Chaperone Requirements for De Novo Folding of Saccharomyces cerevisiae Septins

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)
RE: Manuscript #E22-07-0262
TITLE: “Chaperone Requirements for De Novo Folding of Saccharomyces cerevisiae Septins”

Dear Michael:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

This is an important set of experiments with tremendous relevance for understanding not only septin complex assembly but many stoichiometric quaternary assemblies. I think you did an outstanding job of addressing the review commons reviews and the paper is greatly improved. Thank you for submitting this excellent study to MBoC.

Sincerely,
Amy Gladfelter
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. McMurray:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

Your paper is among those chosen by the Editorial Board for Highlights from MBoC. Highlights from MBoC appears in the ASCB Newsletter and highlights the important articles from the most recent issue of MBoC.

All Highlights papers are also considered for the MBoC Paper of the Year. In order to be eligible for this award, however, the first author of the paper must be a student or postdoc. Please email me to indicate if this paper is eligible for Paper of the Year.

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We are pleased that you chose to publish your work in MBoC.

Sincerely,
Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

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1. General Statements [optional]

We are sincerely grateful to the reviewers for several key comments that led us to correct some mistakes and better appreciate how to put our findings in the context of recently published data. These changes undoubtedly improved the manuscript.

Many other reviewer comments seem to equate chaperone binding with a functional chaperone role in *de novo* folding. These are not the same. Cytosolic chaperones presumably “sample” nearly every protein that is synthesized by cytoplasmic ribosomes. This does not mean that every such protein would misfold if even one of those chaperones failed to bind it. If we want to understand what chaperone mutations might cause human disease due to septin misfolding, for example, it will not be enough to catalog all the chaperones that bind septins. We have already done that. **What will help** is to understand which chaperones make functional contributions to septin folding and complex assembly. **Our study is the first to experimentally address** chaperone roles in *de novo* septin folding, period. We take responsibility for not being sufficiently clear about the goals of our work, and, to emphasize these points, we added one sentence to the Introduction and revised another.
Another consistent criticism was that the use of the E. coli system, both in vivo and in vitro, limited our ability to gain insight into the folding of septins in eukaryotic cells and led to a “tessellated view”. For example, reviewers claimed that our model about translation elongation rates for Cdc12 were “based mainly on the E. coli system and bioinformatics analysis”. We disagree with this interpretation. Key evidence in support of our model come from published data in yeast, specifically the much higher density of ribosomes on Cdc12 and the accumulation of ribosomes on the Pro-rich cluster near the Cdc12 N terminus. These are precisely the kinds of “more stringent analysis” in “authentic yeast” (to use Reviewers’ language) that we would have wanted to do to test our model, had they not already been done by others. Without specific suggestions, we struggle to imagine what other kinds of experiments the Reviewers have in mind, apart from a eukaryotic version of a reconstituted cell-free translation system, which Reviewer #1 admits “would be substantially difficult” and “time consuming”. While we are intrigued by the reconstituted eukaryotic cell-free translation system that was published last year (which we mentioned on lines 994-995) and look forward to exploring it in future studies, it is not commercially available and we agree that the amount of effort required to prepare it ourselves is unrealistic for the current study. Most importantly, we do not find in the critiques provided any specific reason why our E. coli-based systems experiments are intrinsically less “stringent” or “rigorous”.

Accordingly, we think that, together with the results of multiple new experiments (detailed below), the extensive re-writing and re-ordering that we have done in the revised manuscript will be enough to better emphasize the importance and rigor of our findings and thus to address all of the Reviewers’ specific concerns.

2. Point-by-point description of the revisions

Reviewer 1 thought that our manuscript “does not even provide new information, since the involvement of CCT and the Hsp70 system is not novel” and thought that “the key finding of this manuscript is how chaperones are involved in the de novo folding of septins, which is not conceptually new because of previous findings, including those of the authors”. Reviewer #3 also stated that “the function of Tric/CCT in septin folding and assembly is well documented”. We were quite surprised at this reaction, since we dedicated a significant portion of the original manuscript (lines 68-76 and 319-322) to explicitly discussing the only other paper in the literature that specifically addresses the question of whether or not CCT is required for de novo septin folding. As a reminder, that paper explicitly stated that “it is unlikely that CCT is required to fold septins de novo” and “septins probably do not need CCT for biogenesis or folding”. With regard to involvement of the Hsp70 system, the only existing evidence in the literature on this subject is the aggregation of some septins in ssb1Δ ssb2Δ cells. Like the CCT study, that study did not distinguish whether this was a result of problems during septin synthesis and before septin complex assembly, or, alternatively, whether pre-folded and assembled septins were subject to disassembly, misfolding, and aggregation. Our experiments specifically test the fate of newly-synthesized septins prior to assembly in living cells. Our previous findings documented physical interactions between wild-type septins and multiple chaperones but did not address whether these interactions had any functional relevance. We previously reported functional effects of interactions between chaperones and MUTANT
septins but, again, these studies did not address functional chaperone requirements for WILD-TYPE septins. While we did our best to highlight these points in the original document without devoting excessive amounts of text, we accept responsibility for not making these points sufficiently clear and to address this issue we added additional text, including the text quoted above, to the Introduction.

While Reviewer #3 commented that the manuscript “is overall well presented”, Reviewer 1 thought that the manuscript was “complicated to read” with “no logical connections, just a list of many results” and mentioned that part of the difficulty was “that it contains many negative results”.

In addition to reorganizing the manuscript, as suggested by the reviewers, we added more text at the beginning and end of nearly every section to even more explicitly state the logical connections between results. In our opinion, negative results of properly controlled experiments are valuable to the research community, and we do not understand what it is about negative results that makes them difficult to read about. Many of the extra experiments we performed were in anticipation of being asked to perform them by reviewers, some of which generated negative results. We are reluctant to remove negative results unless there is a more compelling reason. For example, to address another reviewer concern, we did remove the negative results with the Ydj1–Ssa2 compensatory mutants.

Reviewer #2: “4) Figure 2: The labeling on the protein structure makes it seem like the exact region for Ydj1 and Hsp70 was experimentally identified, when it hasn’t.”

We acknowledge that the first sentence of the figure legend (“the colored ribbon follows the color scheme in the sequences at right for overlapping β-aggregation, Ydj1 and Hsp70-binding sites”) could be misinterpreted, since only in the second sentence does it say “Sequence alignments show predicted binding sites”. We corrected this mistake, and added the text “Predicted chaperone binding sites” as the first words in the legend to this figure.

Reviewer #2: “8) The authors confusingly jump back and forth between different Septins and different chaperone (Ssa1-4, Ydj1, Sis1, Hsp104). We would ask the authors to re-arrange the manuscript, collating all the yeast work in one section and bacterial work in another.”

We re-arranged the manuscript and put all the yeast work in one section and all the bacterial work in another, with the exception of the studies of individually purified Cdc3 and Cdc12, which we put in between the yeast studies of the kinetics of de novo assembly and the yeast studies of post-translational assembly. Our reasoning is that the studies with the purified proteins demonstrate challenges with maintaining native conformations in the absence of chaperones and other septins, which flows naturally into the yeast studies asking about the ability of “excess” septins to maintain oligomerization-competent conformations in the absence of other septins and when we experimentally eliminate specific chaperones. All of the work actually manipulating *E. coli* genes/proteins is now together.

Reviewer #3: “1. The co-translational binding of CCT to nascent polypeptide chains has been studied (Stein et al., Mol Cell 2019). While the authors indicate that septin subunits are engaged co-translationally, they do not comment which ones are interacting with CCT and at which state of translation. This information is crucial and should also be mentioned in the discussion section.”
We are grateful to the Reviewer for bringing up this point, which we had overlooked. We hadn’t noticed that, in the end, only Cdc3 met the CCT confidence threshold to be included in the supplemental data of the Stein et al. paper. All septins co-purified with CCT in an earlier Dekker et al proteomic study, so we strongly suspect that the failure of the other septins to meet the confidence threshold in the Stein et al paper reflects the sensitivity of that assay, rather than a significant difference in how septin GTPase domains interact with CCT. We also hadn’t appreciated that according to that study, the main sites in the Cdc3 GTPase domain bound by CCT and Ssb are the same. Hence our statement that Ssb bound to septins “earlier” during translation, and CCT bound “later” was wrong. Instead, the overlapping Ssb and CCT site in Cdc3 turns out to be remarkably consistent with a conclusion from Stein et al paper, that CCT binds Rossmann-fold proteins like septins at sites where “early” beta strands have been translated and expose a chaperone-binding surface that later becomes buried by an alpha helix. We corrected our mistake in the text and in our model figure and added: (1) a new supplemental figure with predicted septin structures and a sequence alignment indicating where CCT and Ssb bound; and (2) text discussing the confidence thresholds for “calling” septin-CCT interaction, the Rossmann-fold binding, and how we interpret Ssb and CCT binding to the same site.

Reviewer #3 “3. Figure 3: It is recommended to also follow Cdc10-GFP and Cdc12-GFP fluorescence. This will on the one hand generalize the presented findings and provide a direct link to other parts of the study (e.g. crosslinking analysis of Cdc10).

We carried out the requested experiment for Cdc12, using Cdc12-mCherry rather than Cdc12-GFP because of the formation of non-native foci that we observed with Cdc12-GFP. We also attempted to analyze Cdc10, using an existing GAL1/10-promoter-driven Cdc10-mCherry plasmid that we’d made a few years ago, but it did not behave as expected, with high expression even in the absence of galactose (not shown), which prevented us from performing the requested experiment. We have a Cdc10-GFP plasmid with the inducible MET15 promoter, but this promoter does not provide sufficiently low levels of expression in repressive conditions, so there would be too much expression at the beginning of the experiment for us to accurately follow accumulation thereafter. Instead, we tried the only other plasmid we had with the GAL1/10-promoter controlling a tagged septin: Cdc11-GFP. Above a certain threshold of expression, Cdc11-GFP formed unexpected cortical foci, but we were still able to perform the analysis and found a clear delay in septin ring signal in cct4 cells, providing the requested generalization to other septins, if not Cdc10.

Reviewer #3 “5. Figure 4C: The finding that only ssb1 but not ssb2 knockouts have an effect on joining of free Cdc12-mCherry subunits into septin rings is puzzling. Similarly, Ssb1 largely acts co-translationally, while in this assay post-translational septin ring assembly is monitored. The authors need to comment on these two points.”

We did not examine ssb2 knockouts, so we do not know to what the Reviewer is referring in the first point. If the Reviewer means that they are puzzled by the fact that we saw a phenotype in cells in which only SSB1 was deleted and SSB2 remained, we offer two explanations. As can be seen in the Saccharomyces Genome Database entry for SSB1 (https://yeastgenome.org/locus/S000002388/phenotype), there are at least a dozen known phenotypes associated with deletion of SSB1 in cells with wild-type SSB2. We even showed a very clear septin misfolding/mislocalization phenotype in Supplemental Figure 4D. Thus while our findings are new and provide novel insights into Ssb function, they are not unprecedented. The Reviewer is correct that most Ssb is ribosome-bound and thus Ssb1 “largely acts co-translationally” but ~25% of Ssb is not ribosome-associated (PMID: 1394434). Furthermore, the
lack of a strong phenotype for ssb1Δ cells in our new kinetics-of-folding experiment (see below), plus the realization that Ssb and CCT both bind the same site in Cdc3, leads us to a new model: Ssb acts both co- and post-translationally in septin folding, but only the post-translational function is associated with a phenotype in ssb1Δ cells, because in that assay we drastically overexpress a tagged septin and thereby exceed the Ssb chaperone capacity that remains when we delete SSB1. This logic also explains the first ssb1Δ phenotype we saw, when overexpressing Cdc10(D182N)-GFP. In the kinetics-of-folding assay, on the other hand, tagged septin expression is much lower and reducing the amount of total Ssb by ~50% (via SSB1 deletion) likely does not compromise Ssb function in folding the tagged septin. We therefore removed our statement that “Ssb dysfunction leaves nascent septins in non-native conformations that are aggregation-prone and unrecognizable to CCT”, revised our model figure accordingly, and added new text and citations to explain our new model.

Reviewer #3 “Additionally, they should test whether the appearance of septin ring fluorescence is slowed down in ssb1 mutants (as shown for cct4-1 mutant cells in Figure 3B).”

We agree that slower septin folding in ssb1Δ cells is a prediction of our model, and we performed the requested experiment and include the results in our revised manuscript. The new data show that the appearance of septin ring fluorescence is not delayed in ssb1Δ mutants, which is easily explained by the ability of Ssb2 to chaperone the folding of the low levels of tagged septin that we express in these kinds of experiments (see above).

Reviewer #3: “7. Figure 5G: The data is not convincing. This reviewer cannot detect a specific Cdc12 band accumulating in presence of GroEL/ES.”

We re-ran the reactions again with fresh reagents and this time ran the gel longer to reduce excess signal from free fluorescent puromycin and the bright Cdc10 bands. We now see a very clear band for full-length Cdc12 in the reaction with added GroEL/ES, fully consistent with our mass spectrometry results. We updated the figure with the new results.

Reviewer #3: “Furthermore, the activity tests done for the chaperonin system are confusing (Supplemental Figure 7). The ATPase rate (slope!) of GroEL/GroES seems higher as compared to GroEL but according to the authors it should be opposite.”

In our assays, the ATPase activity is so fast that for our “time 0” timepoint, much of it has already occurred by the time the reaction can be physically stopped and measured. In other words, the handling time is such that we can’t visualize what happened in the earliest stages of the reaction, where the rates could accurately be estimated as slopes. This is obvious from the fact that at time 0, the absorbance for the “GroEL alone” reaction is already more than twice the absorbance for GroEL+ES. We added clarifying text to the figure legend.

Reviewer #3: “The refolding assay using Rhodanese as substrate is also confusing: What is the activity of native Rhodanese? The aggregated Rhodanese sample seems to have substantial activity that is not too different from a GroEL/ES-treated one. From the presented data it is not clear to the reviewer to which extend GroEL/ES prevents aggregation and supports folding of denatured Rhodanese.”

We thank the Reviewer for bringing this to our attention, because made we mistakenly left out the values for native Rhodanese with the reporter. With regard to the aggregated Rhodanese, we failed to note that this sample contains urea. When the urea absorbance is subtracted, it
is clear that the GroEL/ES-treated sample has higher activity. Furthermore, some native enzyme is likely still active within the aggregated sample, explaining the “substantial activity” that the Reviewer correctly notes. We corrected the figure and added clarifying text to the figure legend.

Reviewer #3: “the study goes astray following aspects that does not seem relevant to this reviewer (e.g. the role of N-terminal proline residues for Cdc12 translation, Fig. 5E/F).”

We acknowledge that we did a poor job of introducing the N-terminal Pro-rich cluster in Cdc12 with relation to our model of slow Cdc12 translation. Instead, we have revised and reorganized the manuscript to set up these experiments as a direct test of our model: if ribosome collisions on the body of the ORF drive mRNA decay, then decreasing the spacing of those ribosomes should exacerbate the problem, and eliminating the Pro-rich cluster (where published yeast data already show ribosomes accumulate) is the most logical way to test the prediction. Far from being irrelevant, the results fit the prediction perfectly and thus support the model. We expect that this change will highlight the importance of these experiments for the reader.

Reviewer #2: “1) Fig. 1 Is the folding of Cdc3 being measured in cells lacking chaperones mentioned towards the end of the paper or are the authors referring to the lack of yeast proteins?”

We are unclear as to what the Reviewer is asking here. The title of Figure 1 states that these are “purified yeast septins” and the figure legend further emphasizes this fact. Additionally, the Coomassie-stained gel in Figure 1A shows a single band, corresponding to purified 6xHis-Cdc3. The proteins were purified from wild-type E. coli cells, so all E. coli chaperones were present when Cdc3 initially folded, but chaperones and all other proteins were removed during the purification and prior to the analysis. We do not know what change to make.

Reviewer #2 asked “How do the authors account for the septin defect in Ssa4 delete cells in unstressed conditions where Ssa4 would be very low already? According to the authors previous work, Ssa2 and 3 should be able to compensate.”

We explicitly addressed this point in the original manuscript (lines 893-898). Again, we think here the Reviewer is equating chaperone binding with chaperone function. According to our previous work, Ssa2 and Ssa3 are able to bind septins, but this does not mean that they can fold septins the same way as Ssa4. We cite several papers that discuss the distinct functional roles for the different Ssa proteins. We do not think that additional clarification of this point would strengthen the manuscript.

Reviewer #3: “6. Figure 5B: It is unclear why Cdc3 is observed in the pulldown of His-tagged Cdc12 (37˚C), although no Cdc12 was isolated under these conditions. How is that possible?”

That is not possible. As we indicate in the figure legend and with the red asterisk, the only band appearing in that lane is a non-specific band that cross-reacts with the anti-Cdc3 and/or anti-Cdc11 antibodies. This is why it is also present in the “No septins” control lanes. We made the asterisk larger to help accentuate this point.

Reviewer #3: “Furthermore, the authors observe a specific effect on Cdc12-Cdc11 assembly in the E. coli groEL mutant. How do they rationalize this specific effect as Cdc12-Cdc3 assembly remained unchanged? This observation also seems in conflict with the suggestion of the
authors that Cdc12 preferentially recruits Cdc11 before interacting with Cdc3 (page 45, lane 1024)."

Cdc11 was not expressed in the groEL mutants because no Cdc11 gene was present in those cells, as explained in the body text and indicated in the labeling above the lanes in Figure 5A. The band near the size of Cdc11 is a non-septin protein that bound to the beads in the groEL-mutant cells, as is shown in the immunoblot using anti-Cdc11 antibodies in Figure 5B. Thus there is no conflict to rationalize.

Reviewer #1: “The only evidence that CCT binds to septin is the list of LC-MS/MS. Western blotting would provide more solid data.” and “2) The cross-linking experiments appears not to have been successful. Why are the Ssas, Ydjs etc not detected here? “

First, CCT subunits are relatively low-abundance, expressed at 5- to 50-fold lower levels than other chaperone families in the yeast cytosol (see PMID: 23420633). To the Reviewer’s second point, we did in fact detect other chaperones in our crosslinking mass spectrometry experiments, including Ydj1, multiple Ssa and Ssb chaperones, Hsp104, etc., as can be seen in Table S1. However, they were also detected in negative control experiments. This is not surprising, given that these chaperones are among the most common “contaminants” of affinity-based purification schemes (see the CRAPome database at https://reprint-apms.org/). It was for this reason we had to perform so many negative control experiments, which likely produced some false negative results, as some “real” interactions were likely discarded when the same chaperone showed up in our controls. We added a figure panel with a Venn diagram of overlap between experimental and control samples, and text pointing out this caveat of our approach.

Second, in this experiment we attempted to identify proteins that transiently interact with a specific region of Cdc10 that will later become buried in a septin-septin oligomerization interface. Due to the transient nature of the interaction, we do not expect to detect high levels of crosslinked chaperones. Mass spectrometry is significantly more sensitive than immunoblotting, so there is no guarantee that we would be able to detect a band even if the crosslinking works as desired. Indeed, the crosslinked bands we saw by immunoblot for GroEL were quite faint (see Figure 2F), despite the fact that GroEL and the T7-promoter-driven Cdc10 were among the most abundant proteins in those E. coli cells.

Third, there is no commercially available, verified antibody recognizing yeast Cct3 for which to perform the requested immunoblot experiment. Since both the N and C termini of CCT subunits project into the folding chamber, it is unwise to use a standard epitope tagging approach, as the tags may compromise function. Indeed, for purification purposes others inserted an affinity tag in an internal loop in Cct3 (PMID: 16762366). We have a yeast strain with Cct6 tagged in an analogous way, but to perform the requested immunoblot experiment with Cct3 would require creating or obtaining the Cct3-tagged strain, deleting NAM1/UPF1, and introducing our Bpa tRNA/synthetase and GST-6xHis-Cdc10 plasmids. Given the sensitivity of detection concerns stated above, we doubt this would help.

In summary, we prefer not to attempt the requested immunoblot experiments.

Reviewer #1: “-Fig. 3B ant related Figures: The experiment to see if GFP-tagged septin accumulates in the bud neck is important, but only the graphs after the analysis are shown. The authors should provide the readers with representative examples from imaging data.”
We are confused, because the images at the bottom of Figure 3A already show what the Reviewer requests. As stated in the figure legend, these are representative examples of the imaging data from a middle timepoint of one of the experiments. It would be nearly impossible (for space reasons) to provide representative images for all of the timepoints for all of the genotypes for all of the experiments. Since in our new experiments we introduce new tagged septins (Cdc11-GFP and Cdc12-mCherry), we also now include representative images of cells expressing these proteins, as well.

Reviewer #2: “3) If the authors had evidence of chaperone interaction from their previous study, why did they not simply do IPs with fragments of the septins/chaperones?”

We are unclear why the Reviewer is suggesting IPs after referring to our previous study. IPs are a poor choice for transient interactions, which is why we mostly avoided them in previous studies, and instead used a novel approach (BiFC) to “trap” chaperone–septin interactions. Moreover, we seek to identify chaperones that bind wild-type septins at future septin-septin interfaces on the path towards the native conformation. Fragments of septin proteins would likely misfold and would therefore likely attract chaperones that wouldn’t normally bind the full-length septin. Indeed, our previous studies demonstrated that even a single non-conservative amino acid substitution was sufficient to alter chaperone-septin binding. Thus IPs with fragments of septins or chaperones would be highly unlikely to yield informative results for the questions we seek to answer. We strongly prefer not to attempt these suggested experiments.

Reviewer #2: “5) While differences between Ssa paralogs are highly interesting, using deletions of Ssas is not useful, given that yeast compensate by overexpressing other paralogs. The yeast GFP Septin assays should be repeated in yeast lacking all Ssas and expressing one paralog on a constitutive promoter (See numerous papers by Sharma and Masison).”

We disagree that ssa deletions are “not useful”, since if the overexpressed paralogs cannot fulfill the same function as the deleted SSA, then we will see a phenotype. Which we do. Furthermore, we had already obtained and thoroughly tested a strain like the ones mentioned by the reviewer (ECY487, a.k.a. JN516, from Betty Craig’s lab, with ssa2Δ ssa3Δ ssa4Δ and SSA1, which is constitutively expressed, PMID: 8754838), but we found that, as published, it divides slightly more slowly even under the most permissive of conditions. The requested strain cannot be analyzed using our method, because slow accumulation of ring fluorescence could be attributed to other defects unrelated to septin folding. Thus we strongly prefer not to attempt the suggested experiments.

Reviewer #2: “7) The authors need to clarify the experiment with the Ydj1 D36N and Ssa2 R169H. In Reidy et al, they never fully biochemically test this system and it was never examined for Ssa2-Ydj1. The authors would need to do some fundamental experiments to demonstrate the validity and functionality of this double mutant in yeast.”

Given that this experiment was unable to generate meaningful data, since the mutations affected the kinetics of induction of the GAL1/10 promoter, we do not think the requested biochemical experiments would add any value to the study. Instead, we removed these studies from the manuscript.

Reviewer #3: “4. Figure 3B: The difference between wt and cct4-1 cells in appearance of septin ring fluorescence is observed at one timepoint. Since this experiment is considered highly
relevant, the authors are asked to include another timepoint to bolster the conclusion that Cdc3-GFP folding and thus septin ring assembly is delayed in the CCT mutant.”

We carried out new experiments with cct4-1 cells using Cdc12-mCherry and Cdc11-GFP with more timepoints than in our original cct4-1 experiments with Cdc3-GFP. Since these experiments provide the same kinds of results, but at multiple timepoints, we do not see the value in repeating the Cdc3-GFP experiment.

Reviewer #3: “If Ssb1 functions to maintain Cdc12 in an assembly competent state preventing misfolding, one would expect either enhanced degradation or aggregation of Cdc12-mCherry in ssb1 mutant cells. Did the authors check for such scenario? Septin aggregation has been shown in a ssb1 ssb2 double deletion strain (Willmund et al., 2013), yet the data shown here predict that aggregation might already occur in single ssb1 mutants.”

We already examined septin aggregation in single ssb1 mutants and showed these data (Supplementary Figure 4D). Indeed, this phenotype was the rationale for testing post-translational septin assembly in ssb1 single mutants. We have seen no evidence of septin degradation in any context (as we mentioned on line 889), so we would not expect it here. While we added new text and a very new citation showing that many “misfolded” conformations of wild-type *E. coli* proteins avoid aggregation and degradation, we do not think that the suggested experiments would add enough value to the current study to justify the effort, time and expense.

Reviewer #3: “Fig. 3C: The figure showing septin ring fluorescence does not include error bars. This is crucial, also because the difference between wt and ssa4 mutant cells is not large.”

There are, in fact, error bars included in the figure, as can be most clearly seen for the final timepoint for the ssa4∆ cells. For most of the other timepoints the error bars are smaller than the data point symbols (the circles and squares). We do not think that adjusting the size or opacity of the symbols to better show the error bars will be sufficiently valuable to justify the effort.
1st Editorial Decision

July 21, 2022

RE: Manuscript #E22-07-0262
TITLE: “Chaperone Requirements for De Novo Folding of Saccharomyces cerevisiae Septins”

Dear Michael:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

This is an important set of experiments with tremendous relevance for understanding not only septin complex assembly but many stoichiometric quaternary assemblies. I think you did an outstanding job of addressing the review commons reviews and the paper is greatly improved. Thank you for submitting this excellent study to MBoC.

Sincerely,
Amy Gladfelter
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. McMurray:

Congratulations on the acceptance of your manuscript. A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

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