Lipid saturation induces degradation of squalene epoxidase for sterol homeostasis and cell survival

Rey-Huei Chen and Leng-Jie Huang
DOI: https://doi.org/10.26508/lsa.202201612

Corresponding author(s): Rey-Huei Chen, Institute of Molecular Biology, Academia Sinica

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.)

August 11, 2022

Re: Life Science Alliance manuscript #LSA-2022-01612-T

Dr. Rey-Huei Chen
Institute of Molecular Biology, Academia Sinica
Institute of Molecular Biology
128 Sec. 2, Academia Rd.
Taipei 11529
Taiwan

Dear Dr. Chen,

Thank you for submitting your manuscript entitled "Lipid saturation induces degradation of squalene epoxidase for sterol homeostasis and cell survival" to Life Science Alliance. The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revised manuscript addressing the Reviewer comments.

To upload the revised version of your manuscript, please log in to your account: https://lsa.msubmit.net/cgi-bin/main.plex

You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

While you are revising your manuscript, please also attend to the below editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

The typical timeframe for revisions is three months. Please note that papers are generally considered through only one revision cycle, so strong support from the referees on the revised version is needed for acceptance.

When submitting the revision, please include a letter addressing the reviewers' comments point by point.

We hope that the comments below will prove constructive as your work progresses.

Thank you for this interesting contribution to Life Science Alliance. We are looking forward to receiving your revised manuscript.

Sincerely,

Novella Guidi, PhD
Scientific Editor
Life Science Alliance

A. THESE ITEMS ARE REQUIRED FOR REVISIONS

-- A letter addressing the reviewers' comments point by point.

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure, supplementary figure and video files uploaded as individual files: See our detailed guidelines for preparing your production-ready images, https://www.life-science-alliance.org/authors

-- Summary blurb (enter in submission system): A short text summarizing in a single sentence the study (max. 200 characters including spaces). This text is used in conjunction with the titles of papers, hence should be informative and complementary to the title and running title. It should describe the context and significance of the findings for a general readership; it should be written in the present tense and refer to the work in the third person. Author names should not be mentioned.

-- By submitting a revision, you attest that you are aware of our payment policies found here: https://www.life-science-alliance.org/copyright-license-fee

B. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, https://www.life-science-alliance.org/authors

We encourage our authors to provide original source data, particularly uncropped/-processed electrophoretic blots and spreadsheets for the main figures of the manuscript. If you would like to add source data, we would welcome one PDF/Excel-file per figure for this information. These files will be linked online as supplementary "Source Data" files.

IMPORTANT: It is Life Science Alliance policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Reviewer #1 (Comments to the Authors (Required)):

In this manuscript Huang and Chen explore the effects of phospholipid saturation on stability of a model membrane protein in yeast, Erg 1. To control saturation, they generated temperature-sensitive mutants in the desaturase Ole1, and they obtained a mutant that accumulates saturated fatty acids at high temperature that is toxic to cell survival. Authors found that Erg1, a protein in the ergosterol biosynthetic pathway, is unstable stable in this ole1ts mutant at high temperature. Supplementation with oleic acid (OA) yields stable Erg1. They found that Doa1 and Ubc7, proteins of the ERAD complex, is involved in the degradation of Erg1 upon lipid saturation. The authors then identified Erg1 mutants resistant to degradation upon lipid saturation. They determined cell localization of Erg1 (normally in the ER) and found that it forms foci upon lipid saturation, and that ER morphology was aberrant. The toxic effects of saturation on cells were attributable to these changes in ER morphology, which, in turn, was caused by accumulation of lanosterol. Finally, they demonstrated that addition of glycerol resulted in normal ER morphology and prevention of protein misfolding, presumably due to a chaperone-like effect of this carbon source.

These data are significant in that they establish a new model for tightly temporally regulating phospholipid saturation in yeast, and they demonstrate that folding and stability of membrane proteins, as well as organellar morphology, are exquisitely dependent on this membrane parameter. The paper is easy to read, and the data are appropriately interpreted.

However, there are issues with the manuscript:

(1) It is not clear whether glycerol, used as a source of carbon, can directly affect the expression level of the Erg6 mutants under the Gal promoter. Does glycerol change the morphology of the ER?
(2) The authors showed that the stable Erg1 mutants are toxic for the ole1-20 strain (Fig. 5A), and this is rescued by OA supplementation. Does OA change the localization of Erg1 mutants and wt? What is the ER morphology in the ole1-20 strain during OA supplementation? The authors might observe GFP-Erg1 and mCherry-HDEL-expressed strain in the presence of OA.
(3) The authors use fluconazole and Ro48-8071 to determine lanosterol toxicity. What happens to cells if they are directly fed lanosterol? What is the status of ER morphology and Erg1 and Erg1 variant localization after fluconazole and R048-8071, or exposure to lanosterol?
(4) In Figure 6 the authors tested the status of ER morphology in the ole1-20doa10 strain in the presence and absence of glycerol as chemical chaperone. However, they did not determine in parallel this effect in a ole1-20ubc7Δ mutant. This is important to determine the relative roles of these two degradative proteins in ER morphology.

Minor comments:

(5) Be consistent with spaces (a) between temperature and {degree sign}C (see Fig 2 legend); and (b) between number and "µm" in scale bars (see Fig 6 legend).
(6) In some experiments authors used 34oC and some experiments with 32oC. Is there a justification?
(7) Similarly, "RT" is sometimes used, and 27{degree sign}C is also used. Is there a difference? Please keep uniform.

Reviewer #2 (Comments to the Authors (Required)):

This study investigated phenotypes associated with OLE1 loss and increased lipid saturation in budding yeast, and discovered accelerated degradation of squalene epoxidase (Erg1) under these conditions. Huang & Chen show that this degradation is mediated by the ERAD E3 ubiquitin ligase Doa10 and depends on a conserved proline residue in the membrane-associated domain of Erg1. Interesting, combined OLE1 loss and overexpression of degradation-resistant Erg1 mutants dramatically disrupts ER morphology and impairs cell growth due to the toxic accumulation of lanosterol, suggesting that lipid saturation-induced degradation of Erg1 is necessary to avert this accumulation. Finally, the authors show that loss of Doa10 mimics the effect of Erg1 mutants in causing cell-wide, lanosterol-dependent membrane disruption and toxicity in cells lacking OLE1. Taken together, the study presents a model in which Doa10 is required to clear misfolded proteins from saturated lipid-rich membranes, which in the case of Erg1 prevents aberrant lanosterol accumulation and further ER dysfunction. This finding reveals a new element in the regulation of lipid homeostasis and is likely to be of interest to the journal readership.

Overall, the authors have used a wide range of assays and logical sequence of experiments to support their conclusions. It is convincingly shown that lipid saturation controls Erg1 degradation and that this is required for normal ER function; however, other aspects of the model (e.g., lanosterol accumulation) would benefit from further elaboration.

Major comments

1. The concept of the effect of unsaturated vs saturated fatty acids stabilizing the mammalian equivalent of Erg1 was introduced by Stevenson et al. (2014 Biochem J). This paper was overlooked and should be appropriately discussed here in the context of the present yeast work.

2. Fig. 3C: Erg1 K311R is degraded at the same rate as the WT protein, which seems to conflict with the findings of Foresti et al. (2013 eLife). Furthermore, its degradation in ole1-20 cells appears blunted, suggesting that Lys-311 is involved in the saturated lipid-induced degradation of Erg1. Can the authors please comment on these results in the manuscript?

3. Fig. 2D: the predicted Erg1 conformational change in completely saturated vs. completely unsaturated lipid bilayers is relatively subtle. What is the predicted conformation in a bilayer with a PSPC:YOPC ratio that mimics a 'typical' proportion of saturated and unsaturated lipids? Is a change still apparent?

4. Fig. 5B: the central role of lanosterol accumulation in the proposed mechanism of ER dysfunction is interesting but warrants further clarification. Fig. EV3 (which is not mentioned in the results section) indicates that the levels of downstream ergosterol synthesis intermediates are generally reduced in ole1-20 cells, pointing to a pathway blockage at lanosterol. Why does this occur? One possibility is that lipid saturation also impairs the protein stability or activity of lanosterol demethylase (Erg11), which warrants testing given that the human homologue of Erg11 (LDM) is targeted by the homologue of Doa10 (MARCHF6) (Scott et al. 2021 Biochem J).

5. Fig. 5B: squalene conversion is restored by the addition of glycerol in Erg1 P469L-expressing ole1-20 cells; however, there is no corresponding increase in lanosterol levels. Can the authors please comment on this result? As a point of comparison, this experiment would benefit from including cells expressing WT Erg1 (where the ER is intact) to confirm that this indeed averts or blunts lanosterol accumulation.

6. Fig. 5D: does manipulating lanosterol levels also restore ER morphology? The authors have only considered cell growth. If so, it would help to support their final model.

Minor comments

1. The statement "both mammalian SQLE and Erg1 are destabilized in response to an elevated level of downstream intermediate lanosterol" is incorrect - degradation of mammalian SQLE is unaffected by lanosterol (Gill et al. 2011 Cell Metab.).

2. On page 9, it is stated that "Erg1 structure predicted by AlphaFold2 was similar to SQLE (Fig 3A)." However, no comparison between the two proteins is depicted.

3. Please include methods for the scoring of imaged cells (e.g., Fig. 4B) and tetrad analysis (Fig. 6C).

4. To improve clarity, labels should be added to the icons in Figs. 4A and 6B to explicitly describe the phenotype.

5. The schematic in Fig. 8 is a useful accompaniment to the discussion, but would benefit from inclusion of Erg1, Doa10 and Ubc7 to better integrate the findings of the entire manuscript. The legend does not outline the possible consequences of protein segregation into different membrane phases - presumably this is the likely cause of the membrane dysfunction?

6. Please ensure that figure legends contain full information about temperature(s) at which experiments were performed and any medium supplements (e.g., oleic acid, galactose).

7. Please define the acronyms "TAG" and "NPC" in the main text.

8. Please correct typos in the main text and figures, e.g., "lesser extend" (page 11), "microscopy. Whereas" (page 19), "GFP-P469L" (Fig. 4C), "deleted for" (Fig. 2D legend), "Stutent's" (Fig. 4C legend).

Reviewer #3 (Comments to the Authors (Required)):

In their study, Huang and Chen investigate the cellular consequences of manipulating glycerophospholipid saturation and thus membrane properties in S. cerevisiae. They first generate temperature-sensitive mutant alleles of the single yeast lipid desaturase Ole1, an approach motivated by the fact that the OLE1 gene is essential. These mutant alleles enable them to discover that increasing lipid saturation accelerates ER-associated degradation of the squalene epoxidase Erg1 and thereby impacts the ergosterol biosynthesis pathway. The physiological importance of accelerated Erg1 degradation is revealed when the authors raise the levels of Erg1 under conditions of increased lipid saturation and find a toxic accumulation of the ergosterol synthesis intermediate lanosterol. Lanosterol accumulation under these conditions disrupts ER structure and impairs cells survival, likely because of further increasing lipid order in the ER membrane.

These findings uncover an unanticipated link between glycerophospholipid saturation, ERAD and sterol synthesis. It advances our understanding of lipid homeostasis and the importance of balancing the abundance of saturated and unsaturated glycerophospholipids and sterols. While the exact mechanisms of lipid saturation sensing and subsequent ERAD of Erg1 remains to be elucidated, this is a well-done paper. It is technically convincing throughout and is also nicely written, with a very logical and informative introduction. There are only minor issues to be resolved (see below). The only experimental revision I recommend is to add a true ER transmembrane protein as a control for the subcellular fractionation experiment shown in Figure 3F, which would be a better reference point than the ER-lumenal GFP-HDEL the authors included so far. This would strengthen the claim that Erg1 has a somewhat unusual mode of membrane association. Other than that, the manuscript is suitable for publication very much as is and I congratulate the authors on their fine piece of research.

Minor issues:

P5: When explaining that HMGR becomes misfolded, please cite one of the more recent papers from Randy Hampton, i.e. either Wangeline and Hampton, JBC 2018 or Wangeline and Hampton, JBC 2021.

P6: "... lipotoxicity-related diseases (Schaffer, 2016)." Please give examples of the diseases you are referring to.

P7: "The ole1Δ deletion mutant is lethal". The mutant is not lethal but dead. Hence, more accurate ways of phrasing this would be "The ole1Δ deletion is lethal" or "The ole1Δ deletion mutation is lethal."

P7: For the benefit of orienting the reader, please begin with a statement why OLE1 ts alleles were made.

P7, Figure 1B: In the figure it says "revovered" instead of recovered.

P7, Figure 1D: Please add information to the figure itself that growth is at 34 degrees. This piece of information is in the text and in the figure legend but it would help the reader to also see it in the panel.

P8: The authors mention that SILAC was done to find proteins with differential abundance in WT versus ole1-20 cells but the data are not provided. Please either include the data or add a comment that or why they are not shown.

P9: " ... showed that Erg1 situated on the surface ...". Please edit to: ... Erg1 is situated on ..."

P10, Figure 3F: Please use a transmembrane protein as a reference rather than GFP-HDEL. The GFP-HDEL only shows that carbonate and high salt did not disrupt the ER membrane. However, to illustrate that Erg1 is not a regular integral membrane protein, it is necessary to show that the solubility of a true transmembrane protein is unaffected by carbonate treatment.

P11: "... and to a lesser extend at 27 ...", should be: ... and, to a lesser extent, at 27 ..."

P11: Could the foci of Erg1(P469L) in ole1-20 cells that co-localize with Erg6 but are not lipid droplets (Figure 4) represent ERAD foci (Albert, ... Engel, PNAS 2020)? Please comment in the discussion if you think that this is a reasonable possibility. This is entirely optional, though, the authors do not have to include this idea if they do not think that it is helpful.

P15: "... and for the membrane microdomain of specific functions." Not clear what membrane microdomain is meant here. If you refer to membrane microdomains generally, then edit to: "... for membrane microdomains of specific functions."

Below is the list of new data included in the manuscript, followed by our point-by-point response to reviewers' comments.

New data:

3A, structure of human SQLE protein

3F, differentiation solubilization using integral membrane protein Sec61 as control

5C, sterol analysis of *ole1-20* cells expressing wild-type Erg1 and Erg1 P469L

5G, Ro 48-8071 addition suppressed ER phenotypes in *ole1-20* overexpressing stable Erg1

6E, Ro 48-8071 addition suppressed ER phenotypes in *ole1-20 doa10Δ* double mutant

S2, Erg1 identified in SILAC proteomics

S3, molecular dynamics simulation of Erg1 in lipids mimicking the membranes of WT and *ole1-20*

S6, images of aberrant ER in *ole1-20 ubc7Δ* double mutant

S7, suppression of ER phenotypes by oleic acid treatment

Reviewer #1

(1) It is not clear whether glycerol, used as a source of carbon, can directly affect the expression level of the Erg6 mutants under the Gal promoter. Does glycerol change the morphology of the ER?

The addition of glycerol to cells grown in medium containing glucose or galactose does not normally change ER morphology. Whereas glycerol can restore ER morphology in *ole1* mutant expressing stable Erg1. The effect was not due to suppression of Erg1 expression from the *GAL* promoter. In fact, glycerol addition slightly increases Erg1 abundance, likely due to a stabilizing effect. In addition, the effect of glycerol is independent of its metabolism, because ER morphology is still restored with the deletion of Gut1 that converts glycerol to glycerol-3-phosphate, the first step of glycerol metabolism. We did not include the additional control data in order to save space.

(2) The authors showed that the stable Erg1 mutants are toxic for the *ole1-20* strain (Fig. 5A), and this is rescued by OA supplementation. Does OA change the localization of Erg1 mutants and wt? What is the ER morphology in the ole1-20 strain during OA supplementation? The authors might observe GFP-Erg1 and mCherry-HDEL-expressed strain in the presence of OA.

We have performed the experiments as suggested by the reviewer. As expected, OA addition restored the ER morphology in *ole1-20* overexpressing stable Erg1 mutant and in *ole1-20 doa10* double mutant, supporting our notion that the combination of lipid saturation and lanosterol results in aberrant ER morphology. The data is now shown in Fig S7.

(3) The authors use fluconazole and Ro48-8071 to determine lanosterol toxicity. What happens to cells if they are directly fed lanosterol? What is the status of ER morphology and Erg1 and Erg1 variant localization after fluconazole and R048-8071, or exposure to lanosterol?

  For the effect of Ro 48-8071, we now show that Erg1 distribution and ER morphology are indeed restored by Ro 48-8071 in a dose-dependent manner in *ole1-20* overexpressing stable Erg1 (Fig. 5G) and in *ole1-20 doa10* double mutant (Fig. 6E). For direct lanosterol addition, we did not see perturbed ER morphology in *ole1-20* after incubation with lanosterol at a range of concentrations for 3-5 hr. However, it is unclear whether exogenous lanosterol can be incorporated into the ER, or simply retained in the plasma membrane. It's also unclear whether *ole1-20*, with a relatively normal level of ergosterol, can uptake lanosterol. As to fluconazole, we found that ER morphology was not affected by adding fluconazole to *ole1-20* cells for 3-5 hr at 34°C. We think that the short drug treatment for imaging might not reflect the fluconazole sensitivity of *ole1-20* grown on plates for 2-3 days. Alternatively, the toxicity from fluconazole might be due to some unknown effect of lanosterol accumulation in *ole1-20*, which might not be directly associated with aberrant ER morphology.

(4) In Figure 6 the authors tested the status of ER morphology in the ole1-20doa10Δ strain in the presence and absence of glycerol as chemical chaperone. However, they did not determine in parallel this effect in a ole1-20ubc7Δ mutant. This is important to determine the relative roles of these two degradative proteins in ER morphology.

  We now show that *ole1-20 ubc7Δ* exhibits aberrant ER morphology and clustering of Erg1 and Nup2, albeit to a lesser degree than does *ole1-20 doa10Δ* (Fig. S). The result supports that Doa10-Ubc7 complex acts together to clear misfolded proteins in *ole1-20*, although some unknown E2 enzyme might partially substitute the function of Ubc7.

(5) Be consistent with spaces (a) between temperature and {degree sign}C (see Fig 2 legend); and (b) between number and "μm" in scale bars (see Fig 6 legend).

  We have fixed the spaces accordingly to be consistent throughout the text.

(6) In some experiments authors used 34oC and some experiments with 32oC. Is there a justification?

  The plate assay in Figure 6D was done at 32°C, which is a semi-permissive temperature for *ole1-20*, but nonpermissive for *ole1-20 doa10Δ* and *ole1-20 ubc7Δ* double mutants. The addition of Ro 48-8071 rescued the lethality of the double mutants at 32°C. This experiment was done at 32°C in order to show the difference in temperature sensitivity between *ole1-20* and the double mutants.

(7) Similarly, "RT" is sometimes used, and 27{degree sign}C is also used. Is there a difference? Please keep uniform.

  Due to limited availability of the incubator, we usually placed the plates on the bench for the permissive temperature. "RT" is used, because the temperature in the lab is maintained at 25-27°C, as described in the main text and the materials and methods.

Reviewer #2

Major comments

1. The concept of the effect of unsaturated vs saturated fatty acids stabilizing the mammalian equivalent

of Erg1 was introduced by Stevenson et al. (2014 Biochem J). This paper was overlooked and should be appropriately discussed here in the context of the present yeast work.

We have now discussed the findings from Stevenson et al. (2014 Biochem J) on the stabilization of mammalian equivalent of Erg1 by unsaturated fatty acids in the end of the third paragraph of the discussion section.

2. Fig. 3C: Erg1 K311R is degraded at the same rate as the WT protein, which seems to conflict with the findings of Foresti et al. (2013 eLife). Furthermore, its degradation in ole1-20 cells appears blunted, suggesting that Lys-311 is involved in the saturated lipid-induced degradation of Erg1. Can the authors please comment on these results in the manuscript?

Erg1 K311R is not stabilized in our wild-type cells, which is inconsistent with previous findings of Foresti et al. (2013 eLife). The only explanation we have for this discrepancy is different background strains used in the studies (W303 vs BY4741). K311R mutant is partially stabilized in *ole1-20*, suggesting the K311 is involved in the degradation triggered by lipid saturation. The result also supports that Erg1 might adopt altered conformation and be targeted to degradation via distinct mechanism in saturated lipid membranes. We have now added this information in the third paragraph of the discussion section.

3. Fig. 2D: the predicted Erg1 conformational change in completely saturated vs. completely unsaturated lipid bilayers is relatively subtle. What is the predicted conformation in a bilayer with a PSPC:YOPC ratio that mimics a 'typical' proportion of saturated and unsaturated lipids? Is a change still apparent?

We have now performed molecular dynamics simulation of Erg1 in a mix of saturated and unsaturated lipids that mimics the membranes of WT and *ole1-20* cells. The result still did not show apparent conformational difference (Fig. S3).

4. Fig. 5B: the central role of lanosterol accumulation in the proposed mechanism of ER dysfunction is interesting but warrants further clarification. Fig. EV3 (which is not mentioned in the results section) indicates that the levels of downstream ergosterol synthesis intermediates are generally reduced in ole1-20 cells, pointing to a pathway blockage at lanosterol. Why does this occur? One possibility is that lipid saturation also impairs the protein stability or activity of lanosterol demethylase (Erg11), which warrants testing given that the human homologue of Erg11 (LDM) is targeted by the homologue of Doa10 (MARCHF6) (Scott et al. 2021 Biochem J).

We have now briefly described the results of Fig. EV3 (now Fig S5) in the results section and discussed the possibility of Erg11 downregulation. In fact, we are currently investigating the issue of Erg11 downregulation upon lipid saturation, hoping to complete the study for another manuscript in the future.

5. Fig. 5B: squalene conversion is restored by the addition of glycerol in Erg1 P469L-expressing ole1-20 cells; however, there is no corresponding increase in lanosterol levels. Can the authors please comment on this result? As a point of comparison, this experiment would benefit from including cells expressing WT Erg1 (where the ER is intact) to confirm that this indeed averts or blunts lanosterol accumulation.

There is indeed a small increase of lanosterol in Erg1 P469L-expressing *ole1-20* after glycerol addition (Fig. 5B), although it did not correspond to the reduced amount of squalene. It is possible that glycerol also partially alleviates the blockage of lanosterol turnover, such as by preserving Erg11. We have now included this in the discussion section. We have also added in the new Fig 5C the result of sterol analysis from *ole1-20* expressing WT Erg1 or P469L. The data shows that there is only 2-fold

increase of lanosterol in *ole1-20* expressing WT Erg1, supporting our notion that aberrant ER is caused by the large accumulation of lanosterol from Erg1 P469L expression. WT Erg1 is unable to produce the same effect as P469L mutant, likely because it is unstable in *ole1-20*, unlike P469L.

6. Fig. 5D: does manipulating lanosterol levels also restore ER morphology? The authors have only considered cell growth. If so, it would help to support their final model.

> This question was also raised by review #1 (point 3). As described above, we now show that Erg1 distribution and ER morphology are indeed restored by Ro 48-8071 in a dose-dependent manner in *ole1-20* overexpressing stable Erg1 (Fig. 5G) and in *ole1-20 doa10Δ* double mutant (Fig. 6E).

Minor comments

1. The statement "both mammalian SQLE and Erg1 are destabilized in response to an elevated level of downstream intermediate lanosterol" is incorrect - degradation of mammalian SQLE is unaffected by lanosterol (Gill et al. 2011 Cell Metab.).

> We have corrected the statement.

2. On page 9, it is stated that "Erg1 structure predicted by AlphaFold2 was similar to SQLE (Fig 3A)." However, no comparison between the two proteins is depicted.

> We have now included the structure of human SQLE in Fig 3A.

3. Please include methods for the scoring of imaged cells (e.g., Fig. 4B) and tetrad analysis (Fig. 6C).

> We have now included the methods.

4. To improve clarity, labels should be added to the icons in Figs. 4A and 6B to explicitly describe the phenotype.

> We have now added a few labels in the images to indicate the normal and abnormal patterns. Because the disorganized ER and Erg1 foci are spread throughout the cell, it is hard to label all abnormal structures without messing up and obscuring the pictures. Thus, we only label the representative ones as indicated in the legend.

5. The schematic in Fig. 8 is a useful accompaniment to the discussion, but would benefit from inclusion of Erg1, Doa10 and Ubc7 to better integrate the findings of the entire manuscript. The legend does not outline the possible consequences of protein segregation into different membrane phases - presumably this is the likely cause of the membrane dysfunction?

> We have now included Erg1, Doa10 and Ubc7 in Fig 8, and described in the legend the consequence of aberrant ER morphology and mislocalized and/or dysfunctional ER proteins.

6. Please ensure that figure legends contain full information about temperature(s) at which experiments were performed and any medium supplements (e.g., oleic acid, galactose).

We have ensured the inclusion of the information in the legends.

7. Please define the acronyms "TAG" and "NPC" in the main text.

We have defined these terms as they first appear in the main text.

8. Please correct typos in the main text and figures, e.g., "lesser extend" (page 11), "microscopy. Whereas" (page 19), "GFP-P469L" (Fig. 4C), "deleted for" (Fig. 2D legend), "Stutent's" (Fig. 4C legend).

We have corrected these typos.

Reviewer #3

Minor issues:

P5: When explaining that HMGR becomes misfolded, please cite one of the more recent papers from Randy Hampton, i.e. either Wangeline and Hampton, JBC 2018 or Wangeline and Hampton, JBC 2021.

We have now cited the new paper (Wangeline and Hampton, JBC 2021).

P6: "... lipotoxicity-related diseases (Schaffer, 2016)." Please give examples of the diseases you are referring to.

We have now added diabetes and hepatic steatosis as examples.

P7: "The ole1Δ deletion mutant is lethal". The mutant is not lethal but dead. Hence, more accurate ways of phrasing this would be "The ole1Δ deletion is lethal" or "The ole1Δ deletion mutation is lethal."

The statement is now corrected.

P7: For the benefit of orienting the reader, please begin with a statement why OLE1 ts alleles were made.

We have now added in the beginning of the results section the rationale of generating *ole1ts* mutants.

P7, Figure 1B: In the figure it says "revovered" instead of recovered.

We have now corrected the typo.

P7, Figure 1D: Please add information to the figure itself that growth is at 34 degrees. This piece of information is in the text and in the figure legend but it would help the reader to also see it in the panel.

We have now added this information in Fig 1D.

P8: The authors mention that SILAC was done to find proteins with differential abundance in WT versus ole1-20 cells but the data are not provided. Please either include the data or add a comment that or why they are not shown.

We did not show the SILAC data at the first place, because it was not our intention to describe the overall change in the proteome. Nevertheless, we now include a simple scatter plot of the SILAC proteomics in the new Fig S2 to show that Erg1 is reduced in *ole1-20*. We are currently investigating other candidate proteins.

P9: " ... showed that Erg1 situated on the surface ...". Please edit to: ... Erg1 is situated on ..."

We have changed the wording accordingly.

P10, Figure 3F: Please use a transmembrane protein as a reference rather than GFP-HDEL. The GFP-HDEL only shows that carbonate and high salt did not disrupt the ER membrane. However, to illustrate that Erg1 is not a regular integral membrane protein, it is necessary to show that the solubility of a true transmembrane protein is unaffected by carbonate treatment.

We have now replaced the control using Sec61 to represent an integral membrane protein.

P11: "... and to a lesser extend at 27 ...", should be: ... and, to a lesser extent, at 27 ..."

We have corrected the wording.

P11: Could the foci of Erg1(P469L) in ole1-20 cells that co-localize with Erg6 but are not lipid droplets (Figure 4) represent ERAD foci (Albert, ... Engel, PNAS 2020)? Please comment in the discussion if you think that this is a reasonable possibility. This is entirely optional, though, the authors do not have to include this idea if they do not think that it is helpful.

We thank the reviewer for this interesting idea. However, we decide not to consider this possibility at this point, because the ERAD foci were apparently present in *Chlamydomonas reinhardtii* even without ER stress, which might not be relevant to our observation. Also, it is unclear whether ERAD foci also exist in other organisms, including yeast.

P15: "... and for the membrane microdomain of specific functions." Not clear what membrane microdomain is meant here. If you refer to membrane microdomains generally, then edit to: "... for membrane microdomains of specific functions."

We have changed the wording accordingly.

October 24, 2022

RE: Life Science Alliance Manuscript #LSA-2022-01612-TR

Dr. Rey-Huei Chen
Institute of Molecular Biology, Academia Sinica
Institute of Molecular Biology
128 Sec. 2, Academia Rd.
Taipei 11529
Taiwan

Dear Dr. Chen,

Thank you for submitting your revised manuscript entitled "Lipid saturation induces degradation of squalene epoxidase for sterol homeostasis and cell survival". We would be happy to publish your paper in Life Science Alliance pending final revisions necessary to meet our formatting guidelines.

Along with points mentioned below, please tend to the following:

-please include the supp. Material and Methods section into the main Materials and Methods section

Figure Check:
- Figure 1C need scale bars
- Figure 5 A, E, F, Figure 6D need scale bars

If you are planning a press release on your work, please inform us immediately to allow informing our production team and scheduling a release date.

LSA now encourages authors to provide a 30-60 second video where the study is briefly explained. We will use these videos on social media to promote the published paper and the presenting author (for examples, see https://twitter.com/LSAjournal/timelines/1437405065917124608). Corresponding or first-authors are welcome to submit the video. Please submit only one video per manuscript. The video can be emailed to contact@life-science-alliance.org

To upload the final version of your manuscript, please log in to your account: https://lsa.msubmit.net/cgi-bin/main.plex
You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. FINAL FILES:

These items are required for acceptance.

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure, supplementary figure and video files uploaded as individual files: See our detailed guidelines for preparing your production-ready images, https://www.life-science-alliance.org/authors

-- Summary blurb (enter in submission system): A short text summarizing in a single sentence the study (max. 200 characters including spaces). This text is used in conjunction with the titles of papers, hence should be informative and complementary to the title. It should describe the context and significance of the findings for a general readership; it should be written in the present tense and refer to the work in the third person. Author names should not be mentioned.

B. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, https://www.life-science-alliance.org/authors

We encourage our authors to provide original source data, particularly uncropped/-processed electrophoretic blots and spreadsheets for the main figures of the manuscript. If you would like to add source data, we would welcome one PDF/Excel-file per figure for this information. These files will be linked online as supplementary "Source Data" files.

Submission of a paper that does not conform to Life Science Alliance guidelines will delay the acceptance of your manuscript.

It is Life Science Alliance policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.

The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements.

Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days.

Thank you for this interesting contribution, we look forward to publishing your paper in Life Science Alliance.

Sincerely,

Novella Guidi, PhD
Scientific Editor
Life Science Alliance

Reviewer #1 (Comments to the Authors (Required)):

In this revised manuscript Huang and Chen explore the effects of phospholipid saturation on stability of a model membrane protein in yeast, Erg 1. To control saturation, they generated temperature-sensitive mutants in the desaturase Ole1, and they obtained a mutant that accumulates saturated fatty acids at high temperature that is toxic to cell survival. Authors found that Erg1, a protein in the ergosterol biosynthetic pathway, is unstable stable in this ole1ts mutant at high temperature. Supplementation with oleic acid (OA) yields stable Erg1. They found that Doa1 and Ubc7, proteins of the ERAD complex, is involved in the degradation of Erg1 upon lipid saturation. The authors then identified Erg1 mutants resistant to degradation upon lipid saturation. They determined cell localization of Erg1 (normally in the ER) and found that it forms foci upon lipid saturation, and that ER morphology was aberrant. The toxic effects of saturation on cells were attributable to these changes in ER morphology, which, in turn, was caused by accumulation of lanosterol. Finally, they demonstrated that addition of glycerol resulted in normal ER morphology and prevention of protein misfolding, presumably due to a chaperone-like effect of this carbon source.

These data are significant in that they establish a new model for tightly temporally regulating phospholipid saturation in yeast, and they demonstrate that folding and stability of membrane proteins, as well as organellar morphology, are exquisitely dependent on this membrane parameter. The paper is easy to read, and the data are appropriately interpreted.

I offered some criticisms of the original manuscript. All were sufficiently dealt with in this revision. I have no further critical comments.

Reviewer #2 (Comments to the Authors (Required)):

Congratulations on a very nice piece of work.

Reviewer #3 (Comments to the Authors (Required)):

The minor issues I raised in my original comments have been addressed adequately and I support publication of the manuscript as is.

October 25, 2022

RE: Life Science Alliance Manuscript #LSA-2022-01612-TRR

Dr. Rey-Huei Chen
Institute of Molecular Biology, Academia Sinica
Institute of Molecular Biology
128 Sec. 2, Academia Rd.
Taipei 11529
Taiwan

Dear Dr. Chen,

Thank you for submitting your Research Article entitled "Lipid saturation induces degradation of squalene epoxidase for sterol homeostasis and cell survival". It is a pleasure to let you know that your manuscript is now accepted for publication in Life Science Alliance. Congratulations on this interesting work.

The final published version of your manuscript will be deposited by us to PubMed Central upon online publication.

Your manuscript will now progress through copyediting and proofing. It is journal policy that authors provide original data upon request.

Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.

IMPORTANT: If you will be unreachable at any time, please provide us with the email address of an alternate author. Failure to respond to routine queries may lead to unavoidable delays in publication.

Scheduling details will be available from our production department. You will receive proofs shortly before the publication date. Only essential corrections can be made at the proof stage so if there are any minor final changes you wish to make to the manuscript, please let the journal office know now.

DISTRIBUTION OF MATERIALS:
Authors are required to distribute freely any materials used in experiments published in Life Science Alliance. Authors are encouraged to deposit materials used in their studies to the appropriate repositories for distribution to researchers.

You can contact the journal office with any questions, contact@life-science-alliance.org

Again, congratulations on a very nice paper. I hope you found the review process to be constructive and are pleased with how the manuscript was handled editorially. We look forward to future exciting submissions from your lab.

Sincerely,

Novella Guidi, PhD
Scientific Editor
Life Science Alliance