

# Scw1p Antagonizes the Septation Initiation Network To Regulate Septum Formation and Cell Separation in the Fission Yeast *Schizosaccharomyces pombe*

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**Cytokinesis in the fission yeast *Schizosaccharomyces pombe* is regulated by a signaling pathway termed the septation initiation network (SIN). The SIN is essential for initiation of actomyosin ring constriction and septum formation. In a screen to search for mutations that can rescue the *sid2-250* SIN mutant, we obtained *scw1-18*. Both the *scw1-18* mutant and the *scw1* deletion mutant (*scw1Δ* mutant), have defects in cell separation. Both the *scw1-18* and *scw1Δ* mutations rescue the growth defects of not just the *sid2-250* mutant but also the other temperature-sensitive SIN mutants. Other cytokinesis mutants, such as those defective for actomyosin ring formation, are not rescued by *scw1Δ*. *scw1Δ* does not seem to rescue the SIN by restoring SIN signaling defects. However, *scw1Δ* may function downstream of the SIN to promote septum formation, since *scw1Δ* can rescue the septum formation defects of the *cps1-191β-1,3*-glucan synthase mutant, which is required for synthesis of the primary septum.**

A major function of the cell cycle and mitosis is to achieve accurate allocation of the two sets of duplicated sister chromatids to each daughter cell. At the end of each cell cycle, physical separation of the two daughter cells, a process known as cytokinesis, occurs and marks the completion of the whole cell cycle. It is key for the cell to execute all of these events in the correct order, at the right time, at the right place, and with high fidelity.

The fission yeast *Schizosaccharomyces pombe* provides an excellent eukaryotic model organism for the study of cytokinesis. Recent work with *S. pombe* has shed light on how septum formation and cytokinesis are regulated both spatially and temporally. The timing of cytokinesis in fission yeast is regulated by a signaling pathway termed the septation initiation network (SIN). The SIN is a spindle pole body (SPB)-localized signaling network that transmits a signal to the medial cortex at the end of anaphase to initiate actomyosin ring constriction and septum formation (33). An analogous pathway in *Saccharomyces cerevisiae*, termed the mitotic exit network, is required for mitotic exit and cytokinesis (4, 33). The SIN consists of a number of structural and signaling components (4, 33). Sid4p and Cdc11p form a complex at the SPB that is required for localization of all other SIN components (9, 24, 49). The Spg1p GTPase (43) functions upstream of the three protein kinases Cdc7p (13), Sid1p (19), and Sid2p (47), and both Sid1p and Sid2p have associated factors called Cdc14p (14) and Mob1p (21, 41), respectively. Spg1p is negatively regulated by a two-component GTPase-activating protein complex consisting of Cdc16p and Byr4p (17). Inactivation of the SIN results in failed cytokinesis and the formation of elongated and multinucleated

cells that cannot form a division septum, while cells form several septa when the SIN is hyperactivated by inactivation of Cdc16p (34) or Byr4p (46).

The SIN becomes active in mitosis, and SIN components are recruited to the SPB or cell division site sequentially. The Cdc16p-Byr4p GTPase-activating protein complex localizes to the SPB in interphase (7, 27). As the mitotic spindle forms at metaphase, Cdc16p-Byr4p leaves the SPBs, and Spg1p at both SPBs switches to the active GTP-bound form (7, 27, 45). Cdc7p is then recruited to the SPB(s) by the GTP-bound form of Spg1p (45). During anaphase B, Spg1p is inactivated at one of the two SPBs by Cdc16p-Byr4p (7, 27), which causes loss of Cdc7p from that SPB. Sid1p-Cdc14p localizes to the Cdc7p-containing SPB and is required for activation of Sid2p-Mob1p, which then translocates to the actomyosin ring to trigger ring constriction and septation (19, 47). Targets of Sid2p-Mob1p at the cell division site required for cytokinesis are not known. One candidate target of the SIN, based on mutant phenotypes and genetic interactions, is the  $\beta$ -glucan synthase enzyme Cps1p, which is required for primary septum formation (26, 29).

Septum formation and cell separation require a number of distinct steps, including assembly and constriction of an actomyosin ring as in animal cells, septum formation, and septum disassembly to generate two equal-size daughter cells. The medially placed actomyosin ring structure is assembled in early mitosis (1) and then constricts at the end of anaphase. The division septum is assembled in a centripetal manner concomitant with actomyosin ring constriction. The main component of the *S. pombe* division septum is 1,3- $\beta$ -glucan, which is synthesized by the  $\beta$ -glucan synthase Cps1p, which localizes to the actomyosin contractile ring concomitant with septum synthesis (10, 28). The secondary septum is then synthesized and the primary septum is degraded, allowing cell separation. At present, very little is known at a molecular level about how cell separation is achieved. However, the isolation and character-

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TABLE 1. *S. pombe* strains used in this study

Strain	Genotype	Source
DM1560	<i>scw1-18 sid2-250 ura4-D18 h<sup>+</sup></i>	Lab stock
DM105	<i>leu1-32 ura4-D18 ade6-210 h<sup>-</sup></i>	Lab stock
DM108	<i>leu1-32 ura4-D18 ade6-216 h<sup>-</sup></i>	Lab stock
DM1559	<i>scw1-18 ura4-D18 leu1-32 ade6-210 h<sup>+</sup></i>	This study
DM1300	<i>scw1-18/scw1<sup>+</sup> ade6-210/ade6-216 ura4-D18/ura4-D18 leu1-32/leu1-32 h<sup>+</sup>/h<sup>-</sup></i>	This study
DM1301	<i>scw1-18/scw1Δ::ura4<sup>+</sup> ade6-210/ade6-216 ura4-D18/ura4-D18 leu1-32/leu1-32 h<sup>+</sup>/h<sup>-</sup></i>	This study
DM1274	<i>scw1Δ::ura4<sup>+</sup> leu1-32 ura4-D18 ade6-210 h<sup>-</sup></i>	This study
DM1349	<i>scw1Δ::ura4<sup>+</sup> leu1-32 ura4-D18 ade6 h<sup>+</sup></i>	This study
DM1392	<i>scw1-3HA::kan<sup>R</sup> leu1-32 ura4-D18 ade6-210 h<sup>-</sup></i>	This study
DM1394	<i>scw1-13Myc::kan<sup>R</sup> leu1-32 ura4-D18 ade6-210 h<sup>-</sup></i>	This study
DM115	<i>sid4-A1 leu1-32 ura4-D18 ade6 h<sup>+</sup></i>	Lab stock
DM1322	<i>scw1Δ::ura4<sup>+</sup> sid4-A1 ade6 ura4-D18 leu1-32 h<sup>-</sup></i>	This study
DM274	<i>cdc11-123 ura4-D18 h<sup>+</sup></i>	Lab stock
DM1326	<i>scw1Δ::ura4<sup>+</sup> cdc11-123 ade6-210 ura4-D18 leu1-32 h<sup>-</sup></i>	This study
DM430	<i>spg1-106 ade6-210 ura4-D18 leu1-32 h<sup>+</sup></i>	Lab stock
DM1412	<i>scw1Δ::ura4<sup>+</sup> spg1-106 ade6-210 ura4-D18 leu1-32 h<sup>-</sup></i>	This study
DM1239	<i>cdc7-24 h<sup>+</sup></i>	K. Gould
DM1364	<i>scw1Δ::ura4<sup>+</sup> cdc7-24 leu1-32 h<sup>-</sup></i>	This study
DM458	<i>sid1-125 ade6-210 ura4-D18 leu1-32 h<sup>+</sup></i>	Lab stock
DM1318	<i>scw1Δ::ura4<sup>+</sup> sid1-125 ade6-210 ura4-D18 leu1-32 h<sup>-</sup></i>	This study
DM75	<i>sid1-239 ade6 ura4-D18 leu1-32 h<sup>+</sup></i>	Lab stock
DM1366	<i>scw1Δ::ura4<sup>+</sup> sid1-239 ura4-D18 leu1-32 ade6 h<sup>-</sup></i>	This study
DM436	<i>cdc14-118 ura4-D18 leu1-32 ade6-210 h<sup>+</sup></i>	Lab stock
DM1328	<i>scw1Δ::ura4<sup>+</sup> cdc14-118 ade6-210 ura4-D18 leu1-32 h<sup>+</sup></i>	This study
DM429	<i>sid2-250 ade6 ura4-D18 leu1-32 h<sup>+</sup></i>	Lab stock
DM1320	<i>scw1Δ::ura4<sup>+</sup> sid2-250 ade6 ura4-D18 leu1-32 h<sup>-</sup></i>	This study
DM670	<i>mob1-1 ura4-D18 leu1-32 ade6 his3-D1 + pBGMob1-ts h<sup>-</sup></i>	Lab stock
DM1368	<i>scw1Δ::ura4<sup>+</sup> mob1-1 ura4-D18 leu1-32 ade6 his3-D1 h<sup>-</sup></i>	This study
DM322	<i>cdc12-112 ura4-D18 leu1-32 ade6-210 h<sup>+</sup></i>	Lab stock
DM1370	<i>scw1Δ::ura4<sup>+</sup> cdc12-112 ura4-D18 leu1-32 ade6-210 h<sup>-</sup></i>	This study
DM2	<i>cdc15-140 ura4-D18 h<sup>+</sup></i>	Lab stock
DM1372	<i>scw1Δ::ura4<sup>+</sup> cdc15-140 ura4-D18 ade6-210 h<sup>+</sup></i>	This study
DM916	<i>nda3-KM311 leu1-32 ura4-D18 ade6-21X h<sup>-</sup></i>	Lab stock
DM1268	<i>scw1-18 nda3-KM311 ade6-210 leu1-32 ura4-D18 h<sup>+</sup></i>	This study
DM1459	<i>cdc11-123 GFP-mob1 ade6 ura4-D18 leu1-32 h<sup>+</sup></i>	This study
DM1461	<i>scw1Δ::ura4<sup>+</sup> cdc11-123 GFP-mob1 ade6 ura4-D18 leu1-32 h<sup>-</sup></i>	This study
DM1465	<i>cdc11-123 cdc7-GFP::ura4<sup>+</sup> ade6-210 ura4-D18 leu1-32 h<sup>+</sup></i>	This study
DM1467	<i>scw1Δ::ura4<sup>+</sup> cdc11-123 cdc7-GFP::ura4<sup>+</sup> ade6-210 ura4-D18 leu1-32 h<sup>+</sup></i>	This study
DM497	<i>sid2-13Myc::kan ura4-D18 leu1-32 ade6-210 h<sup>-</sup></i>	Lab Stock
DM1440	<i>scw1Δ::ura4<sup>+</sup> sid2-13Myc::kan<sup>R</sup> ade6 ura4-D18 leu1-32 h<sup>+</sup></i>	This study
DM1443	<i>cdc11-123 sid2-13Myc::kan<sup>R</sup> ade6 ura4-D18 leu1-32 h<sup>+</sup></i>	This study
DM1439	<i>scw1Δ::ura4<sup>+</sup> cdc11-123 sid2-13Myc::kan<sup>R</sup> ade6 ura4-D18 leu1-32 h<sup>+</sup></i>	This study
DM1447	<i>cdc7-24 sid2-13Myc::kan<sup>R</sup> leu1-32 ade6 h<sup>+</sup></i>	This study
DM1445	<i>scw1Δ::ura4<sup>+</sup> cdc7-24 sid2-13Myc::kan<sup>R</sup> leu1-32 h<sup>+</sup></i>	This study
DM1214	<i>cps1-191 leu1-32 lys1-131 ura4-D18 ade6-21X h<sup>-</sup></i>	Lab stock
DM1535	<i>scw1Δ::ura4<sup>+</sup> cps1-191 leu1-32 ura4-D18 ade6-21X h<sup>-</sup></i>	This study
DM1569	<i>cps1-UV1 leu1-32 ura4-D18 ade6-210 h<sup>-</sup></i>	Balasubramanian lab
DM1622	<i>scw1Δ::ura4<sup>+</sup> cps1-UV1 leu1-32 ura4-D18 ade6-21X h<sup>+</sup></i>	This study
DM1570	<i>cps1-UV2 leu1-32 ura4-D18 ade6-216 h<sup>-</sup></i>	Balasubramanian lab
DM1624	<i>scw1Δ::ura4<sup>+</sup> cps1-UV2 leu1-32 ura4-D18 ade6-21X h<sup>-</sup></i>	This study
DM878	<i>sep1-1 leu1-32 ura4-D18 h<sup>-</sup></i>	Sipiczki lab
DM1587	<i>sep1-1 cps1-191 leu1-32 ura4-D18 h<sup>+</sup></i>	This study

ization of one transcription factor, Sep1p, whose mutations interfere with cell separation raised the possibility that expression of certain genes late in the cell cycle is required for efficient cell separation (40).

Presently nothing is known about how the SIN in *S. pombe* transmits the signal to initiate cytokinesis. Because Sid2p and Mob1p localize to the cell division site (21, 41, 47), they presumably transmit the signal to divide to the division machinery. Therefore, we screened for mutations that can suppress the growth defects in *sid2* mutants. Here we describe the characterization of one of these suppressors, *scw1-18*, which on its own causes defects in cell separation. *scw1-18* rescues all

known SIN mutants but does not do so by restoring signaling through the SIN. Thus, the wild-type *scw1<sup>+</sup>* gene may function downstream of or parallel with the SIN in regulating septum formation and stability in the final steps of cytokinesis.

#### MATERIALS AND METHODS

***S. pombe* growth conditions and genetic manipulations.** The fission yeast strains used in this study are listed in Table 1. Genetic crosses and general yeast techniques were performed as previously described (36). *S. pombe* strains were grown in rich medium (yeast extract [YE]) or Edinburgh minimal medium (EMM) with appropriate supplements (36). EMM with 5 µg of thiamine per ml was used to repress expression from the *nmf1<sup>+</sup>* promoter. YE containing 100 mg

of G418 (Calbiochem) per liter was used for selecting *kan<sup>R</sup>*-expressing cells. Microtubule formation was inhibited by the addition of various concentrations of methyl-2-benzimidazolecarbamate (MBC) in solid or liquid media. Synchronous populations of cells were generated by centrifugal elutriation with a Beckman JE 5.0 rotor.

To delete *scw1<sup>+</sup>*, the whole *scw1<sup>+</sup>* open reading frame (ORF) was replaced by the *ura4<sup>+</sup>* gene via a PCR-based procedure (2), using the oligonucleotides 5'-GGT TAC TTT ATC AAC CAC TTT GTC ATT CTT TTT TCT CTT CTT TTC AAT TAC CAT TAT ATA TAA TTT GCA AAC GCC AGG GTT TTC CCA GTC ACG AC-3' and 5'-GGA CCT AAA GTC CTT GCA AGG TAT TGA TGA ATA TGC ATA AAA TGA AGA CGA GAA AAT GCT AGA TGA GCT ATT TGC CAG CGG ATA ACA ATT TCA CAC AGG A-3'.

Strains expressing Scw1p carboxy-terminally tagged with green fluorescent protein (GFP) and 13Myc were generated by PCR-based gene targeting (2) with the oligonucleotides 5'-GAC TCT TTG CTT AAT CAT ACT GGT GGA CAT AAC GAA GTC CAC GCC AGT CCC AGT TGG GGT AAT AAT CTA ATG TAT GGC AAA CGG ATC CCC GGG TTA ATT AA-3' and 5'-GCT TAA CAG ATG GTT AAA GTT GCA TGC AGT CAA AGT GGA ATA GAT CGC AAC TTT TGA TTA ACA AAG AAT CAA TAT GCA AAA CGA ATT CGA GCT CGT TTA AAC-3'. Correct chromosomal integration in the resultant *kan<sup>R</sup>* transformants was confirmed by PCR analysis.

Diploid strains were constructed by crossing haploid strains carrying the *ade6*-complementing mutation *ade6-210* or *ade6-216* and selecting for *ade<sup>+</sup>* white colonies.

**Isolation of *scw1-18* and cloning of *scw1<sup>+</sup>*.** The *scw1* mutation was isolated in a screen for *sid2-250* suppressors. Approximately  $2 \times 10^8$  cells of a *sid2-250 ura4-D18 leu1-32 ade6 h<sup>+</sup>* strain (DM429) were mutagenized for 15 min with nitrosoguanidine as described previously (36) and plated at 36°C. This screen yielded several hundred colonies, of which 125 were initially picked for further characterization. Many of these were discarded after further testing due to poor rescue of the *sid2-250* mutation. From the remaining strains, 43 with representative phenotypes were picked and crossed to the wild type to determine if they represented single mutations and whether they had phenotypes on their own. Twenty-one of these mutants displayed a multiseptate phenotype (Fig. 1) and were kept for further study. The other mutants either had no phenotype on their own or had multiple mutations that contributed to the *sid2-250* suppression. Complementation analysis of the remaining 21 mutants revealed that 19 fell into a single complementation group, which we later named *scw1*. One of these mutants, carrying *scw1-18*, was picked for further study. The other two mutants each fell into separate complementation groups and are not described here.

To clone the *scw1<sup>+</sup>* gene, we first mapped its approximate chromosomal location in a *swi5* mutant background, which reduces recombination frequencies and allows for a crude map position to be determined (31). This analysis demonstrated a weak linkage to the *ura4<sup>+</sup>* locus. Further mapping in a wild-type (non-*swi5*) background was carried out by crossing *scw1-18* to strains bearing mutations in the region of *ura4<sup>+</sup>*. This analysis showed that *scw1-18* was tightly linked (1.1 map units; 44 parental ditype and 1 tetraplate) to the *cut1-205* mutation. We then obtained cosmids in the region of the *cut1<sup>+</sup>* locus from the Sanger Center. The *his7<sup>+</sup>* gene was inserted into these cosmids as previously described (37), and they were transformed into *scw1-18 his7-306* cells and tested for rescue of the multiseptate phenotype of *scw1-18*. Two of these cosmids, c5E4 and c16C4, were able to rescue the *scw1-18* phenotype. Candidate genes from the region of overlap between these two cosmids were cloned into the pREP42 vector and tested for rescue of *scw1-18*. This analysis showed that the SPCC16C4.07 ORF was capable of rescuing *scw1-18*.

To clone *scw1<sup>+</sup>* into the pREP42 vector (5), the coding region for the *scw1<sup>+</sup>* gene was amplified by PCR from the wild-type *S. pombe* genome (using oligonucleotides 5'-CAT GCA TAT GTT TGT GGG ATC ACC G-3' and 5'-CAT GGG ATC CCT ATT TGC CAT ACA TTA G-3'), and the product was digested with *NdeI* and *BamHI* and then subcloned into the pREP42 vector containing the thiamine-repressible *nmt1* promoter (32).

**Microscopy.** Immunofluorescence microscopy was done as described previously (3). For tubulin staining, primary monoclonal antitubulin antibody TAT1 (52) was followed by secondary anti-mouse Texas red or Alexa 594-immunoglobulin G (Molecular Probes). GFP fusion proteins were observed in cells after fixation with 3.7% formaldehyde. DNA was visualized with 4',6-diamidino-2-phenylindole (DAPI) (Sigma) at 2 µg/ml. Photomicrographs were obtained with a Nikon Eclipse E600 fluorescence microscope coupled to a cooled charge-coupled device camera (ORCA-ER; Hamamatsu), and image processing and analysis were carried out with IPLab Spectrum software (Signal Analytics Corporation, Vienna, Va.).

## RESULTS

**Isolation and characterization of *scw1-18*.** In order to identify potential targets and/or regulators of Sid2p, we screened for mutations that could suppress the temperature-sensitive growth defect of *sid2-250* mutant cells (see Materials and Methods). The majority of the suppressors identified had defects in cell separation, resulting in a high percentage of cells with single or multiple septa and multiple nuclei. Complementation analysis of 21 of these suppressors revealed that all but 2 fell into a single complementation group. In the course of these experiments, the same gene was isolated and called *scw1* (23), and hence we have maintained this nomenclature. The other two mutations defined their own complementation groups and have not yet been further characterized. One *scw1* allele, *scw1-18*, was chosen for further analysis. The major phenotypes of *scw1-18* included multiple septa and a relatively high septation index, with 40 to 50% of log-phase cells having one or more septa (Fig. 1). We never observed more than two nuclei in each cell compartment in *scw1-18* mutant cells, suggesting that the placement of the actomyosin ring and septum formation occur normally but the cells have a defect in cell separation. These cells did not show obvious heat or cold sensitivity (data not shown).

**Identification of the *scw1<sup>+</sup>* gene.** The *scw1<sup>+</sup>* gene was cloned through a combination of genetic and physical mapping (see Materials and Methods). Expression of *scw1<sup>+</sup>* in the *scw1-18* strain rescued the cell separation defect in these cells (Fig. 1D). *scw1<sup>+</sup>* is predicted to encode a protein of 561 amino acids with a molecular mass of 60 kDa. A database search revealed that Scw1p shows homology to RNA binding proteins, especially to two budding yeast proteins, WHI3 and WHI4, with the highest identity in the RNA binding domain (39) (Fig. 1E). Interestingly, WHI3 also seems to be involved in cell cycle regulation by causing localized translation of the CLN3 cyclin RNA (18). Deletion of the whole *scw1<sup>+</sup>* ORF showed that the gene is not essential (see Materials and Methods). Closer examination revealed that the *scw1Δ* null mutant showed a cell separation defect similar to that of the *scw1-18* mutant (Fig. 1A and data not shown), and it also could rescue the *sid2-250* mutant at 30 and 36°C (see Fig. 3). Therefore, *scw1-18* and *scw1Δ* behave similarly, suggesting that *scw1-18* is a loss-of-function mutation. To confirm that *scw1-18* represents a mutation in the gene *scw1<sup>+</sup>*, we tested whether *scw1-18* and *scw1Δ* were complementing mutations. We constructed an *scw1-18/scw1Δ* diploid strain to test whether these cells showed a multiseptate phenotype. These diploid cells showed an increased percentage with single and multiple septa compared to the control diploid *scw1-18/scw1<sup>+</sup>* cells or wild-type cells (Fig. 1C), consistent with *scw1-18* being a mutant allele of *scw1<sup>+</sup>*.

**Scw1p localizes to the cytoplasm.** To determine the cellular localization of Scw1p, we tagged Scw1p by fusing the genomic *scw1<sup>+</sup>* ORF to either GFP or 13Myc. Both Scw1p fusions were functional, since the strains expressing them were wild type in morphology, and the tagged alleles were unable to rescue the *sid2-250* mutation (data not shown). Direct visualization of GFP fusion proteins and indirect immunofluorescence of Myc-fused proteins demonstrated that the proteins localized diffusely to the cytoplasm and were excluded from the nucleus at



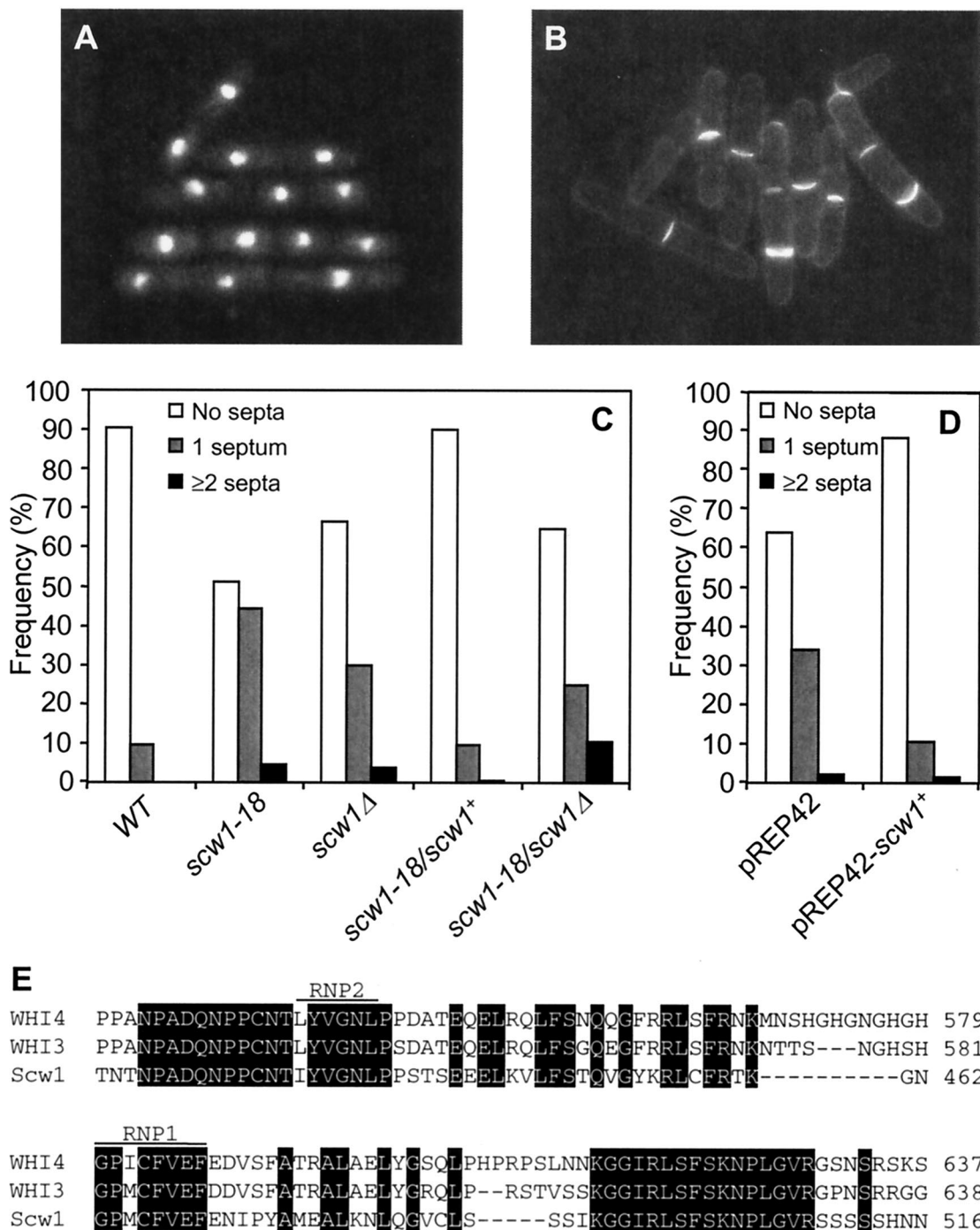


FIG. 1. Characterization of *scw1-18* and *scw1Δ* mutant strains. (A and B) *scw1-18* (DM1559) cells from a log-phase culture at 30°C were fixed and stained with DAPI to visualize nuclei (A) or stained with Calcofluor to visualize cell wall and septa (B). (C) Wild-type (DM105), *scw1-18* (DM1559), *scw1Δ* (DM1274), *scw1-18/scw1<sup>+</sup>* (DM1300), and *scw1-18/scw1Δ* (DM1301) strains were grown in YE to mid-log phase at 30°C and then scored for the septation index. WT, wild type. (D) *scw1-18* mutant cells (DM1559) containing either the pREP42 or pREP42-*scw1<sup>+</sup>* plasmid were grown in EMM without uracil and thiamine for 24 h at 30°C, at which time the septation index was scored. (E) Alignment of the RNP domain of Scw1p with those of two related budding yeast proteins, Whi3 and Whi4. Conserved residues are marked with black boxes. The octamer RNP1 and hexamer RNP2 are labeled as previously defined (39).

all stages of the cell cycle (Fig. 2 and data not shown). Scw1p was not observed at the SPB or cell division site.

***scw1Δ* can rescue all SIN mutants but not actomyosin ring mutants.** We next tested whether the *scw1Δ* mutation specifi-

cally rescued the *sid2-250* mutant or was capable of rescuing other SIN mutants. We constructed double mutants between *scw1-18* or *scw1Δ* and all the other available temperature-sensitive SIN mutants, including the *sid4-A1*, *cdc11-123*, *spg1-*

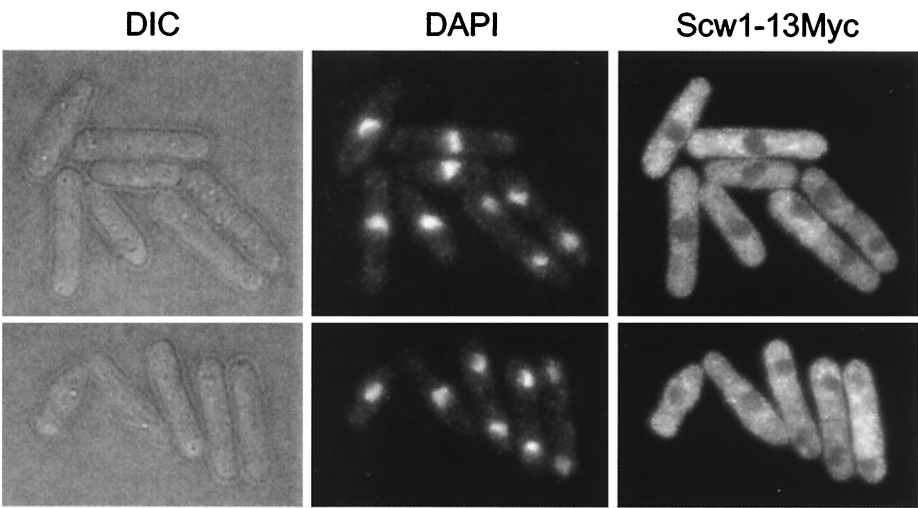


FIG. 2. Intracellular localization of epitope-tagged Scw1p. Wild-type cells expressing *scw1-13Myc* (DM1394) were grown in YE to log phase and then fixed and subjected to indirect immunofluorescence with anti-Myc antibody. DIC, differential interference contrast.

106, *cdc7-24*, *sid1-125*, *sid1-239*, *cdc14-118*, *sid2-250*, and *mob1-1* mutants. Interestingly, serial dilution drop tests on plates at different temperatures showed that both *scw1-18* and *scw1Δ* rescued the growth defects of all of these SIN mutants. The degree of rescue varied depending on the allele, with very strong mutant alleles, such as *sid4-A1* and *sid1-125*, being rescued at 30 but not 36°C (Fig. 3 and data not shown), whereas other mutants were rescued at both 30 and 36°C. This analysis suggested that the *scw1Δ* mutation was not able to bypass the SIN to promote cytokinesis but required some degree of residual SIN signaling to promote rescue. Microscopic examination of double mutant cells (see below) showed a strong correlation between the ability of *scw1Δ* to rescue the temperature-sensitive growth defects of the SIN and its ability to rescue the SIN septation defects. For example, we found that the *scw1Δ* mutation rescues the septation defect of *sid4-A1* and *sid1-125* mutant cells at 30 but not 36°C, consistent with its ability to rescue these mutants growth defects at 30 but not 36°C, (Fig. 3 and data not shown). A similar correlation was observed for other SIN mutants, suggesting that *scw1Δ* rescues the growth defect of SIN mutants by restoring septum formation in these mutants.

Microscopic analysis of these double mutant cells in liquid cultures at 36°C revealed that these cells could form septa, although sometimes not in a very efficient manner, leaving some cell compartments without nuclei while others had multiple nuclei (Fig. 4). In contrast, double mutants between *scw1Δ* and temperature-sensitive actin ring formation mutants, such as the *cdc3-124*, *cdc12-112*, and *cdc15-140* mutants, failed to show any rescue of growth defects at 36°C (Fig. 3 and data not shown) and did not suppress the septum formation defects leading to multiple nuclei (data not shown). In addition, *scw1-18* was also unable to suppress the growth defects of other temperature-sensitive mutants such as the *alp4-1891*, *alp6-719*, and *alp16::ura4<sup>+</sup>* mutants (data not shown) (16, 51), further demonstrating that the suppression of the SIN is specific.

***scw1-18* can stabilize microtubules.** The fact that *scw1Δ* suppresses the SIN implies that wild-type Scw1p antagonizes the SIN. Mutants with mutations in other antagonists of the SIN,

such as *dma1*, *zfs1*, and *cdc16*, have defects in the spindle checkpoint (6, 12, 38), and inappropriate activation of the SIN can cause spindle checkpoint defects (20). Therefore, we tested whether the *scw1-18* mutation, like other SIN suppres-

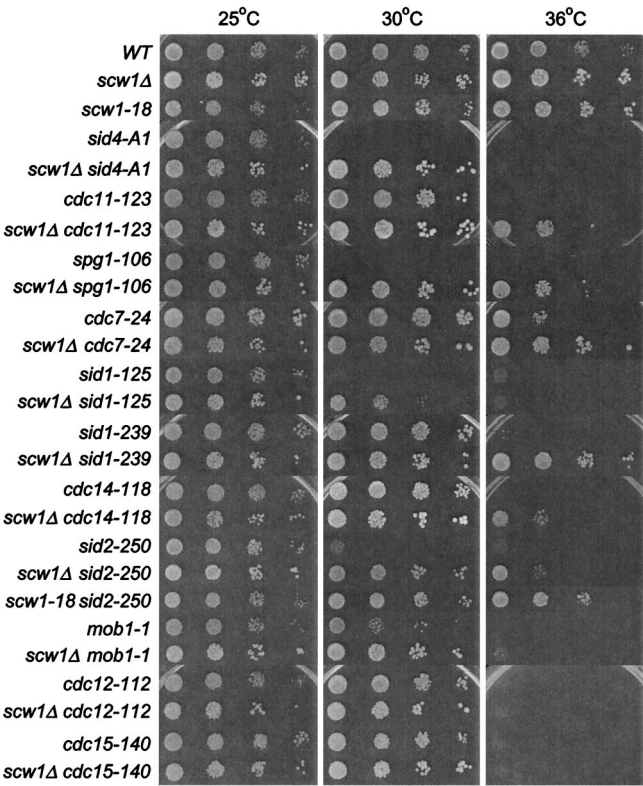


FIG. 3. *scw1Δ* can rescue SIN mutants but not the actomyosin ring mutants. The indicated single and double mutant strains were tested by serial dilution patch test for growth. (Note that the single and double mutant strains used are listed in the same order in Table 1.) Dilutions shown were 10-fold, starting with 10<sup>4</sup> cells. Strains were pregrown in liquid YE at 25°C and then spotted onto YE plates at the indicated temperatures and incubated for 3 to 5 days before photography. WT, wild type.

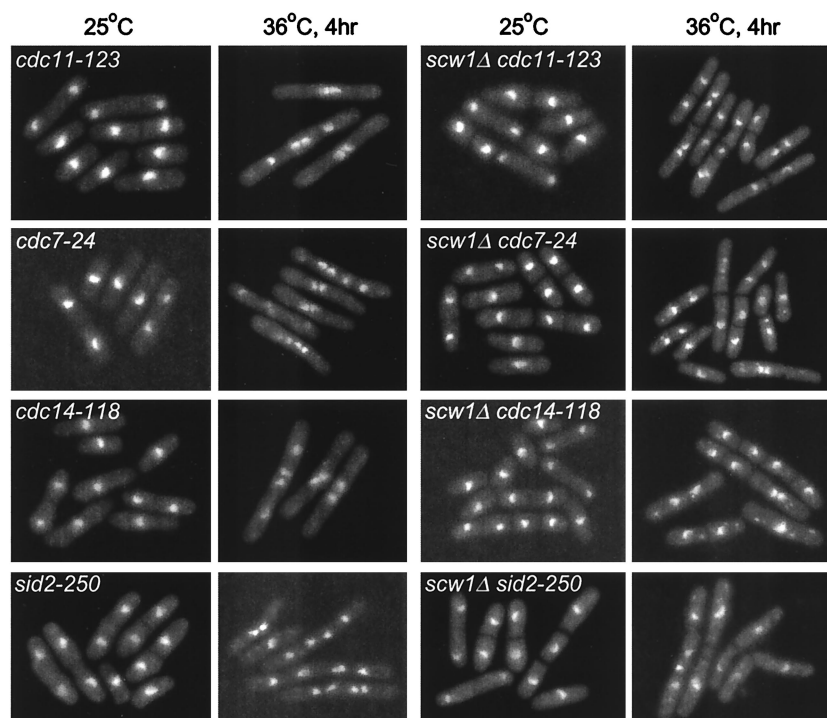


FIG. 4. Microscopic analysis of double mutants between *scw1Δ* and SIN mutations. Cells of the indicated strains were grown in YE at 25°C to log phase and then shifted to 36°C for 4 h before being fixed and stained with DAPI.

sors, also compromises the spindle checkpoint-mediated arrest caused by inactivation of the cold-sensitive *nda3-KM311*  $\beta$ -tubulin mutant (50). The *nda3-KM311* mutant normally arrests in early mitosis at the restrictive temperature due to the failure to form a mitotic spindle and does not exit mitosis and septate although the medial actomyosin ring has been formed (8, 35). We generated synchronous cultures, in early G<sub>2</sub> phase, of both *nda3-KM311* and *scw1-18 nda3-KM311* mutants by elutriation and then shifted them to the restrictive temperature of 19°C. Septation was scored at 1-h intervals for both cultures, as a convenient way to monitor exit from mitosis. As expected, the *nda3-KM311* control cells arrested without a septum and with a single nucleus (Fig. 5A and B). In sharp contrast, the *scw1-18 nda3-KM311* mutant started to accumulate cells with one or more septa after 3 h, and by 9 h 40% of the cells had septated (Fig. 5A and B). Interestingly, the *scw1-18 nda3-KM311* mutant cells after 9 h at 19°C could sometimes accomplish chromosome segregation but failed to fully separate their chromosomes, often resulting in anucleate cell compartments (Fig. 5B). This implies that the *scw1-18* mutation may rescue the *nda3-KM311* cell cycle block by partially restoring microtubule function in these cells. To test this, we examined the microtubules of asynchronous *nda3-KM311* and *scw1-18 nda3-KM311* cells that had been shifted to the restrictive temperature for 6 h. As expected, *nda3-KM311* cells had no microtubules and showed staining only at the SPB. In contrast, *scw1-18 nda3-KM311* cells displayed many short microtubules around or across the nucleus (Fig. 5C), suggesting that the *scw1-18* mutation is not spindle checkpoint defective but partially restores microtubules in the *nda3-KM311* mutation. The increased stability of microtubules in *scw1-18 nda3-KM311* mutant cells is

not sufficient to restore viability of *nda3-KM311* cells at 19°C (Fig. 5E). The increased stability of microtubules in the *scw1-18 nda3-KM311* mutant is not specific to *nda3-KM311* mutant cells, because when we compared wild-type and *scw1-18* cells treated with the microtubule-depolymerizing drug MBC, we found that the *scw1-18* cells displayed more microtubules than wild-type cells (Fig. 5D). However, in terms of cell viability, the *scw1-18* cells were only slightly resistant to MBC compared to wild-type cells (Fig. 5E).

***scw1Δ* does not rescue SIN mutants by restoring SIN protein localization and activity.** Interestingly, *scw1Δ* rescues *cdc11-123* mutants that are defective for localization of downstream SIN components and activation of Sid2p kinase activity (19, 21, 24, 41, 47, 49). We tested whether either of these defects were restored by the *scw1Δ* mutation. We first examined whether the absence of *scw1*<sup>+</sup> restored localization of SIN components in *cdc11-123* mutants. Because the *scw1Δ* mutation was able to rescue *cdc11-123* well only in liquid medium at 33.5°C, localization experiments were carried out at this temperature. Both Cdc7p-GFP and GFP-Mob1p were readily observed at SPBs in late anaphase or telophase wild-type cells at both 25 and 33.5°C (Fig. 6A and B and data not shown). The intensity of Cdc7p-GFP and GFP-Mob1p signals at the SPB in both *cdc11-123* single and *cdc11-123 scw1Δ* double mutants at 25°C was reduced compared to that in the wild type (data not shown). In *cdc11-123* cells incubated at 33.5°C, faint Cdc7p-GFP and GFP-Mob1p signals at the SPB could only occasionally be observed (Fig. 6A and B). Similar results were observed for the *cdc11-123 scw1Δ* double mutant strain, and quantification of the SPB signals showed that the *scw1Δ* mutation was



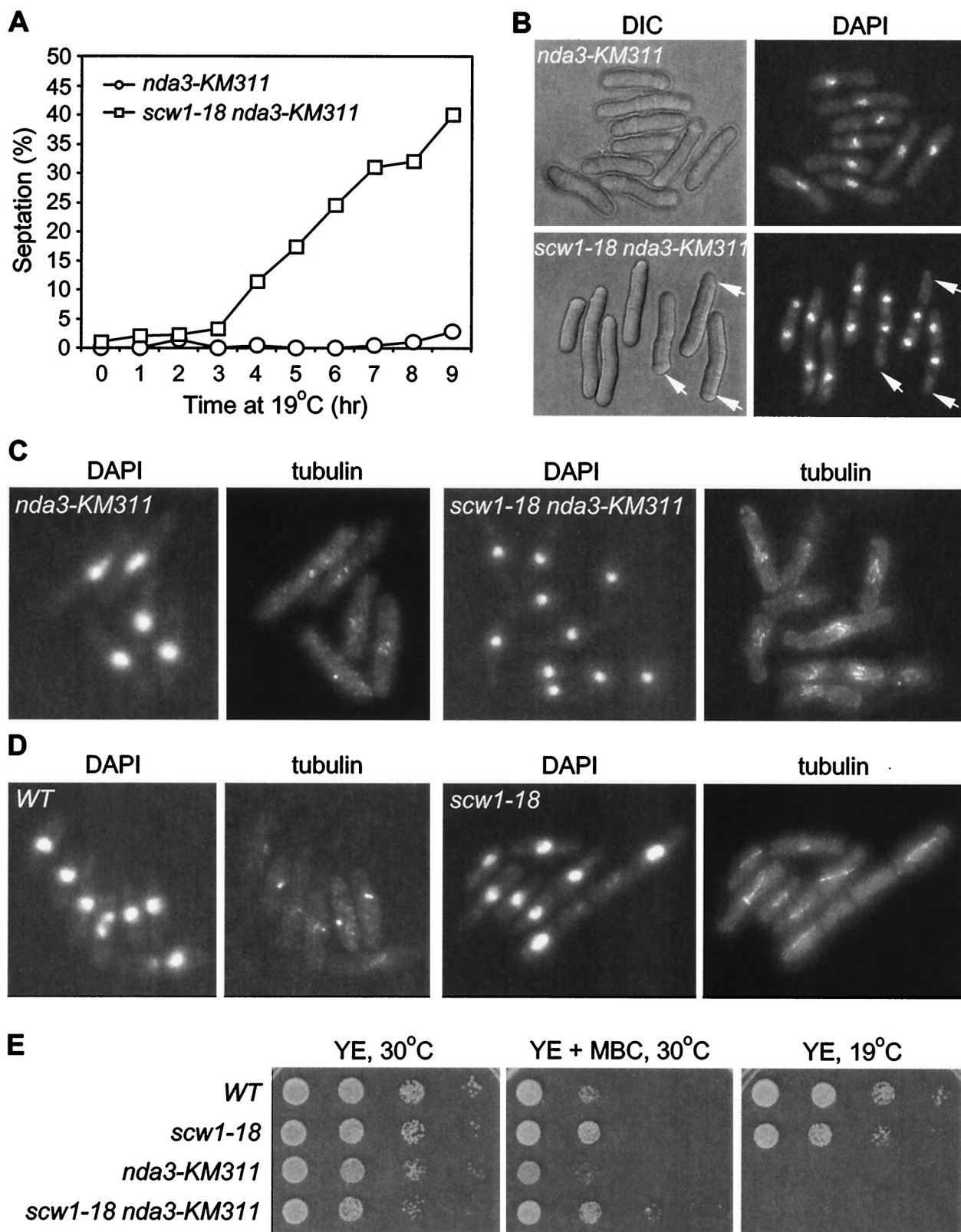


FIG. 5. The *scw1-18* mutation can stabilize microtubules. (A) *nda3-KM311* and *scw1-18 nda3-KM311* cells were synchronized at early G<sub>2</sub> by centrifugal elutriation from log-phase cultures grown at 30°C. Synchronized cells were then shifted to 19°C, and septation was scored for both cultures at 1-h intervals. (B) DAPI-stained cells at the 9-h time point. DIC, differential interference contrast. (C) DAPI and tubulin staining with TAT1 antibody of asynchronous cells of the indicated genotypes 6 h after a shift to 19°C. (D) DAPI and tubulin staining with TAT1 antibody of cells treated with 25 mg of MBC per ml for 2 h at 30°C. WT, wild type. (E) Serial dilution patch test for growth of the indicated single and double mutant strains. Dilutions shown were 10-fold, starting with 10<sup>4</sup> cells. Strains were pregrown in liquid YE at 25°C and then spotted onto YE plates or on YE with 10 mg of MBC per ml and incubated at the indicated temperatures for 3 to 5 days before photography.

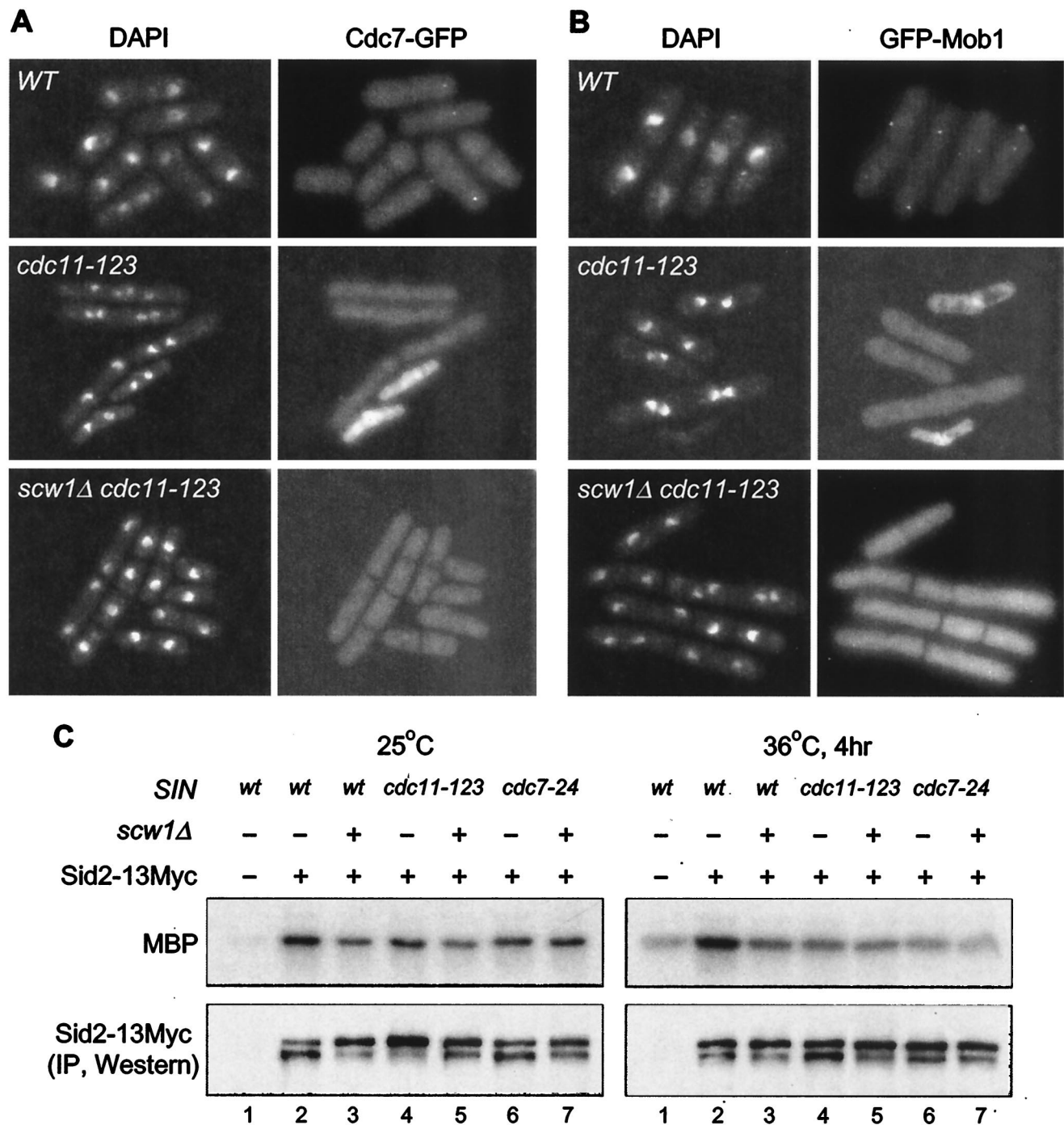


FIG. 6. *scw1Δ* does not rescue SIN mutants by restoring SIN localization and activity. (A and B) The indicated strains were grown in YE at 25°C to log phase and then shifted to 33.5°C for 4 h before being fixed and stained with DAPI and photographed for GFP fluorescence. WT, wild type. (C) The *scw1Δ* mutation does not promote Sid2p kinase activity. Various strains were harvested either in log phase at 25°C or after being shifted for 4 h to 36°C. The presence (+) or absence (–) of either Sid2-13Myc or the *scw1Δ* mutation is indicated, as is the presence of SIN mutations. Immune complexes were prepared from lysates with anti-Myc antibody, and then a kinase assay was performed with MBP as an artificial substrate, as previously described (47). Each sample was split in two, and phosphorylation of MBP (upper panels) or Sid2p-13Myc levels (lower panel) were detected by phosphorimager and Western analysis, respectively. IP, immunoprecipitation.

unable to restore the Cdc7-GFP and GFP-Mob1p SPB localization defect of *cdc11-123* cells (Fig. 6A and B and data not shown). At higher temperatures, *scw1Δ* did not rescue *cdc11-123* cells and no SPB localization of Cdc7p and Mob1p was

observed, consistent with *scw1Δ*-mediated suppression of the SIN requiring a low level of SIN function. Similar results were observed when Sid1p and Sid2p were examined in *cdc11-123* single or *scw1Δ cdc11-123* double mutant cells (data not shown).



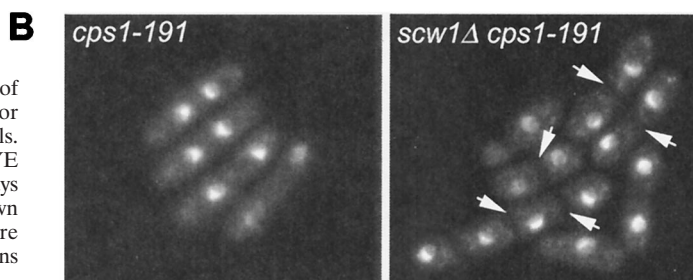
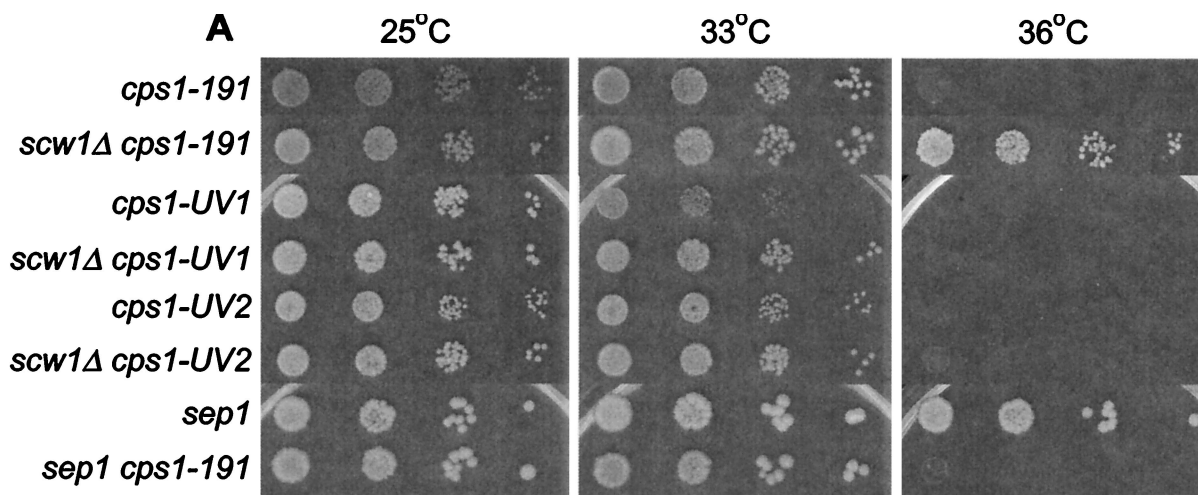


FIG. 7. *scw1Δ* rescues the *cps1-191* mutant strain. (A) Strains of the indicated genotypes were tested by serial dilution patch test for growth. The dilutions shown were 10-fold, starting with  $10^4$  cells. Strains were pregrown in liquid YE at 25°C and then spotted onto YE plates at the indicated temperatures and incubated for 3 to 5 days before photography. (B) Cells of the indicated genotypes were grown in YE at 25°C to log phase and then shifted to 36°C for 4 h before being fixed and stained with DAPI. Septa are apparent as dark regions between nuclei (arrows).

Thus, the *scw1Δ* mutation does not rescue the *cdc11-123* mutation by promoting localization of SIN components to the SPB.

Since the experiments described above suggested that the *scw1Δ* mutation does not rescue SIN mutants by promoting localization of SIN components, we next wanted to test whether it could be functioning by increasing signaling through the pathway. Since Sid2p kinase activity depends on all other SIN proteins (47), we analyzed Sid2p kinase activity in *scw1Δ cdc11-123* and *scw1Δ cdc7-24* mutants. 13Myc epitope-tagged Sid2p was first immunoprecipitated with an anti-Myc antibody, and then in vitro Sid2 kinase assays were performed with myelin basic protein (MBP) as an artificial substrate. Sid2p-13Myc immune complexes were prepared from lysates of cells incubated at the permissive (25°C) and restrictive (36°C) temperatures for the *cdc11-123* and *cdc7-24* mutant strains. As previously observed, Sid2p kinase activity is reduced in *cdc11-123* and *cdc7-24* mutant strains compared to wild-type cells (Fig. 6C, lanes 4, 6, and 2, respectively). The presence of the *scw1Δ* mutation did not restore Sid2p kinase activity to *cdc11-123* and *cdc7-24* mutants (Fig. 6C, lanes 5 and 7, respectively), and in fact, the *scw1Δ* single mutant had somewhat reduced Sid2p kinase activity (Fig. 6C, lane 3). Similar results were obtained at a reduced restrictive temperature, where better rescue by the *scw1Δ* mutation is observed (data not shown). Taken together, these results suggest that the *scw1Δ* mutation does not rescue SIN mutants by restoring localization of SIN proteins or increasing signaling through the SIN.

**The *scw1* mutation restores septum formation in the *cps1-191*  $\beta$ -glucan synthase mutant.** The analysis described above indicated that the *scw1* mutation does not rescue the SIN

mutants by restoring signaling through the SIN but may be promoting septum formation by acting downstream of the SIN. Previous genetic studies have suggested that the  $\beta$ -glucan synthase enzyme Cps1p may function downstream of the SIN to promote septum formation (29). Like SIN mutants, temperature-sensitive *cps1* mutants fail to form septa at the restrictive temperature and arrest as binucleate cells. To test whether Scw1p could be affecting septum formation more directly, we tested whether the *scw1Δ* mutation could rescue the *cps1* mutant strains. Interestingly, the *scw1Δ* mutation could rescue the temperature-sensitive growth defect of *cps1-191* cells (Fig. 7A). This effect was allele specific, since *scw1Δ* was unable to rescue *cps1-UV2* and could only weakly rescue *cps1-UV1* at the reduced restrictive temperature of 33°C (Fig. 7A). Examination of single and double mutant cells after incubation at the restrictive temperature showed that *scw1Δ cps1-191* cells were capable of making septa, unlike *cps1-191* single mutant cells (Fig. 7B). Furthermore, the rescue of *cps1-191* was not an indirect consequence of the cell separation defect in *scw1Δ* cells, since the *sep1-1* cell separation mutant was unable to rescue the *cps1-191* mutant strain (Fig. 7A).

## DISCUSSION

In this study, we have identified the gene *scw1*<sup>+</sup> in a genetic screen for potential regulators and effectors of the SIN pathway in *S. pombe*. An *scw1* deletion mutation can suppress all of the mutations in the SIN pathway and shows a cell separation phenotype on its own. The suppression of the SIN seems to be specific, since the *scw1Δ* mutation does not suppress mutations in other cytokinesis genes, such as those required for actomy-

osin ring formation. How, then, does *scw1* loss of function suppress SIN mutations? First, *scw1Δ* does not seem to bypass the SIN pathway, because *scw1Δ* does not rescue the strongest SIN mutations, such as *sid4-A1* or *sid1-125*, at the highest restrictive temperature. In addition, the *scw1Δ* mutation is unable to suppress *sid2-250 spg1-106* double mutants at 36°C, whereas it can suppress either single mutant at 36°C (data not shown). Together, these results indicate that *scw1Δ* cannot suppress a total loss of function in the SIN pathway. This suggests that *scw1Δ* either acts to enhance weak SIN signaling or removes an inhibitor downstream of the SIN. To study this, we examined *cdc11-123* mutants, which have defects in localizing SIN components and activating Sid2p kinase activity. The *scw1Δ* mutation was unable to rescue the *cdc11-123* defects in localization of SIN components or activation of Sid2p kinase, suggesting that *scw1Δ* does not directly enhance signaling through the SIN. In fact, *scw1Δ* single mutants had reduced Sid2p kinase activity. The reason for this is unclear. However, because the SIN seems to be down regulated once the septum has formed, the persistent presence of septa in *scw1Δ* cells could cause down regulation of Sid2p activity. Alternatively, increased septum-forming activity in *scw1Δ* mutants could inhibit the SIN through a feedback mechanism. Further study will be required to test these possibilities. Interestingly, SIN suppressors such as *cdc16-116* (15) and *par1/pbp1* (22, 25, 48), which are thought to suppress by enhancing signaling through the SIN, do not suppress *cdc11-123*, perhaps because the Cdc11-123p mutant protein does not localize properly to the SPB (24). Thus, the ability of *scw1Δ* to suppress *cdc11-123* is consistent with a model in which it does not suppress by enhancing signaling through the SIN. Together, these results suggest that Scw1p may function as an inhibitor of septum formation, such that its loss of function allows weak SIN signaling to promote septum formation.

Consistent with this model are studies published during the course of this work showing that the *scw1* mutant is resistant to cell wall-degrading enzymes, whereas SIN mutants are sensitive (23). The authors also found that *scw1Δ* rescued SIN mutants, and they proposed that it did so by restoring cell wall synthesis at the septum. Consistent with this model, we have also observed that *scw1Δ* mutants are resistant to Zymolyase treatment (data not shown), and in addition, we found that the *scw1Δ* mutation restored the septum synthesis defects of the *cps1-191* 1,3-β-glucan synthase mutant. 1,3-β-Glucan is the major component of the *S. pombe* division septum and cell wall, and previous studies have suggested that Cps1p may be a target of the SIN (29). Thus, one possible model for Scw1p function could be as a negative regulator of Cps1p, consistent with its loss of function rescuing weak activation of Cps1p by the SIN.

Given the effect of *scw1Δ* on the cell wall, it is interesting that *scw1Δ* mutants have defects in cell separation. It is not clear whether the cell separation defect is a representation of the SIN and *cps1* suppression phenotype or a separate phenotype. It is possible that Scw1p promotes septum degradation leading to cell separation, and thus loss of this function in the *scw1Δ* mutant could rescue the septum synthesis defects of the SIN and *cps1-191* mutants. Another suppressor of the SIN, the B' regulatory subunit of protein phosphatase 2A called *par1*<sup>+</sup>/*pbp1*<sup>+</sup>, also has cell separation defects (22, 25, 48). This may be

coincidental, since *par1Δ* mutations suppress only *cdc7*, *cdc11*, and *spg1* mutations (22, 25), unlike *scw1Δ* mutations, which suppress all SIN mutations. Defects in cell separation alone are unlikely to suppress the SIN, since other mutants with cell separation defects, such as septin mutants (30) and *sep1* mutants, do not suppress the SIN (44) (data not shown).

It is quite possible that Scw1p has multiple functions in the cell. We found that *scw1Δ* mutants could partially restore microtubules to the *nda3-KM311* mutant strain. This effect is not simply from stabilization of the Nda3-KM311 mutant protein, because the *scw1Δ* mutation can partially stabilize microtubules in a wild-type background treated with the microtubule-destabilizing drug MBC. As with the effects of *scw1Δ* on cell separation, it is difficult to tell whether this phenotype is connected to the ability of the *scw1Δ* deletion to suppress the SIN. The SIN seems to be inhibited by microtubule defects, and thus it is possible that stabilization of microtubules could promote signaling through the SIN (20). However, this seems unlikely, since microtubule defects seem to inhibit SIN signaling, whereas *scw1Δ* deletion does not promote signaling through the SIN.

Understanding the relationship between the different phenotypes of the *scw1Δ* deletion mutant will likely depend on characterization of the targets of Scw1p action. Database comparisons revealed that Scw1p shows homology to Whi3p and Whi4p, two *S. cerevisiae* proteins containing RNA binding domains (39). Like Scw1p, Whi3p has also been implicated in cell cycle control. Whi3p specifically binds the G<sub>1</sub> cyclin *CLN3* mRNA and localizes the *CLN3* mRNA into discrete cytoplasmic loci that may locally restrict Cln3p synthesis to modulate cell cycle progression (18). We find that Scw1p localizes to the cytoplasm; however, its localization is more diffuse than that observed for Whi3p. A similar localization pattern has been reported for another putative RNA binding protein, Sce3p, in *S. pombe*, which was isolated as a multicopy suppressor of certain alleles of *cdc7*, *cdc11*, and *sid2* (11, 42). It is possible that Sce3p overproduction and *scw1Δ* deletion could rescue the SIN by affecting a common pathway; however, the genetics suggest that the wild-type gene products would be working in opposition to each other. It will be important in future studies to determine whether Scw1p, like Whi3p, binds specific RNAs and regulates their function. The use of DNA microarray technology may be a powerful approach to address this question.

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