The Scw1 RNA-Binding Domain Protein Regulates Septation and Cell-Wall Structure in Fission Yeast

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ABSTRACT

Loss of the nonessential RNA-binding domain protein, Scw1, increases resistance to cell-wall-degrading enzymes in fission yeast. Surprisingly, *scw1* null mutations also suppress the lethality of mutations (*cdc11-136*, *cdc7-24*, *cdc14-118*, *sid1-239*, *sid2-250*, *sid3-106*, *sid4-A1*, and *mob1-1*) at all levels of the *sid* pathway. This pathway forms part of the septation initiation network (SIN), which regulates the onset of septum formation and ensures the proper coupling of mitosis to cytokinesis. In contrast, *scw1⁻* mutations do not suppress ts alleles of the *rng* genes, *cdc12* or *cdc15*. These mutations also prevent the formation of a septum and in addition block assembly and/or function of the contractile acto-myosin ring. *sid* mutants exhibit a hyper-sensitivity to cell-wall-degrading enzymes that is suppressed by loss of Scw1. Furthermore, *scw1⁻*-mediated rescue of *sid* mutants is abolished in the presence of calcofluor white, a compound that interferes with cell-wall synthesis. These data suggest that Scw1 acts in opposition to the SIN as a negative regulator of cell-wall/septum deposition. Unlike components of the SIN, Scw1 is predominantly a cytoplasmic protein and is not localized to the spindle pole body.

CYTOKINESIS requires a large network of genes that function in a highly coordinated manner (Le Goff *et al.* 1999; Balasubramanian *et al.* 2000). One of our best models of cytokinesis is the fission yeast *Schizosaccharomyces pombe*, in which the process of cytoplasmic division can be separated into four phases: (1) the selection of the site of division; (2) the assembly of an acto-myosin-based medial ring; (3) contraction of the ring concurrent with the deposition of a primary septum and two flanking secondary septa; and (4) degradation of the primary septum leading to the physical separation of two daughter cells.

Mutants specifically defective in each of these processes have been identified and constitute the *pos*, *mid*, and *pom* mutants defective in positioning of the division site (Edamatsu and Toyoshima 1996; Sohrmann *et al.* 1996; Bahler and Pringle 1998; Bahler *et al.* 1998a; Paoletti and Chang 2000); the *rng* mutants defective in assembly of the medial acto-myosin ring (Balasubramanian *et al.* 1992, 1994, 1998; McCollum *et al.* 1995; Chang *et al.* 1996; Bezanilla *et al.* 1997; Chang *et al.* 1997; Gould and Simanis 1997; Kitayama *et al.* 1997; May *et al.* 1997; Motegi *et al.* 1997; Eng *et al.* 1998; Naqvi *et al.* 1999; Wong *et al.* 2000); the *sid* (*septation initiation defective*) mutants defective in the initiation of septum deposition (Nurse and Thuriaux 1976;

Marks *et al.* 1992; Schmidt *et al.* 1997; Balasubramanian *et al.* 1998; Sparks *et al.* 1999; Chang and Gould 2000; Guertin *et al.* 2000; Hou *et al.* 2000; Salimova *et al.* 2000); and the *sep* mutants defective in cell separation (Sipiczki *et al.* 1993; Grallert *et al.* 1999; Ribar *et al.* 1999; Zilahi *et al.* 2000a,b).

The *sid* class of mutants belongs to a regulatory module (referred to as the septation initiation network, or SIN) that localizes to the spindle pole body and functions in signaling the onset of septum formation once per cell cycle. This network consists of the products of the *cdc16*, *byr4*, *cdc11*, *cdc7*, *cdc14*, *spg1/sid3*, *sid1*, *sid2*, *sid4*, and *mob1* genes (reviewed in Sawin 2000; McCollum and Gould 2001).

Sid4 and Cdc11 are the most upstream members of this network and are localized constitutively to the spindle pole body (SPB) throughout the cell cycle. Sid4 is required for the recruitment of all other tested SIN components (Cdc11, Byr4, Spg1, Cdc7, Sid1, Sid2, Mob1, and Cdc14) to this subcellular location, whereas Cdc11 is required for the recruitment of Spg1, Sid2, Cdc7, Mob1, and Byr4, but not Sid4. Sid4 and Cdc11 physically interact, suggesting that the Sid4-Cdc11 complex may act as a scaffold (Chang and Gould 2000; Sawin 2000; Krapp *et al.* 2001).

The Spg1 GTPase, which acts as an on/off switch for septum formation, is also constitutively localized to the SPB but is kept in an inactive guanosine diphosphate-bound state by the GTPase activating protein (GAP) activity of a complex formed between Byr4 and Cdc16 (Furge *et al.* 1998, 1999). Upon mitotic entry Spg1 is activated at both SPBs and in this form is able to recruit

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the protein kinase Cdc7. Intriguingly, Spg1 becomes deactivated at one SPB during anaphase, leaving only the other SPB colocalized with Cdc7 (SCHMIDT *et al.* 1997; SOHRMANN *et al.* 1998). The Cdc7 kinase is then required, after cyclin proteolysis at the end of anaphase, for the recruitment of the Sid1-Cdc14 protein kinase complex to the SPB (GUERTIN *et al.* 2000). This in turn is required for the translocation of the Sid2-Mob1 protein kinase complex from the SPB to the acto-myosin ring where it is thought to phosphorylate targets that trigger ring constriction and septum deposition (SPARKS *et al.* 1999).

Here we describe the initial characterization of an RNA-binding domain protein, Scw1, whose loss results in increased resistance to cell-wall-degrading enzymes. *scw1* null mutations are also able to suppress the lethality and septation defects associated with *sid* mutants. In this report we demonstrate that *sid* mutants themselves exhibit a decrease in resistance to cell-wall-degrading enzymes and, in addition, that loss of *scw1* counteracts this effect. Furthermore, we show that *scw1*⁻-mediated suppression of *sid* mutants is abolished in the presence of the cell-wall synthesis inhibitor, calcofluor white. These data suggest that *scw1* acts as a negative regulator of cell-wall/septum deposition acting in opposition to the SIN.

MATERIALS AND METHODS

Strains, media, and growth conditions: All *S. pombe* strains used in this study (Table 1) were derived from strains 972 *h*⁻ or 975 *h*⁺. Cells were grown in yeast extract medium supplemented with adenine (YEA; ALFA *et al.* 1993) or in appropriately supplemented Edinburgh minimal medium (EMM) adjusted to pH 5.5 or 3.5 (SALEKI *et al.* 1997). Strains were streaked to YEA supplemented with 0.5 mg/ml calcofluor white M2R (Polysciences, No. 4359) where indicated in the text. All genetic crosses and general yeast techniques were performed using standard methods (MORENO *et al.* 1991).

Isolation and cloning of the *scw1* gene: The *scw1-1* (*s*trong *c*ell *w*all) mutation was isolated in a screen analogous to SALEKI *et al.* (1997) aimed at identifying mutants blocked for cell cycle progression under conditions of lowered extracellular pH. The *scw1* gene was cloned by functional complementation of the cell size and colony morphology phenotype displayed by a *scw1-1 leu1-32 h*⁺ strain when plated on EMM pH 3.5 at 36°. Cells were transformed with a *Hin*dIII partial genomic library in pWH5 (P. G. YOUNG and D. BEACH, unpublished data) and a single leu⁺ transformant displaying a wild-type colony morphology was isolated after visually screening ~18,000 transformants. Plasmid co-loss experiments showed that the complementing activity was due to the presence of the plasmid (p5ARES) and integrative mapping showed that the plasmid had integrated at or near the *scw1* locus (indicating that it contained the *scw1*⁺ gene and not a multicopy suppressor). Recovery of the plasmid, followed by sequencing from either end of the genomic insert, comparison to Sanger Centre sequence, subcloning, and plasmid complementation, identified the *scw1*⁺ open reading frame (ORF) as SPCC16C4.07.

Disruption of the *scw1* gene: The *scw1* gene was disrupted using the one-step gene disruption method (ROTHSTEIN 1983). A 2.1-kb *Sal*I-*Sph*I fragment containing the *scw1*⁺ gene

was cloned into the unique *Sal*I and *Sph*I sites of the pGEMT plasmid (Promega, Madison, WI). The *Hin*dIII-*Nco*I fragment encompassing the translational start and 85% of the *scw1* open reading frame (including the highly conserved RNP1 and RNP2 subdomains required for RNA binding) was then replaced with a 1.8-kb *ura4*⁺ fragment PCR amplified to incorporate *Hin*dIII and *Nco*I sites at its termini (forward: 5′-ggg gga agc tta gct aca aat ccc act gg-3′; reverse: 5′-ggg ggc cat ggg ctt gtg ata ttg acg aaa ctt ttt gac-3′). The plasmid was then digested with *Sal*I and *Sph*I releasing the disruption construct, which was purified and used to transform a *ura4-D18 h*⁻/*ura4-D18 h*⁺ diploid strain using the LiAc-DMSO method (BAHLER *et al.* 1998b). Stable ura⁺ transformants were sporulated and the progeny examined by tetrad analysis. One clone displaying 2:2 segregation of wild type (associated with uracil auxotrophy) to a mutant phenotype indistinguishable from the original *scw1-1* mutant (associated with uracil prototrophy) was isolated and analyzed by PCR (forward: 5′-gta ttc tag gtt gtt gcc ctt cta att-3′; reverse: 5′-gta tcc taa aaa ttc ttg tat gta agc-3′) to confirm the disruption (data not shown). The disruption of the *scw1* gene was also confirmed genetically by constructing a *scw1-1/scw1::ura4*⁺ diploid (which was observed to display a *scw1*⁻ phenotype; data not shown).

Overexpression of the *scw1* gene: The *scw1* gene was PCR amplified (forward: 5′-gga att cca tat gtt tgt ggg atc acc gag c-3′; reverse: 5′-acg cgt cga cct att tgc cat aca tta gat tat tac ccc-3′) using the Expand high-fidelity PCR system (Roche, Indianapolis) and cloned into the unique *Nde*I and *Sal*I sites of the pREP1, pREP41, or pREP81 vectors (BASI *et al.* 1993; MAUNDRELL 1993) using standard molecular techniques (AUSUBEL *et al.* 1995). Plasmids were transformed into both *scw1-1 leu1-32* and *leu1-32* strains and leu⁺ transformants examined on EMM media in the presence or absence of 10 μM thiamine added after autoclaving.

Fluorescence microscopy: For 4′,6-diamidino-2-phenylindole [DAPI; Sigma (St. Louis) D1388] and calcofluor staining (Polysciences, No. 4359) cells were grown to mid-log phase, collected by centrifugation (3000 rpm, 5 min), washed, and then fixed by adding one-tenth volume 37% formaldehyde to cells resuspended in 1× PEM (100 mM PIPES, 1 mM EGTA, and 1 mM MgSO₄). Cells were incubated with rotation for 20 min, collected by centrifugation, and then washed and resuspended in 1× PEM. One microliter of cell suspension was then mixed on a microscope slide, with 1 μl of DAPI at a concentration of 1 μg/ml and 1 μl of calcofluor at a concentration of 2 μg/ml. Images were acquired using a Leitz DMRB fluorescence microscope (Leica Microsystems) and a high-performance cooled CCD camera (Cooke Sensicam) operated by Slidebook image analysis software (Intelligent Image Innovations). Methyl blue (Sigma M6900) staining was performed according to the protocol of KIPPERT and LLOYD (1995). Cells were collected (3000 rpm, 5 min) and washed with 10 mM Tris pH 7.6 buffer. Methyl blue was then added to the cell suspension at a concentration of 0.5 mg/ml. Images were acquired as described above. Alternatively, cells were fixed (by adding 2 volumes of ice-cold methanol to 1 volume of culture) before being washed with 10 mM Tris pH 7.6 buffer.

Scw1-GFP fusion: The *scw1* gene was PCR amplified (forward: 5′-gga att cca tat gtt tgt ggg atc acc gag c-3′; reverse: 5′-acg cgt cga cga ttt gcc ata cat tag att att acc cca ac-3′) using the Expand high-fidelity PCR system (Roche) and cloned into the unique *Nde*I and *Sal*I sites of the pREP41-GFP(S65T) plasmid (TARICANI *et al.* 2002) to create a C-terminal Scw1-green fluorescent protein (GFP) fusion. The construct was transformed into a *scw1-1 leu1-32* strain and was able to complement the strong cell-wall, colony morphology, cell length at septation, and increased septation index phenotypes of the mutant in the absence of thiamine (data not shown). The plasmid

TABLE 1

Strain list

Strain	Genotype	Source
Q250	*972 h⁻*	Lab stock
Q2106	*scw1-1 leu1-32 h⁺*	This study
Q2107	*scw1::ura4⁺ ura4-D18 ade6-216 h⁺*	This study
Q2108	*scw1-1 leu1-32 h⁺* (p5ARES int.)	This study
Q2109	*scw1::ura4⁺ ura4-D18 leu1-32 ade6-210 h⁻* (pREP81-*scw1⁺*)	This study
Q2110	*leu1-32* (pREP1) *h⁻*	This study
Q2111	*leu1-32* (pREP41-*scw1⁺*) *h⁻*	This study
Q2112	*leu1-32* (pREP1-*scw1⁺*) *h⁻*	This study
Q2113	*cdc11-136 ura4-D18 h⁺*	Lab stock
Q2114	*cdc7-24 ura4-D18 leu1-32 h⁺*	Lab stock
YDM670	*mob1-1 ura4-D18 leu1-32 ade6-216 his3-D1* (pBE*mob1*-ts) *h⁻*	D. McCollum
Q2115	*cdc12-112 ura4-D18 ade6-210 h⁻*	Lab stock
Q2116	*cdc15-140 ura4-D18 leu1-32 h⁻*	Lab stock
Q2117	*cdc14-118 ura4-D18 ade6-216 h⁻*	Lab stock
MBY 152	*sid1-239 ade6-210 ura4-D18 leu1-32 h⁺*	M. Balasubramanian
MBY 322	*sid4-A1 ade6-216 ura4-D18 leu1-32 h⁺*	M. Balasubramanian
MBY 338	*sid3-106 ade6-210 ura4-D18 leu1-32 h⁺*	M. Balasubramanian
MBY 503	*sid2-250 ade6-216 leu1-32 ura4-D18 h⁺*	M. Balasubramanian
Q2118	*cdc16-116 ura4-D18 ade6-210 h⁺*	Lab stock
Q2119	*cdc11-136 scw1::ura4⁺ ura4-D18 ade6-210 h⁻*	This study
Q2120	*cdc7-24 scw1::ura4⁺ ura4-D18 leu1-32 ade6-210 h⁺*	This study
Q2121	*mob1-1 scw1::ura4⁺ ura4-D18 his3-D1 h⁻*	This study
Q2122	*cdc12-112 scw1::ura4⁺ ura4-D18 ade6-210 h⁺*	This study
Q2123	*cdc15-140 scw1::ura4⁺ ura4-D18 ade6-216 h⁻*	This study
Q2124	*cdc14-118 scw1::ura4⁺ ura4-D18 ade6-216 h⁻*	This study
Q2125	*sid1-239 scw1::ura4⁺ ura4-D18 ade6-216 h⁻*	This study
Q2126	*sid4-A1 scw1::ura4⁺ ura4-D18 ade6-216 leu1-32 h⁺*	This study
Q2127	*sid3-106 scw1::ura4⁺ ura4-D18 ade6-216 h⁻*	This study
Q2128	*sid2-250 scw1::ura4⁺ ura4-D18 ade6-210 h⁻*	This study
Q2129	*cdc16-116 scw1::ura4⁺ ura4-D18 ade6-216 h⁺*	This study
Q2130	*sid2-250 cdc16-116 scw1::ura4⁺ ura4-D18 ade6-210 h⁻*	This study
Q2131	*cdc7-24 scw1::ura4⁺ ura4-D18 leu1-32 ade6-210 h⁺* (pREP81-*scw1⁺*)	This study
Q2132	*scw1-1 leu1-32* (pREP41-*scw1*GFP int.) *h⁺*	This study
Q2133	*cdc10-129 leu1-32* (pREP3-*cdc7*) *h⁻*	This study
Q2134	*cdc2-33 leu1-32* (pREP3-*cdc7*) *h⁺*	This study
Q2135	*leu1-32* (pREP3-*cdc7*) *h⁻*	This study
Q2136	*scw1-1 leu1-32 h⁺/scw1::ura4⁺ ura4-D18 ade6-210 h⁻*	This study

was integrated by homologous recombination and a stable wild-type leu⁺ clone whose GFP expression was independent of thiamine was chosen for analysis (this selected for clones in which the GFP-tagged version was under control of its native promoter and the mutant version was under control of the *nmt41* promoter; see DECOTTIGNIES *et al.* 2001). Integrative mapping confirmed that the plasmid had integrated at or near the *scw1* locus. Localization was monitored in EMM in the presence of 10 μm thiamine. Images were acquired as described above.

Cell-wall digestion: Cell-wall digestion experiments were performed as described in LEVIN and BISHOP (1990). Cells were grown to mid-log phase in EMM at 30°, collected by centrifugation (3000 rpm, 5 min), washed, lightly sonicated (to prevent cell clumping), and then resuspended to an optical density of ∼1.0 in 50 mM sodium phosphate/50 mM sodium citrate buffer pH 5.6 containing 0.2–0.5 mg/ml Novozym 234 (α-glucanase; Interspex Products) or in TE buffer pH 7.5 (10 mM Tris-HCl, 1 mM EDTA) containing 0.5 mg/ml Zymolyase 20T (β-glucanase; ICN Biomedicals). Digestion buffers contained β-mercaptoethanol at 0.01%. The cell suspension

was then incubated with constant shaking at 36° and cell lysis was monitored by measuring the optical density at 600 nm at the time points indicated in the text. In experiments using temperature-sensitive (ts) SIN mutants, cultures were grown in YEA at 25° and then transferred to 36° for 4 hr prior to suspension in the digestion buffer.

RESULTS

Phenotypic characterization of the *scw1-1* mutant: The recessive *scw1-1* mutation was isolated in a screen for mutants with pH-sensitive cell cycle defects (see MATERIALS AND METHODS). The mutation conferred increased cell size at division as well as an unusual, disorganized colony morphology. The phenotype was exacerbated by increased temperatures and by lowered external pHs (Figure 1A, Table 2). Logarithmically growing populations of *scw1-1* mutants also displayed an abnormally
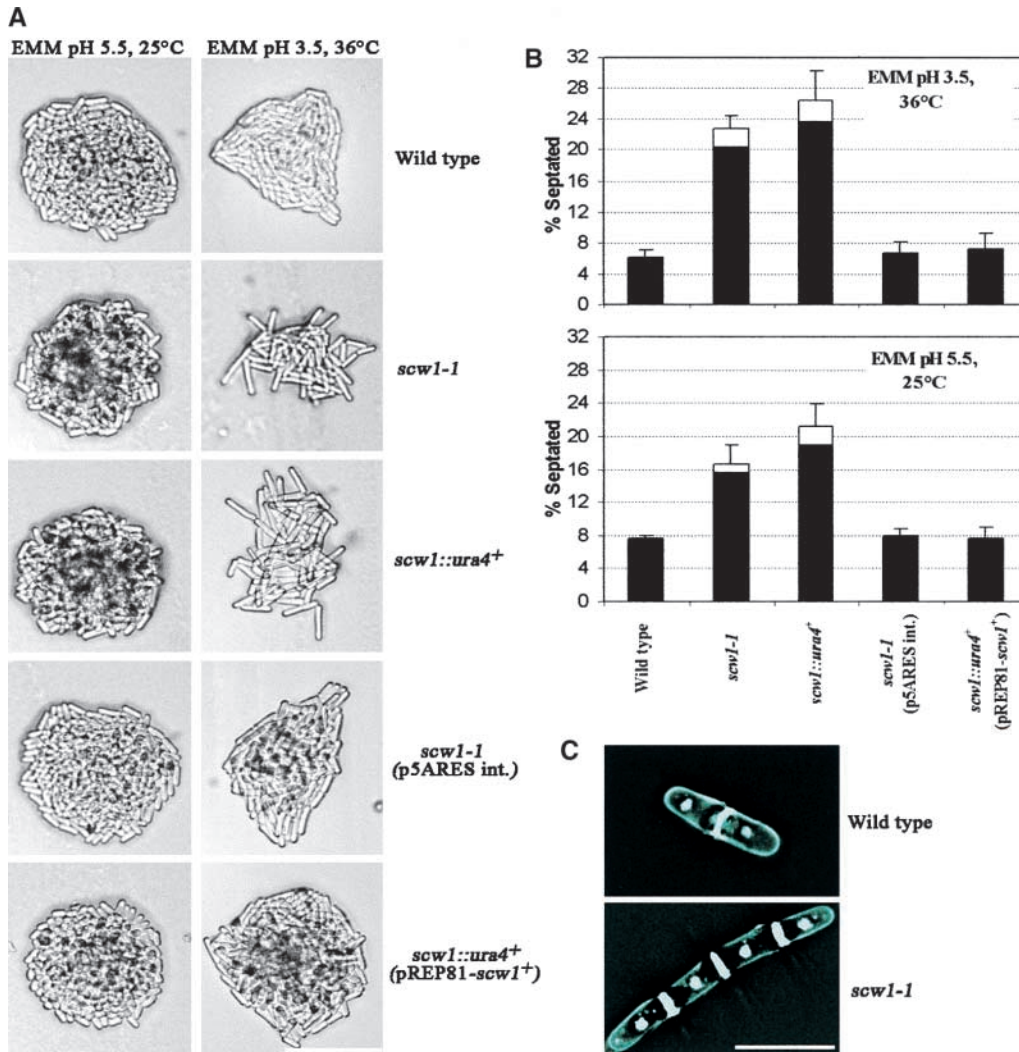
A

Figure 1.—Characterization of *scw1-1* and *scw1::ura4+* mutants. (A) Colony morphology. The indicated strains were freshly streaked to EMM pH 5.5 or EMM pH 3.5 plates and incubated for 24 hr at 25° or 36°, respectively. Unintegrated multicopy plasmids or integrated (int.) plasmids carried by the strains are shown in parentheses following the listed genotype. Bar, 100 μm. (B) *scw1⁻* mutants exhibit an increased septation index as well as cells with multiple septa. Cells of the indicated genotype were grown to mid-log phase in EMM pH 3.5 at 36° (top) or in EMM pH 5.5 at 25° (bottom). The solid portion of the bars indicates the percentage of cells containing a single septum while the open portion of the bars indicates the percentage of cells with multiple septa. Error bars indicate standard deviations. (C) Wild-type and *scw1-1* cells were grown to mid-log phase in EMM pH 3.5 at 36° before septate or multiseptate cells were stained with calcofluor (cell wall and septa) and DAPI (DNA). Bar, 10 μm.

high septation index, as well as a significant proportion of multiseptate cells (Figure 1B). Unlike other multiseptate mutants that display incorrectly placed septa both spatially and temporally (LE GOFF *et al.* 1999), the multiseptate cells in the *scw1-1* mutant invariably contained a single nucleus per compartment and had thickened septa that were symmetrically placed perpendicular to the long axis of the cell (Figure 1C).

Taken together, these data suggest that the fundamental defect in the *scw1-1* mutant was not in the control of cell cycle progression, but rather stemmed from an inability to degrade septal material in a timely manner (*i.e.*, cell separation). Since *S. pombe* grows only by extension in length at its tips this would lead to the appearance of abnormally long, or in more extreme instances (where separation was delayed past the next mitosis), to multiseptate cells.

Septal material is similar in composition to the cell wall (ISHIGURO 1998). Also, degradation of the primary septum is triggered by a localized rupture of the cell wall at the point where the cell wall and the primary septum meet (SIPICZKI and BOZSIK 2000). We therefore examined the ability of wild-type and *scw1-1* mutants to

resist the action of cell-wall-degrading enzymes. Intriguingly, the cell wall of the *scw1-1* strain showed a dramatically increased resistance to both α- and β-glucanases, supporting the hypothesis that the fundamental defect caused by this mutation is in altered cell-wall structure (Figure 2).

Cloning and disruption of the *scw1* gene: The *scw1* gene was cloned by functional complementation and shown to correspond to an open reading frame designated SPCC16C4.07 by the *S. pombe* sequencing project (see MATERIALS AND METHODS; Figure 3A). *scw1* encodes a polypeptide of 561 amino acids and contains an RNA-binding domain in its C terminus (Figure 3B). RNA-binding domains (also referred to as RNA recognition motifs or ribonucleoprotein domains) are a common eukaryotic motif found in proteins with roles in the maturation, transport, stability, localization, and translation of mRNAs (NAGAI *et al.* 1995; VARANI and NAGAI 1998).

scw1 is part of a conserved family of four genes, which includes fission yeast *mde7*, as well as the budding yeast *WHI3* and *WHI4* genes (see DISCUSSION). The four proteins are most highly similar in the RNA-binding do-
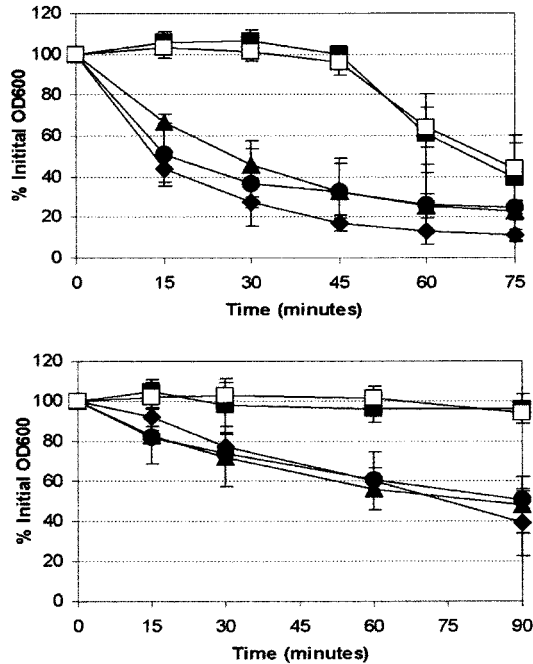
FIGURE 2.—*scw1⁻* mutants exhibit increased resistance to cell-wall-degrading enzymes. Strains were incubated in the presence of either 0.5 mg/ml Novozym 234 (top) or 0.5 mg/ml Zymolyase 20T (bottom). Cell lysis was monitored by measuring the optical density at 600 nm. Error bars indicate standard deviations. (◆) Wild type; (■) *scw1-1*; (□) *scw1::ura4⁺*; (●) wild type transformed with pREP41-*scw1⁺*; (▲) *scw1::ura4⁺* transformed with pREP81-*scw1⁺*.

main, but do show weaker similarity elsewhere in the proteins (Figure 3C, Table 3). *Caenorhabditis elegans* Mec8 also shows significant similarity to Scw1, mainly in the RNA-binding domain. In humans, Scw1 shows highest similarity to poly-pyrimidine tract binding protein. Strains carrying a disruption of the *scw1* ORF (see MATERIALS AND METHODS; Figure 3A) behaved very similarly to *scw1-1* point mutants under all conditions tested (Figure 1, A and B; Figure 2; Table 2).

Overexpression of the *scw1* gene: Overexpression of *scw1* from the strong thiamine repressible *nmt1* promoter (BASI *et al.* 1993; MAUNDRELL 1993) was lethal and could not be rescued by the inclusion of 1.2 M sorbitol to the growth media (data not shown). How-

ever, examination of cells grown for 24 hr under nonrepressing conditions revealed that 11% of the population exhibited cell-wall or septation defects (viability at this point was 21%). Of the defective class 28% exhibited incompletely formed septa (Figure 4e), 41% displayed incompletely formed septa opposite cell-wall ruptures (Figure 4, f and g), and 31% displayed cell-wall ruptures and cell lysis with little or no septal material (Figure 4h). Cells with completely formed septa were also observed and accounted for 5% of the total number of cells examined (Figure 4d).

Logarithmically growing cells expressing *scw1* from the intermediate strength *nmt41* promoter were viable, but expression did result in a slower rate of growth (∼60% that of wild type). Overexpression did not, however, result in septation defects or in a weakened cell wall relative to wild type (Figure 2; data not shown). Since the small percentage of cells exhibiting cell-wall/septation defects could not account for the large drop in viability seen upon strong overexpression, and since moderate overexpression resulted in a slow-growth phenotype in the absence of cell-wall/septation defects, we suggest that *scw1* overexpression may have a dominant negative effect due to a general interference with mRNA metabolism. Expression of *scw1* from the *nmt41* promoter, as well as expression from the weakest *nmt81* promoter, could suppress the colony morphology, cell length at septation, and strengthened cell-wall phenotypes of both the *scw1-1* point mutant and *scw1::ura4⁺* disruption (Figures 1 and 2; Table 2; data not shown).
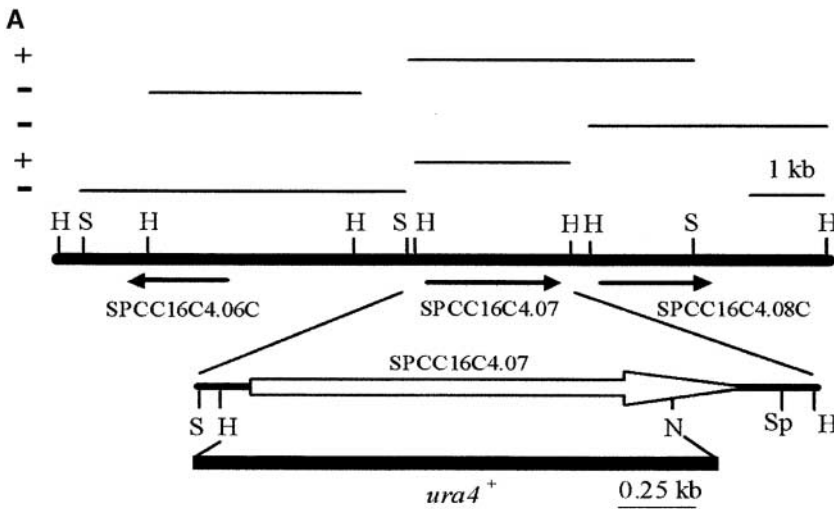
***scw1* null mutations suppress defects in the septation initiation network:** Since *scw1* loss-of-function mutants displayed defects in septation/cell separation, and since strong overexpression resulted in a proportion of cells displaying cell-wall and/or septation defects, we searched for genetic interactions between the *scw1::ura4⁺* disruption and available septation mutants. These mutants included *cdc11-136*, *cdc7-24*, *cdc14-118*, *sid1-239*, *sid2-250*, *sid3-106*, *sid4-A1*, and *mob1-1*. These mutants fall into the class of *sid* mutants, which arrest with an elongated morphology, no septa, and multiple nuclei and which eventually lyse (MARKS *et al.* 1992; BALASUBRAMANIAN *et al.* 1998; CHANG and GOULD 2000; GUERTIN *et al.* 2000; HOU *et al.* 2000). Remarkably, the *scw1::ura4⁺*

TABLE 2

Cell length of logarithmically growing uni-septate cells as a function of temperature and external pH

	EMM pH 5.5		EMM pH 3.5	
	25°	36°	25°	36°
Wild type	14.5 ± 0.8	14.8 ± 1.2	15.2 ± 1.3	15.0 ± 1.0
scw1-1	17.2 ± 3.3	22.8 ± 4.5	20.1 ± 4.6	24.1 ± 5.5
scw1::ura4⁺	18.7 ± 3.0	22.7 ± 5.4	21.4 ± 5.0	24.7 ± 6.2
scw1-1 (p5ARES int.)	14.8 ± 0.9	14.6 ± 1.2	14.9 ± 1.3	15.4 ± 1.4
scw1::ura4⁺ (pREP81-*scw1⁺*)	15.5 ± 1.8	17.9 ± 2.3	17.8 ± 2.9	17.6 ± 1.9

Micrometers ± SD.

FIGURE 3.—*scw1* encodes an RNA-binding domain protein with homology to *S. pombe* Mde7 and *S. cerevisiae* Whi3 and Whi4. (A) Schematic showing subcloning of the original rescuing genomic fragment and disruption of the *scw1* gene (SPCC16-C4.07). (+) a restriction fragment is able to complement a *scw1-1* mutant; (−) no complementation. Arrows indicate a predicted ORF. H, S, Sp, and N indicate *Hin*dIII, *Sal*I, *Sph*I, and *Nco*I restriction sites, respectively. (B) Predicted amino acid sequence of the protein encoded by *scw1*. The RNA-binding domain is indicated by boldface type. The highly conserved RNP2 and RNP1 subdomains are indicated by a single or double underline, respectively. (C) ClustalW alignment of the RNA-binding domains of *S. pombe* Scw1 and Mde7, as well as *S. cerevisiae* Whi3 and Whi4. White type on black background indicates an identity. Boldface type on a shaded background indicates a strongly conserved group (score ≥0.5 using Gonnet PAM250 matrix) whereas regular type on a shaded background indicates a weakly conserved group (a positive score <0.5 using Gonnet PAM 250 matrix).

disruption was able to suppress the lethality and septation defects associated with each of these mutations at the restrictive conditions (Figure 5A; data not shown). Importantly, the double mutants displayed an elongated, multiseptate phenotype similar to that seen in the *scw1::ura4+* single mutant.

TABLE 3

Identity to Scw1 within and outside the RNA-binding domain

	% identity within RBD	% identity outside RBD
Mde7	58	13
Whi3	53	17
Whi4	51	17
Mec8	29	13
Ptb1	18	10

RBD, RNA-binding domain.

The strength of suppression (in terms of growth) varied, depending on which *sid* mutant was tested, but did not show any relationship to the location of the gene within the *sid* pathway. The most complete suppression was seen in *scw1::ura4+ cdc11-136* and *scw1::ura4+ sid1-239* double mutants, which were indistinguishable from *scw1::ura4+* single mutants. The weakest suppression was observed in the *scw1::ura4+ mob1-1* double mutant, which grew relatively poorly and displayed cells that were both wider and more elongated than wild type. All other *scw1::ura4+ sid* double mutants displayed intermediate levels of growth between these two extremes (Figure 5A, data not shown).

Importantly, the *scw1::ura4+* disruption was unable to rescue the phenotypes associated with the *cdc12-112* (Figure 5A), or the *cdc15-140* mutations (data not shown). These mutants also arrest with an elongated morphology and no septa, but are unable to correctly form the acto-myosin ring (Marks *et al.* 1992; Fank-
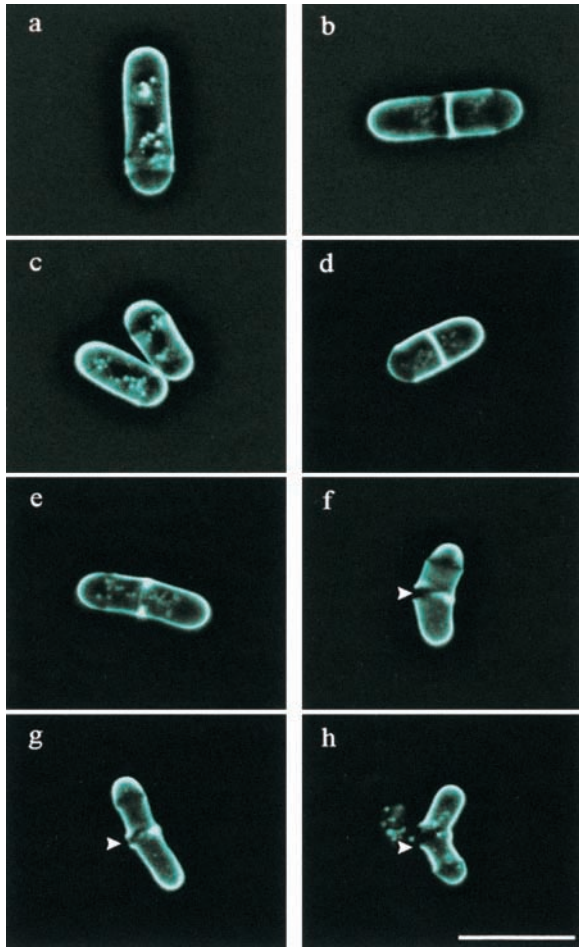
FIGURE 4.—Strong overexpression of *scw1* results in a proportion of cells displaying cell-wall and/or septation defects. Unfixed wild-type cells carrying either the empty pREP1 vector (a and b) or the pREP1-*scw1*[+] vector (c–h) were examined with the cell-wall marker methyl blue after 24 hr growth in nonrepressing conditions. Arrows indicate cell-wall ruptures. Bar, 10 μm.

HAUSER *et al.* 1995; CHANG *et al.* 1997; BALASUBRAMANIAN *et al.* 1998). These data strongly suggest that loss of *scw1* acts at the level of deposition of septal material.

To determine whether the *scw1::ura4*[+] disruption could suppress multiple defects in the SIN we mated *scw1::ura4*[+] *mob1-1* and *scw1::ura4*[+] *sid2-250* strains. Out of 20 analyzed tetrads, 14 displayed a three-to-one segregation of colony- to non-colony-forming ability (at 25°), three tetrads displayed a two-to-two segregation of this phenotype, and the three remaining tetrads displayed a four-to-zero segregation. Microscopic inspection of the non-colony-forming spores revealed that they had germinated and formed *cdc*[−] microcolonies. Genetic analysis of the viable spores in a tetratype tetrad allowed us to infer that the genotype of the *cdc*[−] microcolonies was in fact *scw1::ura4*[+] *mob1-1 sid2-250*, as expected (Figure 5B). Thus the presence of the *scw1::ura4*[+] disruption cannot entirely bypass the requirement of the SIN.

We next constructed *scw1::ura4*[+] *cdc16-116* double

mutants. At 36° *cdc16-116* strains display a terminal phenotype opposite to that of *sid* mutants, arresting with a predominantly multiseptate phenotype (MINET *et al.* 1979; MARKS *et al.* 1992; FANKHAUSER *et al.* 1993). We found that *scw1::ura4*[+] *cdc16-116* double mutants were indistinguishable from the *cdc16-116* single mutant under restrictive conditions and furthermore that the *scw1::ura4*[+] disruption did not lower the restrictive temperature of this ts *cdc16* allele (data not shown).

Interestingly, since the *sid2-250* mutation is epistatic to *cdc16-116* (TANAKA *et al.* 2001), we were also able to determine whether the *scw1::ura4*[+]-mediated suppression of the *sid* phenotype required Cdc16 activity. We found that *scw1::ura4*[+] *sid2-250 cdc16-116* triple mutants were viable under restrictive conditions (Figure 5C). Since suppression occurs in the absence of a fully functional Cdc16, this suggests that *scw1* does not normally operate through modulation of Spg1 GAP activity.

We next examined *scw1::ura4*[+] *sid* double mutants with DAPI and calcofluor 5 hr after shift to restrictive conditions. Although able to form septa, it was clear that *scw1::ura4*[+] *sid* double mutants displayed defects in the coupling of mitosis to cytokinesis. *scw1::ura4*[+] *cdc7-24*, *scw1::ura4*[+] *mob1-1*, *scw1::ura4*[+] *sid2-250*, *scw1::ura4*[+] *sid3-106*, *scw1::ura4*[+] *sid4-A1*, and *scw1::ura4*[+] *cdc14-118* double mutants all exhibited multiseptate cells with anucleate or multinucleate compartments. *scw1::ura4*[+] *cdc11-136* and *scw1::ura4*[+] *sid1-239* double mutants, which were the most strongly suppressed in terms of growth (see above), exhibited these defects to a much lesser extent and appeared similar to *scw1::ura4*[+] single mutants alone (Figure 5D, Table 4). These data demonstrate that the proper coupling of mitosis to cytokinesis can be defective in *scw1*[−] *sid* double mutants even if the lethality and septum formation defects are suppressed.

The SIN is a regulator of cell-wall integrity: Since *scw1*[−] mutants displayed increased resistance to cell-wall-degrading enzymes we examined the ability of *sid* mutants to resist degradation of their cell walls by the α-glucanase Novozym 234. We discovered that after a 4-hr incubation under restrictive conditions *cdc7-24* cells exhibited a marked decrease in resistance to the enzyme relative to wild type while *scw1::ura4*[+] *cdc7-24* cells exhibited similar levels of resistance to those seen in *scw1::ura4*[+] single mutants. Furthermore, hyper-activation of the pathway caused by loss of *cdc16* led to Novozym 234 resistance even greater than that seen in *scw1::ura4*^{+} cells (Figure 6A). These data demonstrate that the SIN module has dosage-dependent effects on cell-wall integrity.

As expected, when experiments were performed using the β-glucanase Zymolyase 20T, we found similar interactions between the *scw1::ura4*[+] disruption and the *cdc7-24* allele (with the exception that *scw1::ura4*[+] *cdc7-24* cells displayed intermediate levels of resistance between *scw1::ura4*[+] and *cdc7-24* single mutants). *cdc16-116* mutants, on the other hand, displayed a profile
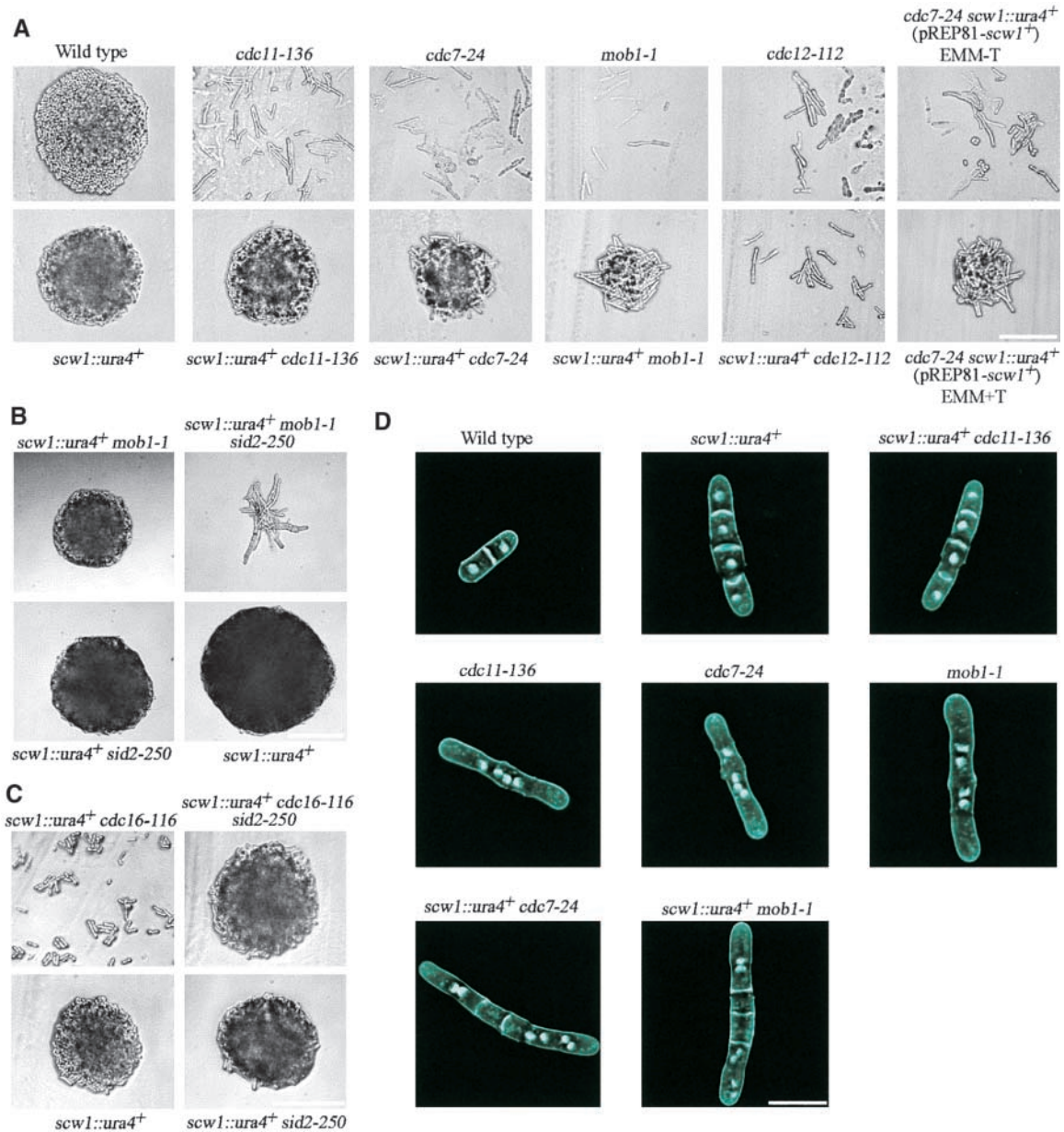
FIGURE 5.—*scw1* null mutations suppress the lethality and septation defects associated with *sid* mutants. (A) Cells of the indicated genotype were freshly streaked to YEA plates and incubated for 24 hr at 36°. As a control *scw1::ura4⁺ cdc7-24* double mutants transformed with the pREP81-*scw1⁺* plasmid were streaked to EMM in the presence (EMM + T) or absence (EMM − T) of 10 μM thiamine (to repress and induce, respectively, *scw1⁺* transcription). Bar, 75 μm. (B) Cells of the indicated genotype (from a typical tetratype tetrad derived from a cross of *scw1::ura4⁺ mob1-1* and *scw1::ura4⁺ sid2-250* strains) were examined 3 days after tetrad dissection at a temperature of 25°. Bar, 100 μm. (C) Cells of the indicated genotype (from a typical tetratype tetrad derived from a cross of *scw1::ura4⁺ sid2-250* and *scw1::ura4⁺ cdc16-116* strains) were freshly streaked to YEA plates and incubated for 24 hr at 36°. Bar, 100 μm. (D) Cells of the indicated genotype were grown to mid-log phase in YEA at 25°, shifted to 36° for 5 hr, and then stained with DAPI and calcofluor. Bar, 10 μm.

similar to wild type (with the exception in this case being that the mutant consistently displayed altered kinetics, *i.e.*, increased sensitivity at earlier time points and increased resistance at later time points). In addition we found that *cdc16-116 scw1::ura4⁺* strains displayed increased resistance over strains carrying the *cdc16-116* mutation alone (Figure 6B). The differential response of the *cdc16-116* mutant to two different cell-wall-degrad-

ing enzymes suggests that the changes in cell-wall structure associated with hyper-activation of the SIN involve more than simple increases in the deposition of cell-wall material. Rather it may involve more complex changes in cell-wall architecture. Similar results to those obtained with the *cdc7-24* mutant were observed with the other *sid* mutants used in this study (data not shown).

Since methyl blue has recently been reported to be

Cells containing anucleate or multinucleate compartments 5 hr after shift to 36° in YEA

	% cells with anucleate compartments	% cells with compartments containing two nuclei	% cells with compartments containing more than two nuclei
Wild type	0	2	0
scw1::ura4[+]	0	4.5	0
scw1::ura4[+] *cdc11-136*	0	9	5
scw1::ura4[+] *cdc7-24*	11	45	26
scw1::ura4[+] *cdc14-114*	2	31	13
scw1::ura4[+] *sid1-239*	1	13	4
scw1::ura4[+] *sid2-250*	2	29	22
scw1::ura4[+] *sid4-A1*	2	45	30
scw1::ura4[+] *sid3-106*	8	29	35
scw1::ura4[+] *mob1-1*	16	31	41

an excellent cell-wall marker (KIPPERT and LLOYD 1995; FEOKTISTOVA *et al.* 2001), we examined *sid* mutants with this dye. Interestingly, when *cdc7-24* cells at the restrictive conditions were fixed with methanol and stained, we noted that ~20% of the cells had lysed. Visible rup-

tures in the cell wall (generally in the medial regions of the cell) and the spilling of cytoplasmic contents were also observed. This phenomenon was not noted in wild-type, *scw1::ura4*[+], or *scw1::ura4*[+] *cdc7-24* cells (Figure 6C). This phenomenon was also not noted in unfixed
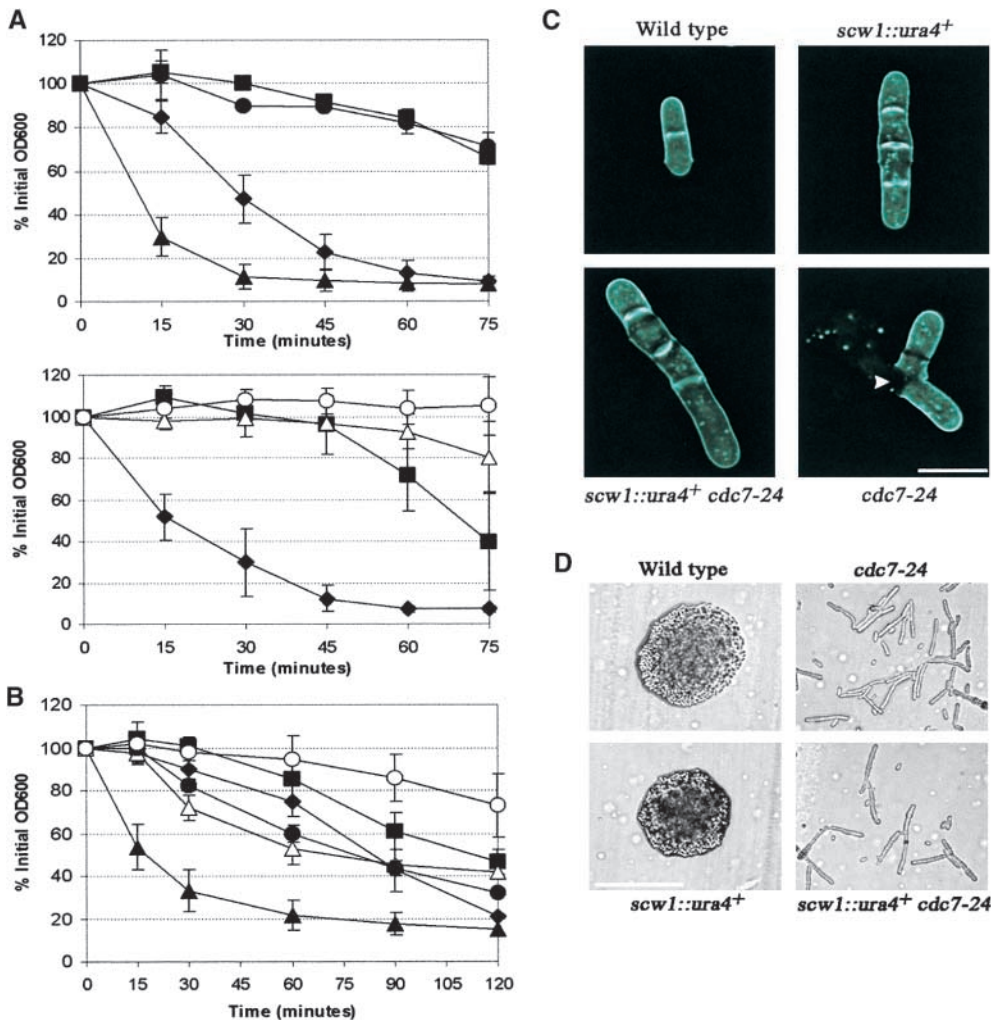

FIGURE 6.—SIN mutants display altered resistance to cell-wall-degrading enzymes. (A and B) Cell lysis was monitored by measuring the optical density at 600 nm. Error bars indicate standard deviations. (◆) Wild type; (■) *scw1::ura4*[+]; (▲) *cdc7-24*; (●) *scw1::ura4*[+] *cdc7-24*; (△) *cdc16-116*; (○) *scw1::ura4*[+] *cdc16-116*. (A) Strains were incubated as described in MATERIALS AND METHODS in the presence of 0.2 mg/ml Novozym 234 (top) or 0.5 mg/ml Novozym 234 (bottom). (B) Strains were incubated as described in MATERIALS AND METHODS in the presence of 0.5 mg/ml Zymolyase 20T. (C) Cells of the indicated genotype were grown to mid-log phase in YEA at 25°, shifted to 36° for 5 hr, fixed with methanol, and then stained with methyl blue. Arrowhead indicates cell-wall rupture. Bar, 10 μm. (D) Cells of the indicated genotype were streaked to YEA plates supplemented with 0.5 mg/ml calcofluor white and incubated for 36 hr at 36°. Bar, 100 μm.

cdc7-24 cells or in *cdc7-24* cells fixed with formaldehyde (data not shown). Therefore, although the phenomenon is artifactual, most likely due to the harsher nature of methanol fixation, it does reveal an inherent weakness in the cell wall of *cdc7-24* cells. Similar results were obtained with the other *sid* mutants used in this study (data not shown). Otherwise, results obtained using methyl blue were similar to those obtained using the more widely used cell-wall marker, calcofluor white (data not shown).

Taking all data together, our results suggested that the stronger cell wall conferred by the *scw1::ura4⁺* disruption played a key role in the observed suppression of *sid* mutations. If this were true, then one would expect conditions that act against cell-wall integrity to negatively affect this rescue. We thus examined the growth of wild-type, *scw1::ura4⁺*, *cdc7-24*, and *scw1::ura4⁺ cdc7-24* cells at 36° in the presence of 0.5 mg/ml calcofluor white. This compound (in addition to acting as a cell-wall marker) inhibits the formation of glucan and chitin microfibrils and thus interferes with cell-wall synthesis (Haigler *et al.* 1980; Roncero *et al.* 1988; Ram *et al.* 1994; Lussier *et al.* 1997). Although growth in media containing calcofluor did not affect the multiseptate phenotype of the *scw1::ura4⁺* mutant (data not shown), *scw1::ura4⁺ cdc7-24* double mutants did behave as *cdc7-24* cells and displayed a lethal *cdc⁻* phenotype (Figure 6D). Similar interactions were observed among the *scw1::ura4⁺* disruption and other *sid* mutants in media containing calcofluor (data not shown). These data strongly suggest that the *scw1::ura4⁺*-mediated rescue of *sid* mutants is achieved through altered cell-wall synthesis and/or structure.

SIN-mediated effects on cell-wall integrity are influenced by cell cycle position: The effects of *cdc7* overexpression on septation are reduced in cells arrested at the G_2/M transition (Fankhauser and Simanis 1994). Furthermore, following mitotic entry, translocation of the Sid1/Cdc14 complex to the SPB is promoted by the inactivation of Cdc2 through cyclin proteolysis (Guertin *et al.* 2000). If activation of the SIN affects cell-wall structure, then changes in cell-wall integrity mediated by *cdc7* overexpression should be influenced by cell cycle position. We thus examined the effects of increased Cdc7 levels on cell-wall integrity in populations arrested at different points of the cell cycle. Wild type, as well as *cdc10-129* and *cdc2-33* mutants (which arrest predominantly at the G_1/S and G_2/M transitions, respectively, at 36°), were transformed with the pREP3-*cdc7* plasmid (which places *cdc7* under control of the *nmt1* promoter).

Consistent with our previous results, hyper-activation of the SIN in exponentially growing cells resulted in increased resistance to Novozym 234. A similar effect was seen in cells arrested at the G_1/S transition. Intriguingly, however, the increased resistance was partially abrogated in cells arrested predominantly in G_2 (Figure 7). When analogous experiments were performed using

Zymolyase 20T we noted that exponentially growing and G_1-arrested cells displayed decreased resistance, but that G_2 arrest partially abrogated this effect (Figure 7). These data show that the effects of SIN hyper-activation on cell-wall integrity are modulated by cell cycle position.

Zymolyase 20T resistance assays were somewhat surprising since *cdc7* loss-of-function mutants displayed a similar resistance to that demonstrated by cells overexpressing *cdc7*. We interpret these results to indicate that gross disturbances of Cdc7 function (either by loss or by strong overexpression) cause drastic changes in cell-wall structure, which manifest as a decrease in Zymolyase 20T resistance. This is to say that although the loss and overexpression of Cdc7 have opposing effects, the net result is a poorly structured cell wall vulnerable to attack by Zymolyase 20T.

Subcellular localization of Scw1: Since members of the SIN localize to the spindle pole body we created a C-terminal Scw1-GFP fusion to determine its subcellular localization (see materials and methods). Unlike members of the SIN, Scw1 was predominantly a cytoplasmic protein (Figure 8). Localization was not altered as a function of cell cycle position by changes in temperature or by external pH conditions (data not shown).

DISCUSSION

The cell wall of *S. pombe* is composed mainly of polymers of 1,3-β-glucan, 1,3-α-glucan, and α-galactomannan. It serves a wide variety of functions including protection from environmental stresses, cell adhesion during conjugation and mating, and the maintenance of cellular morphology (Ishiguro 1998). Many different proteins, acting as regulators of a wide variety of pathways, have been shown to affect cell-wall structure. These include the *rho1* and *rho2* GTP-binding proteins, the *pmk1/spm1* mitogen-activated protein kinase, the *pab1* protein phosphatase 2A regulatory B subunit, and the *pck2* protein kinase C homolog, among many others (Ishiguro 1998).

In this report we show that loss of the *scw1* RNA-binding domain protein also affects the cell wall as evidenced by increased resistance to both α- and β-glucanases. The mechanism by which loss of *scw1* causes these differences is unknown, but on the basis of *scw1* encoding an RNA-binding domain protein, the differences most likely result from an indirect means related to the regulation of mRNAs. RNA-binding domain proteins have been shown to affect many distinct cellular pathways through their effects on mRNA maturation, transport, stability, localization, and translation (see Nagai *et al.* 1995 and Varani and Nagai 1998 for reviews).

The *scw1* RNA-binding domain protein isolated in this study is part of a family of four genes that include *S. pombe mde7*, as well as *S. cerevisiae WHI3* and *WHI4*. *mde7* has not been characterized in detail, but is known to be one of nine meiosis-dependent transcripts under the control of the Mei4 forkhead transcription factor
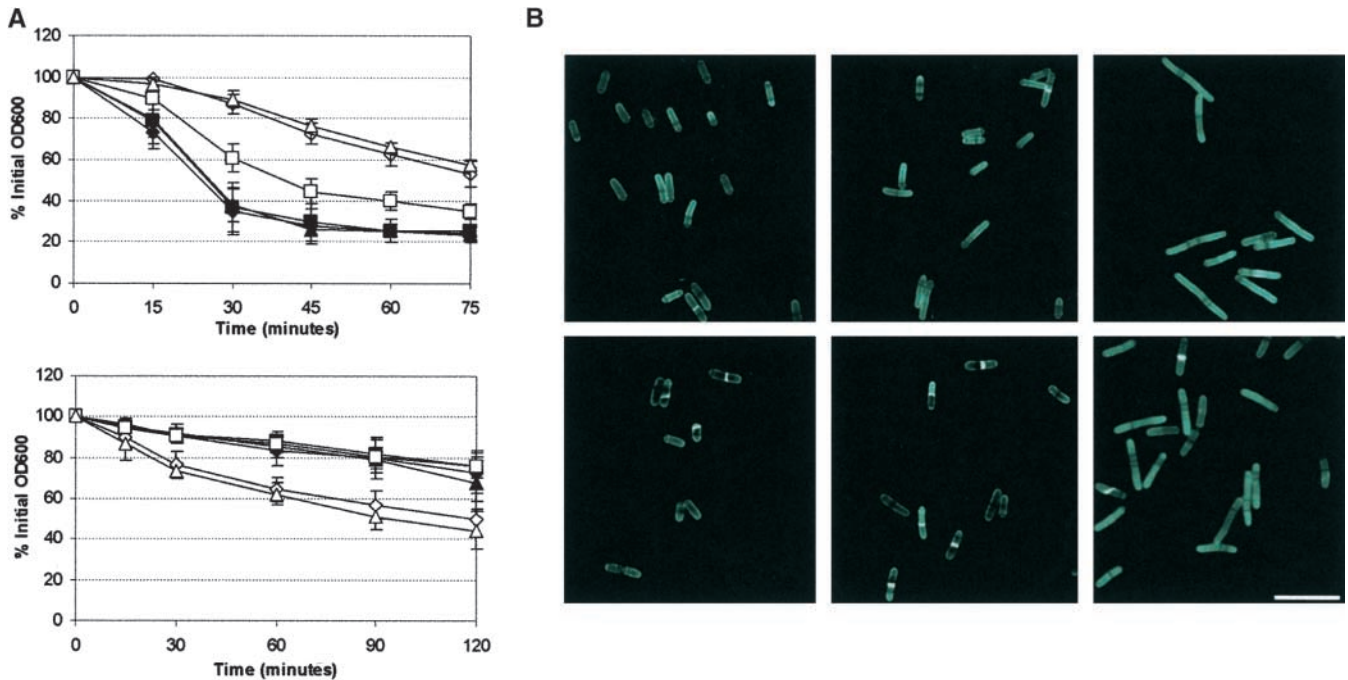
FIGURE 7.—Altered resistance to cell-wall-degrading enzymes mediated by overexpression of Cdc7 is partially abrogated in G_2-arrested cells. (A) Strains carrying the pREP3-*cdc7* plasmid were grown to mid-log phase at 25° in EMM containing 10 μm thiamine to repress *cdc7* transcription. Cells were subsequently centrifuged, washed, and used to reseed EMM cultures containing no thiamine (open symbols) or 10 μm thiamine (solid symbols). Cultures were grown to early-log phase for a further 13 hr at 25° before being shifted to 36° for 4 hr. Strains were then incubated as described in MATERIALS AND METHODS in the presence of either 0.5 mg/ml Novozym 234 (top) or 0.5 mg/ml Zymolyase 20T (bottom). Cell lysis was monitored by measuring the optical density at 600 nm. Error bars indicate standard deviations. (◆ and ◇) Wild type; (■ and □) *cdc2-33*; (▲ and △) *cdc10-129*. (B) Cells treated as in A and then stained with methyl blue 4 hr after the shift to 36°. (Top) Plus thiamine; (bottom) minus thiamine; (left) wild type; (middle) *cdc10-129*; (right) *cdc2-33*. Bar, 30 μm.

(ABE and SHIMODA 2000). In their study, sequence analysis revealed the presence of a conserved *cis*-element GTAAACAAACA(A/T)A (found in either forward or reverse orientation) starting within 26 and 282 bp upstream of the translational start in each of the nine *mde* genes. We examined the *scw1* promoter for this sequence and were able to find only a single copy of GTAAACA in an intergenic region 735 bp upstream of the translational start. Furthermore, no defects in mating or spore formation were observed in *scw1*⁻ mutants (data not shown).

WHI3 was isolated as a dosage-dependent regulator

FIGURE 8.—Scw1 is localized predominantly to the cytoplasm. Scw1-GFP-expressing cells were grown in EMM pH 5.5 to mid-log phase and images taken using either Nomarski optics (left) or fluorescence microscopy (right). Bar, 10 μm.

of cell size in the budding yeast *Saccharomyces cerevisiae* (NASH *et al.* 2001). Whi3 acts as a negative regulator of Cln3 (a G_1 cyclin) through specifically binding *CLN3* mRNA and localizing the transcripts to discrete cytoplasmic foci (GARI *et al.* 2001). *WHI4*, on the other hand, was isolated on the basis of its sequence similarity to *WHI3*. *whi3* null mutants maintain a cell size 78% that of wild type, whereas *whi4* null mutants do not have any cell size or other observable phenotype. *whi3whi4* double mutants are viable, but display an additive interaction (being 64% the size of wild-type cells; NASH *et al.* 2001). No decreased cell size phenotypes were observed for *scw1*⁻ mutants at pHs ranging from 3.5 to 5.5 or at temperatures ranging from 25° to 35°. Taken together these data suggest that (although the RNA-binding domain is conserved) these proteins have evolved unique functions in the more recent evolutionary past. In this respect it is interesting to note that in contrast to the highly conserved RNA-binding domain, flanking sequences accounting for 80% or more of the proteins show much greater levels of divergence (Table 3).

In addition to the characterization of a previously unknown protein, these results are more significant in terms of highlighting the role of cell-wall structure in the onset of septation in *S. pombe*. We are able to show

here that both *scw1*, and the SIN module itself, act as regulators of cell-wall integrity. We further demonstrate that loss-of-function mutations in *scw1*, which counteract the cell-wall changes associated with reduced SIN activity, also allow the formation of septa. Lastly, we show that conditions that negatively regulate cell-wall integrity also abolish the ability of the *scw1::ura4+* disruption to suppress *sid* mutations. These data suggest that *scw1* normally acts in opposition to the SIN as a negative regulator of septum/cell-wall deposition and/or structure. Alternatively, loss of *scw1* may bring about changes in cell-wall architecture that are more permissive for SIN-induced septum formation.

Interestingly, *scw1* is one of several recently identified genes whose loss suppresses defects in the SIN. The first, *zfs1*, encodes a zinc-finger-containing protein, which, when deleted, reduces the restrictive temperatures of ts alleles of *cdc7*, *cdc11*, *cdc14*, *spg1*, *sid1*, *sid2*, and *sid4* (*mob1* was not tested; Beltraminelli *et al.* 1999). *par1* and *par2*, on the other hand, encode B′ regulatory subunits of protein phosphatase 2A (PP2A). Cells deleted for both *par1* and *par2* are able to suppress defects in a loss-of-function allele of *spg1*, but are unable to rescue *sid* mutants downstream of *spg1*. Interestingly, *par1Δ par2Δ* double mutants (like *scw1−* mutants) display a multiseptate phenotype (Jiang and Hallberg 2001).

Whether or not *zfs1*, *par1*, or *par2* act as regulators of cell-wall structure has not been tested explicitly, but loss-of-function mutants of the *pab1* B subunit of PP2A have been shown to display reduced resistance to Zymolyase 100T, demonstrating that the cell wall is sensitive to PP2A activity (Kinoshita *et al.* 1996). Furthermore, overexpression of *pab1* can rescue the *spg1-B8* ts allele (Le Goff *et al.* 2001). These results are made even more intriguing by the fact that B and B′ subunits most likely compete for binding to the core PP2A enzyme (as has been suggested in *S. cerevisiae*; Shu *et al.* 1997; Evans and Hemmings 2000). Thus both loss of *par1* and *par2* or overexpression of *pab1* would be expected to generate PP2A holoenzymes with predominantly B subunits.

The mechanism(s) by which these genes affect the SIN is unknown, but *par1* and *par2* have been proposed to act as negative regulators either at the level of *cdc7* or at a point upstream, whereas *zfs1* has been proposed to act as a negative regulator of a SIN component, an inhibitor of a SIN substrate, or in a parallel pathway whose loss is capable of compensating for reduced SIN signaling (Beltraminelli *et al.* 1999; Jiang and Hallberg 2001; LeGoff *et al.* 2001).

On the basis of genetic analysis *scw1* is clearly acting at the level of septum deposition since *scw1−* mutations are able to suppress ts mutants at all levels of the SIN pathway and since *scw1−* mutations have no ability to suppress either the *cdc12-112* or the *cdc15-140* mutations (which cause defects in acto-myosin ring formation and/or function; Fankhauser *et al.* 1995; Balasubramanian *et al.* 1998). Genetic analysis also suggests that

Scw1 is not acting through modulation of Cdc16/Byr4 since unlike *cdc16-116 sid2-250* double mutants, which display a *sid2-250* terminal phenotype, *scw1::ura4+ cdc16-116 sid2-250* triple mutants are viable at restrictive temperatures. Also, fluorescence-activated cell sorter analysis demonstrated that wild-type and *scw1::ura4+* cells synchronized by nitrogen starvation and released into rich media at 36° progressed into the cell cycle with similar kinetics (our unpublished observations). It is thus unlikely that suppression of the *sid* phenotype is due to an indirect mechanism such as a *scw1::ura4+*-mediated increase in the duration of G_1 (which could have increased the time available to form a septum).

The ability of the *scw1::ura4+* disruption to suppress the *mob1-1* and *sid2-250* mutations singly, but not in combination, also demonstrates that the *scw1::ura4+* disruption does not simply bypass the requirement of the SIN. Instead, *scw1* most likely acts as a negative regulator of the network either at a point upstream of Sid2/Mob1 or possibly in a parallel pathway, which also plays a role in modulating cell-wall structure and counteracts the effects of the SIN. Interestingly, a role for cell-wall structure in regulating septation has been suggested by the cloning and characterization of the *cps1* gene. *cps1* encodes a 1,3-β-glucan synthase subunit whose loss results in an inability to initiate septum formation, but does not affect normal growth (Liu *et al.* 1999).

It is interesting to note that clear functional homologs of many SIN members have not been found in more complex, multicellular eukaryotes to date (Bardin and Amon 2001). Given that the module functions as a regulator of septum formation, this is not surprising since most eukaryotes do not have an analogous structure to the fungal septum or cell wall. In this respect it is interesting to note that *scw1* also has close homologs in budding yeast, but not in humans. Genes homologous to members of the SIN, however, are present in *S. cerevisiae*. These homologs form a regulatory module referred to as the mitotic exit network or MEN (reviewed in Bardin and Amon 2001; McCollum and Gould 2001).

Unlike members of the SIN, loss-of-function mutants of the MEN display a late mitotic arrest. However, a cell lysis phenotype has been observed in the MEN mutant *cdc15-lyt1*, as well as in mutant alleles of DBF2 and TEM1 (homologs of *S. pombe cdc7*, *sid2*, and *spg1*, respectively; Jimenez *et al.* 1998). Furthermore, 28–38% of *cdc15-lyt1/cdc15-lyt1* cells are present in the form of budded chains without septa under prolonged incubation at the restrictive conditions. Each compartment of the chain contains a nucleus due to leakage past the late mitotic block (Jimenez *et al.* 1998). This phenotype (*i.e.*, growth in the absence of septum formation) could be considered analogous to the *sid* phenotype observed in *S. pombe* SIN mutants. Thus, despite the fact that *S. pombe sid* mutants do not display a late mitotic arrest, other phenotypes, including growth in the absence of septum forma-

tion and cell-wall integrity defects, are applicable to at least some MEN mutants.

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