Loss of tolerance to multiple environmental stresses due to limitation of structural diversity of complex sphingolipids

Ayano Koga, Chihiro Takayama, Yohei Ishibashi, Motohiro Tani, Yuji Kono, and Momoko Matsuzaki

Corresponding author(s): Motohiro Tani, Kyushu University

Review Timeline:

Submission Date:	2022-04-06
Editorial Decision:	2022-05-05
Revision Received:	2022-06-27
Accepted:	2022-07-10

Editor-in-Chief: Matthew Welch

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 #E22-04-0117
TITLE: Loss of tolerance to multiple environmental stresses due to limitation of structural diversity of complex sphingolipids

Dear Dr. Tani:

The review of your manuscript is now complete and comments from two expert reviewers follow below. As you will see the reviewers raised important technical issues. There was also some concern from one reviewer about the general significance of the findings.

I believe that a suitably revised manuscript could make a significant contribution to the field and suggest that you carefully address the comments of the reviewers in a revised manuscript. I would send the manuscript to the original reviewers for their approval before making my final decision.

I look forward to receiving a revised manuscript in due course.

Sincerely,

Robert Parton
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Tani,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you haveopted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us at mboc@ascb.org.

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

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

Reviewer #1 (Remarks to the Author):

The complex sphingolipids that make up the plasma membrane are structurally vastly diverse, and understanding their roles is important but extremely difficult due to their complexity. Although the complex sphingolipids of yeast are relatively simple (up to 15 species) compared to those of mammalian cells, there is still a lack of systematic understanding of the physiological function of complex sphingolipids.
In this manuscript, Koga et al. investigate the role of each complex sphingolipid in response to various environmental stresses by generating a library of mutant strains with stepwise defects in complex sphingolipid synthesis and examining the sensitivity of these mutants to various stresses. Some of the sensitivity of ccΔ cells (pH 3.5, CaCl2) could be explained by the accumulation of IPCs, while other sensitivities could be inferred to be caused by loss of production of MIPCs rather than IPCs accumulation. Furthermore, the authors show that Slt2 and Msn2/4 transcription factors play essential roles in the stress response of the ccssΔ mutant strain which is a defect in MIPCs synthesis and hydroxylation of ceramide moiety. The authors demonstrate that the diversity of complex sphingolipids is important for maintaining cell wall and plasma membrane integrity.
This manuscript demonstrates a large amount of good quality data and provides important findings for understanding the role of complex sphingolipids.

Major comments

1. The effects of the suppression of sensitivities by deletion of genes in some data are marginal.
For example, the recovery of pH3.5, CaCl2, or CuCl2 sensitivity of ccΔ by sur2Δ or scs7Δ is very obvious, but the recovery of Cd sensitivity of ccΔ by sur2Δ is very weak (Fig. 2, line 191). Furthermore, ccΔ is less sensitive to caffeine at 5 mM, and the recovery of caffeine sensitivity of ccΔ by sur2Δ is also very weak. Conversely, ccΔ scs7Δ appears to be more sensitive to caffeine than ccΔ (Fig. 2). These results differ from those described in lines 229-230. Can these data be reproduced? How many times were experiments repeated?

2. The sensitivity of ccssΔ to SDS is restored by slt2Δ in Fig. 5. This is described in the discussion (lines 495-497) but not in the results. This result is very interesting and should be focused on and explained in more detail in the results.
Also, the following questions should be addressed in this connection.

2.1 What is the phosphorylation of Slt2 and the expression of downstream genes during SDS treatment in the ccssΔ strain?

2.2 Does deletion of upstream factors of Slt2, such as Bck1 and Rom2, also suppress the SDS sensitivity of ccssΔ?

3. Intriguingly, plasma membrane integrity is abnormal in ccssΔ cells. This reviewer wonders if plasma membrane microdomains, such as eisosomes, are affected in ccssΔ cells. Interestingly, the expression of Pun1, one of the eisosome components, is elevated in ccssΔ cells.

4. Figure 7 concludes that indirect assessment with fluorescent reagents such as aniline blue increases cell wall components at the cell surface. Interestingly, cell wall components remain increased even when Slt2 is deleted in ccssΔ cells, meaning that this increase is not due to Rlm1 and/or Swi4-dependent transcriptional activation associated with constitutive activation of Slt2, as concluded from the RNA-Seq results. Are target genes of Rlm1and/or Swi4 presented in Fig. 6C still upregulated in ccssΔ slt2Δ?

Minor comments

Fig. 1D. The labeling at the bottom is confusing. It looks like some type of addition instead of indicating cell type.

Fig. 1K should be Fig. 1G on line 161.

Fig. 4B should be Fig. 4C on line 354.

Reviewer #2 (Remarks to the Author):

Mammalian cells typically contain hundreds of complex sphingolipid species, and it remains unclear how this tremendous diversity contributes to cell function. This study addresses the role of complex sphingolipid diversity in S. cerevisiae, which produces just 15 subtypes of complex sphingolipids. Making it a simpler, more genetically tractable system to try to understand the roles of complex sphingolipid diversity. A variety of mutants with defects in sphingolipid production are characterized and found to have increased sensitivity to different environmental stresses. The study also assesses the roles of sterols, a MAP kinase, and transcription factors in resistance to environmental stresses. The broad conclusion is that "... the more the structural variation of complex sphingolipids is limited, the more stress sensitivity tends to increase... (lines 460-1). The work is well done

and convincing, though there are few issues listed below. However, my biggest concern about this work is its significance. It has long been known that complex sphingolipids (together with sterols) are critical for maintaining plasma membrane function and cellular resistance to external stressors and toxins. This study examines the role of sphingolipids systematically, but the result is basically what we already knew. Namely, that the roles of various complex sphingolipids in conferring resistance to various stresses is complex. No general principles emerge from the analysis. On the other hand, this study could a useful resource for future work. Overall, the study seems borderline for MBoC. Here are my major concerns.

1. The study would be stronger if there were more characterization of the biophysical properties of the plasma membrane in the mutant strains. This seems especially important since an unstated assumption of the study is that sensitivity to environmental stresses is largely a function of plasma membrane lipid composition. Rhodamine 6G is used to directly assess permeability, but other probes could be used and other membrane properties, such as fluidity, could be assessed.

2. The study make several claims about the structure of the cell wall and how it may change in the mutants, but the composition of the cell wall has not been directly assessed.

3. The weakest part of the study is on the roles of sterols. Changes in nystatin sensitivity do not always correlate with changes in sterol levels in the plasma membrane. There is no evidence in the literature or in this study that cell lacking the sterol-transfer proteins Sip3, Lam1, or Ysp2 have decreased levels of plasma membranes sterols.

4. It would be better to quantitate the results in Fig. 4 by measuring ratio of yeGFP-Rim101 in the cytoplasm and the nucleus.

5. The discussion is quite long. The authors should consider making it more concise.

RE: Manuscript #E22-04-0117R
TITLE: "Loss of tolerance to multiple environmental stresses due to limitation of structural diversity of complex sphingolipids"

Dear Dr. Tani:

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

Sincerely,
Robert Parton
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Tani:

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.

Would you like to see an image related to your accepted manuscript on the cover of MBoC? Please contact the MBoC Editorial Office at mboc@ascb.org to learn how to submit an image.

Authors of Articles and Brief Communications are encouraged to create a short video abstract to accompany their article when it is published. These video abstracts, known as Science Sketches, are up to 2 minutes long and will be published on YouTube and then embedded in the article abstract. Science Sketch Editors on the MBoC Editorial Board will provide guidance as you prepare your video. Information about how to prepare and submit a video abstract is available at www.molbiolcell.org/science-sketches. Please contact mboc@ascb.org if you are interested in creating a Science Sketch.

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

Sincerely,

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

Reviewer #2 (Remarks to the Author):

The authors have done a good job of addressing my concerns.