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Report

Septin Stability and Recycling during Dynamic Structural Transitions in Cell Division and Development

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Summary

Septins are conserved proteins found in hetero-oligomeric complexes that are incorporated into distinct structures during cell division and differentiation; yeast septins Cdc3, Cdc10, Cdc11, and Cdc12 form hetero-octamers and polymerize into filaments, which form a "collar" at the mother-bud neck [1]. Posttranslational modifications, nucleotide binding, and protein-protein and protein-lipid interactions influence assembly and disassembly of septin structures [2], but whether individual septins are used repeatedly to build higher-order assemblies was not known. We used fluorescence-based pulse-chase methods to visualize the fate of pre-existing (old) and newly synthesized (new) molecules of two septins, Cdc10 and Cdc12. They were recycled through multiple mitotic divisions, and old and new molecules were incorporated indistinguishably into the collar. Likewise, old and new subunits intermixed within hetero-octamers, indicating that exchange occurs at this organizational level. Remarkably, in meiosis, Cdc10 made during vegetative growth was reutilized to build sporulation-specific structures and reused again during spore germination for budding and during subsequent mitotic divisions. Although Cdc12 also persisted during sporulation, it was excluded from septin structures and replaced by another subunit, Spr3; only new Cdc12 populated the collar of germinating spores. Thus, mechanisms governing septin incorporation are specific to each subunit and to the developmental state of the cell.

Results and Discussion

To monitor the fate of Cdc12 in time and space in live cells, we constructed a functional Cdc12-AGT chimera. The AGT tag, commercially available as the SNAP-tag, is a domain of human O^6-alkylguanine-DNA alkyltransferase that can covalently couple to benzylguanine (BG) and its derivatives [3]. Cdc12-AGT was fluorescently labeled in vivo via its reaction with fluorophore-conjugated BG-related molecules (either BG-DAF or TMR-*Star*) taken up from the culture medium. Despite a steady-state abundance similar to untagged Cdc12, as judged by immunoblotting of cell extracts (Figure S1, available online), efficient labeling of plasmid-expressed Cdc12-AGT was observed (Figure S1 and Table S1) only when the endogenous *CDC12* gene was absent and extrusion of these dyes was prevented by removal of the genes (*PDR5*, *SNQ2*, and *YOR1*) encoding three different plasma-membrane efflux pumps [4]. For pulse-chase analysis, the *cdc12*Δ *pdr5*Δ *snq2*Δ *yor1*Δ strain expressing Cdc12-AGT was incubated with a fluorescent BG derivative for 30 min (approximately one-third of a cell cycle), washed, and followed through multiple subsequent mitotic divisions in the absence of label. Fluorescent Cdc12-AGT localized to the mother-bud neck as a ring in unbudded and newly budded cells, as an hourglass-shaped collar in cells with medium-sized buds, and as a pair of split rings in large-budded cells (Figure 1 and Figure S1), patterns indistinguishable from those of Cdc12-GFP [5] (Figure S1) and consistent with localization of native Cdc12 determined by indirect immunofluorescence and electron microscopy [6]. Cdc12-AGT labeled during the 30 min pulse was still readily detectable at the bud neck after more than five cell doublings (Figure 1A) and became undetectable only after approximately eight divisions (Figure 1A). The rate of this observed decrease in the level of the fluorescent signal is fully compatible with simple dilution by newly synthesized, unlabeled Cdc12-AGT during the course of each division, rather than with active removal of the older molecules by degradation. Consistent with our findings, the "decay" rate reported for Cdc3 is also well explained by simple growth dilution (see Supplemental Discussion). Also in accord with our results, it has been reported that Cdc3, Cdc10, Cdc11, and Cdc12 molecules labeled with ^{15}N and then chased were not degraded, although these were followed for only one cell cycle [7].

Importantly, regardless of the number of generations after labeling, as long as fluorescence could be visualized, the signal was roughly equivalent in every cell in the population (data not shown; see Figure S2D), indicating that Cdc12-AGT molecules were partitioned into mother and daughter approximately equally. If, for example, old Cdc12-AGT molecules were preferentially localized to the mother side of the bud neck, as proposed by others (see Supplemental Discussion), the fluorescent signal ought to be brightest in the oldest mothers, and the buds of the newest daughters ought to display little or no signal, a pattern we did not observe. Nonetheless, to definitively determine whether old and new septin molecules are segregated within the otherwise symmetrical structures that contain them, we devised and applied a dual pulse-labeling procedure to differentially label pre-existing and newly made septin molecules. Cells expressing Cdc12-AGT were first tagged with a fluorescent BG derivative of one color, washed, chased for two divisions in the absence of label, and then pulse-labeled with an equally reactive fluorescent BG of another color. Essentially every available Cdc12-AGT molecule was fluorescently marked during exposure to the first label because, in the absence of a chase, no labeling with the second label was detectable (Figure S1B). We determined empirically that a chase of approximately two generations allowed for synthesis of a sufficient amount of new Cdc12-AGT molecules to achieve readily detectable labeling with the second dye, while retaining a readily detectable level of the Cdc12-AGT molecules labeled with the first dye. Using these conditions, we found that, regardless of whether Cdc12-AGT was labeled first with BG-DAF and later with TMR-*Star* or vice versa, the pattern of localization of old and new molecules was indistinguishable at both the center and the outer edges of the septin collar at the bud neck

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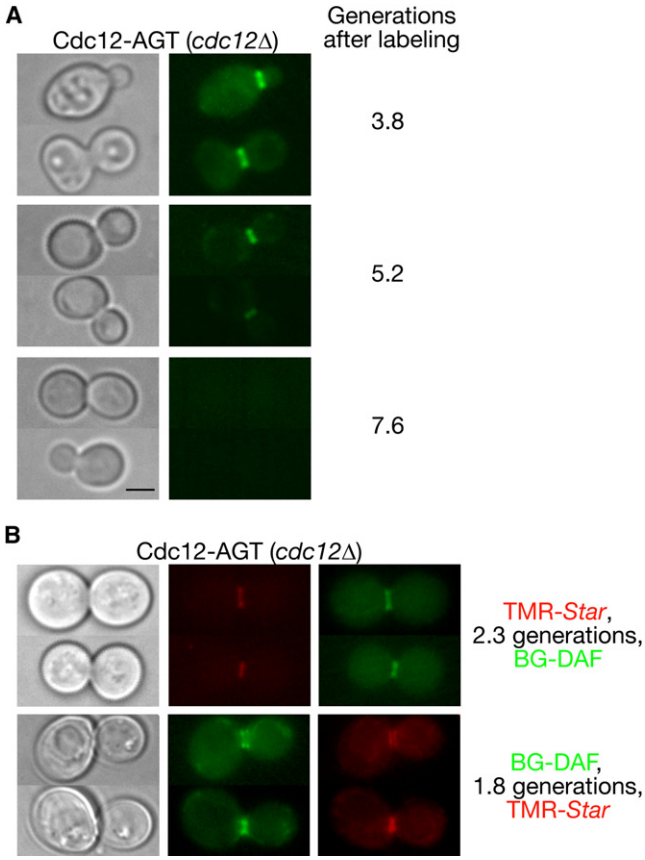

Figure 1. Cdc12-AGT Molecules Persist through Multiple Cell Divisions with Negligible Turnover and Colocalize with Newly Synthesized Cdc12-AGT

(A) Cells (*pdr5*Δ *snq2*Δ *yor1*Δ *cdc12*Δ) carrying YCp*CDC12-AGT* were exposed to 5 μM BG-DAF for 30 min at 30°C, washed thoroughly, incubated in fresh medium for 30 min at 30°C, washed again, and then resuspended in fresh medium and examined by transmitted light (left) or fluorescence microscopy (right) after undergoing the indicated number of doublings.

(B) Cells labeled with TMR-*Star* (upper panels) or BG-DAF (lower panels) and incubated for the indicated number of doublings as in (A) were labeled for 30 min with either BG-DAF (upper panels) or TMR-*Star* (lower panels) before viewing with appropriate filters. Representative cells are shown. The scale bar represents 2 μm.

(Figure 1B). Thus, at this resolution, new Cdc12-AGT and old molecules inherited from prior divisions were incorporated equivalently and isotropically.

The five septins in mitotic cells (Cdc3, Cdc10, Cdc11, Cdc12, and Shs1/Sep7) are homologous and colocalize at the bud neck [1]. Cdc10 is the smallest and, unlike the others, lacks a C-terminal extension with a recognizable coiled-coil domain, but it is nonetheless important for the integrity and stability of higher-order septin structures in vivo and in vitro [8, 9]. To determine whether the behavior we observed for Cdc12-AGT could be generalized, we performed analogous experiments in *pdr5*Δ *snq2*Δ *yor1*Δ cells carrying at the endogenous *CDC10* locus an integrated functional *CDC10-AGT* fusion, which was expressed at a level similar to untagged Cdc10 (Figure S2A). Upon in vivo labeling with BG-DAF (not shown) or TMR-*Star* (Figure S2B), Cdc10-AGT was readily detectable at the bud neck in a pattern equivalent to that previously reported for a functional Cdc10-GFP fusion [10]. After pulse-labeling, subsequent diminution of the signal occurred with kinetics consistent with division-dependent dilution, and Cdc10-AGT were partitioned into mother and daughter in approximately equal amounts (Figure S2D), all as observed for Cdc12-AGT. Likewise, when these cells were subjected to the sequential two-color labeling regimen, old and new Cdc10-AGT molecules were incorporated equivalently into the septin collar (Figure S2C, top).

Although the majority of the Cdc10-AGT molecules were tagged with the first label (TMR-*Star*) during our standard exposure time, some labeling with the second label (BG-DAF) was detectable even in the absence of a chase (Figure S2A), suggesting that labeling of Cdc10-AGT is somewhat slower than that of Cdc12-AGT. Given that we have recently demonstrated that a Cdc10 doublet occupies the central position in every rod-like septin hetero-octamer, whereas Cdc12 occupies the penultimate position at each end of the rod [11], it is possible that in this context, the AGT domain attached to the short Cdc10 C terminus is somewhat less accessible than when attached to the extended C-terminal tail of Cdc12. However, accessibility of Cdc10-AGT and Cdc12-AGT to labeling was not dependent on the nature of the septin structure (i.e., single ring, collar, or split ring) at the time of labeling because septin structures diagnostic of every cell-cycle stage containing either Cdc10-AGT or Cdc12-AGT were labeled upon exposure of cells to the fluorescent dye for just 30 min, a period significantly shorter than a full cell cycle (~90–120 min; data not shown).

Yeast septins are phosphorylated and SUMOylated, modifications correlated with the transitions that septin-containing structures undergo during cell-cycle progression [1]. Some of these modifications and certain septin-associated proteins are found preferentially on one side or the other of the bud neck [12, 13]. Given that our pulse-chase analysis revealed that Cdc10-AGT and Cdc12-AGT are extremely stable and that pre-existing molecules inherited from a previous division are incorporated indistinguishably from the newly made molecules, the striking cell-cycle-dependent changes in the state of septin organization cannot arise from wholesale removal of old molecules and resynthesis of new septin monomers. Rather, our results demand that periodic changes in the supramolecular architecture of septin-containing structures must be driven by cell-cycle-dependent posttranslational modifications that are erased and reimposed with each cell division, as originally suggested by Dobbelaere et al., who used a different and less direct approach [14]. Likewise, observed asymmetries in the association of other bud-neck-localized proteins must be dictated by additional cues and processes aside from the septins themselves.

The nature of the tag itself or the procedure used to study the septin-AGT chimeras could influence septin stability and contribute to the uniform inheritance we observed. To rule out this possibility, we fused the same septins to different tags and devised an independent approach to monitor the persistence of old septins and the fate of newly made septins. This method, which used the *HO* promoter to drive septin expression in a haploid-, cell-cycle-, and mother-cell-specific manner (see Supplemental Data), corroborated the finding that new and old septins are incorporated simultaneously into the filaments in the septin collar at the bud neck. Thus, septins must be recruited from a common pool generated, for old septins, by recruitment from previously existing structures and, for new septins, by their de novo synthesis.

Our finding that old and new septin molecules colocalize may explain the results of fluorescence recovery after photobleaching (FRAP) experiments, which demonstrated rapid

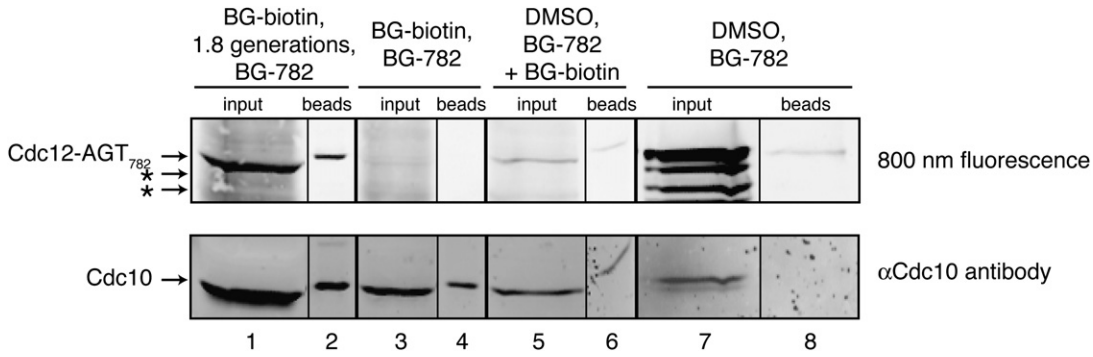

Figure 2. New Cdc12-AGT Molecules Exchange into Premade Hetero-Octameric Septin Complexes

Exponentially growing 5 ml cultures (in YPD) of haploid *pdr5*Δ *snq2*Δ *yor1*Δ *cdc12*Δ cells expressing Cdc12-AGT were treated as follows. BG-biotin (2 μM) was added to one culture and solvent alone (DMSO) was added to three others. After incubation at 26°C for 1 hr, the DMSO-treated cultures were frozen, and the BG-biotin-treated culture was washed, resuspended in fresh growth medium, and incubated at 26°C for a chase period of 1.8 divisions. All cultures were lysed, BG-782 was added to a final concentration of 3.3 μM (and, where indicated, BG-biotin was also added to the same final concentration; lanes 5 and 6), and the labeling was allowed to proceed for 30 min at RT before the NaCl concentration was increased to 1 M and biotinylated complexes were isolated by the addition of streptavidin-coated agarose beads. Bead-bound complexes ("beads") and samples with 10% of the labeled, salt-adjusted lysate ("input") were resolved by SDS-PAGE, transferred to a nitrocellulose filter, and incubated with Cdc10 primary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). After incubation with an infrared-dye-tagged (680 nm emission) anti-goat IgG antibody, an infrared imaging system (Odyssey; LI-COR, Lincoln, NE) was used to visualize immune complexes and 782-labeled Cdc12-AGT. Asterisks indicate two degradation products of Cdc12-AGT. For ease of comparison, intervening lanes were removed from the image. Slight variations in the mobility and appearance of the same protein in different lanes are attributable to differences in the salt concentrations present in the samples.

mobility of GFP-tagged septins prior to and after formation of the septin-filament-containing collar at the bud neck [14, 15]. However, at what level does the intermixing of old and new septins that we observed occur? Are newly made septin subunits able to exchange into a premade hetero-octamer, which we have shown [11] serves as the building block that polymerizes into filaments? Or, once assembled, is a hetero-octamer stable, whereas the filaments are dynamically unstable [16], allowing for reshuffling of new and old hetero-octamers within them? As one approach to address these issues, we used a variation of our Cdc12-AGT pulse-chase method to determine whether newly made Cdc12-AGT molecules can be incorporated into salt-stable hetero-octamers that contain premade Cdc12-AGT subunits.

Cells expressing Cdc12-AGT were pulse-labeled with a BG derivative carrying a biotin moiety, chased, and then lysed. The Cdc12-AGT molecules made during the chase were tagged in the cell lysate with a cell-impermeable BG derivative (BG-782) that is conjugated to a dye absorbing in the near-infrared range. All the available Cdc12-AGT molecules were efficiently biotinylated during the pulse because no incorporation of the infrared dye label was detectable in the lysate in the absence of a prior chase period (Figure 2, lanes 3 and 4). In lysates from cells not previously treated with BG-biotin, coupling of Cdc12-AGT to BG-782 was efficient because after incubation with this label in vitro, no molecules could be subsequently reacted with another cell-impermeable BG derivative conjugated to a different infrared dye (data not shown).

After the sequential-labeling regimen, the lysate was adjusted to 1 M NaCl, a condition under which filaments dissociate into hetero-octamers, which are very stable [8, 11, 17]. Individual hetero-octamers were then recovered via binding of the premade (biotinylated) Cdc12-AGT to streptavidin-coated agarose beads, and the content of newly made (BG-782-tagged) Cdc12-AGT was assessed by SDS-PAGE and infrared scanning of the resulting protein profile (Figure 2). After normalizing to the input and correcting for the background, we found that the amount of infrared-labeled Cdc12-AGT present in the bead-bound complexes (as well the content of other septins, e.g., Cdc10) was comparable between the pulse-chase samples (Figure 2, lanes 1 and 2) and control complexes (Figure 2, lanes 5 and 6), which were prepared by incubating cell lysates in vitro with equal concentrations of BG-biotin and BG-782 simultaneously. Because each hetero-octamer contains two molecules of each of the four core septins (Cdc3, Cdc10, Cdc11, and Cdc12) [11], detection of the new (infrared-tagged) Cdc12-AGT in the purified hetero-octamers containing the old (biotin-tagged) Cdc12-AGT demonstrates that individual subunits must be able to exchange into hetero-octamers in vivo.

Two septins required for mitotic division, Cdc3 and Cdc11, are also found in a series of distinct structures that surround the four spores produced by meiosis in diploids, along with two other septins, Spr3 and Spr28, expressed only during this developmental process [18, 19]. Although *CDC10* transcription is strongly induced during sporulation and *cdc10* mutants are sporulation defective [19, 20], the presence of Cdc10 in sporulation-specific structures had not been examined before. We found that Cdc10-GFP and Cdc10-mCherry localize to the developing prospore membranes in immature asci, as well as around and between mature spores (Figures 3A, 3C, and 3F), consistent with the patterns reported for the other septins in sporulation [18, 19].

At the onset of this developmental transition, the septin complexes made in diploid cells during vegetative growth must be reorganized to accommodate Spr3 and Spr28. Conversely, upon returning to the mitotic division necessary for spore germination, sporulation-specific changes presumably need to be reversed. To determine how individual septins behave through this series of developmental transitions, we also used our pulse-chase approach. Cdc10-AGT was labeled by incubation of vegetatively growing diploids with fluorescent BG and, after washing, the cells were followed through sporulation and germination. Labeled Cdc10-AGT was incorporated into the same spore-associated structures occupied by the other septins that participate in sporulation and, furthermore,

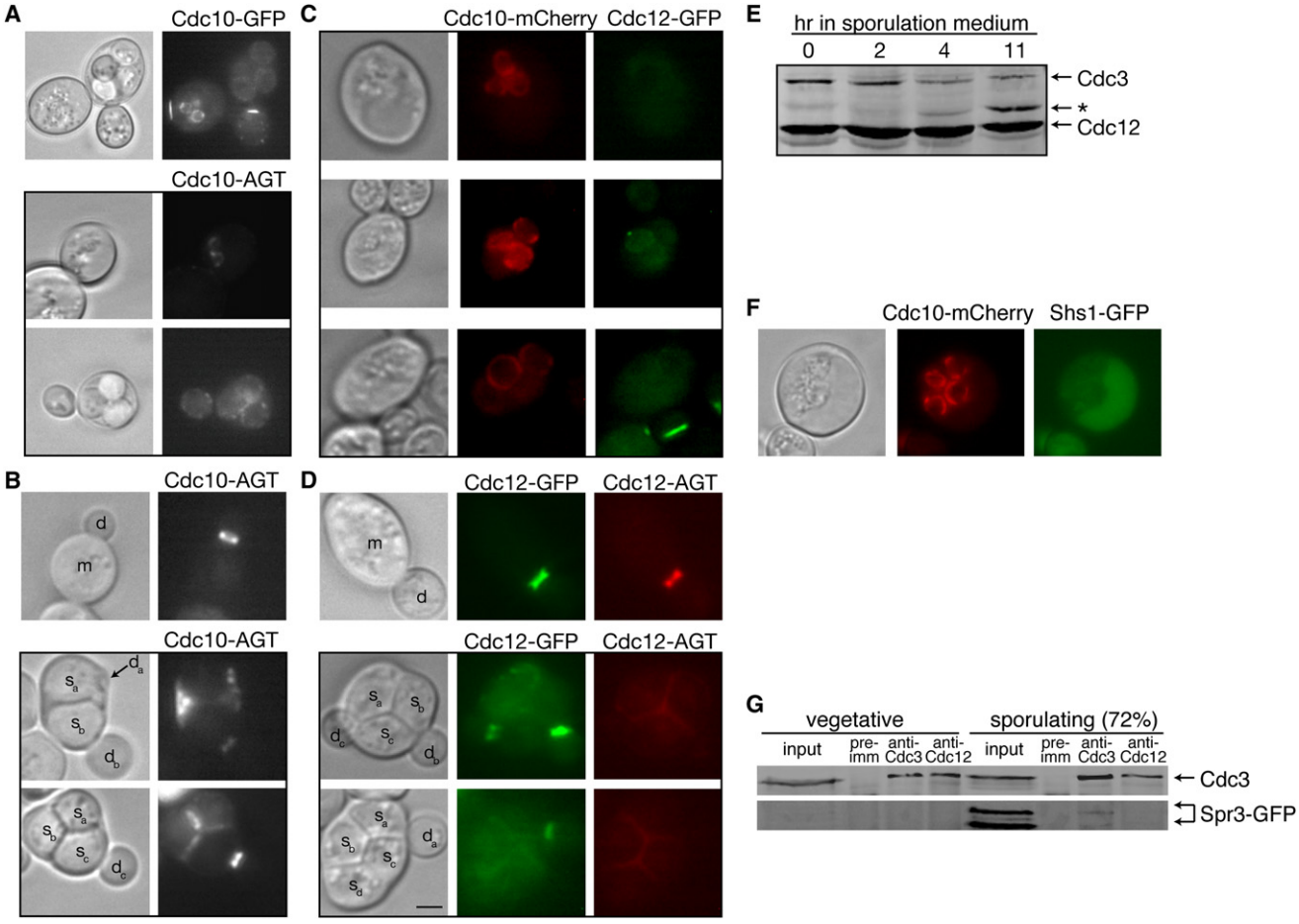

Figure 3. Persistence and Reincorporation of Premade Cdc10-AGT, but Not Cdc12-AGT, from Vegetative Growth to Sporulation and Germination

(A) A culture of diploid cells expressing Cdc10-GFP (top panel) was shifted to sporulation-inducing medium to visualize Cdc10-containing structures at various stages of meiosis and sporulation. A culture of vegetatively growing diploids expressing Cdc10-AGT (bottom panels) was labeled with 5 μM TMR-*Star* for 30 min, washed thoroughly, shifted to sporulation medium, and viewed at various stages of meiosis and sporulation.

(B) Cells from the culture expressing Cdc10-AGT shown in (A) were shifted back to solid growth medium on a microscope slide and incubated at 30°C. The top panel shows a diploid cell (m) that had not undergone meiosis in the sporulation medium and that resumed budding to form a diploid daughter (d). The bottom panels show haploid spores (s_x) that germinated to produce their first haploid daughters (d_x).

(C) As in (A), except the vegetative diploids expressed both Cdc10-mCherry and Cdc12-GFP.

(D) As in (B), except the vegetative diploids expressed both Cdc12-GFP and Cdc12-AGT. The scale bar represents 2 μm.

(E) SK-1-background diploids (JTY4308) were induced to sporulate as described in the Supplemental Experimental Procedures, and the progress of sporulation, monitored by staining of nuclear DNA with 4',6-diamidino-2-phenylindole (DAPI), was found to be complete by 11 hr (data not shown). At the indicated times, 5 ml samples were withdrawn, cells were collected, and lysates were prepared. Portions (~100 μg protein total) of each lysate were resolved by SDS-PAGE, transferred to a nitrocellulose filter, and incubated with rabbit polyclonal Cdc12 antibodies and rabbit polyclonal antibodies directed against a peptide corresponding to the 14 C-terminal residues of Cdc3 (Table S4). Immune complexes were visualized as in Figure 2. For ease of comparison, intervening lanes were removed from the image. The asterisk marks an unidentified molecule (~52 kDa) induced during sporulation and recognized by the Cdc12 antibodies.

(F) As in (A), except the vegetative diploids expressed both Cdc10-mCherry and Shs1-GFP.

(G) Mixed BY4741 × SK1-background diploids expressing Spr3-GFP (JTY4520) were grown exponentially in YPD ("vegetative") or induced to sporulate ("sporulating [72%]") as described in the Supplemental Experimental Procedures; 5 ml or 25 ml of culture, respectively, was collected, and lysates were prepared. After increasing the NaCl concentration of 1 mg aliquots of lysate to 0.5 M, immunoprecipitations were performed with ammonium-sulfate-purified IgG isolated as described in the Supplemental Experimental Procedures from rabbits prior to antigenic stimulation ("pre-imm") or after exposure to purified full-length Cdc3 or the 338 N-terminal residues of Cdc12 (Table S4). Bead-bound complexes and 20% of the input lysate ("input") were resolved and detected as in Figure 2, with the same Cdc3 antibody used for immunoprecipitation and a mouse monoclonal antibody recognizing GFP.

was incorporated into the bud neck of germinating spores (Figure 3). Thus, premade Cdc10-AGT molecules were not only used to construct sporulation-specific structures but were also mobilized thereafter for assembling the bud-neck-associated structures necessary for mitotic division. Thus, the same septin can be reused in multiple organizational contexts and during distinct developmental transitions.

Like Cdc10, the involvement of Cdc12 during sporulation had not been examined previously. No upregulation of *CDC12* transcript level occurs during sporulation [18, 21, 22], suggesting either that the supply of Cdc12 made prior to this developmental transition is sufficient for sporulation-specific events or that Cdc12 is not involved in the assembly of sporulation-specific structures. To distinguish between these possibilities, we examined diploids expressing Cdc12-GFP and co-expressing Cdc10-mCherry as an internal control. Strikingly, little or none of the Cdc12-GFP colocalized with the prominent spore-associated structures marked with Cdc10-mCherry

(Figure 3C). To determine whether the inability to detect Cdc12 in these structures was due to its destruction, we examined diploid cells of the SK-1 lineage, which sporulate quite synchronously and efficiently [23]. We found that Cdc12 was quite stable during sporulation, as judged by immunoblotting (Figure 3E).

Given that the premade Cdc12 persists during sporulation, we next examined whether those molecules could be incorporated into the bud neck of germinating spores. For this purpose, diploid cells expressing Cdc12-AGT were prelabeled with TMR-*Star* during vegetative growth, washed, and then subjected to sporulation. These cells also coexpressed Cdc12-GFP (as a constitutive marker for newly made septin-containing mitotic structures). Upon germination of the resulting spores, no old (TMR-*Star*-labeled) Cdc12-AGT was detectable at the neck of the first buds, which were prominently marked with Cdc12-GFP (presumably made de novo) (Figure 3D, lower panels). The lack of detectable fluorescent Cdc12-AGT was not due to decay of the TMR-*Star* label during the period (up to 5 days) required for sporulation and germination because occasional vegetative diploids remaining in the culture even under sporulation conditions were robustly labeled at their bud necks with both Cdc12-GFP and TMR-*Star*-tagged Cdc12-AGT (Figure 3D, upper panel). Furthermore, the lack of incorporation of premade Cdc12-AGT was not due to any deficiency in the function of this chimeric protein (as compared to Cdc12-GFP) because no TMR-*Star*-tagged Cdc12-AGT was found in spore-associated structures or at the bud neck of germinating spores in cells in which Cdc12-GFP was not expressed (data not shown). Finally, the lack of incorporation of the TMR-*Star*-labeled Cdc12-AGT argues against the possibility that the observed incorporation of TMR-*Star*-tagged Cdc10-AGT (Figure 3A) was the trivial result of inefficient wash-out of the TMR-*Star* dye. Together, these observations indicate that Cdc12 becomes functionally inactivated at some point during the sporulation process.

Unlike that of *CDC3*, *CDC10*, *SPR3*, and *SPR28*, and more akin to that of *CDC12*, *SHS1* expression is repressed during sporulation [21, 22]. Using the same approach just described, we found that, like Cdc12-GFP, Shs1-GFP did not colocalize with the spore-associated structures marked with Cdc10-mCherry (Figure 3F), but rather was present in the ascal cytoplasm, which is not encapsulated within spores [24, 25]. Thus, during sporulation, Cdc12 and Shs1 seem to be sequestered away from the other septins by localization in a distinct cellular compartment.

Our results are consistent with our previous suggestion [1, 26], posited on the basis of sequence similarities and known septin-septin interactions, that Cdc12 is displaced by Spr3 and Shs1 is displaced by Spr28 to form novel sporulation-specific hetero-octamers. Exchange of Spr3 and Spr28 for pre-existing Cdc12 and Shs1 in hetero-octamers in meiotic cells would be analogous to the exchange of old and new Cdc12 molecules we observed in the hetero-octamers made in mitotic cells (Figure 2). Indeed, in agreement with our proposal, we detected Spr3-GFP coimmunoprecipitation with Cdc3, but not with Cdc12, in lysates of a culture of sporulating *SPR3-GFP/+* diploid cells (Figure 3G). The substoichiometric amount of Spr3 recovered by coimmunoprecipitation with Cdc3 may indicate that Spr3-Cdc3 interaction is more salt-labile than the Cdc12-Cdc3 interaction.

In addition, consistent with the fact that Cdc3 and Cdc12 form a stoichiometric complex that is essential for hetero-octamer formation in mitotic cells [9], we found that both Cdc12 antibodies and Cdc3 antibodies coprecipitated equivalent amounts of Cdc3 when lysates were prepared from vegetatively dividing diploid cells, whereas Cdc12 antibodies precipitated only ~25% of the amount of Cdc3 as that precipitated with the Cdc3 antibodies when lysates were prepared from a sporulating culture of diploid cells (Figure 3G and data not shown). Moreover, in the sporulating cultures used for such experiments, 25%–30% of cells did not sporulate, as shown by the fraction of the total cells counted (n = 200 per experiment) that lacked evidence of spore formation visible by transmitted light microscopy or lacked detectable Spr3-GFP fluorescence (data not shown). This level of sporulation proficiency (75%) is nearly identical to that reported by others for this same mixed-strain background [23]. Thus, these data support our conclusion that in sporulating cells, Cdc12 does not associate stably with Cdc3 and exists free of other septins (or is associated with Shs1, which is also excluded from spore-associated structures).

Are similar mechanisms of subunit recycling and exchange at play in the dynamics of other complex cellular structures? Like septins, monomers of actin, tubulin, and certain histones are extremely long-lived proteins [27, 28]. We have now shown that individual old and new septin subunits can be recycled equivalently into different higher-order structures, as is true for actin in microfilaments, tubulin in microtubules, and histones in nucleosomes. However, perhaps unexpectedly, we showed that even the hetero-octameric building block of septin supramolecular structures is quite dynamic in this regard. Most strikingly, we also found that assembly of developmentally specific structures involves the displacement (but not the degradation) of specific septin subunits and their replacement by others. Thus, during development, pre-existing and newly made molecules become differentially marked with regard to their competence for incorporation into larger structures. Similar mechanisms may regulate the assembly of cell-type-specific septin hetero-oligomers from the wide variety of subunits expressed in human cells (see Supplemental Discussion). The approaches we present here could also be used to determine whether exchange in vivo of old and new molecules of tubulin or histones occurs within their respective salt-stable hetero-oligomers and, if so, whether cell-type-specific isoforms of these proteins persist after their displacement.

Supplemental Data

Supplemental Data include Supplemental Results, Supplemental Discussion, Supplemental Experimental Procedures, three figures, and four tables and can be found with this article online at http://www.current-biology.com/cgi/content/full/18/16/1203/DC1/.

Acknowledgments

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