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SNC1, a yeast homolog of the synaptic vesicle-associated membrane protein/synaptobrevin gene family: Genetic interactions with the *RAS* and *CAP* genes

(signal transduction/oncogenes/vesicular trafficking/cAMP)

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ABSTRACT **_SNC1_, a gene from the yeast *Saccharomyces cerevisiae*, encodes a homolog of vertebrate synaptic vesicle-associated membrane proteins (VAMPs) or synaptobrevins. _SNC1_ was isolated by its ability to suppress the loss of CAP function in *S. cerevisiae* strains possessing an activated allele of _RAS2_. CAP is a component of the RAS-responsive *S. cerevisiae* adenylyl cyclase complex. The N-terminal domain of CAP is required for full cellular responsiveness to activated RAS proteins. The C-terminal domain of CAP is required for normal cellular morphology and responsiveness to nutrient extremes. Multicopy plasmids expressing _SNC1_ suppress only the loss of the C-terminal functions of CAP and only in the presence of activated RAS2.**

The yeast *Saccharomyces cerevisiae* contains two *RAS* genes that encode proteins highly homologous to mammalian *RAS* oncogene products (1, 2). These RAS proteins are required to activate *S. cerevisiae* adenylyl cyclase (3, 4) but may have other functions as well (5). The functions of RAS in higher organisms are not known. When expressed in *S. cerevisiae*, mammalian RAS proteins are capable of both activating adenylyl cyclase and suppressing the lethality associated with the loss of endogenous RAS function (3, 5, 6). Thus, some functions of RAS may have been conserved during the course of evolution. To explore this we have begun to characterize the *S. cerevisiae* adenylyl cyclase complex.

We previously identified a protein called CAP that copurifies with a RAS-responsive adenylyl cyclase complex (7). The gene for CAP encodes a 526-residue protein that is required for full cellular responsiveness to activated *RAS* and for normal cellular morphology and responsiveness to nutrient extremes (8, 9). Deletion analysis has shown that CAP is bifunctional (10). Expression of a domain consisting of the N-terminal 168 amino acids is sufficient for full cellular responsiveness to activated RAS, while expression of the C-terminal 160 amino acids is sufficient for normal cellular responses to nutrient extremes (10). At present, it is unclear whether RAS or adenylyl cyclase influences CAP function.

To understand the function of CAP, we have isolated genes that on multicopy plasmids are capable of suppressing loss of C-terminal function. One such gene, *PFY*, encodes profilin, an actin binding protein (11). Another gene, which we have named *SNC1* (suppressor of the null allele of CAP), is described here. It encodes a protein homologous to low molecular weight proteins known as VAMPs (synaptic vesicle-associated membrane proteins) (12, 13) or synaptobrevins (14–16) that are associated with synaptic vesicles and are found in a wide variety of organisms.§

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MATERIALS AND METHODS

Microbial Culture. Yeast strains were grown in rich medium (YPD; yeast extract/Bactopeptone/dextrose), synthetic complete minimal medium (SC), or SC drop-out minimal medium lacking an essential amino acid or nucleotide base. Drop-out minimal medium was used to maintain selection of plasmids. Yeast extract, Bactopeptone, and yeast nitrogen base lacking ammonium sulfate and amino acids (YNB) were purchased from Difco. YPD was prepared according to Sherman *et al.* (17). SC minimal complete and drop-out media were prepared as described by Sherman *et al.* (17) and consisted of 0.7% YNB supplemented with the appropriate auxotrophic requirements and 2% glucose. Yeast medium lacking in amino acids and a nitrogen source (YNB-N) was prepared according to Toda *et al.* (4). Standard methods were used to introduce plasmids into the various yeast strains (17). *Escherichia coli* strains HB101 and DH5α were used for plasmid transformations and plasmid DNA preparations.

Yeast Strains. *cap* yeast strains SKN32 (*Mata leu2 ura3 trp1 ade8 can1 cap::HIS3*) and SKN37 (*Mata leu2 ura3 trp1 ade8 can1 RAS2^{val19} cap::HIS3*) have been described (9). This *cap::HIS3* allele lacks amino acids 78–451 of the coding region of CAP and is a null allele. *cap* strain SKO13 (*Mata leu2 ura3 trp1 ade8 can1 cap::HIS3*) has also been described (9). *cap* strain SKN50 (*Mata leu2 trp1 ade8 can1 ira1::HIS3 cap::URA3*) was created by transforming the *ira1* strain IR-1 (18) with the *Eco*RI fragment of the *CAP* disruption plasmid pUSMN2 as described (9). *cap* strains SKN55 and SKN56 (*Mata ade8 can1 bcy1::LEU2 tpk2::HIS3 tpk3::TRP1 cap::URA3*) were created by transforming the *bcy1 tpk2 tpk3* strain S13-58A (19) with the *Eco*RI fragment of pUSMN2 *CAP* disruption plasmid. *cap* strains SKN58 and SKN59 (*Mata ade8 trp1 can1 pde1::LEU2 pde2::URA3 cap::HIS3*) were created by transforming the *pde1 pde2* strain DJ23-3C (20) with the *Eco*RI fragment of the *CAP* disruption plasmid pHSPN5 as described (9). *HIS3*$^+$ transformants were isolated and the disruption of *CAP* was verified by phenotypic and Southern blot analysis. The *snc1* strain JG4 (*Mata leu2 trp1 ade8 his3 can1 snc1::URA3*) was constructed by transforming the haploid SP1 yeast strain (*Mata leu2 ura3 trp1 ade8 his3 can1*) (4) with the *Sal* I/*Sac* I fragment of the *SNC1* disruption plasmid pORF3U (see below). Integration of this construct at the *SNC1* locus results in an insertion in *SNC1*. The *snc1* strain JG5 (*Mata leu2 trp1 ade8 his3 can1 snc1*Δ::URA3) was created by transforming the haploid SP1 yeast strain with the *Sal* I/*Sac* I fragment of the *SNC1* disruption plasmid pNCSU (see below). Integration of this

Abbreviations: VAMP, synaptic vesicle-associated membrane protein; ORF, open reading frame.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M91157).

construct at the *SNC1* locus results in deletion of the *SNC1* gene. Diploid yeast, formed by mating DC124 (*Matα leu2 ura3 trp1 ade8 his4*) (19) and SP1, were also transformed with the *Sal* I/*Sac* I fragment of pORF3U. Ura$^+$ transformants were sporulated and subjected to tetrad analysis (17). Distribution of the *URA3* marker in haploid strains derived from tetrad analysis was found to be 2:2. In all the cases described above, genotypes were verified by Southern blot analysis.

DNA Manipulations. DNA restriction endonucleases, *Taq* polymerase, and T4 DNA ligase were used as recommended by the suppliers (New England BioLabs and Cetus). Molecular cloning, Southern blotting, and colony hybridization techniques were performed as described by Maniatis *et al.* (21). DNA sequencing was performed by the dideoxynucleotide chain-termination method (22). The polymerase chain reaction (PCR) (23) and subcloning of PCR products were carried out as described (10). Oligonucleotides used to amplify *SNC1* included a forward oligonucleotide bearing a *Sal* I site (5′-AACGTATTCGTCGACCATGTCGTC-3′) and a reverse oligonucleotide bearing a *Sac* I site (5′-CTACATATGGGAGCTCCCTAT-3′). Total RNA was isolated from wild-type yeast (SP1) according to Sherman *et al.* (17). Isolation of poly(A)$^+$ RNA was accomplished with a kit from Stratagene. First-strand cDNA synthesis from yeast poly(A)$^+$ RNA, which was used as a template for PCR, was accomplished with a cDNA synthesis kit from Bethesda Research Laboratories.

Genomic DNA from a YPD$^+$ revertant of the SKO13 *cap* mutant strain (9) was isolated according to Sherman *et al.* (17). The DNA was partially digested with the *Sau*3A restriction endonuclease and size-fractionated by gel electrophoresis. A library was constructed in the yeast expression plasmid YEp13M4 (20) from size-selected DNA cloned into the *Bam*HI site of the vector.

Phenotypic Assays and Selections. *cap* strain SKN37 was transformed with the library and grown for 48 hr on selective medium before replica plating onto YPD plates to assay for growth on rich medium. Of 2000 Leu$^+$ transformants, 30 were YPD$^+$, only one of which was found to grow on rich medium in a plasmid-dependent manner. Plasmids were isolated from yeast according to standard methods (17). Candidate plasmids were then retransformed back into the *cap* SKN37 strain and were examined for their ability to confer growth on YPD. Assays for growth on rich medium, temperature-sensitive growth at 37°C, and sensitivity to nitrogen starvation or heat shock (55°C) were performed as described (10).

Plasmids. Plasmids used in this study included the following vectors: YEp13M4, yeast expression plasmid bearing the *LEU2* selectable marker (20); pAD4Δ, a similar plasmid bearing both the *LEU2* selectable marker and the *ADH1* promoter (18); and pUV2, pTV3, and pAV3, YEp-based plasmids bearing the *URA3*, *TRP1*, or *ADE8* selectable markers, respectively. Previously described plasmids included the following: pADH-CAP, which expresses full-length CAP under the control of the *ADH1* promoter (9); pADH-CAPΔ4, which expresses 237 amino acids of the C terminus of CAP (10); pADH-CAPΔ15, which expresses 283 amino acids of the N terminus of CAP (10); and YEpIRA2, which expresses IRA2 (R.-M. Ballester and M.W., unpublished results).

Other plasmids included the following: YEpSNC1, a YEp13M4 plasmid bearing a 3.4-kilobase (kb) *Sau*3A partial digestion fragment of genomic *SNC1*; YEpTSNC1, a pTV3 plasmid bearing this gene as a *Sal* I/*Sac* I fragment; pUCSNC, a pUC118 plasmid bearing this fragment; pADH-SNC1 and pADH-cSNC1, which contain a 550-base-pair (bp) fragment of genomic *SNC1* or a 370-bp fragment of *SNC1* cDNA, cloned into the *Sal* I and *Sac* I sites of pAD4Δ, respectively; pADH-ASNC1, which bears a 2.3-kb *Bam*HI fragment, containing the *ADH1* promoter and the *SNC1* sequence from pADH-SNC1, cloned into the *Bam*HI site of pAV3; and pADH-ACAPΔ4, which bears a 3.0-kb *Bam*HI fragment, containing the *ADH1* promoter and the *CAP*Δ4 allele (10) from pADH-CAPΔ4, cloned into the *Bam*HI site of pAV3. The modified pAV3 plasmids were used in the transformation of *cap* strains SKN55-56 and SKN58-59.

Two plasmids derived from pUCSNC were used for disruptions of *SNC1*: pORF3U, which has the *URA3* selectable marker cloned into the *Sty* I site (base pair 325) of *SNC1*; and pNCSU, which has *URA3* cloned into the *Spe* I sites, which flank *SNC1* (base pairs −237 and +646, respectively). For pNCSU, pUCSNC was digested with *Spe* I, which removes the entire *SNC1* coding region, and the *URA3* gene was cloned into this site. Both disruption constructs were verified by restriction analysis.

RESULTS

A Yeast Gene That Suppresses Loss of the C-Terminal Functions of CAP. We have screened a yeast genomic library for multicopy suppressors of the *cap* null phenotype of the SKN37 strain. This strain is unable to grow on the standard amino acid-rich medium YPD. Expression of the C-terminal domain of CAP is fully able to complement this defect (10). Of 2000 transformants only one was capable of growth on YPD in a plasmid-dependent manner. This transformant contained YEpSNC1, a plasmid that was found to be as potent a suppressor of rich medium growth defects in SKN37 cells as plasmids expressing either full-length CAP or the C-terminal domain of CAP (Fig. 1). SKN37 cells display other defects associated with loss of the C-terminal function of CAP, including large cell size, temperature-sensitive growth, and inability to withstand nitrogen starvation (9). YEpSNC1 suppressed the abnormal cell size (Fig. 2) and the other growth defects as well as did plasmids expressing the C-terminal domain of CAP (data not shown). In contrast, YEpSNC1 did not suppress loss of the N-terminal functions of CAP, the loss of full RAS responsiveness. Wild-type yeast expressing the activated *RAS2*^{val19} allele are sensitive to heat shock (24). SKN37 cells, which contain the activated *RAS* allele, are resistant to heat shock because of the absence of the N-terminal domain of CAP (10). SKN37 cells containing the YEpSNC1 plasmid remain resistant to heat shock (data not shown).

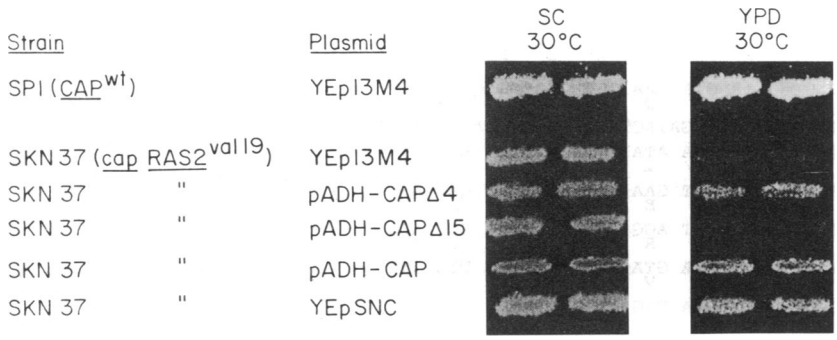

FIG. 1. Effect of *SNC1* on the growth of cap strains on rich medium. *cap* yeast strain SKN37 was transformed with yeast episomal plasmids expressing the C-terminal domain of CAP (pADH-CAPΔ4), the N-terminal domain of CAP (pADH-CAPΔ15), full-length CAP (pADH-CAP), or SNC1 (YEpSNC). Both *CAP*^{wt} (SP1) and *cap* yeast were transformed with an empty vector (YEp13M4) as control. Patches of transformed yeast were grown for 3 days on synthetic minimal medium (SC) before replica plating to rich growth medium (YPD).

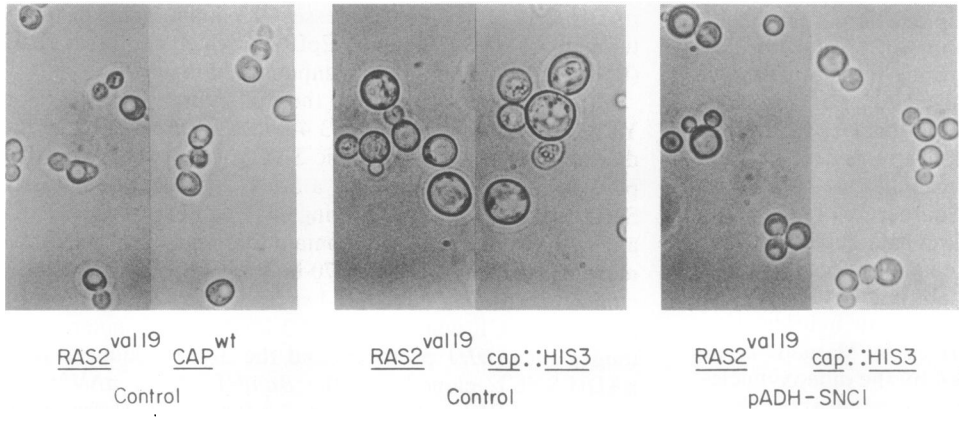

FIG. 2. Effect of *SNC1* on the morphology of *cap* yeast. *cap* yeast strain SKN37 (*RAS2*^{val19} *cap::HIS3*) was transformed with either a plasmid expressing SNC1 (pADH-SNC1) or YEp13M4 as a control. *CAP*^{wt} *RAS2*^{val19} yeast were also transformed with YEp13M4 as a control. Transformed yeast strains were grown in liquid synthetic medium for 2 days before visual inspection. (×1050.)

Sequence of SNC1. The YEpSNC1 plasmid contained an insert of 3.4 kb. Deletion analysis of the plasmid localized the full suppressor activity of YEpSNC1 to a 1-kb fragment. DNA sequencing of this fragment revealed two small open reading frames (ORFs) (Fig. 3). A search for similar DNA sequences revealed a strong homology between these ORFs, when taken together as a single ORF, and members of a class of small, highly conserved proteins [VAMPs (12, 13) or synaptobrevins (14–16)]. These proteins have been identified in a wide variety of organisms (from fish to humans). Their function is unknown, but they are suspected to play a role in vesicle fusion and neurotransmitter release. Two classes of these proteins have been identified—the type 1 and type 2 VAMP/synaptobrevins, which differ slightly in their N-terminal domains.

Alignment between the translated sequence of the ORFs and those of the VAMP/synaptobrevin genes suggested that, together, these ORFs constitute the entire coding region of a yeast homolog. Several lines of evidence confirm this. First, *SNC1* contains sequences that match the consensus sequences for splice donor and acceptor sites in *S. cerevisiae*: the 5′ splice motif (GTANGT) and the 3′ splice motif (YAG, where Y is pyrimidine) (25) are found at the candidate intron–exon junction at base pairs 101–106 and 213–215, respectively. In addition, the TACTAAC box, a consensus motif present in *S. cerevisiae* introns (26), is found at base pairs 146–152. Second, oligonucleotide-directed amplification of *SNC1* from yeast cDNA by PCR generated a product ≈100 bp smaller than that generated when genomic DNA was used as a template (data not shown). DNA sequencing of the PCR product generated from yeast cDNA revealed that base pairs 101–215 were absent. Finally, expression of the PCR-amplified cDNA clone was able to suppress the CAP-deficient phenotypes of SKN37 cells (data not shown). These studies demonstrate not only the existence of an intron but also that we have identified the correct ORF with suppressor activity.

The ATG codon starting at nucleotide 1 is preceded by multiple in-frame stop codons. Given this, we conclude that *SNC1* encodes a protein of 117 amino acids and has a predicted molecular mass of ≈13 kDa. A 115-bp intron sequence connects the region encoding the first 33 amino acids to that encoding the last 84 amino acids.

Homology Between SNC1 and Mammalian VAMPs. The yeast SNC1 protein is highly homologous to both the type 1 and type 2 mammalian VAMP/synaptobrevins (Fig. 4). The amino acid sequence of SNC1 shows ≈40% identity and ≈50% overall homology to either type of protein. The VAMP/synaptobrevin protein has been structurally divided into three distinct regions based on analysis of the secondary structure and tryptic digestion patterns (12–14, 16). The N-terminal 80–90 amino acids constitute a highly charged hydrophilic region that extends into the cytosol (13). The first 20 residues of the N terminus of both the type 1 and type 2 proteins are highly diverged (12, 14). This is the region with the least homology to yeast SNC1. C-terminal to the cytosolic domain is a stretch of 20–25 hydrophobic residues that constitute a transmembrane domain. The last four residues of the C terminus of the protein are thought to constitute an intravesicular domain (12–16). The region of strong homology between SNC1 and the VAMP/synaptobrevins lies downstream of residue 20 and extends throughout the hydrophilic region (residues 21–91) and includes the stretch of ≈20 hydrophobic amino acids. Computer analysis by the method of Klein *et al.* (27) predicts the SNC1 protein to be an integral membrane protein. The membrane-spanning region consists of residues 95–111.

Disruption of the *SNC1* Locus. The chromosomal *SNC1* locus was disrupted by insertion into, or deletion of, the coding region of *SNC1* by transformation (see *Materials and Methods*). Microscopic and phenotypic analysis of confirmed *snc1* strains indicated that the disruption of *SNC1* has no apparent phenotype in yeast (data not shown). *snc1* strains were found to grow and mate in a normal fashion, were morphologically normal, and did not show phenotypes that result from loss of the C-terminal functions of CAP. Haploid *snc1* strains derived from tetrad analysis also had no phenotype. Thus, *SNC1* does not, by itself, provide an essential function.

Genetic Interactions Between *SNC1* and *RAS2*. *SNC1* was isolated as a suppressor of *cap* phenotypes in the yeast SKN37 strain, which contains an activated *RAS2*^{val19} allele. We therefore determined whether SNC1 was also capable of suppressing *cap* defects in the *cap RAS2*^{wt} strains SKN32 and SKN34. In contrast to cells transformed with plasmids expressing either full-length CAP or the C-terminal domain of

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   1 ATG TCG TCA TCT ACT CCC TTT GAC CCT TAT GCT CTA TCC GAG CAC GAT GAA GAA CGA
   1  M   S   S   S   T   P   F   D   P   Y   A   L   S   E   H   D   E   E   R
  58 CCC CAG AAT GTA CAG TCT AAG TCA AGG ACT GCG GAA CTA CAA GCT GTAAGTACAGAAAGC
  20  P   Q   N   V   Q   S   K   S   R   T   A   E   L   Q   A
 118 CACAGAGTACCATCTAGGAAATTAACATTATACTAACTTTCTACATCGTTGATACTTATGCGTATACATTATAT
 193 ACGTTCTTCGTGTTTATTTTTAG GAA ATT GAT GAT ACC GTG GGA ATA ATG AGA GAT AAC ATA
  35                         E   I   D   D   T   V   G   I   M   R   D   N   I
 255 AAT AAA GTA GCA GAA AGA GGT GAA AGA TTA ACG TCC ATT GAA GAT AAA GCC GAT AAC
  48  N   K   V   A   E   R   G   E   R   L   T   S   I   E   D   K   A   D   N
 312 CTA GCG GTC TCA GCC CAA GGC TTT AAG AGG GGT GCC AAT AGG GTC AGA AAA GCC ATG
  67  L   A   V   S   A   Q   G   F   K   R   G   A   N   R   V   R   K   A   M
 369 TGG TAC AAG GAT CTA AAA ATG AAG ATG TGT CTG GCT TTA GTA ATC ATC ATA TTG CTT
  86  W   Y   K   D   L   K   M   K   M   C   L   A   L   V   I   I   I   L   L
 426 GTT GTA ATC ATC GTC CCC ATT GCT GTT CAC TTT AGT CGA TAG
 105  V   V   I   I   V   P   I   A   V   H   F   S   R   *
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FIG. 3. Sequence of the *SNC1* gene and its encoded product. Coordinates for nucleotides and codons are indicated on the left. The single-letter amino acid code for the SNC1 protein is given below each 3-bp codon of the ORFs. The consensus sequences for intron splicing in *S. cerevisiae* are indicated in boldface type. The stop codon is indicated with an asterisk.

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RATVAMP1     1   MSAPA--QPPAEGTE--GAAPGGGPPGPPPNTTSNRRLQQTQAQVEEVVDIIRVNVD
                 ||       | |                 | |  |  |    || ::: | |:| |:
Yeast SNC1   1   MSSSTPFDPYALSEHDEER---------PQNVQSKSRTAELQAEIDDTVGIMRDNIN
                 || :    | |                 | |: |  |    || :|: | ||| |:
RATVAMP2     1   MSATAATVPPA------APAGEGGPPAPPPNLTSNRRLQQTQAQVDEVVDIMRVNVD

53   KVLERDQKLSELDDRADALQAGASVFESSAAKLKRKYWWKNCKMMIMLGAICAIIVVVIVI----YIFT.
     || ||  :|: ::|:|| |   |  |   | ::::  |:|  || : |  :  |::|||::
48   KVAERGERLTSIEDKADNLAVSAQGFKRGANRVRKAMWYKDLKMKMCLALVIIILLVVIIVPIAVHFSR.
     || ||  :|: ::|:|| |   |  |   | ::::  |:| ||| : |  ::  |:|::|||     ||
53   KVLERDQKLSELDDRADALQAGASQFETSAAKLKRKYWWKNLKMMIILGVICAIILIIIIV----YFST.
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FIG. 4. Comparison between SNC1 and the VAMP/synaptobrevin proteins. The amino acid sequence of SNC1 was aligned with the two types of rat VAMP/synaptobrevin proteins. Vertical bars designate identities between pairs; dots represent conservative amino acid substitutions. Amino acid coordinates are on the left.

CAP, SKN32 or SKN34 cells transformed with YEpSNC1 remained incapable of growth on rich medium or on synthetic minimal medium at 37°C and retained their abnormal morphology and sensitivity to nitrogen starvation. These results suggest that activation of the RAS pathway is required for *SNC1* suppressor activity.

To rule out the possibility that undetected genetic differences between the *cap RAS2*^{wt} strains (SKN32 and SKN34) and the *cap RAS2*^{val19} strain (SKN37) might be affecting the suppressor activity of *SNC1*, we created another *cap* null strain, SKN50 (see *Materials and Methods*). This strain bears the *RAS2*^{wt} allele but also has a disrupted *IRA1* locus. The yeast *IRA1* gene is a functional homolog of the mammalian GTPase-activating protein (GAP) (18, 28). An *ira1 CAP*^{wt} strain shows all the phenotypes associated with persistent activation of the RAS pathway (3, 24, 28), including heat shock sensitivity, presumably due to perpetuation of RAS in its activated GTP-bound state (29). SKN50 cells, however, are resistant to heat shock because of loss of the N-terminal functions of CAP and also show the phenotypes associated with loss of the C-terminal functions of CAP (data not shown). The typical *ira1* phenotypes can be restored to SKN50 cells upon expression of full-length CAP. In contrast to the results obtained with the SKN32 and SKN34 strains, but like our results with the SKN37 strain, YEpSNC1 was able to confer robust growth on rich medium in SKN50 cells (Fig. 5*A*). In addition, the expression of *SNC1* was able to correct other defects associated with loss of the C-terminal

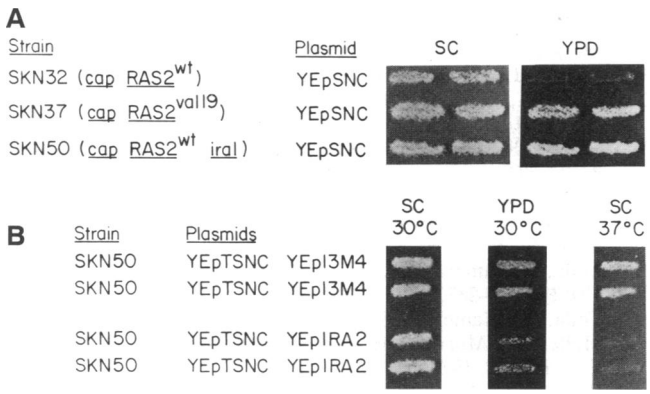

FIG. 5. Effect of *SNC1* in *cap* strains having a disruption of the *IRA1* locus. (*A*) *cap* strains SKN32 (*RAS2*^{wt}), SKN37 (*RAS2*^{val19}), and SKN50 (*RAS2*^{wt} *ira1*) were transformed with a plasmid (YEpSNC) expressing SNC1. These strains were also transformed with an empty vector (YEp13M4) as control (data not shown). After growth for 3 days on synthetic medium (SC), patches of transformed yeast were replica plated onto YPD plates to test for growth on rich medium (YPD). (*B*) *cap* strain SKN50 (*RAS2*^{wt} *ira1*) transformed with a plasmid (YEpTSNC) expressing SNC1 was transformed with a second plasmid (YEpIRA2) expressing either *IRA2* or a control vector (YEp13M4). Double transformants were grown for 3 days on synthetic medium (SC) before replica plating onto YPD plates to test for growth on rich medium (YPD; 30°C) or to prewarmed synthetic medium to test for temperature-sensitive growth at 37°C (SC; 37°C).

functions of CAP—namely, growth at 37°C (Fig. 5*B*) and resistance to nitrogen starvation.

To verify further that the functional activity of *SNC1* results from activation of RAS, we coexpressed the yeast *IRA2* gene from a multicopy plasmid containing the *LEU2* selectable marker YEpIRA2 and *SNC1* from a multicopy plasmid containing the *TRP1* selectable marker YEpTSNC1 in SKN50 cells. The *IRA2* gene encodes a second GAP-like protein and is able to functionally complement the loss of *IRA1* (30). SKN50 cells transformed with both *SNC1* and *IRA2* plasmids were found to grow slowly on YPD and were unable to grow altogether at 37°C under conditions selective for the plasmids (Fig. 5*B*). In contrast, SKN50 cells transformed with the *SNC1* plasmid and a control plasmid bearing the *LEU2* marker YEp13M4 were capable of strong growth either on YPD or on synthetic medium at 37°C. Thus, the overexpression of *IRA2* appears to inhibit the ability of *SNC1* to suppress the loss of CAP functions in SKN50 cells. This conclusion was further confirmed by plasmid segregation analysis (data not shown).

Genetic Interactions Between *SNC1* and Genes Encoding Components of the cAMP Pathway. Evidently expression of *SNC1* suppresses the phenotype resulting from loss of C-terminal CAP function only in cells that have activated RAS. One possible explanation for this phenomenon is that activation of RAS stimulates the cAMP effector pathway even in the absence of CAP. We therefore sought to determine whether the suppressor activity of *SNC1* was dependent on stimulation of the cAMP effector pathway. We created four additional *cap* mutant strains: two, SKN58 and -59, lacked the cAMP phosphodiesterases encoded by *PDE1* and *PDE2* (20, 24); and two, SKN55 and -56, lacked the regulatory subunit of the cAMP-dependent protein kinases encoded by the *BCY1* gene (31) and two of the three catalytic subunit isoforms encoded by the *TPK2* and *TPK3* genes (32). Wild-type yeast lacking the *PDE* genes are unable to hydrolyze cAMP and are sensitive to heat shock (20). Yeast lacking *BCY1*, but expressing at least one of the *TPK* genes, are also heat shock sensitive (31). As expected, disruption of *CAP* in these yeast strains had no effect on their sensitivity to heat shock, because the N-terminal function of CAP is not required in these strains. Like other *cap* disruption strains, SKN55, -56, -58, and -59 were unable to grow on rich medium and displayed the other defects associated with loss of the C-terminal functions of CAP. Overexpression of *SNC1* was unable to confer growth on rich medium in these four *cap* mutant strains. On the basis of these experiments, it appears that the functional activity of SNC1 in *cap* mutant strains is dependent on the activated state of RAS but not on the level of cAMP or cAMP-dependent protein kinase activity.

DISCUSSION

The *SNC1* gene encodes a protein of 117 amino acids that is highly homologous to members of the VAMP/synaptobrevin family (12–16), which, because of their subcellular localization, are presumed to be involved in targeting and fusion of synaptic vesicles with the presynaptic membrane (12–16).

These proteins have a distinct structure, composed of a C-terminal transmembrane domain and a small cytoplasmic N-terminal domain. Other proteins of similar overall structure but with larger cytoplasmic domains are known to function in the secretory pathway of *S. cerevisiae*. SNC1 is very weakly homologous to these other yeast proteins [e.g., SLY12/BET1 (33, 34), SLY2 (33), and BOS1 (35)].

A phenotypic assay for the VAMP/synaptobrevins in yeast would aid in evaluation of their function. Unfortunately, despite the high degree of conservation between SNC1 and the VAMP/synaptobrevins, expression of either the type 1 or type 2 rat *VAMP* genes does not complement defects seen in *cap* cells (data not shown). Suppression of the loss of *SNC1* function could in principle provide an assay for VAMP/synaptobrevin function. Unfortunately, disruption of the *SNC1* locus does not cause a discernible phenotype. Recently, we have found clear evidence for a second VAMP/synaptobrevin homolog in yeast. Disruption of both homologs may result in a phenotype that would allow analysis of SNC1 function and also provide a way to use yeast as a model organism for study of VAMP/synaptobrevin functions.

SNC1 is one of two yeast genes that we have described that were isolated by their ability to complement the cellular defects associated with loss of the C-terminal functions of CAP. The first of these, *PFY* (11), encodes profilin, an actin/phospholipid binding protein that is presumed to be involved in cytoskeletal organization (36) but that might also be involved in inositol phospholipid metabolism (37–39). We do not understand how profilin suppresses the loss of CAP function, nor do we understand the mechanism for SNC1 suppression. We have postulated that profilin alters phospholipid metabolism (11), and it is conceivable that SNC1 may directly or indirectly alter phospholipid content or distribution within cellular compartments. We are unlikely to resolve this problem without understanding the normal function of SNC1.

We and others (8, 9) have shown that yeast with a disrupted *CAP* locus is at least partly uncoupled from activated RAS proteins: cells without CAP function do not display the phenotypes normally associated with the expression of activated RAS. Our experiments with SNC1 suggest that *cap* strains are in fact at least partly responsive to activated RAS, although apparently in a manner independent of the RAS–adenylyl cyclase interaction. *SNC1* is capable of suppressing defects associated with loss of the C-terminal function of CAP only in strains with constitutively activated RAS2 protein. *SNC1* is unable to confer suppressor activity in *cap* strains bearing the $RAS2^{wt}$ allele, even when other negative regulators of the cAMP effector pathway are absent. Thus, the suppressor activity of *SNC1* appears to be independent of the presently known downstream effectors of RAS in yeast. This result confirms that RAS proteins in the yeast *S. cerevisiae* have functions in addition to regulation of adenylyl cyclase (5). Moreover, these functions do not depend on CAP.

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