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Upstream Region of the *SUC2* Gene Confers Regulated Expression to a Heterologous Gene in *Saccharomyces cerevisiae*

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The *SUC2* gene produces two differently regulated mRNAs that encode two forms of invertase. The 1.9-kilobase mRNA encoding secreted invertase is regulated by glucose (carbon catabolite) repression, and the 1.8-kilobase mRNA encoding intracellular invertase is synthesized constitutively. Previous work has shown that the 5′ noncoding region between −650 and −418 is required for derepression of secreted invertase in response to glucose deprivation. We show here that this upstream region can confer glucose-repressible expression to a heterologous gene, a *LEU2-lacZ* gene fusion, that is not normally regulated by glucose repression. This expression was found to respond appropriately to mutations in *trans*-acting genes that affect regulation of *SUC2* expression. Mutations in the *SNF1* through *SNF6* loci reduced derepression of β-galactosidase, and a mutation at the *SSN6* locus caused constitutive expression. These findings indicate that the *SUC2* upstream region mediates the regulatory effects of these genes and suggest that regulation occurs at the level of transcription. In addition, the upstream region was partially active in the inverted orientation.

The *SUC2* gene of *Saccharomyces cerevisiae* encodes two forms of the enzyme invertase synthesized from two mRNAs. A 1.9-kilobase (kb) mRNA encodes a signal-peptide-containing precursor to the secreted, glycosylated invertase (5, 8, 24), which is responsible for the extracellular hydrolysis of sucrose. The production of this mRNA is regulated by glucose (carbon catabolite) repression, and the secreted enzyme is produced only under conditions of glucose deprivation. A second, 1.8-kb mRNA, which lacks the signal sequence at its 5′ end (8), encodes a nonglycosylated, intracellular form of invertase that has no known physiological function (5). This mRNA is produced constitutively at low levels (5).

Sequences required for expression of the *SUC2* gene were previously identified by deletion analysis of the *SUC2* 5′ noncoding region (26). This study revealed the presence of a region located between −650 and −418, relative to the translational start site, that was required for derepression of secreted invertase synthesis. The 3′ boundary of this region was near −418; the 5′ boundary was not sharply defined, but the most crucial sequences appeared to lie downstream from −496 because a deletion extending from −1900 to −496 resulted in only a threefold decrease in derepression. No sequences essential for derepression lie between −418 and −140; the TATA box is at position −133 and the 5′ end of the 1.9-kb mRNA is at −40 (8). Sequences between −1900 and −86 are not important for the constitutive synthesis of the 1.8-kb mRNA and can be deleted without effect.

What is the role of the upstream region in derepression of *SUC2*? One possibility is that this region mediates the regulation of *SUC2* expression in response to the availability of glucose. However, the alternative possibility that this region is required for expression per se but plays no role in regulation has not been excluded; for example, the regulatory sequences could lie downstream of position −140, near the TATA box, and the upstream region could be required simply for promoter function or for enhancement of transcription. It is also possible that this region includes sequences with both regulatory and promoter or enhancer functions. To determine whether the *SUC2* upstream region plays a regulatory role, we investigated its ability to confer glucose-repressible expression to a heterologous gene that is not normally regulated by glucose repression, in this case a *LEU2-lacZ* gene fusion. We found that *SUC2* upstream sequences do confer glucose-repressible expression to the *LEU2-lacZ* fusion and, moreover, are partially functional when present in the inverted orientation.

We also examined whether this upstream region mediates the action of *trans*-acting genes that are necessary for regulation of *SUC2* expression. The *SNF1* through *SNF6* (sucrose-nonfermenting) genes appear to act as positive regulators of *SUC2* expression; mutations in these genes cause defects in derepression of secreted invertase (6, 21). The *snf1*, *snf2*, *snf4*, and *snf5* mutations also cause pleiotropic defects in expression of other glucose-repressible genes. Mutations at the *SSN6* (suppressor of *snf*) locus cause constitutive synthesis of secreted invertase, that is, synthesis under conditions of glucose repression (7). The *ssn6* mutations also suppress defects in derepression of invertase caused by *snf* mutations (7, 21). We examined the effects of these mutations on the expression of *SUC2-LEU2-lacZ* fusions, and our findings suggest that the *SUC2* upstream region mediates the regulatory effects of the *SNF* and *SSN6* genes.

MATERIALS AND METHODS

Preparation and analysis of DNA. Plasmid DNAs and yeast genomic DNAs were prepared as described previously (26). Gel electrophoresis, purification of DNA fragments from gels, blot hybridization, and enzymatic manipulations of DNA were carried out by standard procedures as described previously (9, 26).

Construction of plasmids. Plasmids pLS6 and pLS7 were constructed by inserting an *Nco*I-*Eco*RI fragment containing the *SUC2* upstream region between positions −384 and about −900 into the *Eco*RI site of pMC2019 (18) in both orientations. Nucleotide positions are relative to the *SUC2* translational start site for secreted invertase. The *SUC2*

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DNA fragment was prepared by digesting plasmid pLS27 (26) with *Nco*I, which cleaves at several sites within the plasmid, including a site at position −384. Fragment ends were blunted with the Klenow fragment of *Escherichia coli* DNA polymerase as described previously (17). *Eco*RI linkers were added, and the fragment was purified as described previously (26). The fragment was then digested with *Eco*RI to activate the linkers and to cleave at a site at about position −900. The resulting 0.5-kb fragment was then ligated to plasmid pMC2019 DNA (gift of M. Casadaban; formerly called pMC1876Δ2 [18]) that had been linearized by *Eco*RI digestion. The ligated DNA was used to transform *E. coli* HB101 to ampicillin resistance by the $CaCl_2$ procedure (17), and plasmid pLS6 was recovered. Plasmid pLS7 was constructed by subcloning the 0.5-kb *Eco*RI fragment from pLS6 into pMC2019; this two-step procedure was used to demonstrate that the *SUC2* DNA sequence in pLS6 was functional when cloned in the correct orientation relative to the gene fusion. The orientation of the inserted *Eco*RI fragment was determined from the position of an asymmetrically placed *Ava*I site.

The integrative plasmid pLS9 was derived from pMC2019 by replacing a *Hin*dIII-*Eco*RI fragment containing the *ARS* sequence and part of the *TRP1* gene with a *Hin*dIII-*Eco*RI fragment carrying the *URA3* gene. The *URA3* DNA fragment was obtained from pAC100, a pUC18 (22) derivative carrying the 1.1-kb *Hin*dIII fragment of *URA3* cloned into the *Xba*I site of the polylinker (E. Abrams and M. Carlson, unpublished data). Plasmids pLS10 and pLS11 were constructed by subcloning the 0.5-kb *Eco*RI fragment containing *SUC2* 5′ noncoding DNA from pLS7 into the *Eco*RI site of pLS9.

Yeast strains and genetic methods. All *S. cerevisiae* strains used in this study were isogenic or congenic to S288C and were constructed by standard genetic methods (27). All alleles have been described previously (6, 7, 21) except for *snf1*::*HIS3*, which is an insertion of the *HIS3* gene within the *SNF1* coding region (J. Celenza and M. Carlson, unpublished data), *snf4-Δ1*, which is a deletion of part of the *SNF4* gene (F. Eng and M. Carlson, unpublished data), and Δ*trp1* and Δ*his3*, which were obtained from P. Hieter and R. W. Davis. Media and methods for scoring markers have been described previously (21). The *ssn6* mutation was scored by its phenotype of allowing growth on sucrose in the presence of 200 μg of 2-deoxyglucose per ml (J. Celenza and M. Carlson, unpublished observation), a glucose analog that causes repression of secreted invertase synthesis.

Construction of yeast strains carrying *LEU2-lacZ* fusions. Strains carrying plasmids pLS9, pLS10, and pLS11 integrated into the genome were constructed by transforming (15) MCY638 (*MAT***a** *ura3-52 his4-539 lys2-801 SUC2*) to uracil prototrophy. Integration was targeted (23) to the *URA3* locus by cleaving the plasmid DNA at the unique *Nco*I site within the *URA3* gene before transformation. Transformants carrying a single copy of the plasmid integrated at the *URA3* locus were identified by blot hybridization analysis (28) of genomic DNA; digestion of the DNA with *Bam*HI and hybridization with a probe prepared by nick translation (25) of YIp5 (4) provided a diagnostic pattern of fragments. To examine the effect of regulatory mutations on expression of the *SUC2-LEU2-lacZ* fusion in the integrated pLS11, a single transformant was crossed to strains carrying each of the *snf* and *ssn6* mutations, and meiotic segregants carrying both the gene fusion and the mutation of interest were identified. Yeast strains carrying *ssn6* and an integrated copy of pLS9 were constructed by a similar procedure.

Yeast strains carrying the episomal plasmids pMC2019, pLS6, and pLS7 were obtained by transforming (15) cells carrying the Δ*trp1* mutation with each plasmid DNA and selecting tryptophan prototrophs. The wild-type host was strain MCY730 (*MAT***a** Δ*trp1 ura3-52 lys2-801 ade2-101* Δ*his3 SUC2*). To examine the effects of *snf* and *ssn6* mutations, the plasmids were used to transform mutant strains to tryptophan prototrophy.

β-Galactosidase and invertase assays. Repressed and derepressed cells were prepared as previously described (9). For strains carrying integrated plasmids, glucose-repressed cells were prepared by growth to the exponential phase in yeast extract-peptone rich medium (YEP) containing 2% glucose, and derepressed cultures were prepared by shifting such repressed cells to YEP containing 0.05% glucose for 2.5 h. For strains carrying episomal plasmids, a similar procedure was followed, except that selection for the *TRP1* marker was maintained by growing cells in synthetic dextrose minimal medium (SD) containing the appropriate supplements minus tryptophan. β-Galactosidase activity was assayed in cells permeabilized with SDS and chloroform as described previously (19). Cell density was determined by measuring the dry weight of cells from strains carrying *ssn6* (7). Secreted invertase was assayed in whole cells as previously described (9).

RESULTS

Construction of *SUC2-LEU2-lacZ* gene fusions. We previously identified by deletion analysis a region between positions −650 and −418 that is required for regulated expression of the *SUC2* gene; the most crucial sequences appear to lie between −496 and −418 (26). To determine whether this region can confer glucose-repressible expression to a heterologous gene that is not normally regulated by glucose repression, we inserted *SUC2* upstream sequences, extending from about −900 to −384, immediately 5′ to the *LEU2-lacZ* gene fusion in plasmid pMC2019 (formerly pMC1876Δ2 [18]). This gene fusion contains *LEU2* DNA sequences extending from position −125 to +39, relative to the translational start site, fused in frame to the eighth codon of the *E. coli lacZ* gene. The *LEU2* sequence includes both the *LEU2* transcriptional and translational start sites but lacks sequences required for leucine-specific regulation of the fusion gene (18); the 5′ end of the major *LEU2* RNA species maps to position −16 (1). *LEU2* gene expression is not glucose repressible (13, 18). The *SUC2* upstream region is not sufficient to allow derepression of the *SUC2* gene when sequences between −180 and −86, which include the TATA box, are deleted (26).

The *SUC2* 5′ noncoding sequence was inserted in both orientations in pMC2019, as described in Materials and Methods; pLS7 carries the *SUC2* sequence in the correct orientation relative to the *LEU2-lacZ* fusion, while pLS6 carries the *SUC2* sequence in the opposite orientation (Fig. 1). An integrative plasmid, pLS9, was derived from pMC2019 by substitution of the yeast *URA3* gene for most of the *TRP1-ARS1* sequence. The *SUC2* DNA fragment was inserted into pLS9 in both orientations at the same position relative to the *LEU2-lacZ* fusion as in pMC2019; pLS11 carries the fragment in the correct orientation, and pLS10 has the fragment in the inverted orientation (Fig. 1).

The *SUC2* upstream sequence is approximately 230 base pairs closer to the major *LEU2* transcriptional start site in pLS7 and pLS11 than it is to the start site for the 1.9-kb *SUC2* mRNA at the *SUC2* locus. The distance between the relevant *SUC2* sequences and the transcriptional start site is

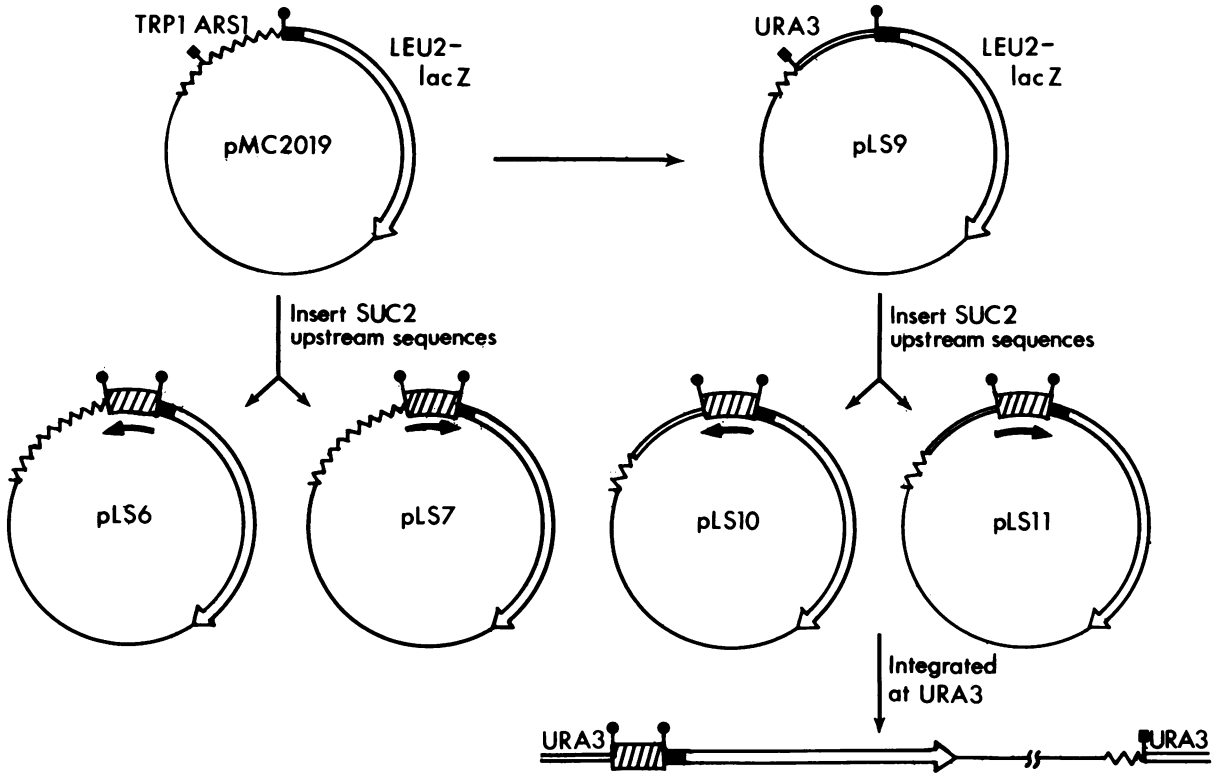

FIG. 1. Construction of plasmids. Plasmids were constructed as described in Materials and Methods. The structure of the *URA3* locus on chromosome V of *S. cerevisiae* after integration of a single copy of plasmid pLS10 or pLS11 is shown. The dark bar and the open bar represent *LEU2* and *lacZ* DNA, respectively, and the arrow indicates the direction of transcription. The hatched bar represents *SUC2* sequences, and the arrow indicates the orientation. The thin line is the pBR322 sequence, and the jagged line and thin bar are yeast DNA, as labeled. Symbols: ●, *Eco*RI; ■, *Hin*dIII.

more difficult to assess in pLS6 and pLS10 because the *SUC2* sequence is inverted; however, nucleotide −500, an arbitrary point within the upstream region, is about 55 base pairs farther away in pLS6 and pLS10 than at the *SUC2* locus.

***SUC2* upstream region confers glucose-regulated expression to the *LEU2-lacZ* fusion.** Yeast strains carrying a single copy of plasmid pLS11, or its parent pLS9, integrated at the chromosomal *URA3* locus were constructed as described in Materials and Methods. These strains were grown under glucose-repressing or -derepressing conditions and then assayed for β-galactosidase activity (Table 1). Strains carrying pLS9 showed no increase in β-galactosidase activity upon derepression, whereas strains harboring pLS11 showed a 35-fold increase. These results indicate that the *SUC2* upstream region is able to confer glucose-repressible expression to the *LEU2-lacZ* gene fusion.

We also examined the effect of the *SUC2* upstream region on *LEU2-lacZ* expression from the multicopy episomal plasmid pLS7. Glucose-repressed and -derepressed cultures of strains carrying the episomal plasmid pLS7 or the parent vector pMC2019 were assayed for β-galactosidase activity (Table 1). In this case, the control strains carrying pMC2019 showed some derepression of β-galactosidase; pMC2019 differs from the integrative vector pLS9 in that pMC2019 contains the *TRP1-ARS1* sequence 5′ to the gene fusion rather than the *URA3* gene. Nonetheless, a much more dramatic, 300-fold derepression occurred in cells carrying pLS7. The 10-fold-higher derepressed activity observed in strains carrying the multicopy episome pLS7 as compared with strains carrying a single integrated copy of pLS11 is consistent with the difference in copy number (16).

Effects of mutations in *trans*-acting regulatory genes on *SUC2-LEU2-lacZ* fusion expression. Previous genetic analysis has identified *trans*-acting genes required for normal regulation of *SUC2* gene expression; these genes are *SNF1* through *SNF6* (6, 21) and *SSN6* (7). We carried out experiments to determine whether the *SUC2* upstream regulatory

TABLE 1. Effects of the *SUC2* upstream region on *LEU2-lacZ* fusion expression

Relevant genotype	β-Galactosidase activity[a]	
	Glucose repressed	Glucose derepressed
ura3::pLS9	1.5	1.5
ura3::pLS11	1.1	38
ura3::pLS10	1.0	3.6
pMC2019	0.4	7
pLS7	1.4	380
pLS6	0.3	14
ura3::pLS9 *ssn6-1*	1.5	1.3
ura3::pLS11 *ssn6-1*	16	50
ura3::pLS10 *ssn6-1*	17	22

[a] Values are the average from assays of at least three independent transformants or segregants, except that values for pLS9 are the average from assays of two strains. Units of activity normalized for the OD_{600} of the culture were calculated as described by Miller (19). Standard errors were <20% for integrated plasmids and <25% for episomal plasmids; errors were lower for determinations giving values >2.

TABLE 2. Effects of regulatory mutations on *SUC2-LEU2-lacZ* fusion expression

Relevant genotype	Invertase activity[a]		β-Galactosidase activity[b] with:					
			ura3::pLS11		pLS7		pMC2019	
	R[c]	D	R	D	R	D	R	D
Wild type	<1	200	0.7[d]	43[d]	1.4	380	0.4	7
snf1-28	<1	<1	0.7	1.1	0.5	0.8[e]	0.6	0.8[e]
snf2-50	<1	3	2	8	1.2	13	0.3	0.6
snf3-39	40	70	8	23	7	30	0.6	3
snf3-72	14	50	1.4	40	ND[f]	ND	ND	ND
snf3-217	5	25	1.2	50	6	50	0.4	0.8
snf4-319	<1	1	0.7	1.1[g]	3	4	0.4	0.4
snf5-18	<1	4	0.6	1.2	ND	ND	ND	ND
snf6-719	<1	20	1.0	15	ND	ND	ND	ND
ssn6-1	300	380	16	50	130	130	3	3

[a] Values were taken from the work of Neigeborn and Carlson (21). Units are micromoles of glucose released per minute per 100 mg (dry weight) of cells.

[b] In each case, at least two spore clones or two transformants were assayed. Units of activity normalized for the OD_{600} of the culture were calculated as described by Miller (19). Standard errors were <25% for determinations with values >2 and <35% for values ≤2, with one exception (62% for pLS7, *snf3-217*, R).

[c] R, Glucose repressed; D, derepressed.

[d] Values are the average for six wild-type segregants from crosses of one transformant to mutants and are therefore different from the values in Table 1; standard errors were <10%.

[e] The *snf1*::*HIS3* allele was used in these experiments.

[f] ND, Not determined.

[g] The *snf4-Δ1* allele was used in this experiment.

region mediates the regulatory actions of these genes and, if so, to show thereby that these genes most likely act to regulate gene expression at the level of transcription. First, a series of strains carrying a *snf* or *ssn6* mutation and one copy of pLS11 integrated at *URA3* were derived from a single strain carrying the integrated plasmid, as described in Materials and Methods. To determine the effect of the mutation on expression of the *SUC2-LEU2-lacZ* fusion, each strain was grown under conditions of glucose repression or derepression and assayed for β-galactosidase activity (Table 2). For comparison, Table 2 also shows the effects of these mutations on expression of secreted invertase from the *SUC2* gene (7, 21).

The *snf1*, *snf4*, *snf5*, and *snf6* mutations caused defects in derepression of β-galactosidase activity from the integrated pLS11 of approximately the same magnitude as the defects in derepression of secreted invertase. The *snf2* mutation reduced the expression of β-galactosidase fivefold; this decrease is substantial but is less dramatic than the decrease in invertase expression caused by *snf2*. Three *snf3* mutations were tested because different alleles affect *SUC2* expression to different extents, and none of the mutations is known to be a null mutation. Two of the *snf3* alleles did not reduce the derepression of β-galactosidase, but *snf3-39* affected β-galactosidase expression in much the same way that it affects invertase expression, causing reduced derepression and some constitutivity. Thus, expression of the *SUC2-LEU2-lacZ* fusion was sensitive to mutations in all of the *SNF* genes.

Expression of the *SUC2-LEU2-lacZ* fusion from the integrated pLS11 also responded appropriately to mutations in a negative regulatory element, *SSN6*. An *ssn6* mutation which causes high-level constitutive invertase production also caused constitutive β-galactosidase synthesis (Table 2). In the control experiment, the *ssn6* mutation had no effect on expression of the *LEU2-lacZ* gene fusion in the parent vector pLS9 (Table 1).

We next examined the effects of some of these regulatory mutations on the expression of the *SUC2-LEU2-lacZ* gene fusion carried on the multicopy episome pLS7. For these experiments, the plasmid was introduced into a series of mutant strains by transformation, and β-galactosidase activity was assayed in glucose-repressed and -derepressed cells (Table 2). The results were similar to those obtained with the integrated plasmid pLS11. The *snf1*, *snf2*, and *snf4* mutations prevented high-level derepression of β-galactosidase activity, and in this case, both *snf3-39* and *snf3-217* caused defects in derepression. The *ssn6* mutation caused high-level constitutive expression; both repressed and derepressed values were approximately one-third the wild-type level. Thus, expression of the *SUC2-LEU2-lacZ* fusion from a multicopy plasmid was appropriately affected by mutations in *trans*-acting regulatory genes.

We also examined the effects of some of these mutations on the low-level derepression of the *LEU2-lacZ* fusion from plasmid pMC2019, which lacks the *SUC2* upstream sequences. We transformed *snf1*, *snf2*, *snf3*, *snf4*, and *ssn6* mutants with pMC2019, and in each case the mutation affected *LEU2-lacZ* fusion expression (Table 2). These findings suggest that pMC2019 carries sequences, presumably within the *TRP1-ARS1* region, that act in conjunction with these *trans*-acting regulatory genes to cause derepression of the *LEU2* promoter.

Activity of *SUC2* upstream region in inverted orientation 5′ to the *LEU2-lacZ* fusion. To determine whether the *SUC2* upstream region can confer regulated expression to the *LEU2-lacZ* fusion when it is in the inverted orientation relative to the gene, we first examined the expression of the *LEU2-lacZ* fusion from a copy of pLS10 integrated into the yeast genome at the *URA3* locus. Plasmid pLS10 carries the *SUC2* upstream region, including sequences between about −900 and −384, inserted 5′ to the *LEU2-lacZ* gene fusion in the orientation opposite to its normal orientation relative to *SUC2* (Fig. 1). The sequence surrounding nucleotide −500, an arbitrary point within the regulatory region, is about 280 base pairs farther from the *LEU2-lacZ* fusion in pLS10 than in the comparable plasmid pLS11, in which the upstream region is correctly oriented.

Three independent transformants carrying pLS10 integrated at *URA3* were assayed for production of β-galactosidase under conditions of glucose repression and derepression (Table 1). The presence of the inverted *SUC2* sequences had no significant effect on the repressed level of *LEU2-lacZ* fusion expression. All three transformants, however, derepressed β-galactosidase activity more than threefold, with a mean value of 3.6-fold. Three spore clones containing the integrated plasmid, which were derived from two crosses, were also assayed, and each showed greater than fourfold derepression of β-galactosidase (data not shown).

To evaluate further the significance of this derepression from pLS10, we crossed an *ssn6* mutation into strains carrying integrated pLS10 or the parent vector pLS9. The *ssn6* mutation had no effect on expression of the *LEU2-lacZ* fusion from pLS9 (Table 1). In contrast, *ssn6* significantly augmented the derepression of β-galactosidase from pLS10; *ssn6* strains produced about sixfold-higher β-galactosidase activity than wild-type (*SSN6*$^+$) strains carrying pLS10 (Table 1). Although *ssn6* also increased derepression of *SUC2* (Table 2) and the *LEU2-lacZ* fusion on pLS11 (Table 1), the effect was much less dramatic. Moreover, the consti-

tutive level of β-galactosidase synthesis from pLS10 was as high as that from pLS11 in *ssn6* strains. These findings support the idea that the inverted *SUC2* upstream region regulates *LEU2-lacZ* fusion expression.

A similar analysis of the effects of an inverted *SUC2* upstream region was carried out with the episomal plasmid pLS6, in which the relative positions of the *SUC2* and *LEU2-lacZ* fusion sequences are the same as in pLS10 (Fig. 1). Strains carrying pLS6 showed only a twofold increase in the derepressed levels of β-galactosidase activity relative to strains carrying the parent vector lacking *SUC2* sequences, pMC2019 (Table 1). However, pLS6 differs from pMC2019 in that the inserted *SUC2* DNA displaces the *TRP1-ARS1* sequences previously adjacent in the *LEU2* promoter to a position 0.5 kb farther upstream. If these sequences are responsible for the derepression observed from pMC2019 and their effect is attenuated by the increased distance from the *LEU2* promoter, then the inverted *SUC2* region could in fact be solely responsible for the nearly 50-fold derepression. Nonetheless, the *SUC2* upstream region is clearly more effective in conferring glucose-repressible expression to the *LEU2-lacZ* fusion on the episome when it is present in the correct orientation.

DISCUSSION

These studies show that the upstream region of the *SUC2* gene is able to confer glucose-repressible expression to a heterologous gene. This finding confirms that the region is responsible for regulation and, because deletion of the region prevents derepression of *SUC2* (26), implies that *SUC2* is regulated by positive control. The regulation of *SUC2* expression has previously been shown to occur by regulation of the level of stable mRNA (5). These studies suggest that this regulation is at the level of transcription rather than at that of mRNA stabilization, as the *LEU2-lacZ* fusion transcript is unlikely to bear any functionally significant resemblance to the *SUC2* mRNA.

Derepression of β-galactosidase activity in response to glucose limitation was observed both when the *SUC2-LEU2-lacZ* fusion was present on an episomal plasmid and when it was integrated into the chromosome at the *URA3* locus. Thus, the regulation did not depend on special properties of the chromosomal *SUC2* locus.

Trans-acting genes that affect the regulation of *SUC2* gene expression include *SNF1* through *SNF6* and *SSN6* (6, 7, 21). To determine whether the *SUC2* upstream region mediates the regulatory functions of these genes, we examined the effects of these mutations on expression of the *SUC2-LEU2-lacZ* fusion. The *snf1*, *snf2*, *snf4*, *snf5*, and *snf6* mutations, which prevent normal derepression of secreted invertase, caused analogous defects in derepression of β-galactosidase, although *snf2* impaired expression of the gene fusion to a lesser extent than is observed with the *SUC2* gene. It is worth noting that a *snf2* ochre allele does not completely prevent derepression of *SUC2* (21), suggesting that the *SNF2* gene product is not absolutely required for *SUC2* expression. It is possible that the *SUC2-LEU2-lacZ* construct has a less stringent requirement for the *SNF2* gene product than does *SUC2*. We also tested the effects of several *snf3* alleles on expression of the integrated and episomal fusions. Although defects were not detected in all instances, probably because the mutations are leaky, significant defects in derepression were observed in three cases. The findings that the *SNF1* through *SNF6* genes are required for regulated expression of the *SUC2-LEU2-lacZ* gene fusion suggest that the *SNF* gene products exert their positive regulatory effects on *SUC2* expression by interacting or influencing interactions with the upstream region. These results also suggest that these gene products affect regulation at the transcriptional level. The failures of *snf1* mutants (5) and *snf2* mutants (L. Neigeborn and M. Carlson, unpublished data) to derepress secreted invertase have been shown to result from defects in derepression of the mRNA.

The *SSN6* gene is a *trans*-acting negative regulator of *SUC2* (7). An *ssn6* mutation was found to cause high-level constitutive expression of the *SUC2-LEU2-lacZ* fusion, suggesting that the *SUC2* upstream region mediates the repressive effect of the *SSN6* gene product on transcription. Previous studies have also suggested that *ssn6* does not simply permit a bypass of the regulatory mechanisms involving the upstream region; the *SUC2* upstream region has been found to be required for *SUC2* expression in *ssn6* mutants (26).

Related studies have shown that upstream sequences of other yeast genes can confer appropriately regulated expression to heterologous genes that are normally controlled in response to different signals. The region between *GAL1* and *GAL10*, in conjunction with the *GAL4* and *GAL80* regulatory gene products, has been shown to mediate galactose induction of a heterologous gene (14). The upstream activation site of the *CYC1* gene confers regulation by catabolite repression and heme to the same *LEU2-lacZ* fusion used in these studies and appears to mediate the action of *trans*-acting regulatory genes (13). The *LEU2* upstream regulatory element has been shown to mediate leucine-specific regulation (18).

We also examined the ability of the *SUC2* upstream region to function in the inverted orientation. Analysis of the expression of β-galactosidase from the integrated plasmid pLS10 suggested that the region is active in the inverted orientation but does not function as effectively as it does when oriented correctly. A surprising result was obtained when the effects of an *ssn6* mutation on expression from the inverted construct in pLS10 were examined: *ssn6* not only caused high-level constitutive expression of β-galactosidase but also increased the derepressed level of expression sixfold relative to that in an *SSN6*$^+$ background. This evidence supports the conclusion that the upstream region functions in the inverted orientation, and it raises the question of why *ssn6* so dramatically affects the expression conferred by the inverted region. The *ssn6* mutations also caused increased derepression of the *SUC2* gene, but the effect was less than twofold. Interestingly, *ssn6* has an equally dramatic effect on expression of a *SUC2* gene with a deletion of the sequence between −1900 and −436: *ssn6* causes an increase in synthesis of secreted invertase from 3 to 40% of the wild-type level (26). One possible explanation is that the magnitude of the effect of *ssn6* is related to the degree of activity of upstream sequences in derepressing expression rather than to their orientation.

The evidence that the *SUC2* upstream region is partially active when inverted suggests that the region resembles in some respects the enhancer elements found in higher eucaryotes. Enhancers augment expression of heterologous genes and are active in both orientations (3, 20); some enhancers mediate hormonal regulation or cell-type specificity of expression (2, 10, 11). In related studies, Guarente and Hoar (12) have shown that the upstream activation site of the *CYC1* gene of *S. cerevisiae* functions when inverted.

Our finding that the upstream region was less active when inverted than when correctly oriented may reflect the particulars of our constructs rather than an intrinsic property of

the region. It is possible that the reduced effectiveness of the inverted sequence is due not to its orientation but rather to interference by sequences that are ordinarily located 5′ to the regulatory region but are positioned between the regulatory region and the *LEU2* promoter in the inverted construct. In addition, the regulatory region is placed somewhat farther from the *LEU2* promoter in the inverted constructs. Further experiments to explore the ability of inverted upstream sequences to regulate expression of the *SUC2* gene at its normal chromosomal locus are under way.

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