC5314		Suc$^+$ Mal$^+$	Clinical isolate, E. R. Squibb & Sons (13)
C. stellatoidea			
1481P	*CASUC1/CASUC1*	Suc$^+$ Mal$^+$	This study
B-4365P	*ade1/ade1 casuc1/casuc1*	Suc$^-$ Mal$^+$	40
S. cerevisiae			
TCY-137	*MAL13::LEU2 ura3-52 leu2-3,-112*	Suc$^+$ Mal$^-$	Marmur (7)
2102	*suc2-Δ9 ura3-52 leu2-3,-112 his4-519*	Suc$^-$ Mal$^-$	Emr (10)

of a *C. albicans* gene (*CASUC1*) that complements the sucrose utilization defect of type II *C. stellatoidea*. The isolated gene encodes a protein containing a putative zinc finger motif. To our knowledge, this is the first zinc finger-encoding gene, as well as the first regulatory gene, to be identified in *C. albicans*.

MATERIALS AND METHODS

Strains and media. The yeast strains used are listed in Table 1. Strain 1481P was a Suc$^+$ clonal culture derived from B-4365P. Competent cells of *Escherichia coli* DH5α (Bethesda Research Laboratories, Gaithersburg, Md.) were used for plasmid propagation. X-Glu-containing medium contained 7 g of yeast nitrogen base without amino acids, 1 g of glucose, and 20 g of agar per liter. Fifty microliters of a 5-mg/ml solution of X-Glu (5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside; Boehringer Mannheim) dissolved in *N*,*N*-dimethylformamide was spread on the surface of each plate. Sucrose and maltose were filter sterilized and added to the appropriate medium at a final concentration of 2% (wt/vol). GE medium, containing 6.7 g of yeast nitrogen base per liter, 3% glycerol, and 2% ethanol, was supplemented to a final concentration of 2% with either maltose (GEM), sucrose (GES), or maltose and glucose (GEMG). All other media have been described previously and are standard for yeast genetics studies (51).

Plasmids. YEp13, YEp352, and pUC19 have been described previously (6, 16, 49). p1056 contains the *C. albicans ADE1* gene and autonomously replicating sequence (ARS) and was kindly provided by Stewart Scherer (50a). E. R. Squibb & Sons generously provided the *Sau*3A partial digestion library of *C. albicans* genomic DNA in YEp13 (13) and pADE1-1, which contains the *C. albicans ADE1* gene in vector YEp13 (36). pMR42 contains an *S. cerevisiae* maltose-regulatory gene (*MAL63*) cloned into YEp13 and was a gift from Julius Marmur (8).

The isolation of the *C. albicans* clone pCASUC1 by complementation of the *S. cerevisiae suc2* mutant 2102 is described in the Results section. All plasmids were constructed by standard recombinant DNA techniques. Plasmid pRK54 was constructed by subcloning the *Sph*I-*Eco*RI fragment of pCASUC1 containing the *C. albicans* insert into the *Sph*I-*Eco*RI site of pUC19. The plasmids shown in Fig. 1 were constructed by subcloning the fragments indicated into the appropriate site of the multicloning region of YEp352. Plasmid pRK60 was made by blunt ending the 2.3-kb *Sph*I-*Xba*I fragment of pRK54 and ligating it to a *Bam*HI-*Sal*I digest of p1056 that was also blunt ended.

Yeast transformation. *S. cerevisiae* was transformed by the spheroplast procedure of Beggs (3) or by the lithium acetate procedure of Ito et al. (23). *C. stellatoidea* was transformed by the *Saccharomyces* protocol described previously for *C. albicans* (35).

Nucleic acid isolations and hybridizations. Plasmid DNAs were isolated from minilysates of *E. coli* by the boiling procedure of Holmes and Quigley (19) and from large-scale preparations by an alkaline lysis method (49). *C. albicans* genomic DNA was isolated by a method for *S. cerevisiae* described by Hoffman and Winston (18). Total RNA was prepared by standard methods (51) from cultures grown to mid-log phase on minimal medium containing the carbon source designated in the legend to Fig. 8. DNA slot blots were prepared by applying 5 μg of each sample to a Minifold II slot blot apparatus (Schleicher and Schuell) according to the directions of the manufacturer. The blots were prehybridized at 68°C in 5× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate)–5× Denhardt's solution (49)–0.15 M sodium phosphate buffer (pH 6.7)–0.5% sodium dodecyl sulfate–100 μg of salmon sperm DNA per ml. The hybridization conditions were the same, except that the Denhardt's solution was used at 1×. Preparation of the radiolabeled probe and washing conditions have been described previously (28). Southern and Northern (RNA) blot hybridizations were performed as described previously (28, 29).

Nucleotide sequence analysis. The nucleotide sequence of both strands of the 2.3-kb *Sph*I-*Xba*I fragment of pRK54 was determined by the method of Sanger et al. (50). Restriction fragments were subcloned into M13mp18 and M13mp19 (49), and sequencing was initiated from the universal primer region. As *C. albicans* sequences were identified, new primers were prepared to extend the sequence. The sequence was analyzed with the University of Wisconsin Genetics Computer Group sequence analysis software package (9) at the National Cancer Institute Supercomputing Facility, Frederick, Md.

Enzymatic assay of α-glucosidase. α-Glucosidase activity was assayed in whole cells of the two *Candida* species grown to mid-log phase in YEP broth supplemented with 2% sugar by a procedure similar to a previously reported method (37). The cultures were harvested by centrifugation and washed two times in phosphate-buffered saline (0.01 M sodium phosphate, 0.14 M NaCl [pH 7.4]). The cells were resuspended in 1 ml of phosphate-buffered saline, and the cell number was determined microscopically. An aliquot of cell suspension containing 5×10^8 cells (0.5 ml) was added to 0.5 ml of substrate solution (0.385 mg of *p*-nitrophenyl-α-D-glucopyranoside dissolved in phosphate-buffered saline). The mixture was incubated at 37°C, and the reaction was terminated by removing 0.2-ml aliquots to 0.8 ml of 0.1 M sodium borate, pH 10.0. The samples were immediately chilled, and the supernatant was clarified by two successive centrifugations. Subsequently, the A_{400} was determined spectrophotometrically.

α-Glucosidase activity was determined in permeabilized cells of logarithmically growing *S. cerevisiae* in GE broth supplemented with 2% sugar by a procedure similar to that of Zimmerman and Eaton (61). The cells were collected by centrifugation and washed two times in 0.05 M potassium phosphate buffer (pH 6.8)–1 mM β-mercaptoethanol. The cell pellet was frozen and thawed five times and resuspended in 1 ml of buffer. An aliquot of cell suspension containing 2.5×10^7 cells (0.5 ml) was added to 0.5 ml of substrate solution

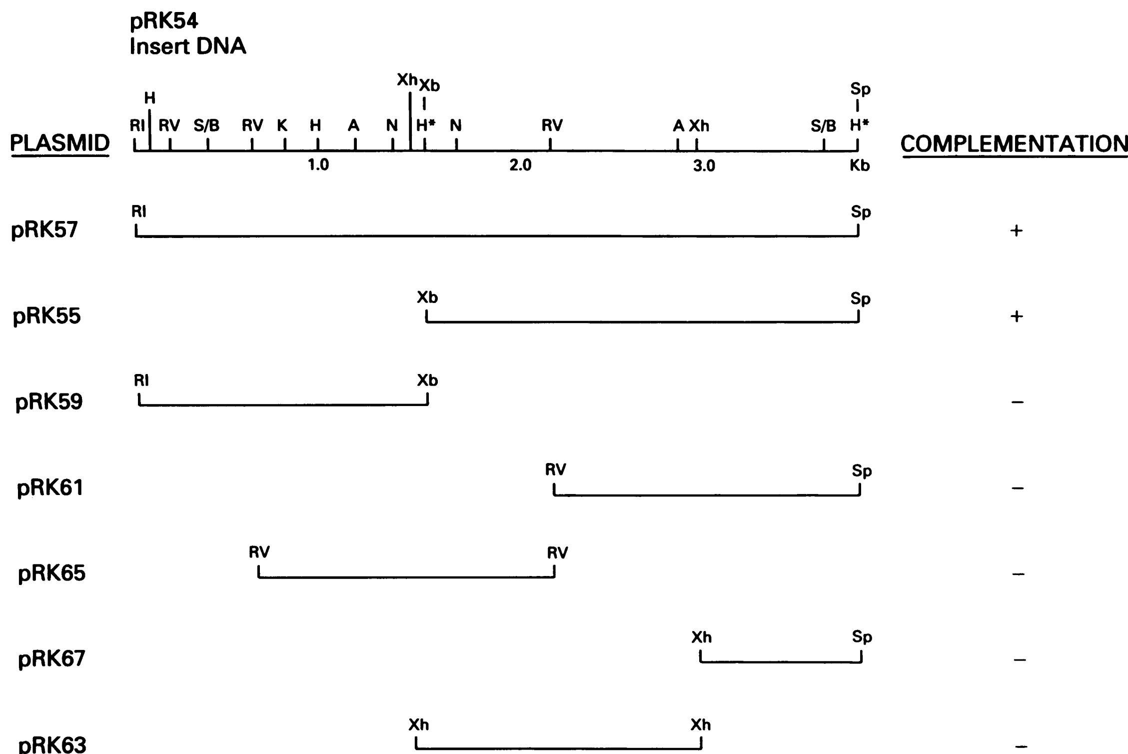

FIG. 1. Restriction map of *C. albicans CASUC1* insert in pRK54. RI, *Eco*RI; H, *Hin*dIII; RV, *Eco*RV; K, *Kpn*I; A, *Ava*I; N, *Nde*I; Xh, *Xho*I; Xb, *Xba*I; Sp, *Sph*I; S/B, *Sau*3A-*Bam*HI. S/B denotes the junction of ligation of *Sau*3A inserts into the *Bam*HI site of the vector. The asterisk designates *Hin*dIII fragment described in the text. The *Hin*dIII site furthest to the right is actually in the multicloning site of the vector pUC19 (49). The lower part of the figure shows subclones constructed in YEp352 that were tested for complementation of the *S. cerevisiae suc2* mutation in strain 2102 as described in the Results section.

(0.75 mg of *p*-nitrophenyl-α-D-glucopyranoside dissolved in buffer) and incubated at 30°C. The samples were processed as described for the previous assay. Specific activity is expressed as nanomoles of *p*-nitrophenyl-α-D-glucopyranoside hydrolyzed per minute per milligram (dry weight). All values represent the average of at least two independent determinations. Dry weight was determined in duplicate on 160-μl aliquots of cell suspension. Both of these assays measure total α-glucosidase and do not discriminate between maltase and α-methylglucosidase activity.

RESULTS

Isolation of a *C. albicans SUC* gene. Since several *C. albicans* genes have been cloned by their expression in *S. cerevisiae*, we sought to clone a *C. albicans SUC* gene by complementation of the sucrose utilization deficiency of an *S. cerevisiae suc2* mutant. *S. cerevisiae* 2102, a strain containing a complete deletion of the *SUC2* gene (encoding invertase) and no *SUC* genes at other loci (10), was transformed with a library of *C. albicans* genomic DNA in vector YEp13. The transformants were first plated to select leucine prototrophs and subsequently to select sucrose prototrophs. Sufficient transformants were plated (10^6) to ensure a 99.99% probability of recovering any given gene. Sucrose prototrophs were recovered at a frequency of approximately 1 in 1,000 transformants. All 20 of the Suc$^+$ transformants selected demonstrated plasmid-mediated Suc$^+$ prototrophy. Restriction analysis of the plasmid isolates indicated that a single type (pCASUC1) was recovered, containing an insert of approximately 3.3 kb. It was unusual to recover only one type of plasmid with such a small insert, since the library consisted of *Sau*3A partial fragments (13). Perhaps larger plasmids containing sequences adjacent to the insert are unstable or confer toxicity to *E. coli* or *S. cerevisiae* when present on a high-copy-number vector. Alternatively, overexpression of sequences adjacent to the insert could inhibit expression of *CASUC1* in *S. cerevisiae*.

The insert of pCASUC1 is colinear with the genome of *C. albicans*, as determined by Southern blot analysis, and also hybridized to genomic blots of type I and type II *C. stellatoidea* DNA (data not presented). Subsequently, the insert was subcloned into pUC19 (pRK54), a restriction map was determined (Fig. 1), and the Suc$^+$ complementing activity was defined by subcloning experiments. The smallest insert capable of complementation was a 2.3-kb *Sph*I-*Xba*I fragment (Fig. 1). Transformants containing this insert were also able to utilize maltose and gave a positive reaction on media containing X-Glu. When X-Glu is cleaved by an α-glucosidase, a blue reaction product is produced (33). Complementation of both the sucrose and maltose utilization defects of *S. cerevisiae* 2102 was also achieved with a single-copy vector containing the 2.3-kb *Sph*I-*Xba*I fragment (data not presented).

Complementation of type II *C. stellatoidea* with the *CASUC1* gene. In order to determine whether the cloned *CASUC1* gene is involved in sucrose utilization in *Candida* spp., it was tested for the ability to complement the sucrose utilization defect of type II *C. stellatoidea*. As mentioned earlier, type II *C. stellatoidea* has been shown to be a sucrose-negative

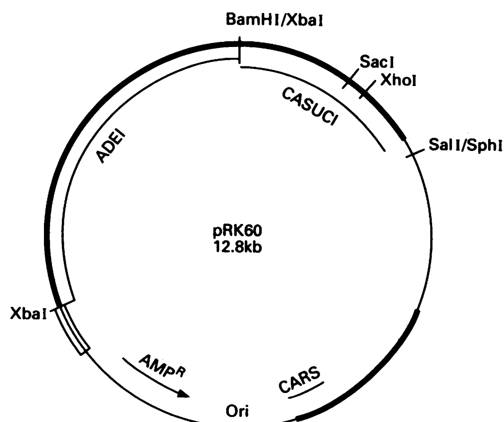

FIG. 2. Partial restriction map of autonomously replicating *CASUC1* vector for *C. albicans*. Symbols: thin lines, pBR322 sequences; heavy lines, *C. albicans* sequences; box, *S. cerevisiae* sequences. (It should be noted that the *ADE1* gene has not yet been definitively mapped.)

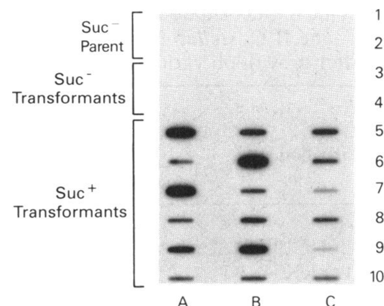

FIG. 3. Autoradiogram of DNA slot blot hybridization of genomic DNA from B-4365P (Suc$^-$ parent) and Ade$^+$ transformants of B-4365P obtained with pRK60. Ade$^+$ transformants are designated Suc$^+$ or Suc$^-$. The probe was radiolabeled pBR322 DNA.

mutant of *C. albicans*. A red adenine-requiring type II *C. stellatoidea* mutant, B-4365P, had been isolated previously (40). Red adenine-requiring mutants of fungi are usually *ade1* or *ade2* mutants (52). Strain B-4365P could be transformed to Ade$^+$ with plasmids containing two independently isolated putative *ADE1* genes, pADE1-1 and p1056, but not with a construct containing the *C. albicans ADE2* gene, pSM7. Therefore, *CASUC1* was subcloned into p1056 (which also contains the *C. albicans* ARS), and the resultant plasmid, pRK60 (Fig. 2), was used to transform B-4365P to Ade$^+$. Two classes of Ade$^+$ transformants were observed by Kurtz et al. in studies with *ADE2* ARS-containing vectors (36). They showed that ARS-containing plasmids will either replicate as a large multimeric, unstable plasmid or integrate stably into the genome at various copy numbers. Similarly, we obtained two types of Ade$^+$ prototrophs: large white colonies stable for the Ade$^+$ phenotype, and small pink unstable transformants. All 24 of the small Ade$^+$ transformants tested sucrose positive, and curing experiments demonstrated that the Suc$^+$ and Ade$^+$ phenotypes were plasmid-borne. Eighteen of 24 large colonies were sucrose positive. To confirm that these Suc$^+$ prototrophs were true Suc$^+$ transformants, they were assayed for sequences unique to the transforming plasmid by DNA slot blot analysis. The blot was hybridized to pBR322 DNA, since pRK60 contains a portion of this vector (refer to Fig. 2). As shown in Fig. 3, DNA from the Suc$^+$ transformants hybridized with various degrees of intensity to the pBR322 DNA probe, but none of the Suc$^-$ transformants or the parent strain demonstrated homology.

Frequently in *S. cerevisiae*, genes coding for supressors will complement a mutation when present on a plasmid with a high copy number but not when on a single-copy vector (17, 34). Therefore, *CASUC1* was introduced into B-4365P on a single-copy vector. This was achieved by cotransformation of B-4365P to Ade$^+$ with 5 μg of pADE1-1 DNA and 15 μg of pRK54 DNA. Nineteen of 48 Ade$^+$ cotransformants were sucrose positive. No sucrose-positive isolates arose when B-4365P was cotransformed with pADE-1 and vector pUC19. Southern blot analysis was performed to determine whether the Suc$^+$ strains contained transforming DNA. The presence of integrated pRK54 can be determined by the introduction of a new *Hin*dIII fragment extending from a *Hin*dIII site in the vector portion of pRK54 to the first *Hin*dIII site in the gene (denoted by asterisks in Fig. 1). The results obtained with genomic digests of *Hin*dIII are shown in Fig. 4. Transformant 5-4, a control Suc$^-$ cotransformant obtained by cotransformation with pUC19, displayed a single 3.9-kb *Hin*dIII fragment (lane 1). A typical Suc$^+$ cotransformant, 3-1, contains this band as well as a new 2.1-kb fragment (lane 2). The 2.1-kb fragment is the same size as that obtained when plasmid pRK54 is digested with *Hin*dIII (lane 3). From the intensity of the new band, it is likely that the integrated plasmid is present in low copy number.

It was recently shown that an α-glucosidase activity is present at negligible levels in type II *C. stellatoidea* but is expressed at high levels in Suc$^+$ revertants and wild-type *C. albicans* cultivated on sucrose-containing media (37). To determine whether *CASUC1* increased α-glucosidase activity in Suc$^+$ transformants of type II *C. stellatoidea*, enzyme activity was assayed in whole cells after cultivation on various carbon sources as described in the Materials and Methods section. The transformants assayed included the Ade$^+$ Suc$^+$ cotransformant described above (3-1) and several Ade$^+$ Suc$^+$ stable transformants obtained with pRK60 (9A, 9B, 9C, and 5A; see Fig. 2), as well as Ade$^+$ Suc$^-$ transformants selected with the vector. As shown in Table 2, the Suc$^-$ Ade$^+$ transformants 2-1 and 5-4 demonstrated low activity regardless of the carbon source. All of the Suc$^+$

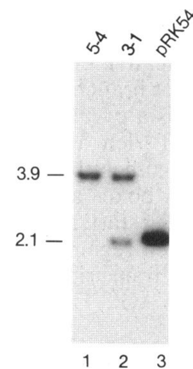

FIG. 4. Autoradiogram of Southern blot hybridization of *Hin*dIII digests of genomic DNA obtained from B-4365P cotransformed to Ade$^+$ with pADE1-1 and pUC19 (lane 1) or pADE1-1 and pRK54 (lane 2). Lane 3 contains a *Hin*dIII digest of pRK54 DNA. The blot was hybridized with the radiolabeled *Sph*I-*Xba*I *CASUC1* fragment of pRK54. Sizes are shown in kilobases.

TABLE 2. Specific α-glucosidase activities in *C. albicans* and transformants of type II *C. stellatoidea* containing the *CASUC1* gene after growth on various carbon sources

Strain (plasmid)/designation	α-Glucosidase sp act[a] (nmol of PNPG hydrolyzed/min/mg [dry wt])		
	Sucrose	Maltose	Glucose
B-4365P(p1056)/2-1	14	ND[b]	10
B-4365P(pADE-1, pUC19)/5-4	12	17	10
B-4365P(pADE1-1, pRK54)/3-1	257	132	10
B-4365P(pRK60)/9C	217	136	13
B-4365P(pRK60)/5A	220	ND	14
B-4365P(pRK60)/9A	307	ND	ND
B-4365P(pRK60)/9B	369	ND	ND
1481P[c]	343	ND	5
SC5314[d]	492	276	9

[a] Specific α-glucosidase activity was determined as described in the Materials and Methods section. PNPG, *p*-nitrophenyl-α-D-glucopyranoside.
[b] ND, not determined.
[c] Suc$^+$ revertant.
[d] Wild-type *C. albicans*.

transformants demonstrated a significant increase in enzyme activity when cultivated on either sucrose- or maltose-containing medium versus glucose-containing medium. When sucrose was the carbon source, the Suc$^+$ cotransformant 3-1 had a specific activity of 257 nmol/min/mg, similar to the specific activity of the Suc$^+$ transformants 9C and 5A. Transformant 5A showed strong hybridization to pBR322 in a slot blot assay, whereas 9C gave the weakest signal. Each of these transformants had a specific activity about two-thirds of that determined for 1481P, a Suc$^+$ revertant of B-4365P, and about one-half of the activity found in the wild-type strain from which the gene was isolated (SC5314). Two additional transformants that exhibited stronger hybridization than the other isolates, 9A and 9B, had specific activities of 307 and 369 nmol/min/mg, respectively. The specific activity of all of the Suc$^+$ strains tested after cultivation on sucrose-containing medium was approximately twice that obtained from cultures grown on maltose-containing medium.

DNA sequence determination. The DNA sequence of the 2.3-kb complementing region was determined, and the *Candida* sequence is presented in Fig. 5. The sequence contains a single open reading frame encoding 501 amino acids and encodes a protein with a predicted molecular mass of 57.2 kDa. No sequences indicative of *S. cerevisiae* introns, such as a TACTAAC/T box for branch formation or a 5′ splice site (GTAPyGT, where Py is a pyrimidine), were found. The 5′ noncoding region is AT rich and has the sequence CTT repeated directly seven times, beginning at position −126. Two overlapping TATA boxes are located upstream of the presumptive translational start site, at positions −103 and −101. The 3′ untranslated region contains the *S. cerevisiae* consensus sequence for transcription termination described by Zaret and Sherman, TAG...TAGT....TTT (60), beginning at nucleotide 1583.

It was initially thought that *CASUC1* might encode an α-glucosidaselike enzyme, since it affected α-glucosidase activity in *C. stellatoidea* and *S. cerevisiae* (the latter based on testing with X-Glu). An enzyme such as maltase was a good candidate, as *CASUC1* enabled *S. cerevisiae* 2102 to use maltose as well as sucrose; however, no significant identity was found when the product of the open reading frame of *CASUC1* was compared with the maltase of *S. cerevisiae* (20). At least 15 α-glucosidases from diverse organisms have the amino acid sequence asparagine-histidine-aspartic acid at the active site (54), but this sequence was not present in *CASUC1*, nor was any identity found between the amino acid sequences of *S. cerevisiae* invertase (a β-fructosidase that hydrolyzes sucrose [55]) and *CASUC1*. If *CASUC1* encoded a secreted enzyme that hydrolyzed sucrose, one might expect to find a signal peptide, but no such sequence was apparent. A search of the Protein Identification Resource data base did not detect any proteins with extensive identity to *CASUC1*. The most striking feature of the protein sequence was the presence of a single zinc finger motif located near the N terminus and beginning at amino acid residue 13 (Fig. 5 and 6). The zinc finger appears to be structurally related to the zinc finger of fungal transcriptional activator proteins containing six conserved cysteines. Many of these transcription factors have been shown to bind nuclear DNA and subsequently to activate transcription (11). The nuclear localization signals of proteins from several organisms have been reported (26, 44). These sequences are short (6 to 10 residues) and are rich in lysine and arginine. A similar sequence in *CASUC1* includes amino acid residues 45 to 53.

***CASUC1* complements maltose-regulatory mutants of *S. cerevisiae*.** The presence of a zinc finger motif in the *CASUC1* coding region strongly suggested that *CASUC1* encodes a regulatory protein (11). It seemed plausible that expression of *CASUC1* enabled the *S. cerevisiae suc2* mutant to activate a cryptic structural gene that allows sucrose utilization. It is unlikely that this gene was that for invertase, as the *suc2* mutant contained a deletion of *SUC2* and lacked *SUC* genes at other loci (10). In addition, the Suc$^+$ transformants utilized maltose, a substrate of the α-glucosidase maltase, but did not use raffinose, an expected substrate for invertase.

S. cerevisiae has been shown to utilize sucrose in vivo if maltase is expressed constitutively (31). Normally, maltase is induced in *S. cerevisiae* by maltose but not by sucrose. The maltase of *S. cerevisiae* is a cytoplasmic α-glucosidase whose expression requires two additional gene products, a maltose permease as well as a maltose-regulatory gene (for a review, see reference 56). The three corresponding genes are linked and comprise a *MAL* locus. The ability of *Saccharomyces* spp. to ferment maltose depends upon the presence of any one of five unlinked *MAL* loci (*MAL1* through *MAL4* and *MAL6*). All *Saccharomyces* strains contain the *MAL1* locus or one of its alleles and may contain additional *MAL* loci (most Mal$^-$ mutants are derived from strains containing only *MAL1*). The *MAL1* and *MAL6* loci exhibit extensive sequence homology as well as functional identity and are used interchangeably. We hypothesized that the *S. cerevisiae suc2* mutant contained a defective maltose-regulatory gene and that *CASUC1* corrected this defect. To test this hypothesis directly, an *S. cerevisiae* Mal$^-$ strain (TCY-137) containing a disruption of the maltose-regulatory gene at *MAL1* (denoted *MAL13*) was transformed with *CASUC1*. TCY-137 (7) was constructed from a strain containing only a *MAL1* locus.

Ura$^+$ transformants of TCY-137 containing either pRK55 (*CASUC1*) or the *URA3* vector YEp352 were selected and tested for the ability to use maltose. Transformants containing *CASUC1* but not YEp352 were Mal$^+$. To determine whether α-glucosidase activity was increased in the transformants, the enzyme was assayed in permeabilized cells from cultures grown on maltose-containing medium as described in the Materials and Methods section. A transform-

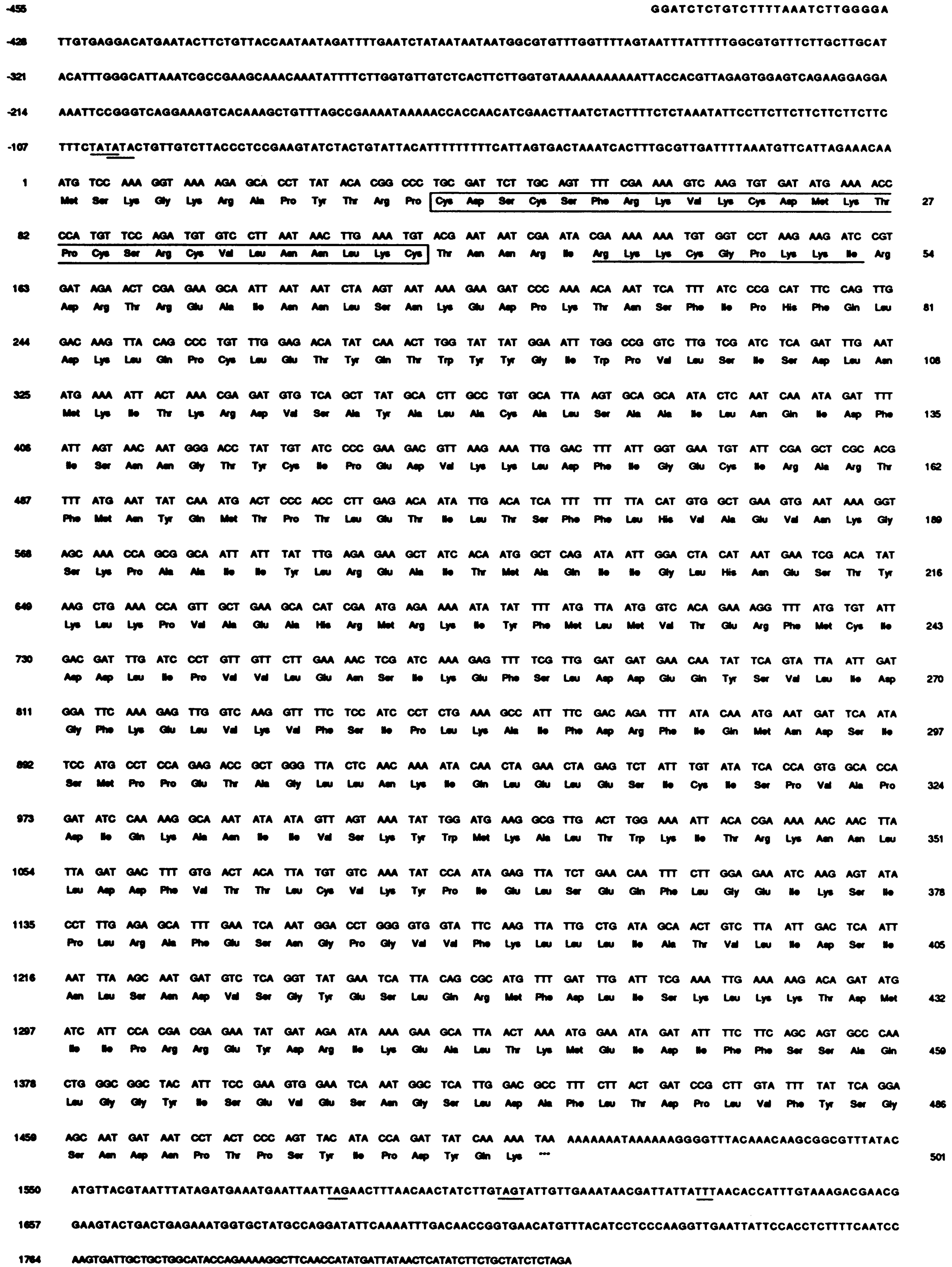

FIG. 5. Complete nucleotide sequence and deduced amino acid sequence of *CASUC1*. Putative TATA sequences, nuclear localization signal, and transcription termination signal are underlined. A putative zinc finger motif that matches the consensus for C_6 zinc fingers is indicated by a box.

								Lys	Arg												Lys																					
Consensus	Ala	Cys	Asp	X	Cys	Arg	X	Arg	Lys	Pho	Lys	Cys	Asp	X	X	X	Pro	X	Cys	X	Arg	Cys	X	Lys	X	Asn	Pho	X	Cys													
CASUC1	Pro	Cys	Asp	Ser	Cys	Ser	Phe	Arg	Lys	Val	Lys	Cys	Asp	Met	Lys	Thr	Pro	—	Cys	Ser	Arg	Cys	Val	Leu	Asn	Asn	Leu	Lys	Cys	Thr	Asn	Asn	Arg	Ile	Arg	Lys	Lys	Cys	Gly	Pro	Lys	Lys
MAL63	Ser	Cys	Asp	Cys	Cys	Arg	Val	Arg	Arg	Val	Lys	Cys	Asp	Arg	Asn	Lys	Pro	—	Cys	Asn	Arg	Cys	Ile	Gln	Arg	Asn	Leu	Asn	Cys	Thr	Tyr	Leu	Gln	Pro	Leu	Lys	Lys	Arg	Gly	Pro	Lys	Ser
LAC9	Ala	Cys	Asp	Ala	Cys	Arg	Lys	Lys	Lys	Trp	Lys	Cys	Ser	Lys	Thr	Val	Pro	Thr	Cys	Thr	Asn	Cys	Leu	Lys	Tyr	Asn	Leu	Asp	Cys	Val	Tyr	Ser	Pro	Gln	Val	Val	Arg	Thr	Pro	Leu	Thr	Arg
GAL4	Ala	Cys	Asp	Ile	Cys	Arg	Leu	Lys	Lys	Leu	Lys	Cys	Ser	Lys	Glu	Lys	Pro	Lys	Cys	Ala	Lys	Cys	Leu	Lys	Asn	Asn	Trp	Glu	Cys	Arg	Tyr	Ser	Pro	Lys	Thr	Lys	Arg	Ser	Pro	Leu	Thr	Arg

FIG. 6. Homologies among the cysteine zinc fingers of *CASUC1*, *MAL63* (32, 53), *LAC9* (58), and *GAL4* (41). This is a selected representation of the zinc finger regions of yeast transcriptional activation proteins containing C_6 zinc fingers. The consensus sequence was derived from these zinc fingers and additional fungal C_6 fingers, including those in *LEU3* (12), *PPR1* (27), *HAP1* (47), *ARGRII* (42), *PDR1* (1), *QUTA* (5), and qa-1F (2). Pho, hydrophobic amino acid; X, any amino acid. Amino acids in the top line are found less frequently in these positions.

ant containing pRK55 had a specific activity of 9,500 nmol/min/mg, whereas a transformant containing vector alone had a value of 830 nmol/min/mg. No activity was obtained when the cells were not permeabilized.

Confirmation that the *S. cerevisiae suc2* mutant used to select *CASUC1* contained a defective maltose-regulatory gene was obtained by transforming it with pMR42, a plasmid containing the *S. cerevisiae* maltose-regulatory gene *MAL63* (*MAL63* denotes the regulatory gene at the *MAL6* locus). The Leu^+ transformants were able to use both maltose and sucrose, similar to the transformants obtained with pCASUC1. To determine whether the transformants contained α-glucosidase activity that was inducible by sucrose or maltose, the enzyme was assayed after growth on different carbon sources, as described earlier. As shown in Table 3, the specific activity of α-glucosidase in transformants containing either pCASUC1 or pMR42 grown under noninduced nonrepressed conditions (GE medium) was essentially the same as and just slightly higher than the basal activity obtained with vector YEp13. When maltose was added to the medium, transformants containing pMR42 and pCASUC1 demonstrated a 15- and 5-fold increase in specific activity, respectively. The results obtained when sucrose was added to the GE medium were not clear-cut, since a transformant containing the vector alone exhibited a sixfold increase in activity. This increase cannot be attributed to the presence of vector sequences, as the untransformed mutant exhibited a similar increase in activity. Transformants containing either gene showed specific activities of 6,000 to 7,000 nmol/min/mg when cultivated on sucrose-supplemented medium. This represents an approximately 12-fold increase in activity compared with growth on GE; however, this increase is only 2-fold higher than what was obtained with the vector alone. When glucose was added to the GEM medium, the specific activity of the enzyme in transformants containing either gene was negligible.

Comparison of *CASUC1* and a maltose-regulatory gene of *S. cerevisiae*. Each of the functional *MAL* loci of *S. cerevisiae* includes a maltose-regulatory gene. To date, only the sequence of the gene at the *MAL6* locus (denoted as either *MAL63* or *MAL6R*) has been reported (32, 53). This gene encodes a protein of 470 amino acids, similar in size to that encoded by the open reading frame of *CASUC1* (501 amino acids). The products of both *MAL63* and *CASUC1* contain a single zinc finger motif beginning at amino acid residues 8 and 13, respectively. When the open reading frames of *CASUC1* and *MAL63* were aligned, 28% identity and 50% similarity were found (refer to the legend to Fig. 7 for a description of similarity). Three regions with more extensive homology were identified. One of these regions includes the zinc finger motif and is shown in Fig. 6. This 41-amino-acid stretch exhibited 51% identity and 60% similarity. The zinc fingers of *CASUC1* and *MAL63* are of the general form Cys-Xaa_2-Cys-Xaa_6-Cys-$Xaa_{variable}$-Cys-Xaa_2-Cys-Xaa_6-Cys (where Xaa_n indicates a stretch of the indicated number of any amino acids), and both contain five amino acids in the variable region. Hence, a gap must be introduced after the proline in order for these sequences to conform to the consensus. The other two regions of *CASUC1* and *MAL63* with extensive homology are shown in Fig. 7. Together, all of these regions account for 28% of the protein.

It was reported that *MAL63* hybridizes to a constitutive 1.6-kb mRNA in *S. cerevisiae* (56). *CASUC1* hybridizes to a

TABLE 3. Specific α-glucosidase activities in transformants of the *S. cerevisiae suc2* mutant 2102 after growth on various carbon sources

Plasmid in strain 2102	α-Glucosidase sp act[a] (nmol of PNPG hydrolyzed/min/mg [dry wt])			
	GE	GEM	GES	GEMG
pMR42	590	8,800	7,100	0
pCASUC1	570	2,600	6,300	0
YEp13	220	280	1,300	0
None	150	ND[b]	800	ND

[a] See Table 2, footnote *a*.
[b] ND, not determined.

Homology Region A

```
MAL63    95 YVIWPMLSYDDLHKLLEEKYDDRCAYWFLVSLSAATLSDLQIEIEYEEGV 144
            I III:II  II:   :   :   I :II :    IIII :    II::  ::I
CASUCI   96 YGIWPVLSISDLNMKITKR..DVSAYALACALSAAILN..QIDFISNNGT 141

           145 TFTGEQLCTL  154
                   I::   I
           142 YCIPEDVKKL  151
```

Homology Region B

```
MAL63   198 EAVGLIKIAGFHREETYEFLPFGEQQLRRKVYYLLLMTERF  238
            II: :   I I:I I II : I :I  :    II:I::I::IIII
CASUCI  200 EAITMAQIIGLHNESTYKLKPVAEAHRMRKIYFMLMVTERF  240
```

	Percent Identity	Percent Similarity
Region A	38	59
Region B	44	71

FIG. 7. Amino acid sequence homologies between the *CASUC1* and *MAL63* proteins. The amino acid sequences were compared with the GAP program, which uses the alignment method of Needleman and Wunsch (45). Percent identity and similarity were calculated by the program. Identity is indicated by straight lines, and similarity is shown by two dots. A similarity is scored when the "similarity threshold" (the symbol comparison value for a pair of amino acids) is greater than or equal to 0.5. A symbol comparison table based on the evolutionary distance between the amino acids, with a match registering a value of 1.5 and mismatches yielding a mean value of −0.17, was employed (15).

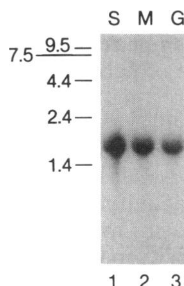

FIG. 8. Northern blot hybridization of total RNA isolated from SC5314 cultivated on the following carbon sources: lane 1, sucrose; lane 2, maltose; lane 3, glucose. The probe was a radiolabeled *Ava*I-*Eco*RV fragment of pRK54. Sizes are shown in kilobases.

C. albicans constitutive RNA that is similar in size (1.75 kb), as determined by Northern blot analysis (Fig. 8). The transcript is found in RNA isolated from *C. albicans* grown on either maltose or sucrose as well as glucose.

Codon usage is thought to reflect the level of gene expression and may be correlated with the relative abundance of the isoacceptor tRNAs (4). Unlike highly expressed genes, which use a preferred set of codons, the maltose-regulatory gene of *S. cerevisiae* does not exhibit codon bias (59). Codon usage in *CASUC1* is like that in the *MAL63* gene; 60 of 61 possible codons were utilized, an indication that this gene is not highly expressed. Using the calculation described by Bennetzen and Hall for *S. cerevisiae* genes (4), the *CASUC1* gene has a codon bias index of −0.02, compared with 0.04 for *MAL63*. These values are indicative of a random selection of codons.

DISCUSSION

To date, many *C. albicans* genes which encode enzymes have been cloned by heterologous expression in *S. cerevisiae*, usually by complementation of auxotrophic mutations (reviewed by Kurtz et al. [36]). Here we report the isolation of a *C. albicans* putative regulatory gene involved in sucrose utilization (*CASUC1*) by expression in an *S. cerevisiae* mutant devoid of invertase genes. It was initially surprising to find that a gene encoding a zinc finger motif corrected the sucrose utilization defects of both type II *C. stellatoidea*, a mutant of *C. albicans* which is unable to utilize sucrose, and an *S. cerevisiae* invertase deletion mutant. Proteins that contain such zinc finger motifs are usually involved in regulation (11). The data suggest that *CASUC1* encodes a regulatory protein which in turn activated a cryptic α-glucosidase structural gene in both type II *C. stellatoidea* and the *S. cerevisiae suc2* mutant, thus enabling them to grow on sucrose. *CASUC1* has genetic and biochemical properties, as well as structural features, in common with a maltose-regulatory gene of *S. cerevisiae*. The *S. cerevisiae* gene encodes a protein containing a zinc finger motif which binds to an upstream activation sequence located upstream of two divergently transcribed genes for maltose utilization, a permease and a structural gene (21, 46). All three of these genes are essential for maltose utilization in *S. cerevisiae*. The maltase of *S. cerevisiae*, as mentioned earlier, can hydrolyze sucrose in vivo if it is expressed constitutively (31). We demonstrated that *CASUC1* corrected the maltose utilization defect of an *S. cerevisiae* strain containing a defined mutation in a maltose-regulatory gene and that the ability to use maltose correlated with a significant increase in α-glucosidase activity in a Mal^+ transformant. The enzyme assay detects maltase and α-methylglucosidase activities. Since the transformants grow well on maltose, it is likely that a large proportion of the total α-glucosidase activity represents maltase.

The hypothesis that *CASUC1* complemented a mutation in a maltose-regulatory gene of the *S. cerevisiae suc2* mutant 2102 (the strain used to select *CASUC1*) was strengthened by confirming that this strain could also be transformed to Mal^+ with the *S. cerevisiae* maltose-regulatory gene *MAL63*. We originally thought that *MAL63* would not complement the sucrose utilization defect of this strain. This belief was based on observations that the maltase of *S. cerevisiae* can hydrolyze sucrose in vivo only if it is expressed constitutively (31) and that overexpression of the *S. cerevisiae* maltose-regulatory gene does not lead to constitutive maltase activity (14); however, the *S. cerevisiae* Mal^+ transformants obtained with *MAL63* were also Suc^+, refuting our notion that only *CASUC1* responded to sucrose. The data from our enzyme assays suggest that α-glucosidase is inducible by both maltose and sucrose in transformants containing either *CASUC1* or *MAL63* (Table 3). The 12-fold induction observed in transformants containing either *CASUC1* or *MAL63* cultivated on sucrose-containing medium may in part reflect endogenous activity. Activity in the recipient is induced sixfold by sucrose in the presence or absence of YEp13, while the presence of *CASUC1* or *MAL63* results in a further twofold increase. On the other hand, the magnitude of the induction obtained with *CASUC1* and *MAL63* may be largely independent of the endogenous induction. If this is the case, a 15-fold induction can be determined by subtracting the specific activities obtained with vector alone from the values obtained with each gene. The source of the endogenous sucrose-inducible activity found in the mutant is not known. Perhaps this strain contains a partially active maltose-regulatory gene that confers a slight induction by sucrose. Some strains of *S. cerevisiae* contain partially active *MAL* loci (56).

Our observation that sucrose as a carbon source results in increased α-glucosidase activity is in agreement with in vitro studies demonstrating hydrolysis of the sugar by maltase and α-methylglucosidase in *S. cerevisiae* (30). It is also consistent with a report showing in vivo utilization of sucrose by maltase (31). An undocumented observation regarding "sucrose induction" runs counter to our data (31).

It is widely accepted that invertase is responsible for sucrose utilization in *S. cerevisiae*, and many studies have been carried out with the assumption that sucrose hydrolysis by this secreted enzyme takes place extracellularly. Little consideration has been given to the possibility that sucrose may be transported into the cell or that hydrolysis can occur via an alternative enzyme. It is highly unlikely that the sucrose-positive phenotype of transformants of *S. cerevisiae* 2102 is due to activation of a cryptic invertase gene, as this strain is devoid of such genes (10). In addition, the transformants do not use raffinose, as would be expected of cells expressing invertase. As no homology was found between *CASUC1* and other carbohydrate-utilizing enzymes, *CASUC1* probably does not encode a bifunctional protein which serves as a regulatory molecule and also hydrolyzes sucrose. Future *Saccharomyces* studies of sucrose hydrolysis via mechanisms other than invertase should prove very interesting.

The open reading frame of *CASUC1* is approximately the same size as that of the maltose-regulatory gene of *S. cerevisiae* and is 28% identical and 50% similar at the amino

acid level. Although the homology is not extensive, it is similar to what has been reported for other regulatory proteins which function heterologously. For example, *LAC9* of *Kluyveromyces lactis* and *GAL4* of *S. cerevisiae* encode regulatory proteins displaying 32% identity and interchangeable function (58). In general, gene activation does not require extensive sequence conservation. Transcriptional activators usually comprise a DNA-binding component and an "activating region," which has been postulated to interact with either a general transcription factor or an RNA polymerase (48).

Three corresponding regions of *CASUC1* and *MAL63* exhibit more extensive identity and are probably responsible for specific functions common to these proteins. One of the regions includes the zinc finger motif and several adjacent amino acids on the carboxyl side (depicted in Fig. 6). Finger-swapping experiments have demonstrated that the zinc finger of *MAL63* participates in DNA binding (25), and it can be inferred that *CASUC1* functions in the same manner. C_6 zinc fingers are of the general form Cys-Xaa_2-Cys-Xaa_6-Cys-$Xaa_{variable}$-Cys-Xaa_2-Cys-Xaa_6-Cys (where Xaa_n indicates a stretch of the indicated number of any amino acids) and exhibit a high degree of conservation. Most C_6 zinc fingers contain six amino acids in the variable region, but interestingly, both *CASUC1* and *MAL63* contain five. A unique feature of the *CASUC1* zinc finger is the substitution of a serine in place of the first arginine, a conserved assignment in all other C_6 zinc fingers. Thus, strong conservation of the arginine might not be essential for zinc finger function. A high degree of sequence conservation is also found between *CASUC1* and *MAL63* adjacent to the zinc finger motif but not between *CASUC1* and other regulatory genes (Fig. 6, not all data presented). Thus, it is possible that amino acids in this region contribute to the DNA-binding specificity of *CASUC1* and *MAL63*. The other regions of *CASUC1* and *MAL63* with extensive homology (depicted in Fig. 7) may interact with the inducer or confer repression by glucose (Table 3). It should be possible to exploit the similarities and differences between *CASUC1* and *MAL63* to better ascertain how gene expression is regulated by these proteins.

The ability of *CASUC1* to correct the sucrose utilization defect of type II *C. stellatoidea* and restore α-glucosidase activity in sucrose-grown cells suggests that type II contains a mutation in a regulatory gene and is consistent with previous evidence that type II *C. stellatoidea* is simply a mutant of *C. albicans* that does not express sucrose-inducible α-glucosidase (37). The basal level of α-glucosidase activity found in type II *C. stellatoidea* is also indicative of a regulatory mutant. Enzymatic analysis of the transformants of type II *C. stellatoidea* containing *CASUC1* suggested that it functions as a regulatory molecule in *Candida* spp. Overexpression of the *CASUC1* gene did not lead to a significant overexpression of α-glucosidase activity, as would be expected if an enzyme were encoded. The α-glucosidase activity in two multiple-copy transformants demonstrated a 1.3- to 1.6-fold increase compared with that in a low-copy-number transformant. This was only marginally higher than that of a Suc^+ revertant and is consistent with data reported for *S. cerevisiae*. Overexpression of the *S. cerevisiae* maltose-regulatory gene led to a 1.5- to 2-fold increase in maltase activity (14), whereas overexpression of the structural gene yielded a 4- to 5-fold increase.

It is intriguing that *CASUC1* affects a cytoplasmic α-glucosidase activity in *S. cerevisiae* and an activity from *Candida* spp. that appears to be extracellular. It was previously shown that the sucrose-inducible α-glucosidase activity of *C. albicans* was released by β-mercaptoethanol treatment, a characteristic of periplasmic and cell wall enzymes (37). It will be interesting to determine whether the *S. cerevisiae* maltose-regulatory gene corrects the sucrose utilization defect of type II *C. stellatoidea*. It should be noted that type II *C. stellatoidea* is capable of assimilating maltose, probably due to the activity of an additional α-glucosidase located in the cytoplasm, as we were not able to detect a maltose-inducible activity in whole-cell assays. These assays detect only extracellular enzyme.

It is our hypothesis that the sucrase of *C. albicans* resembles the maltase of *S. cerevisiae* but, in addition, possesses a signal peptide. This type of α-glucosidase has been found in the Egyptian mosquito, *Aaedes aegypti*, and its proposed function is to assist the mosquito in its sugar-feeding capabilities (24). One could speculate that the inability of type I *C. stellatoidea* to use sucrose is due to a defective structural sucrase gene and that type I and type II *C. stellatoidea* are sucrose nonutilizers analogous to *S. cerevisiae* malp and malg strains. These *Saccharomyces* strains are naturally occurring maltose nonfermenters that complement one another for maltose utilization (56). Malp designates the maltose regulatory gene, and malg encodes the permease and structural genes. Subsequent cloning and analysis of the *C. albicans* sucrase gene would address these possibilities and facilitate studies to determine how *CASUC1* regulates sucrose utilization in *C. albicans*, if that is indeed its function.

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