

SSN Genes That Affect Transcriptional Repression in *Saccharomyces cerevisiae* Encode SIN4, ROX3, and SRB Proteins Associated with RNA Polymerase II

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The RNA polymerase II of *Saccharomyces cerevisiae* exists in holoenzyme forms containing a complex, known as the mediator, associated with the carboxyl-terminal domain. The mediator includes several SRB proteins and is required for transcriptional activation. Previous work showed that a cyclin-dependent kinase-cyclin pair encoded by *SSN3* and *SSN8*, two members of the *SSN* suppressor family, are identical to two SRB proteins in the mediator. Here we have identified the remaining *SSN* genes by cloning and genetic analysis. *SSN2* and *SSN5* are identical to *SRB9* and *SRB8*, respectively, which encode additional components of the mediator. Genetic evidence implicates the *SSN* genes in transcriptional repression. Thus, these identities provide genetic insight into mediator and carboxyl-terminal domain function, strongly suggesting a role in mediating transcriptional repression as well as activation. We also show that *SSN4* and *SSN7* are the same as *SIN4* and *ROX3*, respectively, raising the possibility that these genes also encode mediator proteins.

Studies of the *Saccharomyces cerevisiae* RNA polymerase II have led to the identification of a holoenzyme form containing a large multiprotein complex associated with the carboxyl-terminal repeat domain (CTD) of the largest subunit and a subset of general transcription factors (22–24, 44). The CTD-associated complex contains many components, including GAL11, SUG1, and SRB proteins. The association of SRB proteins with the CTD is consistent with genetic evidence regarding function: mutations in the *SRB* genes were isolated as suppressors of cold sensitivity caused by truncation of the CTD (31, 44). Evidence indicates that this complex is required to mediate transcriptional activation, and it has been designated the mediator (15, 22, 23).

Our studies of genes that contribute to glucose repression have unexpectedly led us to two proteins in the mediator. We recently identified a new cyclin-dependent kinase-cyclin pair encoded by *SSN3* and *SSN8* (25). Sequence comparison indicates that *SSN3* and *SSN8* are identical to *SRB10* (also known as *UME5* and *ARE1* [43, 52]) and *SRB11*, respectively, which are new members of the *SRB* suppressor family encoding proteins in the mediator complex (27). Mutations in *SSN3* and *SSN8* are members of a set of suppressors of *snf1*, designated *ssn1* through *ssn8* (5). These *ssn* mutations suppress growth defects of a mutant lacking the SNF1 protein kinase, which is required to relieve glucose repression of gene expression (6). The identities between these two pairs of *SSN* and *SRB* genes link two genetically defined sets of suppressors and provide an unanticipated functional connection between the *SSN* family and RNA polymerase II.

The functions of two genes in the *SSN* family have been extensively characterized. *SSN1*, which is the same as *MIG1* (51), and *SSN6* have been directly implicated in transcriptional repression. The *SSN6* protein, together with TUP1, forms a complex (54) that represses transcription of many genes (38,

47). The *SSN6*-TUP1 complex is tethered to differently regulated promoters by specific DNA-binding proteins such as $\alpha 2$ and MIG1 (21, 46, 48, 49). MIG1 is a zinc finger protein that mediates glucose repression of *SUC*, *GAL*, and other genes, and MIG1 may be a target of the SNF1 kinase (20, 28, 29, 37, 46).

The roles of the other *SSN* genes in transcriptional control are less clear. The remaining *ssn* mutations (*ssn2* through *ssn5*, *ssn7*, and *ssn8*) cause similar phenotypes and exhibit similar genetic behavior. They resemble *ssn6* and *tup1* in causing severe flocculence, but they are only weak suppressors of *snf1*, and none significantly affects glucose repression in strains wild-type for *SNF1* and *MIG1* (5). Strikingly, however, each acts synergistically with *mig1* to relieve glucose repression of the *SUC2* (invertase) gene (51). The strong synergy observed in the double mutants suggests that all of these *SSN* proteins contribute to negative regulation of transcription. The identity of *SSN3* and *SSN8* with *SRB* genes raises the possibility that other *SSN* genes are also functionally connected to RNA polymerase II.

Here we have identified the other *SSN* genes by cloning and genetic analysis. We present evidence that *SSN2* and *SSN5* are identical to *SRB9* and *SRB8*, respectively, which have recently been shown to encode additional components of the CTD-associated mediator (15). We also show that *SSN4* and *SSN7* are identical to *SIN4* (*TSF3*) (7, 18) and *ROX3* (35), respectively. The genetic evidence that *SSN2*, *SSN3*, *SSN5*, and *SSN8* affect transcriptional repression, combined with biochemical evidence that all four proteins are associated with the mediator, strongly suggests a role for the mediator complex in mediating transcriptional repression as well as activation.

MATERIALS AND METHODS

Strains and genetic methods. The *S. cerevisiae* strains used are listed in Table 1. Standard methods for yeast genetic analysis (34) and transformation (16) were followed. Rich medium was yeast extract-peptone containing 2% glucose (YPD). Scoring for utilization of other carbon sources was done as described previously

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TABLE 1. List of *S. cerevisiae* strains

Strain ^a	Genotype
MCY447.....	<i>MATα snf1-28 ssn2-4 ade2-101 ura3-52 his4-539</i>
MCY829.....	<i>MATα his3Δ200 ura3-52 lys2-801</i>
MCY1250.....	<i>MATα snf2Δ1::HIS3 his3Δ200 ura3-52 lys2-801</i>
MCY2099.....	<i>MATα snf5Δ2 his3Δ200 ura3-52 ade2-101</i>
MCY2578.....	<i>MATα suc2Δ-1900/-400 ura3-52 his4-539</i>
MCY3304.....	<i>MATα ssn2-4 snf1 mig1Δ2::LEU2 ura3-52 ade2-101 his4-539</i>
MCY3311.....	<i>MATα ssn4-1 snf1 mig1Δ2::LEU2 ura3-52 his4-539 lys2-801</i>
MCY3312.....	<i>MATα ssn4-1 snf1 mig1Δ2::LEU2 ura3-52 his4-539 lys2-801 ade2-101</i>
MCY3316.....	<i>MATα ssn5-4 snf1 mig1Δ2::LEU2 ura3-52 his4-539</i>
MCY3319.....	<i>MATα ssn7-1 snf1 mig1Δ2::LEU2 ura3-52 his4-539</i>
MCY3641.....	<i>MATα mig1Δ2::LEU2 his3Δ200 ura3-52 leu2-3,112 lys2-801</i>
SLY40 ^b	<i>MATα srb8Δ1::URA3,hisG his3Δ200 leu2-3,112 ura3-52</i>
FY250 ^c	<i>MATα his3Δ200 ura3-52 leu2Δ1 trp1Δ63</i>
CTY10.5d ^d	<i>MATα ade2 trp1-901 leu2-3,112 his3Δ200 gal4 gal80 URA3::lexAop-lacZ</i>

^a MCY strains have the S288C background and carry *SUC2*; *snf1* alleles are either *snf1 Δ 3* or *snf1-28*.

^b Obtained from R. Young.

^c Obtained from F. Winston.

^d Constructed by R. Sternglanz.

(51). Selective synthetic complete (SC) medium was used to maintain selection for plasmids. The *Escherichia coli* strain used was XL1-Blue.

Cloning of *SSN2* and *SSN7*. A genomic library in the centromere vector YCp50 (33) was used to transform MCY3304 (*snf1 mig1 ssn2*) or MCY3319 (*snf1 mig1 ssn7*). Nonflocculent Ura⁺ transformants were enriched by differential sedimentation (38) and tested for recovery of the flocculent phenotype after selection on 5-fluoro-orotic acid for plasmid loss. Plasmids were then isolated by passage through bacteria and retested for complementation. pWS9-1 and pWS10-1 were derived from pWS8 by deletion of the indicated *MluI* and *BamHI* fragments. pIT218 and pIT225 are subclones in YCp50 and pRS306 (39), respectively.

Sequence analysis. For *SSN2*, the *BamHI* and *EcoRI* fragments spanning the gene were cloned into pRS316 (39). For pIT200, three *XbaI* and *XbaI-SalI* fragments were subcloned into pBluescript vectors (Stratagene). Partial nucleotide sequences were determined and compared with the sequences in GenBank by using the BLAST programs at the National Center for Biotechnology Information (1).

Disruption of chromosomal *SSN2* locus. pWS44-11 contains the *BamHI-SalI* fragment cloned into pRS316, with the *EcoRI* fragments replaced with the *URA3* gene. pWS45-15 was derived from pWS44-11 by replacing the *BglII-MscI* frag-

ment with the *LEU2* gene. The *BanI* fragment from pWS44-11 and the *XhoI-SalI* fragment from pWS45-15 were released and used to transform yeast strains to prototrophy. Disruption of the chromosomal locus was confirmed by Southern blot. The resulting alleles were designated *ssn2 Δ 1::URA3* and *ssn2 Δ 2::LEU2*, respectively.

Construction of LexA fusions. To construct pLexA-SSN2, the N-terminal *SSN2* coding sequence (nucleotides 1 to 161) was amplified from pWS8 by PCR. The primers were oligonucleotides SO-4 (5'-CGGGATCCTTATGAGTTCTGACGCTTCCACGTAC-3') and SO-10 (5'-CTTTAGATCTTGCTTCCGTAGTACG-3'). These primers insert a *BamHI* site 5' to the ATG and change the *BamHI* site at position 160 to a *BglII* site, respectively. The PCR product was digested with *BamHI* plus *BglII* and inserted in the correct orientation into a plasmid containing the 6.2-kb *SSN2 BamHI-SalI* fragment cloned in pRS316. The *BamHI-SalI* fragment from the resulting plasmid, containing the complete *SSN2* coding sequence, was inserted into pSH2-1 (14).

To construct pLexA-ROX3, the *ROX3* sequence was amplified from pIT218 by PCR with primers OL70 (5'-CCCCGAATTCATGGCTTCTAGAGTGGAC-3') and OL71 (5'-CCCCCTCGAGCTACTCCAGCCTCC-3'). The product was cut by *EcoRI* plus *XhoI* and introduced into pSH2-1.

Invertase assay. Glucose-repressed cultures were grown to mid-log phase in selective SC-2% glucose medium; derepressed cultures were prepared by shifting cells to SC-0.05% glucose for 3 h. Invertase activity was assayed as previously described (50).

β -Galactosidase assay. Transformants were grown to mid-log phase in selective SC-2% glucose. β -Galactosidase activity was assayed in permeabilized cells and is expressed in Miller units (13).

RESULTS

Cloning of the *SSN2* gene and identity with *SRB9*. The *SSN2* gene was cloned by complementation of the flocculent phenotype caused by the *ssn2-4* mutation. Two overlapping plasmids, pWS7 and pWS8 (Fig. 1), were recovered, and both also complemented *ssn2* for synergy with *mig1* in relieving glucose repression of *SUC2* (Table 2). Previously, *ssn2* was mapped to a locus linked to *snf1* (5) and localized to a lambda clone containing DNA from this region (10). A labeled 1.4-kb *EcoRI* fragment from pWS8 hybridized to the appropriate lambda clone.

The *SSN2* gene was localized to one end of pWS8 by constructing deletions (Fig. 1). Sequence analysis of this region revealed a long open reading frame beginning 0.5 kb from the vector junction and extending beyond the four *EcoRI* sites. Comparison with sequences in GenBank (release 83.0, June 9, 1994) showed identity to *SCA1*, encoding a 1,420-amino-acid

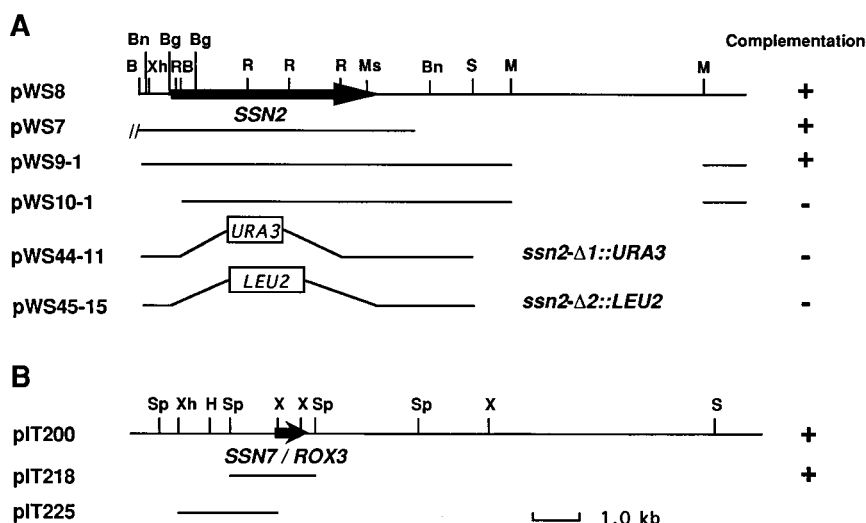


FIG. 1. Maps of *SSN2* and *SSN7/ROX3* plasmids. Only the inserted yeast DNA is shown. Heavy arrows indicate the *SSN* coding region and direction of transcription. The ability to complement the cognate *snf1 mig1 ssn* mutant for flocculence and glucose-resistant invertase expression is shown to the right. (A) *SSN2* plasmids. The insert in pWS7 extends farther to the left. Restriction sites: B, *BamHI*; Bg, *BglII*; Bn, *BanI*; M, *MluI*; Ms, *MscI*; R, *EcoRI*; S, *SalI*; Xh, *XhoI*. Not all *BglII* sites are shown. (B) *SSN7/ROX3* plasmids. The only *HindIII* site shown is the site in pIT225 that was used to target integration to the homologous chromosomal locus. Restriction sites: H, *HindIII*; S, *SalI*; Sp, *SphI*; X, *XbaI*; Xh, *XhoI*.

TABLE 2. Complementation of *ssn2* and *ssn7* for synergy with *mig1* in relieving glucose repression

Genotype	Plasmid	Invertase activity ^a
<i>snf1 mig1</i>	None	4
<i>snf1 ssn2</i>	None	1
<i>snf1 mig1 ssn2</i>	YCp50	53
	pWS8	2
	pWS7	3
<i>snf1 mig1 ssn7</i>	YCp50	45
	pIT200	1

^a Invertase activity is expressed as micromoles of glucose released per minute per 100 mg (dry weight) of cells. Transformants were grown in selective SC-2% glucose to maintain plasmids.

protein. *SCA1* was isolated as a suppressor of lethal substitution mutations in the C-terminal domain of the largest subunit of RNA polymerase II (A. Yuryev and J. L. Corden; cited in GenBank). At this time, we became aware of the identities of *SSN3* and *SSN8* with *SRB10* and *SRB11*, respectively (25, 27). We therefore compared *SSN2* with other *SRB* genes cloned in the laboratory of R. Young. *SSN2* is the same as *SRB9*, the sequence of which has now been published (15).

Disruption of the chromosomal *SSN2* locus. To determine the phenotype of a null mutation in *SSN2*, we disrupted the chromosomal locus. The *ssn2Δ1::URA3* and *ssn2Δ2::LEU2* mutations (Fig. 1) were introduced into haploid strain FY250, yielding flocculent disruptants. The *ssn2Δ1::URA3* mutant showed slightly impaired growth on galactose but no apparent defect on glucose at high (37°C) or low (16°C) temperatures. In addition, regulation of *SUC2* expression was nearly normal (Table 3). We also confirmed that the null mutation did not complement *ssn2-4*.

The original *ssn2* mutations were isolated as suppressors of the sucrose and raffinose growth defects of an *snf1* mutant. To determine whether the null mutation also suppresses *snf1*, we disrupted *SSN2* in an *snf1Δ10* mutant. The *ssn2Δ1::URA3* mutation caused a slight improvement in growth on sucrose and raffinose in four disruptants and a slight elevation in *SUC2* expression similar to that reported for the point mutation (5). *ssn2Δ1::URA3* showed strong synergy with a *mig1Δ* mutation in relieving glucose repression of *SUC2* expression (Table 3).

***SSN5* is the same as *SRB8*.** The establishment of identity for three pairs of *SSN* and *SRB* genes prompted us to test for further identities. The *SRB8* gene seemed a likely candidate because a mutation in it causes flocculence (15). We therefore transformed *ssn4*, *ssn5*, and *ssn7* mutants with a centromeric plasmid carrying *SRB8* (pSL301 [15]). This plasmid complemented the flocculent phenotype of the *ssn5* mutant. An *srb8Δ1* mutant (SLY40) was then crossed to the *ssn5* mutant MCY3316. The *srb8* and *ssn5* mutations did not complement with respect to flocculence, and tetrad analysis of the diploid yielded only flocculent segregants from 13 complete tetrads. Thus, these mutations fail to complement and are tightly linked, indicating identity of the *SSN5* and *SRB8* genes. *SSN5* is also the same as *ARE2* (51a).

***SSN4* is the same as *SIN4*.** Mutations in *SIN4* (*TSF3*) cause pleiotropic phenotypes similar to those of *ssn* mutations, including flocculence and partial release of glucose repression (7, 8, 18). We therefore tested a multicopy *SIN4* plasmid (YEp-SIN4; gift of A. Mitchell, Columbia University) for complementation of the two remaining *ssn* mutations, *ssn4* and *ssn7*. Transformants of the *ssn4-1* mutant MCY3312 were nonflocculent. To test allelism, we introduced the *sin4Δ::TRP1* disruption (18) into the S288C genetic background. The *ssn4-1* and

sin4Δ::TRP1 mutations did not complement for flocculence. Because the *ssn4-1/sin4Δ::TRP1* diploid did not sporulate efficiently, we introduced YEp-SIN4. After tetrad analysis of this diploid, spore clones were grown on 5-fluoro-orotic acid to select for plasmid loss. All segregants from 13 tetrads were flocculent. Thus, *SSN4* and *SIN4* are the same gene.

Cloning of *SSN7* and identity with *ROX3*. To characterize *SSN7*, we cloned the gene from a low-copy-number library by complementation of the flocculent phenotype conferred by the *ssn7-1* mutation. Plasmid pIT200 was recovered and shown to also complement *ssn7* for synergy with *mig1* in relieving glucose repression and suppressing *snf1*. Sequence analysis localized the clone to a region of chromosome II containing the *ROX3* gene and 10 other open reading frames.

Genetic evidence suggested the possible identity of *SSN7* and *ROX3*. The *rox3* mutations were isolated under circumstances reminiscent of those in which the *ssn* suppressors were recovered. Mutations in *ROX3* were identified by selecting for increased *CYC7* (iso-2-cytochrome *c*) expression, which has also yielded mutations in *SSN6*, *TUP1*, and *ROX1* (35). *ROX1* mediates heme repression of hypoxic gene expression and is analogous to *MIG1* (*SSN1*) in that it encodes a DNA-binding repressor protein that functions with *SSN6* (2, 56). *ROX3* encodes a 220-amino-acid protein with an essential function (35).

To determine whether *ROX3* was responsible for the observed complementation, we constructed a subclone containing only the *ROX3* coding region in a centromeric vector. This plasmid, pIT218 (Fig. 1B), complemented *ssn7* for flocculence. To test for genetic linkage of the cloned DNA to the *ssn7* locus, we constructed an integrating *URA3* plasmid (pIT225 [Fig. 1B]). *HindIII*-cleaved DNA was integrated into the homologous chromosomal locus of MCY829, as confirmed by Southern blot analysis, and two *Ura*⁺ integrants were crossed to an *ssn7 ura3* mutant (MCY3319). Tetrad analysis of the resulting diploids yielded only *Ura*⁺ nonflocculent and *Ura*⁻ flocculent segregants in 14 of 15 complete tetrads and in six tetrads with three viable spores. One tetrad appeared to be recombinant. These genetic data indicate tight linkage of *ROX3* and *SSN7* and confirm their identity.

Requirement for UASs in *ssn* mutants. Mutations in *SIN4* allow expression of several promoters in the absence of upstream activation sequences (UASs), including *GAL1*, *HO*, *CYC1*, and *PHO5* (8, 18). For example, expression of a *GAL1-lacZ* fusion lacking the UAS (plasmid pLR1Δ1 [53]) was six-fold higher in *sin4Δ* mutants than in the wild type during growth in glucose (18). We tested *ssn* mutants for a similar phenotype. In comparable experiments, the *ssn2Δ* mutant showed no significant elevation of β-galactosidase expression

TABLE 3. Effects of *ssn2Δ* on invertase activity^a

Genotype	Invertase activity	
	Repressed	Derepressed
Wild type	1	130
<i>ssn2Δ</i>	3	130
<i>mig1Δ</i>	18	240
<i>mig1Δ ssn2Δ</i>	200	630
<i>snf2Δ</i>	4	11
<i>snf2Δ ssn2Δ</i>	2	21

^a The *ssn2Δ1::URA3* mutation was introduced into strains FY250 (wild type), MCY3641 (*mig1Δ1::LEU2*), and MCY1250 (*snf2Δ1::HIS3*). Invertase activity is expressed as micromoles of glucose released per minute per 100 mg of cells (dry weight). Values are averages for assays of three disruptants or two assays of the parent strains. Cultures were grown in SC-3% glucose (repressed) and shifted to SC-0.05% glucose for 3 h (derepressed). Standard errors were <10%.

from pLR1Δ1 relative to the wild type (0.38 and 0.23 Miller units, respectively). We also assayed *ssn3Δ* and *ssn8Δ* single and double mutants after growth in galactose but again found no significant differences from the wild type (*ssn3Δ*, 0.35 U; *ssn8Δ*, 0.30 U; *ssn3Δ ssn8Δ*, 0.16 U; wild type, 0.11 U).

We next tested for expression of a *SUC2* gene deleted for the UAS (Δ-1900/-400) (36). Neither *ssn2Δ*, *ssn3Δ*, nor *ssn8Δ* noticeably suppressed the growth defect on raffinose caused by this UAS deletion, and we detected no significant invertase activity in assays of the *ssn2Δ* mutants.

Suppression of *snf2* and *snf5* by *ssn* mutations. The SNF/SWI complex is required for transcriptional activation of many genes, including *SUC2* (reviewed in reference 55). The complex contains SWI1/ADR6, SNF2/SWI2, SWI3, SNF5, SNF6, SNF11, and at least five other proteins (4, 32, 45). It is thought to activate transcription by altering chromatin structure and thereby facilitating binding of specific and general transcription factors. Previous studies showed that two mutations in the *ssn* series suppress *snf/swi* mutant defects: *ssn6* suppresses *snf2*, *snf5*, and *snf6* with respect to *SUC2* expression (11, 30), and *snf4* partially suppresses the *snf2/swi2* mutant defects in *HO::lacZ* expression and growth on sucrose (18).

To assess the genetic relationship between other *ssn* and *snf/swi* mutations, we constructed several different mutant combinations. First, we disrupted *SSN2* in an *snf2Δ1::HIS3* mutant. The *ssn2Δ1::URA3* mutation weakly suppressed the growth defect of the *snf2Δ* mutant on sucrose and raffinose and increased derepression of *SUC2* expression twofold (Table 3). Next, both *ssn3Δ snf5Δ* and *ssn8Δ snf5Δ* double mutants were constructed by genetic crossing. Analysis of tetrads revealed that each *ssn* mutation slightly improved the growth of *snf5* segregants on raffinose, sucrose, and glycerol. Thus, *ssn2*, *ssn3*, and *ssn8* weakly suppress defects in the function of the SNF/SWI complex, but none of them are strong suppressors.

LexA-SSN2 and LexA-ROX3 fusion proteins activate transcription. Previous studies have shown that SIN4-LexA and LexA-SSN8 fusion proteins activate transcription when bound to DNA near a promoter, whereas LexA-SSN3 has no substantial effect (18, 25). To test if SSN2 and ROX3 activate transcription, we constructed fusions to the LexA DNA-binding domain (residues 1 to 87). Both LexA-SSN2 and LexA-ROX3, expressed in strain CTY10.5d, activated transcription of a *GAL1-lacZ* reporter with LexA binding sites (β-galactosidase activity, 287 and 104 U, respectively). In contrast, a peptide consisting of LexA residues 1 to 87 expressed from plasmid pSH2-1 had <1 U of activity. (These activity values are averages for at least four transformants; standard errors were <5%). Thus, four members of the SSN family can activate transcription when artificially bound to a promoter. In contrast, LexA-MIG1 and LexA-SSN6 function as repressors when bound to DNA, although MIG1 can also activate transcription in mutants lacking SSN6 (21, 46).

DISCUSSION

We have here completed the identification of all members of the SSN family of suppressors (Table 4). We show that *SSN2* and *SSN5* are identical to *SRB9* and *SRB8*, respectively, giving a total of four SSN genes that are also SRB genes, encoding components of the mediator associated with the RNA polymerase II CTD (called SSN/SRB genes in this discussion). These identities provide new genetic insight into mediator function, strongly suggesting a role in transcriptional repression as well as activation. We also show that *SSN4* and *SSN7* are the same as *SIN4* and *ROX3*, respectively, raising the

TABLE 4. Other names of SSN genes

SSN gene	Alternate name(s)
<i>SSN1</i>	<i>MIG1</i>
<i>SSN2</i>	<i>SRB9, SCA1</i>
<i>SSN3</i>	<i>SRB10, UME5, ARE1</i>
<i>SSN4</i>	<i>SIN4, TSF3</i>
<i>SSN5</i>	<i>SRB8, ARE2</i>
<i>SSN6</i>	<i>CYC8</i>
<i>SSN7</i>	<i>ROX3</i>
<i>SSN8</i>	<i>SRB11</i>

possibility that these genes also encode components of the mediator.

The mediator has previously been shown to be required for transcriptional activation. Biochemical studies demonstrate a requirement for the mediator for transcriptional activation in vitro, and mutations in *SSN/SRB* genes affect transcriptional activation in vivo (15, 22, 23, 25, 27). However, workers in this laboratory initially characterized the *ssn* alleles with respect to their phenotype of relieving glucose repression of gene expression, and considerable genetic evidence now indicates that the four SSN/SRB genes are involved in transcriptional repression of diversely regulated genes. Mutations in all four show strong synergy with *mig1* in relieving glucose repression of *SUC2* (51), and mutations in *SSN3* and *SSN8* partially relieve phosphate repression of acid phosphatase (25). Mutations in *SSN3* have also been independently isolated by their effects on repression of meiotic genes in vegetative cells (*ume5*) (42, 43) and on repression of α-specific genes in α cells, known as α2 repression (*are1*) (52). Thus, these SSN/SRB genes affect the repression of a broad spectrum of differently regulated genes. In addition, a negative regulatory role in transcription has also been attributed to another gene encoding a mediator component, *GAL11* (7, 12).

This genetic evidence implicates the mediator in transcriptional repression. One possibility is that the mediator is involved in mediating the response of the transcriptional apparatus to repressor proteins, such as SSN6-TUP1. The mediator is a large multiprotein complex, so it is easily conceivable that it mediates responses to numerous regulatory proteins, including both activators and repressors. While this model is attractive, other explanations could also account for the genetic data. For example, it is possible that the defects in repression result indirectly from defects in activation of genes encoding repressors of these various, differently regulated genes. It is also possible that all four of these SSN/SRB proteins have dual roles in the cell, such that they participate in transcriptional activation as components of the mediator and participate in transcriptional repression by some other mechanism. However, the simple model is that the mediator is involved not only in transcriptional activation but also in transcriptional repression.

The synergy of *ssn* mutations with *mig1* in relieving glucose repression of *SUC2* deserves comment. Previous work showed that other proteins besides MIG1 are required for the full repressive effect of SSN6-TUP1 on *SUC2* expression (51). We imagine that another DNA-binding protein, X, exists which can recruit SSN6-TUP1 to the *SUC2* promoter or to the promoter(s) of the gene(s) that regulates *SUC2*. If the SSN/SRB proteins contribute to repression by both MIG1-SSN6-TUP1 and X-SSN6-TUP1, this would account for the observed synergy.

Are SIN4 and ROX3 components of the mediator? Our recovery of the cognate genes as members of the SSN family

raises this possibility. The *SIN4* (*TSF3*) gene has been extensively characterized. *SIN4* has been implicated in transcriptional activation and repression of a broad spectrum of genes, consistent with a role in general transcriptional control (7–9, 18, 19, 40). It is worth noting that *SIN4* displays somewhat different genetic properties than the *SSN/SRB* genes characterized here. Mutations in *SIN4* allow expression of various promoters in the absence of UASs (8, 18), whereas *ssn2*, *ssn3*, and *ssn8* did not bypass the requirement for a UAS for the promoters tested here. In addition, *sin4* has been reported to suppress the *snf2* defects in *HO::lacZ* expression and growth on sucrose (18). Only very weak suppression of *snf2* or *snf5* mutant defects by *ssn2*, *ssn3*, and *ssn8* was detected in this study. Moreover, Jiang and Stillman (18) have presented evidence that *SIN4* affects chromatin structure, although the effect could be indirect. These data suggest that *SIN4* plays a somewhat different role from the *SSN/SRB* proteins in transcriptional control. However, different proteins in the mediator clearly have different functions (for example, some *SRB* proteins are essential for viability [44]), so the genetic evidence can be viewed as compatible with the idea that *SIN4* is a component of the mediator. Finally, there are unidentified proteins of appropriate size in the purified mediator complex (15, 22). If *SIN4* does prove to be associated with the mediator, it is interesting that several lines of evidence suggest a functional relationship between *SIN4* and *RGR1* (9, 17, 41).

ROX3 is also a candidate for a component of the mediator, based on its identity as an *SSN* family member. Previous studies lend some supporting evidence. The protein is localized in the nucleus and essential for viability, and the mutant phenotypes are consistent with a general role in transcriptional control (35). The viable *rox3* mutations that were recovered by Rosenblum-Vos et al. (35) increased the expression of some heme-regulated genes and decreased the expression of others, and the *ssn7-1* allele affected glucose repression of *SUC2* (51). In addition, the mediator complex (15, 22) contains unidentified proteins that could correspond to *ROX3*. Biochemical studies will be required to resolve this issue.

Four of the *SSN* proteins (*SSN2/SRB9*, *SIN4*, *ROX3*, and *SSN8/SRB11*) function as transcriptional activators when artificially bound to a promoter as a LexA fusion protein. These activities most likely reflect physiologically relevant functions because evidence indicates that these proteins affect transcriptional activation in vivo. However, it seems unlikely that these proteins contain activation domains analogous to those of gene-specific DNA-binding activator proteins. The stimulation of transcription in this assay may rather reflect other roles in the activation process. The association of the two *SSN/SRB* proteins with the mediator raises the possibility that these LexA fusion proteins simply serve to recruit RNA polymerase II holoenzyme to the target promoter, thereby stimulating transcription. A similar model has been proposed to account for activation by a LexA fusion to *GAL11*, another component of the holoenzyme (3). If this proves to be true, then *SSN3/SRB10* may be less tightly associated with the mediator than *SSN2/SRB9* or *SSN8/SRB11*, as LexA-*SSN3* provides *SSN3* function in vivo but does not activate in this assay (25).

Why does the *SSN* set of suppressors have so many genes in common with the *SRB* family? The *ssn* suppressors were selected to bypass the requirement for the SNF1 protein kinase for expression of glucose-repressed genes. The two strongest suppressors of the *snf1* defect in *SUC2* expression, *mig1* (*ssn1*) and *ssn6*, relieve transcriptional repression by the MIG1-*SSN6*-TUP1 complex; the function of this repressor complex is most likely regulated by SNF1 (20, 37, 46, 51). The other *ssn* mutations are much weaker suppressors, although all show

synergy with *mig1* in suppressing *snf1* (51). In our view, it is likely that the four *ssn/srb* mutations partially bypass the requirement for SNF1 by virtue of attenuating transcriptional repression. Alternatively, it is possible that the recovery of *ssn/srb* mutations as suppressors of *snf1* reflects a direct regulatory interaction between the SNF1 protein kinase and RNA polymerase II holoenzyme.

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