SUMO Modification of Septin-interacting Proteins in Candida albicans

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The initiation of bud and hyphal growth in the opportunistic fungal pathogen Candida albicans both involve polarized morphogenesis. However, there are many differences including the function of the septin proteins, a family of proteins involved in membrane organization in a wide range of organisms. Septins form a characteristic ring on the inner surface of the plasma membrane at the bud neck, whereas the septins are diffusely localized across emerging hyphal tips. In addition, septin rings are maintained at sites of septum formation in hyphae rather than being disassembled immediately after cytokinesis. The possibility that C. albicans septins are regulated by the small ubiquitin-like protein SUMO was examined in this study because the Saccharomyces cerevisiae septins were shown previously to be modified by SUMO (10, 26). However, SUMO conjugates to septins was not detected during budding or hyphal morphogenesis in C. albicans. These results are supported by the lack of conserved SUMO consensus motifs between septins from the two organisms even after adjusting the predicted Cdc3p and Cdc12p septin sequences to account for mRNA splicing in C. albicans. Interestingly, a homolog of the Smt3p SUMO was identified in the C. albicans genome, and an epitope tagged version of Smt3p was conjugated to a variety of proteins. Immunofluorescence analysis showed prominent Smt3p SUMO localization at bud necks and sites of septum formation in hyphae similar to the septins. However, Smt3p was primarily detected on the mother cell side of the septin ring. A subset of these Smt3p-modified proteins co-immunoprecipitated with the septin Cdc11p. These results indicate that septin-associated proteins and not the septins themselves are the key target of SUMO modification at the bud neck in C. albicans.

Candida albicans is a dimorphous yeast capable of growing in various morphologies ranging from round buds to elongated hyphae (1). The ability of C. albicans to switch between different morphologies is a significant virulence factor for this opportunistic fungal pathogen (2). Investigation of the signaling pathways has identified many components involved in regulating hyphal growth (3–8). One interesting target in these pathways is the septin family of cytoskeletal proteins (9). Septins comprise a distinct family of GTPases involved in dynamic membrane events in many eukaryotic organisms except plants (10–13). The septins were originally identified in Saccharomyces cerevisiae as genes affecting cell cycle progression and cytokinesis (14, 15). More recently, they have also been implicated in sporulation and mating pheromone-induced morphogenesis (16–20). One general function of the septins is to act as a scaffold to recruit other proteins to the bud neck including proteins required for chitin ring formation, a checkpoint for morphogenesis and cell size, regulation of cell cycle progression, and septum formation (10). The septins can also act as boundary domains that restrict protein distribution between mother and daughter cells (21, 22).

The septins have been studied most extensively in S. cerevisiae, where they display distinct patterns of localization during vegetative growth and are often used as a spatial landmark for cell cycle progression (10, 12). In unbudded cells, five septins (Cdc3p, Cdc10p, Cdc11p, Cdc12p, and Sep7p/Shs1p) coalesce into a ring on the inner surface of the plasma membrane at the site where future bud emergence will occur. As budding progresses, the septin ring extends across the mother-bud junction and then appears to split into two rings as the septum forms between the mother and daughter. The septin rings disassemble rapidly after cytokinesis and reassemble at the site of the next bud emergence. An identical pattern of septin localization was observed during budding of C. albicans (9, 23, 24). However, septins behave differently during filamentous growth of C. albicans. Rather than forming tight rings, they coalesce into a more diffuse structure at the base of emerging germ tubes and form a cap across growing hyphal tips (9, 24). As the hypha matures, characteristic double rings do form at sites of septum formation. Significantly, these rings do not disassemble, as evidenced by the appearance of multiple septin rings along the length of a hypha, each marking a septation site.

Septin protein abundance does not change significantly during budding or hyphal growth, indicating that their assembly and disassembly is regulated by other mechanisms. One possibility was suggested by the observation that three S. cerevisiae septins, Cdc3p, Cdc11p, and Sep7p/Shs1p, are modified by the Smt3p late in the budding cell cycle, at the G2/M transition (25, 26). Smt3p is a member of the SUMO family of small ubiquitin-like proteins that are conjugated to lysine residues of target proteins within the consensus sequence (I/L/V)(D/E)X(D/E) (27–29). Unlike ubiquitin, SUMO does not promote protein degradation. Rather, it typically affects protein-protein interactions and subcellular distribution (27–29). Interestingly, a mutant strain of S. cerevisiae in which the attachment sites in the septins were mutated to prevent sumoylation, septin ring disassembly was delayed, suggesting that SUMO modification...
affects septin ring stability (25). Furthermore, it was of interest to examine the role of SUMO for cytoplasmic proteins since most research to date has focused on the role of SUMO regulation of nuclear proteins. Therefore, in this study we investigated whether septin sumoylation contributed to the different pattern of septin localization and stability seen during budding versus hyphal morphogenesis in C. albicans. Interestingly, septin sumoylation was not detected in C. albicans. However, a SUMO homolog is produced in C. albicans and localizes at septation sites as it does in S. cerevisiae (25). This suggests that, despite the differences, there is a conserved function for SUMO at bud necks in these two species.

**EXPERIMENTAL PROCEDURES**

| Strain | Genotype |
|--------|----------|
| BWP17* | ura3Δ::XMM434/ura3::XMM434 his1::HIS3/arg4::HIS3 (C. albicans) |
| ySM5*  | yAW7 (cdc11D) except pHA-SMT3 |
| ySM16  | BWP17 except CDC3::pCDC3-HA |
| ySN17  | BWP17 except CDC11::pCDC11-HA |
| ySM18  | BWP17 except CDC12::pCDC12-HA |
| ySM21  | BWP17 except CDC10::pCDC10-HA |
| ySM22  | BWP17 except SMT3::pHA-SUMO |
| ySM24  | BWP17 except SEPT7::pSEPT7-HA |
| ySM26  | BWP17 except SEPT7-GFP::URA3 |
| W303-1a| MATa ade2-1 can1-100 his3-11 leu2-3,112 trpl1, ura3-1 (S. cerevisiae) |

* Wilson et al. (30).

| Strain | Genotype |
|--------|----------|
| ySM15  | BWP17 except CDC3::pCDC3-HA |
| ySM17  | BWP17 except CDC11::pCDC11-HA |
| ySM18  | BWP17 except CDC12::pCDC12-HA |
| ySM21  | BWP17 except CDC10::pCDC10-HA |
| ySM22  | BWP17 except SMT3::pHA-SUMO |
| ySM24  | BWP17 except SEPT7::pSEPT7-HA |
| ySM26  | BWP17 except SEPT7-GFP::URA3 |
| W303-1a| MATa ade2-1 can1-100 his3-11 leu2-3,112 trpl1, ura3-1 (S. cerevisiae) |

| Strain | Genotype |
|--------|----------|
| ySM15  | BWP17 except CDC3::pCDC3-HA |
| ySM17  | BWP17 except CDC11::pCDC11-HA |
| ySM18  | BWP17 except CDC12::pCDC12-HA |
| ySM21  | BWP17 except CDC10::pCDC10-HA |
| ySM22  | BWP17 except SMT3::pHA-SUMO |
| ySM24  | BWP17 except SEPT7::pSEPT7-HA |
| ySM26  | BWP17 except SEPT7-GFP::URA3 |
| W303-1a| MATa ade2-1 can1-100 his3-11 leu2-3,112 trpl1, ura3-1 (S. cerevisiae) |

* Warenda and Konopka (9).

**TABLE I**

| Strain Genotype |
|----------------|
| BWP17* ura3Δ::XMM434/ura3::XMM434 his1::HIS3/arg4::HIS3 (C. albicans) |
| ySM5* yAW7 (cdc11D) except pHA-SMT3 |
| ySM16 BWP17 except CDC3::pCDC3-HA |
| ySM17 BWP17 except CDC11::pCDC11-HA |
| ySM18 BWP17 except CDC12::pCDC12-HA |
| ySM21 BWP17 except CDC10::pCDC10-HA |
| ySM22 BWP17 except SMT3::pHA-SUMO |
| ySM24 BWP17 except SEPT7::pSEPT7-HA |
| ySM26 BWP17 except SEPT7-GFP::URA3 |
| W303-1a MATa ade2-1 can1-100 his3-11 leu2-3,112 trpl1, ura3-1 (S. cerevisiae) |

* The abbreviations used are: YPD, yeast extract/peptone/dextrose; HA, hemagglutinin; GFP, green fluorescent protein.
Whole lysates of *C. albicans* or *E. coli* expressing CDC3-HA were analyzed on a Western blot probed with α-HA monoclonal antibody 12CA5. Samples analyzed included *C. albicans* strain ySM16 that expresses CDC3-HA (lane 1), *E. coli* carrying plasmid pET-CDC3-HA (lane 2), and *E. coli* carrying pET-CDC3-HA-corrected in which the CDC3 open reading frame was adjusted to account for mRNA splicing (lane 3). Lanes 4 and 5 show control *C. albicans* strain BWP17 and control *E. coli* cells carrying the control vector pET28, respectively. The relative positions of protein standards with the indicated mass in kDa are shown to the left.

**Fig. 1. Comparison of yeast and bacterially produced Cdc3p-HA.** Whole lysates of *C. albicans* or *E. coli* expressing CDC3-HA were analyzed on a Western blot probed with α-HA monoclonal antibody 12CA5. Samples analyzed included *C. albicans* strain ySM16 that expresses CDC3-HA (lane 1), *E. coli* carrying plasmid pET-CDC3-HA (lane 2), and *E. coli* carrying pET-CDC3-HA-corrected in which the CDC3 open reading frame was adjusted to account for mRNA splicing (lane 3). Lanes 4 and 5 show control *C. albicans* strain BWP17 and control *E. coli* cells carrying the control vector pET28, respectively. The relative positions of protein standards with the indicated mass in kDa are shown to the left.

RESULTS

*C. albicans* Septins CDC3 and CDC12 Are Spliced—Comparison of the septin sequences revealed that the predicted *C. albicans* Cdc3p lacked the N-terminal ~150 amino acids found in its *S. cerevisiae* homolog. However, a C-terminally tagged Cdc3-HA protein produced in *C. albicans* was ~6 kDa larger than the bacterially produced form of Cdc3p-HA that initiated from the first ATG of the major open reading frame (Fig. 1). N-terminal protein sequencing demonstrated that the bacterially produced protein was not degraded (data not shown), suggesting that the *C. albicans* CDC3 open reading frame was longer than initially predicted.

A portion of the missing N-terminal sequences appeared to be present in an open reading frame upstream of the predicted start site for the *C. albicans* CDC3. To determine whether the CDC3 open reading frame was extended by mRNA splicing, a cDNA clone of CDC3 was obtained using a rapid amplification of cDNA ends procedure (see “Experimental Procedures”). DNA sequencing indicated that CDC3 mRNA was spliced in a manner that extended the open reading frame at the 5′ end by 50 codons (Fig. 2A). Donor and acceptor consensus splice sites were identified in the genome sequence at positions corresponding to the splice junction detected in the cDNA clone, revealing the presence of a 113-bp intron. Correction of the bacterial expression construct to include the N-terminal extension yielded a Cdc3p-HA that comigrated with the yeast form (Fig. 1). Thus, in contrast to its *S. cerevisiae* homolog, CDC3 is spliced in *C. albicans*.

Inspection of the other *C. albicans* septin genes indicated that both CDC12 and SPR28 contained splicing consensus sites. The open reading frame for CDC12 is likely extended at the 5′ end by 31 codons after excision of a 150-bp intron (Fig. 2B). The predicted SPR28 splice sites are in the 5′-untranslated region such that its open reading frame should not be affected if mRNA splicing occurs.

**SEP7 Is Expressed in C. albicans and Localizes at Bud Necks and Hyphal Septae—** Epitope-tagged versions of four of *C. albicans* septins (Cdc3p-HA, Cdc10p-HA, Cdc11p-HA, and Cdc12p-HA) showed gel mobilities consistent with their predicted molecular weight. A fifth septin, Sep7p-HA, migrated ~40 kDa higher than its predicted molecular mass of 76 kDa (Fig. 3A). Sep7p-HA produced in bacteria also migrated at an unusually high molecular weight (Fig. 3A). Similar unexpected gel mobility was also detected for Sep7-GFP, which was constructed independently by direct tagging of genomic SEP7 at the C terminus with GFP (see “Experimental Procedures”; data not shown). These data suggested that the primary structure of Sep7p caused the aberrant gel mobility. Consistent with this interpretation, Sep7p has an unusual amino acid composition relative to other *C. albicans* septins, including three stretches of six or more asparagines (see “Discussion”).

Although bacterially produced Sep7p-HA exhibited unusually slow migration on polyacrylamide gels, it was still ~10-kDa smaller than the yeast-produced protein (Fig. 3A). N-terminal protein sequencing indicated that the bacterially produced protein was not degraded (data not shown). One possibility is that Sep7p-HA gel mobility is modified by phosphorylation, although no cell cycle-regulated shift in mobility was observed for Sep7p-HA. Another possibility is that this minor difference is due to the deviation from the standard genetic code in *C. albicans* in which CUG codons code for serine instead of leucine (34, 35). The *C. albicans* and bacterially produced versions of Sep7p-HA are expected to differ at four positions. Perhaps these differences influence the structural properties that cause the aberrant mobility of Sep7p (see “Discussion”).

To determine whether Sep7p localized to the same sites as other septins during budding and filamentous growth of *C. albicans*, a strain expressing the SEP7-GFP was observed by fluorescence microscopy. As shown in Fig. 3B, Sep7p-GFP localized as distinct rings at the necks of budding cells in a pattern identical to that reported for other septins in *C. albicans* and *S. cerevisiae* (9, 10, 24). In serum-induced hyphal cells, Sep7p-GFP localized as a band at the base of emerging germ tubes in 80% (n = 72) of cells examined (Fig. 3C). Later in hyphal growth, Sep7p-GFP localized as distinct rings at sites of septation in 92% (n = 100) of cells examined (Fig. 3D). Thus, Sep7p localizes in an identical pattern to other septins during filamentous growth (9, 24).

**C. albicans Septins Are Not Detectably Sumoylated during G2 Arrest—** Because previous studies demonstrated that the *S. cerevisiae* Cdc3p could be sumoylated at four different sites in *G*2 (25, 26), we investigated whether *C. albicans* Cdc3p-HA was also sumoylated under the same conditions. To test this, a logarithmic culture was arrested with nocodazole for 3 h, and then cell lysates were analyzed by Western blot. SUMO is ~10 kDa, and its conjugation to a target protein is readily detected by a gel mobility shift. Western blot analysis found no evidence for SUMO modification of Cdc3p-HA in nocodazole-arrested cells (Fig. 4A). As a control, we showed that sumoylation of Cdc11p was readily detectable from a nocodazole-arrested culture of *S. cerevisiae* (Fig. 4B), in agreement with previous work. This indicates that the failure to detect sumoylation of *C. albicans* Cdc3p-HA was not due to an inability to detect SUMO modification.

To test whether any of the other *C. albicans* septins is sumoylated in *G*2, Western blots were performed to analyze HA-tagged Cdc10p, Cdc11p, Cdc12p, and Sep7p from nocodazole-arrested cultures (Fig. 4A). Surprisingly, and in contrast to *S. cerevisiae*, we did not detect gel mobility shifts large enough to be caused by SUMO for any of the septins tested, even upon overexposure of the blots. Control experiments to assess the sensitivity showed that septin proteins were readily detected on Western blots even after diluting the samples 1:500, indicating that SUMO modification on even a minor fraction of the protein would have been detected (data not shown). Consistent with our inability to detect SUMO modification of *C. albicans* septins, the SUMO sites used in *S. cerevisiae* septins are not conserved in the *C. albicans* homologs (see “Discussion”).

**C. albicans Septins Are Not Detectably Sumoylated during Hyphal Growth—** SUMO modification of septins was next examined during hyphal growth to determine whether this could account for either the diffuse localization of septins at the base of the emerging germ tubes and across the growing hyphal tip...
or the greater stability of the septin rings at sites of septation in mature hyphae (9, 24). For this analysis, a fresh overnight culture of cells expressing HA-tagged septins was induced with serum to form hyphae at 37°C for times between 0 and 180 min that correspond to specific stages of hyphal growth and septin localization (Fig. 5). At 0 min, cells have not formed germ tubes, and septin localization was not detectable within the cell. At 30–60 min, most cells formed germ tubes with septins diffusely localized at the base and across the growing hyphal tip. After 90–120 min, most hyphae formed at least one septum marked by a distinct septin ring. By 180 min, mature hyphae with multiple septin rings, each marking a septation site, were present. Western blot analysis of extracts of the corresponding cells did not detect SUMO modification, as measured by gel mobility shift of any of the septins at any of the times examined (Fig. 5). Smaller gel mobility shifts were sometimes observed, particularly at lower exposures of the blots (not shown), suggesting that other modifications such as phosphorylation might occur.

**C. albicans Smt3p Is Produced and Localizes at the Bud Neck**—The lack of detectable septin sumoylation led us to investigate whether SUMO protein was produced in *C. albicans*. A *C. albicans* homolog of *SMT3*, the *S. cerevisiae* gene encoding SUMO (36), was identified using a tBLASTn search against the Stanford Genome Technology center website at www-sequencce.stanford.edu/group/candida. Alignment of the predicted protein sequence revealed 62 and 48% amino acid identity with *S. cerevisiae* Smt3p and human SUMO-1, respectively (Fig. 6). Importantly, *C. albicans* Smt3p contains the essential GG motif at its C terminus (27). No other open reading frames with...
significant homology were identified.

*C. albicans* SMT3 was tagged at its N terminus with the triple-HA epitope and expressed in the wild type strain BWP17. Western blot analysis showed a ladder of proteins representing multiple targets for Smt3p modification, as expected (Fig. 7). Interestingly, differences in the pattern of Smt3p-modified proteins were noticed in nocodazole-arrested and serum-stimulated cultures (Fig. 7). For example, a band appeared at ~45 kDa during G2 arrest with nocodazole, and a 50-kDa band disappeared during serum induction. These differences could result from cell cycle-regulated changes in sumoylation of target proteins or from changes in target protein production and stability. Both of these proteins migrate lower than the predicted M, for a SUMO-modified protein.

Because septins are not obvious targets of Smt3p in *C. albicans*, we examined whether Smt3p would still localize to the bud neck of G2-phase cells as in *S. cerevisiae* (25, 26). Interestingly, immunofluorescence analysis detected HA-Smt3p at the bud neck in 73.5% (n = 219) of cells that were arrested in G2 with nocodazole for 3 h (Fig. 8, B and C). Large-budded cells from a logarithmic culture also showed HA-Smt3p staining at the neck, demonstrating that its localization was not an effect of nocodazole treatment (data not shown). Control experiments showed that this staining was not observed in strains lacking the HA-Smt3p construct (Fig. 8A). Significantly, HA-Smt3p was found primarily on the mother side of the junction. Staining on the daughter side was detected occasionally, but the staining was always significantly stronger on the mother side (Fig. 8, compare B and C). These results are consistent with the observed pattern of Smt3p localization in *S. cerevisiae* (25, 26).

Analysis of HA-Smt3p localization in serum-induced hyphal cells failed to detect significant staining at the base of germ tubes in early hyphae at the site where septins localize (data not shown). However, clear rings of Smt3p localization were observed at sites of septation in more mature hyphae in a pattern reminiscent of septin localization during budding (Fig. 8E). No signal was detected in strains lacking the HA-Smt3p construct (Fig. 8D). Thus, the *C. albicans* Smt3p SUMO only appeared to colocalize with septins when they are assembled into a tight ring.

**Modification of Septin-associated Proteins by Smt3p**—The similar bud neck localization of Smt3p-modified proteins and the septins suggested the possibility that they interact directly. To test this, we examined whether Smt3p-modified proteins would co-immunoprecipitate with the Cdc11p septin. Cell extracts from log-phase cells were immunoprecipitated with anti-Cdc11p antibodies and then analyzed on a Western blot probed with anti-HA to detect several Smt3p-modified proteins including a prominent ~90-kDa band (Fig. 9A). A similar band was also detected in nocodazole-arrested cells and in cells that were induced to form hyphae in Lee’s medium (45). Control experiments showed that no Smt3p-modified proteins were detected in samples prepared from *cdc11Δ* cells that lack Cdc11p (Fig. 9B). Thus, a subset of the Smt3p-modified proteins co-precipitated with Cdc11p.

**DISCUSSION**

**Septin mRNA Splicing in C. albicans**—Analysis of *C. albicans* septins revealed that CDC3 undergoes mRNA splicing and that the CDC12 and SPR28 septin genes contain consensus splice sites and are likely to undergo mRNA splicing as well. This was unexpected in that the corresponding homologs in *S. cerevisiae* are not spliced. The open reading frames of CDC3 and CDC12 are extended by 50 and 31 codons, respectively, whereas the SPR28 coding sequence is unaffected. The introns in the *C. albicans* septins are all predicted to be at the 5' ends of the genes, similar to most spliced genes in *S. cerevisiae* (37). It has been proposed that the 5' bias of intron position in *S. cerevisiae* genes might result from homologous integration of reverse-transcribed pseudogenes (37). Given that *C. albicans* and *S. cerevisiae* are relatively close species, it seems likely that a similar pattern will emerge for spliced genes in *C. albicans*.

Analysis of *C. albicans* SEP7—Septin—*C. albicans* septins differed from the other *C. albicans* septins in that it migrated on gels at ~40-kDa higher than its predicted M,. Unexpectedly slow gel mobility was also seen for Sep7p-GFP and for Sep7p-HA produced in bacteria, suggesting that aberrant migration is an intrinsic property of the protein and is not likely to result from mRNA splicing or post-translational processing. Consistent with this, Sep7p has an unusual amino acid sequence (Ref. 9 and GenBankTM accession number AY112707). For example, it contains three separate stretches of six or more asparagine residues. Furthermore, the *S. cerevisiae* Sep7p/Shs1p does not share the unusual sequence characteristics of *C. albicans* Sep7p, and it migrates close to its predicted molecular mass of ~63 kDa (25, 38). Despite this unusual gel mobility, a GFP-tagged version of *C. albicans* Sep7p localized in a pattern identical to other septins in both budding and hyphal cells (Fig. 3). This distinctive property of the Sep7p does not correlate with a key functional property in vivo since previous gene deletion studies demonstrated that SEP7 does not play an obvious role in *C. albicans* morphogenesis as do the CDC3, CDC10, CDC11, and CDC12 septins (9).

**Role of SUMO at Bud Necks**—Septin protein levels remain relatively constant during the different phases of budding and hyphal growth (Figs. 4 and 5), suggesting that other mechanisms such as post-translational modification regulate septin activity. One known form of post-translational modification of
septins in \textit{S. cerevisiae} is sumoylation (25). However, our analysis found no evidence for SUMO modification of septin proteins in \textit{C. albicans} either during G2 arrest or hyphal growth (Figs. 4 and 5). Thus, it seems likely that other factors may regulate septin function. For example, smaller gel mobility shifts of septins observed during serum induction (data not shown) suggest the possibility that phosphorylation could contribute to septin regulation. Consistent with this, phosphorylation of Cdc3p, Cdc10p, and Sep7p in \textit{S. cerevisiae} has been reported (38–40). Septins might also be regulated by virtue of their interactions with other proteins. For example, Int1p is a septin-binding protein that plays a role in controlling hyphal growth in \textit{C. albicans} (23).

Failure to detect septin sumoylation in \textit{C. albicans} is consistent with the lack of conservation of SUMO modification consensus sites between the \textit{S. cerevisiae} and \textit{C. albicans} homologs. In \textit{S. cerevisiae}, Cdc3p contains four SUMO sites within its N terminus, whereas Cdc11p and Sep7p are each sumoylated once (25, 26). Significantly, \textit{C. albicans} Cdc3p lacks the sequences corresponding to the SUMO sites in the N terminus of \textit{S. cerevisiae} Cdc3p (Fig. 2) and does not contain any other SUMO consensus sequences. The \textit{C. albicans} Sep7p also lacks SUMO consensus sites. Cdc11p possesses two SUMO consensus sequences (IK\textsuperscript{315}LE and LK\textsuperscript{379}FD), but their sequence and position are not strictly conserved with the \textit{S. cerevisiae} Cdc11p site (IK\textsuperscript{412}QE). Interestingly, Cdc12p contains a SUMO consensus sequence (VK\textsuperscript{355}AE) that is conserved in its \textit{S. cerevisiae} homolog (VK\textsuperscript{358}AE). However, these sites are not detectably used in either organism (Fig. 4 and Ref. 25).

Inability to detect sumoylation of \textit{C. albicans} septins is also consistent with the apparent dispensability of this modification for \textit{S. cerevisiae} septin function. Although SUMO gene SMT3 is required for viability of \textit{S. cerevisiae}, septin sumoylation is not essential. Mutation of the SUMO attachment sites in the septins to prevent their sumoylation caused a delay in post-cytokinesis ring disassembly but not obvious defects in growth rate or in cellular morphology (25). In addition, deletion of two E3 ligases that mediate SUMO conjugation in \textit{S. cerevisiae}, SIZ1 and SIZ2, appeared to abolish septin sumoylation but caused...
Septin Function in C. albicans

Fig. 9. Coimmunoprecipitation of a SUMO-modified protein with Cdc11p. A, lysates from log phase, nocodazole-arrested, or Lee’s medium-induced cultures of control strain BPW17 (lanes 1, 3, and 5) or strain ySM22 expressing HA-SMT3 (lanes 2, 4, and 6) were immunoprecipitated using α-Cdc11p polyclonal antibody and analyzed by Western blot using α-HA monoclonal antibody 12CA5. B, α-Cdc11p immunoprecipitation and α-HA Western blot from wild type and cdc11Δ strains expressing HA-SMT3.

only mild growth defects at low temperature (41–43°C). Thus, the functional significance of septin sumoylation in S. cerevisiae is unclear.

It was interesting that in C. albicans, HA-Smt3p still localized to bud necks and at septation sites in hyphae despite the fact that the septins are not detectably modified by Smt3p. This indicates that another bud neck protein(s) is the main target of SUMO modification. In fact, several Smt3p-modified proteins co-immunoprecipitated with Cdc11p, including a major band at ~90 kDa. Attempts to identify this band by mass spectrometry approaches were not successful. The difficulty in identifying this protein may be due in part to the fact that the C. albicans genome has not been fully annotated yet so it is not clear to what extent the identified open reading frames are affected by splicing or DNA sequence errors. Another complication is that septin-binding proteins are either not very abundant or do not copurify efficiently, as demonstrated by previous purification of septin complexes, which do not contain other readily observed proteins (44). Immunoprecipitation of HA-Smt3p with anti-HA antibodies revealed that only a minor fraction (0.5–1%) of Cdc11p coprecipitated with SUMO-modified proteins under the conditions used (data not shown). One possibility is that the SUMO-modified protein interacts with only a minor fraction of the total Cdc11p present at the bud neck. Alternatively, the SUMO-modified protein might interact preferentially with filamentous septin complexes that are disrupted under the detergent conditions used in the immunoprecipitation.

As an alternative approach to examine the role of SUMO modification of bud neck proteins, we inspected the C. albicans homologs of the more that 40 bud neck proteins that have been identified in S. cerevisiae (10). Consensus sites for SUMO modification are present in several proteins including Gin4p, Bni4p, and Cdc6p. These proteins are also interesting candidates in that they are detected at the mother cell side of the bud junctions, similar to Smt3p early in the cell cycle. At later stages these proteins are found on both sides of the bud junction, suggesting that a potential function of Smt3p may be to regulate this change in localization. The estimated Mₐ of C. albicans Cdc6p most closely matched the predicted Mₐ of the prominent Smt3p-modified band that co-precipitated with Cdc11p. However, we were not successful in obtaining convincing evidence of Smt3p modification of Cdc6p, perhaps because only a fraction of the Cdc6p localizes to the bud neck. Nonetheless, these results are significant in that they indicate a role for SUMO in the regulation of cytoplasmic proteins, whereas most studies to date have focused on the regulation of nuclear proteins by SUMO. The absence of detectable SUMO modification of septins in C. albicans will likely be an asset for future studies in that novel SUMO targets can be identified and analyzed without mutating the septins themselves.

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