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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 cps1-191Δ-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 β-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 mediially 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-β-glucan, which is synthesized by the β-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-
TABLE 1. *S. pombe* strains used in this study

| Strain  | Genotype                              | Source       |
|---------|---------------------------------------|--------------|
| DM1560  | scw1-18 sid2-250 ura4-D18 h            | Lab stock    |
| DM105   | leu1-32 ura4-D18 ade6-210 h            | Lab stock    |
| DM108   | leu1-32 ura4-D18 ade6-216 h            | Lab stock    |
| DM1589  | scw1-18 ura4-D18 leu1-32 ade6-210 h    | This study   |
| DM1300  | scw1-18/scw1 sid2-216 ura4-D18/ura4-D18 leu1-32/leu1-32 h | This study   |
| DM1274  | scw1::ara4 leu1-32 ura4-D18 ade6-210 h | This study   |
| DM1349  | scw1::ara4 leu1-32 ura4-D18 ade6 h    | This study   |
| DM1392  | scw1-3HA::kan6 leu1-32 ura4-D18 ade6-210 h | This study   |
| DM1394  | scw1-L5Myc::kan6 leu1-32 ura4-D18 ade6-210 h | This study   |
| DM1115  | sid4-1A leu1-32 ura4-D18 ade6 h        | Lab stock    |
| DM1322  | scw1::ara4 sid4-1A ade6 ura4-D18 leu1-32 h | This study   |
| DM274   | cdc11-123 ura4-D18 h                   | Lab stock    |
| DM1326  | scw1::ara4 cdc11-123 ade6-210 ura4-D18 leu1-32 h | This study   |
| DM430   | spg1-106 ade6-210 ura4-D18 leu1-32 h   | Lab stock    |
| DM1412  | scw1::ara4 spg1-106 ade6-210 ura4-D18 leu1-32 h | This study   |
| DM1239  | cdc7-24 h                             | This study   |
| DM1364  | scw1::ara4 cdc7-24 leu1-32 h          | This study   |
| DM458   | sid1-125 ade6-210 ura4-D18 leu1-32 h   | Lab stock    |
| DM1318  | scw1::ara4 sid1-125 ade6-210 ura4-D18 leu1-32 h | This study   |
| DM1285  | sid1-239 ade6 ura4-D18 leu1-32 h       | Lab stock    |
| DM1366  | scw1::ara4 sid1-239 ura4-D18 leu1-32 ade6 h | This study   |
| DM436   | cdc14-118 ura4-D18 leu1-32 ade6-210 h  | This study   |
| DM1328  | scw1::ara4 cdc14-118 ade6-210 ura4-D18 leu1-32 h | This study   |
| DM429   | sid2-250 ura4-D18 leu1-32 h           | This study   |
| DM1320  | scw1::ara4 sid2-250 ade6 ura4-D18 leu1-32 h | This study   |
| DM670   | mob1::1 ura4-D18 ade6 his3-D1 + pBGMob1-ts h   | Lab stock    |
| DM1368  | scw1::ara4 mob1-1 ura4-D18 leu1-32 ade6 his3-D1 h | This study   |
| DM322   | cdc12-112 ura4-D18 leu1-32 ade6-210 h  | This study   |
| DM1370  | scw1::ara4 cdc12-112 ura4-D18 leu1-32 ade6-210 h | This study   |
| DM1372  | cdc15-140 ura4-D18 h                   | This study   |
| DM1372  | cdc15-140 ura4-D18 ade6-210 h          | This study   |
| DM1376  | cdc1-250 ade6 ura4-D18 leu1-32 h       | This study   |
| DM1268  | nda3::KM311 leu1-32 ura4-D18 ade6-210 h | This study   |
| DM1459  | cdc11-123 GFP-mob1 ade6 ura4-D18 leu1-32 h | This study   |
| DM1461  | scw1::ara4 cdc11-123 GFP-mob1 ade6 ura4-D18 leu1-32 h | This study   |
| DM1465  | cdc11-123 cdc7::GFP::ara4 ade6-210 ura4-D18 leu1-32 h | This study   |
| DM1467  | scw1::ara4 cdc11-123 cdc7::GFP::ara4 ade6-210 ura4-D18 leu1-32 h | This study   |
| DM1440  | sid2::L5Myc::kan ade6 ura4-D18 leu1-32 h | This study   |
| DM1444  | scw1::ara4 sid2::L5Myc::kan6 ade6 ura4-D18 leu1-32 h | This study   |
| DM1439  | scw1::ara4 cdc11-123 sid2::L5Myc::kan6 ade6 ura4-D18 leu1-32 h | This study   |
| DM1447  | cdc7-24 sid2::L5Myc::kan6 ade6-210 h   | This study   |
| DM1445  | scw1::ara4 cdc7-24 sid2::L5Myc::kan6 ade6-210 h | This study   |
| DM1444  | cdc15-101 ade6 ura4-D18 leu1-32 h      | This study   |
| DM1535  | cdc15-101 cdc7-24 ade6 ura4-D18 ade6-210 h | This study   |
| DM1569  | cdc7-24 ade6 ura4-D18 ade6-210 h       | This study   |
| DM1622  | cdc15-101 cdc7-24 ade6 ura4-D18 ade6-210 h | This study   |
| DM1624  | cdc15-101 ade6 ura4-D18 ade6-210 h     | This study   |
| DM1587  | cdc15-101 ade6 ura4-D18 ade6-210 h     | This study   |

The role of Scw1p in cytokinesis is demonstrated by the fact that it rescues all known SIN mutants but does not do so by restoring signaling through the SIN. Thus, the wild-type *scw1Δ* 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’s minimal medium (EMM) with appropriate supplements (36). EMM with 5 μg of thiamine per ml was used to repress expression from the *nmt1* promoter. YE containing 100 mg...
of G418 (Calbiochem) per liter was used for selecting kan<sup>+</sup>-expressing cells. Microtubule formation was inhibited by the addition of various concentrations of methyl-2-benzimidazolocarboxylate (MBC) in solid or liquid media. Synchronous populations of cells were generated by centrifugal elutriation with a Beckmann 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 TTC AAT CAC CTT TTC AAG TAT TTA AAA TTA GTG TCA AAC TCC GAC ACG ACC AGG GTT TGC CCA GTC ACG AC-3' and 5'-GGA CTT ATT GGT TGA TTA ATG ATA AAA TTA GGA AGA AAA GAT CTT AGA TGA CTT ATT TGC CAG CCG GTA ACA ATT TCA CAC AGG A-3'.

Strains expressing Swcp1 carboxy-terminally tagged with green fluorescent protein (GFP) and 13Myc were generated by PCR-based gene targeting (2) with the oligonucleotides 5'-GAC TCT TTT CTT ATT CAT ACT GTG GTA CAT AAT TGA GCC ATG CCC AGG TTA ATT AA-3' and 5'-GCT TAA CAG ATG GTT AAA GTT GCA TGC AGT CAA AGT GGA ATA GAT CGC CTA AAT CAA TCA CAC AGG A-3'.

Isolation of scw1<sup>+</sup> and cloning of scw1<sup>−</sup>. The scw1<sup>−</sup> mutation was isolated in a screen for slow-growing, multiseptate (phenotype of scw1<sup>−</sup>) transformants. Approximately 2 × 10<sup>6</sup> nitroreductase-positive (nrd2:G418<sup>−</sup>) transformants was 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 nrd2<sup>−</sup> mutation. From the remaining 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 nrd2<sup>−</sup> suppression. Complementation analysis of the remaining 21 mutants revealed that 19 fell into a single complementation group, which we later named scw1<sup>−</sup>. One of these mutants, carrying scw1<sup>−</sup>, 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 swc5<sup>−</sup> mutant background, which reduces recombination frequencies and allows for a crude map position to be determined (31). This analysis demonstrated a weaker linkage to the ura4<sup>+</sup> locus. Further mapping in a wild-type (non-sw5<sup>−</sup>) background was carried out by crossing scw1<sup>−</sup> to strains bearing mutations in the region of ura4<sup>+</sup>. This analysis showed that scw1<sup>−</sup> was tightly linked (1.1 map units; 44 parental ditype and 1 tetraploidy) to the cut1<sup>−</sup> mutation (32). Deletion of the whole scw1<sup>−</sup> ORF showed that the scw1<sup>−</sup> mutation had a cell growth defect of 25% similar to that of the nrd2<sup>−</sup> 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<sup>−</sup> 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 scw1<sup>−</sup> null mutant showed a cell separation defect similar to that of the scw1<sup>−</sup> mutant (Fig. 1A and data not shown), and it also could rescue the nrd2<sup>−</sup> mutant at 30 and 36°C (see Fig. 3). Therefore, scw1<sup>−</sup> and scw1<sup>Δ</sup> behave similarly, suggesting that scw1<sup>−</sup> is a loss-of-function mutation. To confirm that scw1<sup>−</sup> represents a mutation of scw1<sup>−</sup>, we tested whether scw1<sup>−</sup> and scw1<sup>Δ</sup> were complementing mutations. We constructed an scw1<sup>−</sup>/scw1<sup>Δ</sup> 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<sup>−</sup>/scw1<sup>−</sup> cells or wild-type cells (Fig. 1C), consistent with scw1<sup>−</sup> 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 nrd2<sup>−</sup> 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
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 specifically 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-
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+ 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-
sors, also compromises the spindle checkpoint-mediated arrest caused by inactivation of the cold-sensitive nda3-KM311 β-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 G2 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 scw1-18 mutant normally arrests without a septum and with a single nucleus (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 scw1Δ 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 Cdc7-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 G₂ 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. *scw1*-18 *nda3*-KM311 mutant cells often only partially segregated their DNA, leading to anucleate cell compartments (arrows). 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 10⁴ 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.
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).
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 β-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 β-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 scw1Δ 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 spo1-106 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/php1 (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 cpsi-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 Cpsi1p may be a target of the SIN (29). Thus, one possible model for Scw1p function could be as a negative regulator of Cpsi1p, consistent with its loss of function rescuing weak activation of Cpsi1p 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 cpsi1 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 cpsi1-191 mutants. Another suppressor of the SIN, the B’ regulatory subunit of protein phosphatase 2A called par1+/*php1+, also has cell separation defects (22, 25, 48). This may be coincidental, since par1Δ mutations suppress only cdc7, cdc11, and spo1 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 G1 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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