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Genetics

Cyclin-dependent protein kinase and cyclin homologs SSN3 and SSN8 contribute to transcriptional control in yeast

SERGEI KUCHIN, PAULA YEGHIAYAN, AND MARIAN CARLSON

Departments of Genetics and Development and Microbiology, College of Physicians and Surgeons, Columbia University, New York, NY 10032

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ABSTRACT **The *SSN3* and *SSN8* genes of *Saccharomyces cerevisiae* were identified by mutations that suppress a defect in SNF1, a protein kinase required for release from glucose repression. Mutations in *SSN3* and *SSN8* also act synergistically with a mutation of the MIG1 repressor protein to relieve glucose repression. We have cloned the *SSN3* and *SSN8* genes. *SSN3* encodes a cyclin-dependent protein kinase (cdk) homolog and is identical to *UME5*. *SSN8* encodes a cyclin homolog 35% identical to human cyclin C. SSN3 and SSN8 fusion proteins interact in the two-hybrid system and coimmunoprecipitate from yeast cell extracts. Using an immune complex assay, we detected protein kinase activity that depends on both SSN3 and SSN8. Thus, the two SSN proteins are likely to function as a cdk–cyclin pair. Genetic analysis indicates that the SSN3–SSN8 complex contributes to transcriptional repression of diversely regulated genes and also affects induction of the *GAL1* promoter.**

In the budding yeast *Saccharomyces cerevisiae*, expression of glucose-repressed genes in response to glucose starvation requires the SNF1 protein-serine/threonine kinase (1). The SNF1 kinase is widely conserved, and its mammalian counterpart, AMP-activated protein kinase, is involved in regulation of lipid metabolism and cellular stress responses (2). In yeast, genetic evidence indicates that one of the functions of SNF1 is to relieve transcriptional repression mediated by the SSN6–TUP1 repressor complex. This complex is tethered to glucose-repressed promoters, including that of *SUC2* (encoding invertase), by the DNA-binding protein MIG1 (3–5). Mutations in *SSN6* or *TUP1* completely relieve glucose repression of *SUC2* and bypass the requirement for the SNF1 kinase. A *mig1* mutation partially relieves repression, suggesting that other DNA-binding proteins are also involved (6).

Mutations in the *SSN3* and *SSN8* genes were isolated as suppressors of the *snf1* mutant defect in *SUC2* derepression (7). This selection also yielded mutations in six other *SSN* genes, including *MIG1* (= *SSN1*) and *SSN6*. The *ssn3* and *ssn8* mutations are weak suppressors alone but show strong synergy with *mig1*. Moreover, in strains wild type for *SNF1*, both *ssn3* and *ssn8* act synergistically with *mig1* to relieve glucose repression of *SUC2* (6). These findings implicate *SSN3* and *SSN8* in negative regulation of *SUC2* expression. Both mutations also cause flocculence.

To explore the regulatory roles of *SSN3* and *SSN8*, we have cloned the genes by complementation. Sequence analysis showed homology to the cyclin-dependent protein kinase (cdk) family and cyclin C, respectively. Many protein kinases involved in cell cycle control are composed of a catalytic subunit, the cdk, and an activating/targeting subunit, the cyclin. We present genetic and biochemical evidence that SSN3 and SSN8 constitute a cdk–cyclin pair. Genetic evidence suggests a general role for SSN3–SSN8 in transcriptional control.*

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MATERIALS AND METHODS

Strains and Genetic Methods. *S. cerevisiae* strains were derivatives of S288C except for CTY10-5d (gift of R. Sternglanz, State University of New York, Stony Brook). CMY604 [*MAT***a** *cln1::TRP1 cln2::HIS3 Δcln3 leu2 ade1 ura3 trp1 his3* (YCp50-CLN3)] was a gift of C. Mann (Centre d'Etudes Nucleaires, Saclay, France). Genetic methods were as described (8). Gene disruptions were confirmed by Southern blot. *Escherichia coli* XL1-Blue was the plasmid host.

Cloning of *SSN3* and *SSN8*. Strains MCY3309 (*MATα ssn3-1 mig1Δ2::LEU2 snf1-Δ3* or *-28 ura3-52 his4-539 ade2-101*) and MCY3322 (*MAT***a** *ssn8-1 mig1Δ2::LEU2 snf1-Δ3* or *-28 ura3-52 his4-539*) were transformed with a genomic library in the centromere vector YCp50 (9). Nonflocculent transformants were selected by differential sedimentation (10) and then tested for restoration of flocculence after growth on 5-fluoroorotic acid, which selects for plasmid loss. Plasmid DNAs were isolated by passage through bacteria and reintroduced into the corresponding yeast mutant. Complementation of the glucose repression defect was then tested by assaying invertase activity.

Plasmids. pPY18 and pPY21 are in YCp50. pPY23a is in YCp50-XR, which lacks *Xho* I and *Eco*R I sites. *SSN3* sequence was amplified from pPY24 by PCR using primers 5′-CGGGATCCTAATGTATAATGGCAAGGATAGAGC-3′ and 5′-GACGGATCCTGAATGTTGCAGACTTGC-3′ (*Bam*HI sites and ATG underlined) and Vent DNA polymerase (New England Biolabs). The cloned *Bam*HI PCR fragment was expressed from the *ADH1* promoter and complements *ssn3*. This fragment was used to construct pPY30, pSK39, and pSK40, which are derivatives of pRS315 (11), pSH2-1 (12), and pACTII (13), respectively. pACTII expresses a hemagglutinin (HA) epitope tag 3′ to the GAL4 activation domain (GAD).

pSK8 contains the *Xho* I/*Cla* I fragment in YCp50-XR. pSK10 is derived from pSK5. pSK11-1 contains an *Sph* I fragment in YCp50-XR. pSK12-2, pSK23, and pSK46 are in pRS316 (11). *SSN8* sequence was amplified from pSK12-2 by PCR by using primers 5′-CGGGATCCTAATGTCGGGGAGCTTCTGGAC-3′ and 5′-TAATACGACTCACTATAGGGAGA-3′ (vector sequence), and the cloned *Bam*HI PCR fragment was used in the following constructions. pSK31 is a centromeric plasmid that expresses SSN8 from the *ADH1* promoter and complements *ssn8*. pSK32, pSK36, and pSK41 are derived from pSH2-1, pACTII, and pACTII deleted for GAD, respectively.

pGAD-HcycC_{21-303} (pSK38) contains the 3.0-kb *Nco* I/*Bam*HI fragment from a human cyclin C clone [provided by S. Reed (14)] in pACTII.

Sequence Analysis. DNA sequence was determined (15) on both strands with a Sequenase kit (United States Biochemical)

Abbreviations: cdk, cyclin-dependent protein kinase; HA, hemagglutinin; GAD, GAL4 activation domain.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. U20635).

and subcloned fragments. Data base searches were performed using BLASTN and BLASTP (16).

Enzyme Assays. Cultures were grown to midlogarithmic phase in synthetic complete (SC) medium (8). β-Galactosidase activity was assayed in permeabilized cells and expressed in Miller units (17). Invertase activity was assayed in whole cells (6).

Immunoprecipitation and Kinase Assays. Procedures were as described (18), except Immobilized rProtein A (RepliGen) was used. For immunoblot analysis, antibodies were detected by enhanced chemiluminescence with ECL reagents (Amersham). Primary antibodies were anti-LexA (gift of C. Denis, University of New Hampshire, Durham), anti-SNF1 (1), and monoclonal HA antibody (Boehringer Mannheim).

RESULTS

***SSN3* Encodes a cdk Homolog.** The *SSN3* gene was cloned by complementation of the flocculence and glucose repression defects of an *ssn3-1 mig1 snf1* triple mutant. Two plasmids with overlapping inserts were identified (pPY24 and pPY25; Fig. 1*A*), and subcloning experiments showed that the complementing gene crosses the *Sph* I site. DNA sequence analysis of 0.7 kb near the *Sph* I site revealed identity to the *UME5* gene, which has a role in repressing meiotic gene expression in vegetative cells (19, 20). *UME5* encodes a 63-kDa protein with homology to members of the cdk family—for example, 38% identity to CDC28 of *S. cerevisiae* (20).

To confirm that *SSN3* and *UME5* are the same, we replaced codons 19–434 with *HIS3* (Fig. 1*A*) and disrupted the chromosomal locus in a haploid strain [the gene is not essential for viability (20)]. Three His^+, flocculent disruptants were crossed to an *ssn3-1* mutant, and the resulting diploids were flocculent, indicating that the disruption and *ssn3-1* do not complement. Tetrad analysis of the three diploids showed $4^+:0^-$ segregations for the flocculent phenotype in all 21 tetrads examined,

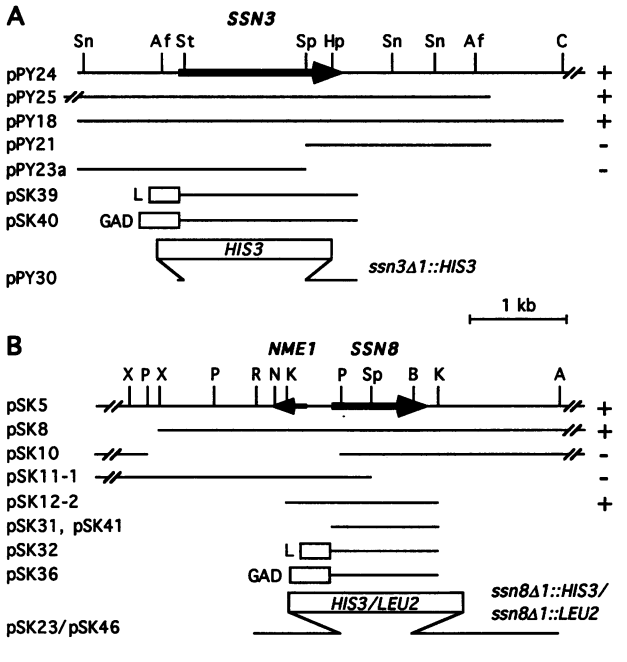

FIG. 1. Restriction maps of *SSN3* and *SSN8* plasmids. Arrows designate coding regions. Ability of plasmids to complement *ssn3-1* or *ssn8-1* is indicated. L, $LexA_{1-87}$. (*A*) *SSN3* plasmids. Broken lines represent 3 kb in pPY24 and 5 kb in pPY25. (*B*) *SSN8* plasmids. Broken lines on the right represent 2 kb and on the left represent 4 kb; those in pSK11-1 represent 2.4 kb. A, *Apa* I; Af, *Afl* II; B, *Bgl* II; C, *Cla* I; Hp, *Hpa* I; K, *Kpn* I; N, *Nco* I; P, *Pvu* II; R, *Eco*RI; Sn, *Sna*BI; Sp, *Sph* I; St, *Stu* I; X, *Xho* I.

confirming that the two mutations are tightly linked. These data show the identity of the cloned cdk gene with *SSN3*.

***SSN8* Encodes a Cyclin C Homolog.** The *SSN8* gene was similarly cloned by complementation of the flocculence and glucose repression defects of an *ssn8-1 mig1 snf1* mutant. The cloned DNA (pSK5; Fig. 1*B*) was shown to be tightly linked to the chromosomal *SSN8* locus, and subcloning experiments located the complementing gene on the 1.6-kb *Kpn* I fragment. DNA sequence analysis of this fragment identified an open reading frame of 323 codons encoding a M_r 37,766 protein, located near the *NME1* gene (21) on chromosome XIV. The predicted SSN8 protein is homologous to cyclin C from human and *Drosophila* (35% and 39% identity, respectively; Fig. 2) (14, 22). The regions of maximal similarity include the cyclin box [residues 74–185 (23)]. SSN8 is more distantly related to CCL1 of *S. cerevisiae* (24), mcs2 of *Schizosaccharomyces pombe* (25), and human cyclin H (26, 27). The role of cyclin C in cell cycle control is not clear (14, 22).

To construct null mutations, we replaced codons 35–281 of *SSN8* with *HIS3* or *LEU2* (Fig. 1*B*). The *ssn8Δ1::HIS3* mutation was used to disrupt the chromosomal locus in a wild-type diploid, and tetrad analysis of two heterozygous transformants showed that *SSN8* is not essential for viability. To confirm the identity of the cloned gene, a mutant segregant was crossed to an *ssn8-1* strain. The resulting diploid was flocculent, as were all segregants from 27 tetrads. Thus, the cloned cyclin homolog is indeed *SSN8*.

SSN3 and SSN8 Interact in the Two-Hybrid System. Previous genetic evidence suggests that *SSN3* and *SSN8* are functionally related (6). To test for interaction between the two proteins *in vivo*, we used the two-hybrid system (28). We expressed hybrid proteins containing the LexA DNA-binding domain ($LexA_{1-87}$) fused to SSN3 and the GAD fused to SSN8 (Fig. 1). Interaction between the two fusion proteins was monitored by activation of β-galactosidase expression from a *lexAop–GAL1–lacZ* target gene. In combination, LexA–SSN3 and GAD–SSN8 stimulated β-galactosidase expression >100-fold (Table 1). Interaction was detected in cells grown in either glucose-repressing or derepressing conditions. A LexA–SSN8 fusion was also expressed, but it alone activated transcription of the target gene (data not shown). Consistent with this result, the interaction of LexA–SSN3 with SSN8 (not fused to GAD) also activated transcription (Table 1).

SSN3 and SSN8 Fusion Proteins Coimmunoprecipitate. To provide biochemical evidence that the two SSN proteins interact, we carried out coimmunoprecipitation experiments (Fig. 3). Extracts were prepared from cells expressing either LexA–SSN3 and GAD–SSN8 or LexA–SSN8 and GAD–SSN3. The GAD fusion proteins carry a HA epitope tag. The LexA proteins were immunoprecipitated with LexA antibody, and the precipitates were analyzed by Western blotting for the presence of the GAD fusion protein using monoclonal HA antibody. In each case, the SSN3 and SSN8 fusion proteins coimmunoprecipitated. Control experiments with LexA–SNF1 and GAD–SNF1 fusions ruled out artifactual interactions involving the LexA or GAD moieties. These results, together with the genetic evidence, suggest that SSN3 and SSN8 are partners in a cdk–cyclin pair.

Kinase Activity in an Immune Complex Assay Depends on SSN3 and SSN8. To assay for protein kinase activity dependent on SSN3 and SSN8, we used an immune complex kinase assay. Cell extracts were prepared from wild type expressing LexA–SSN3 or $LexA_{1-87}$ and an *ssn8Δ* mutant expressing LexA–SSN3. The LexA proteins were immunoprecipitated with anti-LexA antibody, and the precipitates were incubated with [γ-^{32}P]ATP in kinase buffer. Phosphorylated products were separated by SDS/PAGE and detected by autoradiography (Fig. 4*A*). Several products, including a major phosphoprotein of ≈220 kDa, were present in assays of wild-type extracts containing LexA–SSN3 but not in control assay mix-

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SSN8                                MSGSFWTSTQ RHHWQYTKAS LAKERQKLWL   30
HCYCC  MVAPRPLRRV VLFYQGKLCS MAGNFWQSSH YLQWILDKQD LLKERQKDLK   50
DCYCC                        MAGNFWQSSH SQQWILDKPD LLRERQHDLL   30

SSN8   LECQLFPQGL NIVMDSKQNG IEQSITKNIP ITHRDLHYDK DYNLRIYCYF   80
HCYCC  F.......... .......... .......... .....LSEEE YWKLQIFFTN   66
DCYCC  A.......... .......... .......... .....LNEDE YQKVFIFFAN   46

SSN8   LIMKLGRRLN IRQYALATAH IYLSRFLIKA SVREINLYML VTTCVYLACK  130
HCYCC  VIQALGEHLK LRQQVIATAT VYFKRFYARY SLKSIDPVLM APTCVFLASK  116
DCYCC  VIQVLGEQLK LRQQVIATAT VYFKRFYARN SLKNIDPLLL APTCILLASK   96

SSN8   VEECPQYIRT .LVSEARTL. ........WPE FIPPDPTKVT EFEFYLLEEL  171
HCYCC  VEEFGVVSNT RLIAAATSVL KTRFSYAFPK EFPYRMNHIL ECEFYLLELM  166
DCYCC  VEEFGVISNS RLISICQSAI KTKFSYAYAQ EFPYRTNHIL ECEFYLLENL  146

SSN8   ESYLIVHHPY QSLKQIVQVL KQPPFQITLS SDDLQNCWSL INDSYINDVH  221
HCYCC  DCCLIVYHPY RPLLQYVQDM GQEDMLLPLA .......WRI VNDTYRTDLC  209
DCYCC  DCCLIVYQPY RPLLQLVQDM GQEDQLLTLS .......WRI VNDSLRTDVC  189

SSN8   LLYPPHIIAV ACLFITISIH GKPTKGSSLA SAASEAIRDP KNSSSPVQIA  271
HCYCC  LLYPPFMIAL ACLHVACVVQ QKD.......  ..........  .........A  233
DCYCC  LLYPPYQIAI ACLQIACVIL QKD.......  ..........  .........A  213

SSN8   FNRFMAESLV DLEEVMDTIQ EQITLYDHWD KYHEQWIKFL LHTLYLRPAS  321
HCYCC  .RQWFAELSV DMEKILEIIR VILKLYEQWK NFDERKEMAT ILSKMPKPKP  282
DCYCC  TKQWFAELNV DLDKVQEIVR AIVNLYELWK DWKEKDEIQM LLSKIPKPKP  263

SSN8   AI                                                     323
HCYCC  PPNSEGEQGP NGSQNSSYSQ S                                 303
DCYCC  PPQR                                                    267
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FIG. 2. Sequence comparison of SSN8 and cyclin C. Predicted amino acid sequences of SSN8 and cyclin C from human (HCYCC) and *Drosophila* (DCYCC) were aligned using the GCG sequence analysis software package (University of Wisconsin Genetics Computer Group). Identical residues are shown in reverse contrast.

tures containing $LexA_{1-87}$. Moreover, the 220-kDa phosphorylated product was not detected in the *ssn8Δ* mutant assay, although more LexA–SSN3 was precipitated from the mutant than from wild type (Fig. 4*B*). Thus, phosphorylation of the 220-kDa protein in the immune complex assay clearly depends on both SSN3 and SSN8. It is possible that the SSN3–SSN8 complex does not directly phosphorylate this protein; for example, SSN3–SSN8 could activate another kinase in the immune complex. We also cannot exclude that the 220-kDa protein is specifically associated with SSN3–SSN8 but is phosphorylated by an unrelated kinase present in the immunoprecipitate.

***SSN3* and *SSN8* Function Together in Transcriptional Control of Diverse Genes.** To extend previous genetic evidence regarding *SSN3* and *SSN8* function, we examined the phenotypes of the null mutants. Both *ssn3Δ* and *ssn8Δ* weakly suppressed the *snf1* growth defects on sucrose and raffinose (substrates of invertase). The null mutations partially relieved glucose repression of *SUC2* in a wild-type *SNF1* background (Fig. 5*A*) and showed synergy with *mig1* (Table 2). Repression of a *SUC2–LEU2–lacZ* fusion, under control of the *SUC2* regulatory region and the *LEU2* promoter, was also relieved, indicating that the defect is primarily at the transcriptional level (Fig. 5*B*). In limiting glucose, both mutants derepressed

Table 1. SSN3 interacts with SSN8 and human cyclin C in the two-hybrid system

DNA-binding protein	Activating protein	β-Gal activity, units	
		Glucose	Raffinose
$LexA_{1-87}$	GAD	<1	<1
$LexA_{1-87}$	GAD–SSN8	<1	<1
LexA–SSN3	GAD	1.5	3.3
LexA–SSN3	GAD–SSN8	190	250
LexA–SSN3	SSN8	70	110
LexA–SSN3	GAD–$HcycC_{21-303}$	85	52
$LexA_{1-87}$	GAD–$HcycC_{21-303}$	<1	<1

Transformants of CTY10-5d (*MATa gal4 gal80 URA3::lexAop–lacZ ade2 his3 leu2 trp1*) were grown in selective SC/2% glucose or SC/2% raffinose (derepressed). Values are averages for three transformants; standard errors were <13%. SSN8 was expressed from pSK41. β-Gal, β-galactosidase; HcycC, human cyclin C.

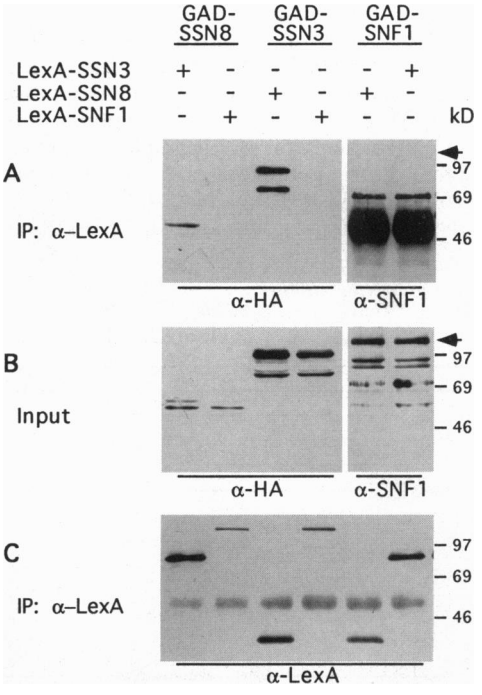

FIG. 3. Coimmunoprecipitation of SSN3 and SSN8 fusion proteins. Protein extracts (250 μg) were prepared from glucose-grown wild-type strains expressing the indicated proteins. LexA fusions were immunoprecipitated (IP) with anti(α)-LexA antibody. (*A*) Precipitated proteins were separated by SDS/PAGE and immunoblotted with monoclonal α-HA or rabbit polyclonal α-SNF1 antibody, as indicated. GAD–SSN3 and GAD–SSN8 contain HA epitope tags. The smaller GAD–SSN3 species is presumably a degradation product. Arrow marks position of GAD–SNF1; an unidentified 69-kDa protein is detected with this α-SNF1 antiserum. (*B*) Similar analysis of the input protein (25 μg). α-SNF1 antiserum detects GAD–SNF1 (arrow), a degradation product, native SNF1, and unidentified 69- and 55-kDa proteins. (*C*) Immunoblot shown in *A* was reprobed with α-LexA antibody to confirm precipitation of LexA fusions. In another experiment, LexA–SNF1 was more highly expressed. LexA–SNF1 and GAD–SNF1 were expressed from pRJ55 (R. Jiang and M.C., unpublished data) and pSG1 (gift of Z. Xue and T. Melese, Columbia University, New York, NY).

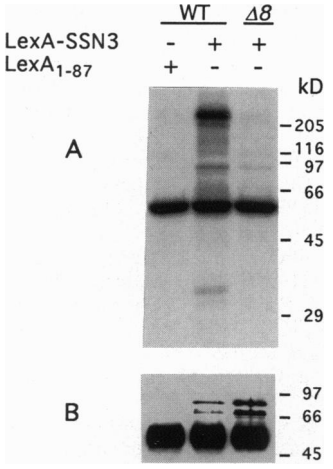

FIG. 4. Immune complex kinase assays. Protein extracts (250 μg) were prepared from wild-type (WT) and *ssn8*Δ strains expressing $LexA_{1-87}$ or LexA–SSN3. LexA proteins were immunoprecipitated with anti-LexA antibody. (*A*) Precipitated proteins (4/5 of the sample) were incubated with [γ-^{32}P]ATP in kinase buffer, separated by SDS/PAGE, and subjected to autoradiography. (*B*) Remainder of precipitate was analyzed by immunoblotting with anti-LexA antibody.

SUC2 and *SUC2–LEU2–lacZ* expression almost as well as wild type (within 2-fold; data not shown).

We noted that both mutants grew slowly on galactose. Consistent with this growth defect, they failed to induce fully the expression of a *GAL1–lacZ* reporter, and β-galactosidase activity was 5-fold lower than in wild type (Fig. 5*C*). Glucose repression of this reporter was also slightly relieved, and during growth on galactose plus glucose, β-galactosidase activity was 5-fold higher in mutants (1.6 units for *ssn3*Δ; 2.1 units for *ssn3*Δ *ssn8*Δ) than wild type (0.33 unit).

An *ssn3* (*ume5*) mutant was previously shown to express early meiotic genes in the absence of appropriate signals for starvation and cell type (*MAT***a**/*MAT*α) (19). The presence of the *SPO13–lacZ* promoter fusion on plasmid p(spo13)28 caused blue colony color. To test the *ssn8*Δ mutant for this phenotype, we introduced p(spo13)28. Under mitotic growth conditions β-galactosidase was expressed in the haploid mutant but not in wild type.

We also examined regulation of *PHO5* (acid phosphatase) gene expression. In high phosphate medium, both *ssn3*Δ and *ssn8*Δ mutants showed 2.5- to 3.0-fold higher acid phosphatase activity (31) than wild type (6.7 units for wild type, 18 units for *ssn3*Δ, and 19 units for *ssn8*Δ; units are nmol of *p*-nitrophenol released per min per A_{600} of cells, and values are averages for three segregants). These findings suggest that the *ssn* mutations allow a partial release from phosphate repression.

Table 2. Cyclin C does not complement *ssn8* for synergy with *mig1*

Expressed protein	Invertase activity, units	
	*mig1*Δ	*mig1*Δ *ssn8*Δ
None	6.0	95
$HcycC_{1-303}$	5.5	100
$HcycC_{21-303}$	4.5	73

Transformants of *mig1*Δ and *mig1*Δ *ssn8*Δ strains were grown in selective SC/2% glucose. Multicopy plasmids expressed full-length or truncated human cyclin C (HcycC; residues indicated) (14). Vector pAB23BX expressed no protein (None). Values are averages for three transformants; standard errors were <20%.

If *SSN3* and *SSN8* function together, mutations in the two genes should cause no additional phenotype when combined. We therefore constructed *ssn3*Δ *ssn8*Δ strains by genetic crossing. The double mutants grew as well as the single mutants on glucose and showed no exacerbation of the defects in growth on galactose or *GAL1–lacZ* expression (Fig. 5*C*). Furthermore, the two *ssn* mutations showed no synergy in relieving repression of *SUC2* (Fig. 5 *A* and *B*) or acid phosphatase (18 units for *ssn3*Δ *ssn8*Δ).

These genetic data support the biochemical evidence that the *SSN3* and *SSN8* gene products function together and suggest a role in transcriptional control. The mutations affect transcriptional repression of a spectrum of diversely regulated genes and also prevent full induction of *GAL1–lacZ* transcription.

Functional Comparison of SSN8 and Human Cyclin C. Human and *Drosophila* cyclins C were cloned by complementation of the G1 cyclin deficiency of a yeast *cln1 cln2 cln3* mutant (14, 22). We therefore tested whether overexpression of SSN8 could likewise rescue this triple mutant. The mutant CMY604, carrying a *CLN3-URA3* plasmid, was transformed with pSK31 or pSK41, which are *LEU2*-marked plasmids expressing *SSN8* from the strong *ADH1* promoter. The transformants remained dependent on the *CLN3-URA3* plasmid, as judged by their failure to grow on 5-fluoroorotic acid, suggesting that overexpression of SSN8 was not sufficient to provide G1 cyclin function.

To test the ability of human cyclin C to provide SSN8 function, we used two plasmids expressing full-length human cyclin C cDNA or a truncated version that complements the yeast triple *cln* mutant (14). Neither plasmid complemented *ssn8*Δ for growth on galactose, flocculence, or synergy with *mig1* in relieving glucose repression (Table 2).

Although SSN8 and human cyclin C do not appear to be functionally interchangeable, human cyclin C nonetheless interacted effectively with SSN3 in the two-hybrid system (Table 1). Thus, sequences responsible for interaction with SSN3 have been conserved from yeast to human.

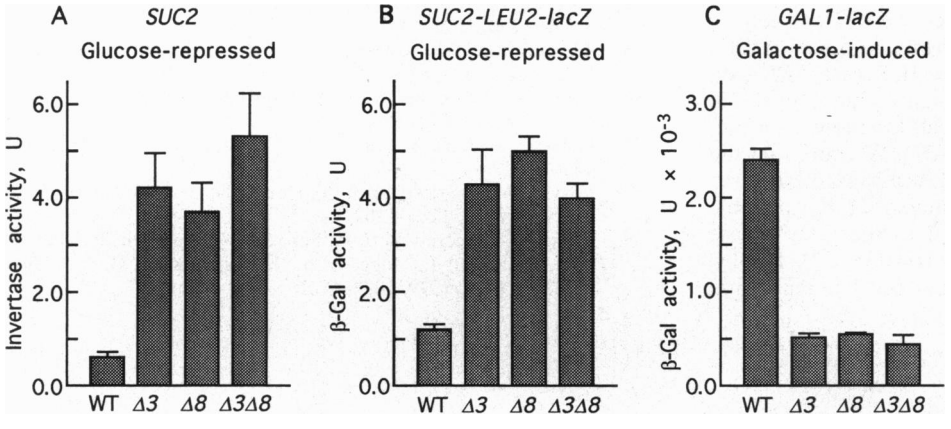

FIG. 5. Effect of *ssn3* and *ssn8* mutations on regulation of gene expression. Relevant genotypes are indicated. WT, wild type. (*A*) Invertase activity in glucose-repressed cultures (grown in 2% glucose). Values are averages for three segregants. Wild-type derepressed activity was 400 units. (*B*) β-Galactosidase activity in glucose-repressed strains carrying integrated *SUC2–LEU2–lacZ* plasmid pLS11 (29). Values are averages for three to six transformants. Wild-type derepressed activity was 37 units. (*C*) β-Galactosidase activity in strains carrying integrated *GAL1–lacZ* reporter pRY171 (30). Cultures were grown in 2% galactose. Values are averages for two to four transformants.

DISCUSSION

We report here the identification of a pair of cdk–cyclin homologs in *S. cerevisiae*. We have cloned two genes that were originally identified by mutations that suppress a defect in the SNF1 protein kinase. *SSN3* encodes a cdk homolog, and *SSN8* encodes a cyclin homolog related to cyclin C. Both biochemical and genetic evidence indicates that SSN3 and SSN8 function together. First, SSN3 and SSN8 fusion proteins interact strongly in the two-hybrid system. Second, the two fusion proteins coimmunoprecipitate from yeast cell extracts. Third, protein kinase activity that depends on SSN3 and SSN8 was detected in an immune complex assay. Finally, *ssn3* and *ssn8* mutations cause the same array of phenotypes and show no additive effects when combined in a double mutant. Taken together, these findings strongly suggest that SSN3 and SSN8 function as a cdk–cyclin complex.

Genetic evidence implicates the SSN3–SSN8 complex in transcriptional control of diversely regulated genes. The null mutations cause partial defects in glucose repression of *SUC2* transcription. Moreover, mutations in both genes synergize strongly with *mig1* in relieving repression: glucose-grown *ssn mig1* double mutants show >10-fold higher *SUC2* expression than *mig1* mutants (ref. 6; Table 2). The effects of *ssn3* and *ssn8* are not limited to glucose repression, as these mutations also partially relieve repression of the meiotic gene reporter *SPO13–lacZ* in vegetative haploid cells (19) and phosphate repression of acid phosphatase. In addition, *ssn3* mutations (called *are1*) have been shown to affect α2 repression of a-specific genes (M. Wahi and A. Johnson, personal communication). Thus, SSN3 and SSN8 are required for complete repression of a spectrum of genes. Our analysis of *lacZ* fusions strongly suggests that the primary effect is on transcription.

The SSN3–SSN8 complex also appears to affect transcription in a positive manner. In exploring the basis for the growth defect on galactose, we found that expression of β-galactosidase activity from *GAL1–lacZ* was 5-fold reduced in *ssn3* and *ssn8* mutants. Thus, the SSN3–SSN8 activity is required for full induction of the *GAL1* promoter.

Cyclins and cdks typically function in cell cycle control. We have no evidence that SSN3–SSN8 affects the cell cycle. However, it is possible that the kinase has a role in coordinating regulation of transcription and cell cycle progression in response to nutrient availability, as has been proposed for PHO85 (32–34).

What is the relationship of SSN3–SSN8 to the SNF1 protein kinase? These *ssn* mutations alone are weak suppressors of *snf1*, but they synergize with *mig1* to give effective suppression of the invertase defect in *snf1* mutants (6). Further experiments will be required to determine whether this suppression reflects a direct regulatory interaction between SSN3–SSN8 and the SNF1 kinase.

Recent work from Young and colleagues (S.-M. Liao, J. Zhang, and R. A. Young, personal communication) showed that *SSN3* and *SSN8* are identical to *SRB10* and *SRB11*, respectively. Mutations in these genes were identified as suppressors of a truncation in the C-terminal domain (CTD) of the largest subunit of RNA polymerase II. Several *SRB* gene products have been shown to be components of a multiprotein complex associated with the CTD, which functions as a mediator of transcriptional activation (35–37). We note that the major protein phosphorylated in our kinase assay is close to the size of the largest subunit of RNA polymerase II. It is possible that SSN3–SSN8 is functionally related to the CTD kinase associated with transcription factor TFIIH (38, 39). Finally, our genetic evidence for a role of SSN3 and SSN8 in repression may implicate the CTD-associated complex in transcriptional repression as well as activation.

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