

Inner-Nuclear-Membrane-Associated Degradation Employs Dfm1-Independent Retrotranslocation and Alleviates Misfolded Transmembrane-Protein Toxicity

Matthew Flagg, Margaret Wangeline, Sarah Holland, Sascha Duttke, Christopher Benner, Sonya Neal, and Randolph Hampton

Corresponding author(s): Randolph Hampton, UCSD

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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 #E20-11-0720

TITLE: "Inner-Nuclear-Membrane-Associated Degradation Employs Dfm1-Independent Retrotranslocation and Alleviates Misfolded Transmembrane-Protein Toxicity"

Dear Dr. Hampton:

Thank you for submitting your manuscript entitled "Inner-Nuclear-Membrane-Associated Degradation Employs Dfm1-Independent Retrotranslocation and Alleviates Misfolded Transmembrane-Protein Toxicity" to Molecular Biology of the Cell. I have received reports from two experts in the field that have carefully read your manuscript.

As you will see from the attached reports, both reviewers indicate that the study is carefully conducted and described, of broad interest to the membrane protein quality control field, and contributes an important advance related to the distinct mechanisms of protein quality control employed in the ER and the inner nuclear membrane. Both reviewers provide useful comments and suggestions. Reviewer #2 raises some concerns, such as the failure to fully address the underlying mechanism of suppression following chromosome duplication. I feel that carefully addressing these comments and concerns would be beneficial and improve the manuscript.

Therefore, we would be happy to consider a revised manuscript that satisfies the joint concerns of the reviewers. We look forward to receiving your revised manuscript, together with a letter indicating the changes you have made and the responses to the reviewers' comments.

Sincerely,
James Olzmann
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Hampton,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) 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 have opted out of publishing the review history.

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

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 version, and figures, please use this link (please enable cookies, or cut and paste URL): [Link Not Available](#)

Authors of Articles and Brief Communications whose manuscripts have returned for minor revision ("revise only") 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.

Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

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

Reviewer #1 (Remarks to the Author):

In this investigation, Flagg and colleagues have evaluated retrotranslocation of inner nuclear membrane (INM)-associated degradation (INMAD) substrates. Unlike Hrd1- and Doa10-mediated endoplasmic reticulum-associated degradation (ERAD), Asi- and Doa10-mediated INMAD of three substrates (sec61-2-GFP, Erg11, and Asi2) proceeds independently of Dfm1. Asi-dependent ubiquitylation and retrotranslocation of sec61-2-GFP also proceeds independently of Dfm1. Finally, overexpression of ERAD/INMAD substrate sec61-2-GFP is toxic in the context of impaired ERAD and INMAD. This toxicity is likely emblematic of a novel form of misfolded INM protein stress and is suppressed by ASI1 overexpression. Spontaneous suppresses were identified that possessed duplication of chromosome V and XIV (in three cases) and just of chromosome V (in one case). While the precise mechanism of suppression remains uncharacterized, it is distinct from previously characterized suppression of mutant ERAD phenotypes (in which HRD1 is overexpressed).

This paper was a delight to read. The work will be an important contribution to INMAD/ERAD research communities - it reveals critical mechanistic differences between quality control pathways in contiguous, yet distinct, INM and ER membranes. This manuscript will be likely be of broad

interest to the membrane protein quality control field. The data in this paper are compelling. Experiments are carefully described and analyzed. The conclusions are supported by the results.

I only have minor comments/suggestions:

- Paragraph 2 of Introduction: Authors describe the "conical" ERAD pathways. I assume they mean to say "canonical".
- In the first sentence of the penultimate paragraph of the Introduction, the authors write, "Here, we demonstrate that classical ERAD-M retrotranslocation of full-length multispansing INMAD substrates occurs, and that Dfm1 is not involved in this process."
 - o This sentence is a little confusing. I think the authors mean to say that in INMAD, full-length protein is retrotranslocated, similar to what happens in ERAD-M. However, to describe what happens in INMAD as "classical ERAD-M retrotranslocation" suggests to me a similar mechanism of membrane extraction. The authors show that this is decidedly not the case.
- In results section "Dfm1 was not required for INMAD retrotranslocation": The authors write that *asi1*^Δ *hrd1*^Δ cells display no ("none") ubiquitination of sec61-2-GFP. This is not what is depicted in Figure 3. It appears that there is minimal sec61-2-GFP ubiquitination in *asi1*^Δ *hrd1*^Δ cells that does not increase upon MG132 addition.
- In results section, "Sec61-2 toxicity can be suppressed by aneuploidy":
 - o 1st paragraph, penultimate sentence: *dfm1*^Δ is misspelled as *dmf1*^Δ.
 - o 3rd paragraph, last sentence: The authors have shown the *asi1*^Δ *hrd1*^Δ suppresses do not degrade the sec61-2-GFP INMAD substrate. Based on this observation, they write "...suggesting no additional modes of INMAD retrotranslocation were available to cells". This may be true (it seems likely). However, the authors have not analyzed retrotranslocation in the suppresses (just degradation). One plausible route to suppression could be ASI-independent retrotranslocation without degradation.
- Figure 2B strain labels to right of graph appear out of order and do not include symbol key.

A few minor comments regarding items on the MBoC Author Checklist:

Section I

- Legends for Figures 5 and 6A: A statement of number of times these experiments were performed is missing. (I note that several key observations in Figure 5 are replicated throughout the growth assays in this figure, and that the experiment in Figure 6A validates experiments in Figure 5. These observations provide increased confidence in the central results.)

Section II

- Meets all criteria.

Section III

- The checklist indicates that full sequence information for all recombinant DNA constructs and synthetic oligonucleotides used in the study should be provided. The authors do not include this information. However, it is not standard for plasmid sequences to be included in these types of studies, nor is inclusion of oligonucleotide sequences a universal practice. The authors confirm that plasmids were sequence-verified and primer sequences are available upon request.

Section IV

- Meets all criteria.

Reviewer #2 (Remarks to the Author):

OVERVIEW

In this manuscript, the authors report that Dfm1, a protein in essential for retrotranslocation of misfolded protein during ER-associated degradation (ERAD) in yeast, does not participate in the degradation processes at the inner nuclear membrane (INMAD), even though those substrates are indeed retrotranslocated. The authors find evidence for aneuploidy in the face of losing both ERAD and INMAD pathways when a misfolded membrane protein accumulates to toxic levels. The authors clearly show that a model INMAD and ERAD substrate Sec61-2 is degraded in a process that depends both on Hrd1 and Asi1 (Figure 1D), as well as Cdc48 (Figure 1E). Sec61-2 degradation also requires Dfm1, which mostly phenocopies the deletion of Hrd1 (Figure 2A,B), confirming both work within the same ERAD process. In contrast, degradation of Erg11, a validated substrate of the Asi complex, does not require Dfm1 (Figure 2C). Moreover, degradation of the INM-localised Asi2 requires both Asi1 and Doa10 (Figure 2E), but not Dfm1 (Figure 2D,E), indicating that Dfm1 is not involved in either of the 2 degradation routes in the INM. The authors go on to show both Hrd1 and Asi ubiquitinate and retrotranslocate Sec61-2 (Figure 3, 4B) but that only Δ Dfm1 in combination with Δ Asi and not Δ Hrd1 can fully prevent retrotranslocation, comparable to Cdc48-2 mutant, providing further evidence that retrotranslocation of INM substrates by Asi occurs via a process that does not involve Dfm1. They concluded that INM factors must serve the role that Dfm1 does in the ER but do not identify those factors. The authors then show that both INMAD (dependent on Δ Asi1 or Δ Asi3 but not Asi2) and ERAD (by Hrd1) working in parallel are required to detoxify cells from an accumulating misfolded membrane protein Sec61-2 (Figure 5A-E). This also depends on the ubiquitination by Asi1 (Figure 5F). Finally, the authors used a clever strategy to reveal suppressors of Sec61-2-GFP lethality in Δ Hrd1 Δ Asi1 strains and found aneuploidic strains hallmarked by duplication of ChrV and ChrXIV. The presence of Asi3 on ChrXIV was postulated as a potential source of the suppression but this was not followed up upon.

SUMMARY

The manuscript is fairly concise, clearly written and presents some interesting observations regarding the conservation of membrane protein retrotranslocation mechanisms as well as the consequences and adaptation of cells to loss of parallel quality control pathways. Understanding the interplay between INMAD and ERAD in terms of a proteostasis network is of great interest to this field and many others and so is of general interest. The figures are of good quality, clearly presented with sufficient controls, replicates and statistics included. Overall the manuscript is suitable in detail and scope for the journal.

While the manuscript does provide some interesting findings, it does feel a little bit preliminary to this reviewer. While it was not known whether Dfm1 would be necessary for INMAD, its lack of involvement is clear and that might not have been unexpected. Not all mechanisms share a common element and it is clear that retrotranslocation is one of those cases. The finding that Dfm1 is used differentially with INM and ER Doa10 substrates is particularly noteworthy but does not seem to get enough attention here. Moreover, perhaps the most interesting finding is the chromosomal duplication that results in viable Δ Hrd1 Δ Asi1 clones and the observation that that is not the same solution found with the Δ Dfm1 Δ Hrd1 Δ Ire1 case, is striking. The suggestion that Asi3 is on ChrXIV as a possible mechanism is intriguing but as ChrV is duplicated in all suppresseses,

perhaps the answer lies elsewhere. Moreover, as the authors show that degradation is not restored in these suppresses, the mechanism appears to lie in an alternate sphere of homeostatic adjustment other than restored degradation. In this reviewer's opinion, shoring up the potential origin of the suppressive mechanism, as has been done previously by the authors for Dfm1, would substantially improve the impact of the manuscript. There are some interesting ideas here such as the notion of different mechanisms for retrotranslocation being required in the INM vs ER and the idea that E3s such as Doa10 can function with and without Dfm1, but this reviewer is left wanting a bit more clarity and the data presented fall slightly short of pushing those ideas significantly forward.

As the COVID19 outbreak has severely hampered research progress in many labs, this reviewer is sympathetic to the authors and wary of any request for significant additional experiments. If possible, the addition of such data would be welcomed but in lieu of an inability to embark on such endeavours, addressing these points in the text could potentially suffice and could be at the discretion of the reviewing editor and MBoC's current policies.

There are a few issues that require attention.

SELECTED ISSUES/QUERIES

Issue 1. The authors suggest that the presence of Asi3 on duplicated ChrXIV may underpin viability of Δ Hrd1 Δ Asi suppresses in the face of Sec61-2-GFP toxicity. Has the overexpression of Asi3 been tested directly for the ability to decrease toxicity in these double-delete strains as well? If not just Asi3, what other homeostasis-related candidates are there on these chromosomes? This seems to be a very promising observation but without identifying or even testing candidates, the impact is mitigated.

Issue 2. Have any interaction proteomics been done on the Asi complex and was Dfm1 or any other unknown INM factor identified that could support retrotranslocation? Any hints from screening deletion collections? If stronger evidence that either Asi itself (or another INM factor) were responsible for retrotranslocation, that would broaden these findings. Even something like addressing how Cdc-48 is linked to Asi would

Issue 3. Could the authors provide an explanation for the difference in Asi2 degradation dependence on Doa10 with the report of Boban et al. (JCS 2014).

Issue 4. Is HA-Asi2 assembly competent? If so, does it compete with endogenous Asi2 for assembly into a complex? HA-Asi2 turnover appears clear but why would it be in strains with both Asi1 and Asi3? Is Asi2 always in excess and needs to be degraded? HA-Asi2 stabilisation in the Δ Asi1 Δ Doa10 strain would seem to indicate that Asi2 is both a component and a substrate of the Asi complex. Not an unheard-of scenario but perhaps the authors could provide additional context and detail regarding how this might work.

SPECIFIC COMMENTS/QUESTIONS

Comment 1. In Supplementary Figure 1, there is no panel A, as is written on pg. 8 (Fig S1A)

Comment 2. Pg. 10, line 7 should read Asi1-Asi2-Asi3

Comment 3. Pg 10, line 16, should read Asi3 and not Asi2 (in parentheses)

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Comment 5 Pg 22, last line, should be conformation, not confirmation

Comment 6 Pg 23, line 4, should be affects, not effects

Comment 7 Figure 2B, missing the lines demarcations to identify the strains

Comment 8 Pg 19, last line, retrotranslocation is misspelled



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Dear MBoC editors and reviewers,

We thank the managing editor and each of the reviewers for conducting a speedy review with thoughtful edits and criticism. We have carefully read and responded to each suggestion made by each reviewer. Our point-by-point responses are below, alongside each reviewer's commentary.

We have made edits to the text and figures in every case suggested. We thank the reviewers for their feedback and hope that the manuscript is now clearer and more concise.

We also share the second reviewer's opinion that a deeper study of Sec61-2-GFP-induced suppression would improve the impact of the manuscript. Unfortunately, our lab has been essentially closed in the midst of the Covid-19 pandemic, and our ability to pursue additional lab work remains very limited at this time. Therefore, in lieu of additional in-lab experiments, we have used existing data sets to identify genes of interest on the chromosomes duplicated in our suppressesees. In two new tables, we present a number of genes that, alone or in combination, could alter the proteostatic landscape of our suppressesees and thereby allow them to survive Sec61-2-GFP toxicity.

We again thank the managing editors and the reviewers for their ongoing consideration. As always, it has been a pleasure working with MBoC.

Sincerely,

Randolph Hampton and Matthew Flagg

Reviewer One

Paragraph 2 of Introduction: Authors describe the "conical" ERAD pathways. I assume they mean to say "canonical".

Corrected to canonical

In the first sentence of the penultimate paragraph of the Introduction, the authors write, "Here, we demonstrate that classical ERAD-M retrotranslocation of full-length multispansing INMAD substrates occurs, and that Dfm1 is not involved in this process."

This sentence is a little confusing. I think the authors mean to say that in INMAD, full-length protein is retrotranslocated, similar to what happens in ERAD-M. However, to describe what happens in INMAD as "classical ERAD-M retrotranslocation" suggests to me a similar mechanism of membrane extraction. The authors show that this is decidedly not the case.

We have rewritten this sentence to make it clearer.

In results section "Dfm1 was not required for INMAD retrotranslocation": The authors write that *asi1Δ hrd1Δ* cells display no ("none") ubiquitination of sec61-2-GFP. This is not what is depicted in Figure 3. It appears that there is minimal sec61-2-GFP ubiquitination in *asi1Δ hrd1Δ* cells that does not increase upon MG132 addition.

Edited text to better reflect experimental results, as suggested.

In results section, "Sec61-2 toxicity can be suppressed by aneuploidy":
1st paragraph, penultimate sentence: *dfm1Δ* is misspelled as *dmf1Δ*.

Fixed

3rd paragraph, last sentence: The authors have shown the *asi1Δ hrd1Δ* suppresses do not degrade the sec61-2-GFP INMAD substrate. Based on this observation, they write "...suggesting no additional modes of INMAD retrotranslocation were available to cells". This may be true (it seems likely). However, the authors have not analyzed retrotranslocation in the suppresses (just degradation). One plausible route to suppression could be ASI-independent retrotranslocation without degradation.

We have edited the language of this section to reflect our data more closely and conservatively.

Figure 2B strain labels to right of graph appear out of order and do not include symbol key.

Labels have been reordered and symbol key has been added.

A few minor comments regarding items on the MBoC Author Checklist:

Section I

- Legends for Figures 5 and 6A: A statement of number of times these experiments were performed is missing. (I note that several key observations in Figure 5 are replicated throughout the growth assays in this figure, and that the experiment in Figure 6A validates experiments in Figure 5. These observations provide increased confidence in the central results.)

Statements about the number of experimental replicates have been added in all cases in question. We apologize for this oversight in the original manuscript.

Section III

- The checklist indicates that full sequence information for all recombinant DNA constructs and synthetic oligonucleotides used in the study should be provided. The authors do not include this information. However, it is not standard for plasmid sequences to be included in these types of studies, nor is inclusion of oligonucleotide sequences a universal practice. The authors confirm that plasmids were sequence-verified and primer sequences are available upon request.

We are willing to provide any of the above-mentioned sequence information along with the manuscript. As the reviewer notes, oligo sequences are not universally included in MBoC papers, and so were omitted in the original submission.

Reviewer Two

SELECTED ISSUES/QUERIES

Issue 1. The authors suggest that the presence of Asi3 on duplicated ChrXIV may underpin viability of Δ Hrd1 Δ Asi suppresses in the face of Sec61-2-GFP toxicity. Has the overexpression of Asi3 been tested directly for the ability to decrease toxicity in these double-delete strains as well? If not just Asi3, what other homeostasis-related candidates are there on these chromosomes? This seems to be a very promising observation but without identifying or even testing candidates, the impact is mitigated.

We have not directly tested the ability of Asi3 overexpression to alleviate Sec61-2-GFP toxicity and regret our present inability to pursue this and other similar modes of inquiry at this time.

However, in response to these comments we have constructed tables of homeostasis-related candidates on chromosome V and XIV. We discuss some of these candidates in the body of the text and describe our methodology in the materials and methods section.

Issue 2. Have any interaction proteomics been done on the Asi complex and was Dfm1 or any other unknown INM factor identified that could support retrotranslocation? Any hints from screening deletion collections? If stronger evidence that either Asi itself (or another INM factor) were responsible for retrotranslocation, that would broaden these findings. Even something like addressing how Cdc-48 is linked to Asi would

To our knowledge and according to the *Saccharomyces* Genome Database, no high-throughput proteomics have been reported for physical interactions with the Asi complex. Additionally, a split GFP screen for INM localization did not isolate Dfm1 as a hit, further suggesting its exclusion from the INM (Smoyer et al., 2016).

In the case of substrates such as Erg11, it seems likely that the Asi complex is responsible for both Cdc48 recruitment and retrotranslocation. We have more carefully emphasized that Cdc48/Npl4/Ufd1 can facilitate retrotranslocation *in vitro* in a reconstituted system comprised of a substrate, the Asi complex, Ubc4, and Ubc7 (Natarajan 2020). As mentioned in the manuscript, similar *in vitro* studies at least allow the possibility that Doa10 also mediated INMAD retrotranslocation.

In the case of Asi2- and Doa10-independent substrates such as Sec61-2, the literature offers few if any compelling candidates for an additional INMAD retrotranslocon. Perhaps the most direct genetic inquiry into UPS factors involved in such cases of INMAD is presented in Pantazopoulou et al., 2014. In that publication, Pantazopoulou et al. identify Asi1 itself as a target of Asi-complex- and Doa10-independent INMAD, and they use a collection of UPS-related genes curated by the Hochstrasser lab (Xie et al., 2010) to identify factors required for Asi1 degradation. They report no additional Dfm1-like transmembrane factors that could mediate retrotranslocation. In fact, they rule out several intriguing factors that access the INM, such as Ubx2 (Smoyer et al., 2016). In short, there are few if any concrete data pointing to another INM retrotranslocon, which underlines the value of crossing a *hrd1Δ* Sec61-2-GFP strain to the deletion collection.

Issue 3. Could the authors provide an explanation for the difference in Asi2 degradation dependence on Doa10 with the report of Boban et al. (JCS 2014).

We clarified the language in this part of the manuscript and offered explanations for the minor differences between our own studies of Asi2 and those reported in JCS.

Issue 4. Is HA-Asi2 assembly competent? If so, does it compete with endogenous Asi2 for assembly into a complex? HA-Asi2 turnover appears clear but why would it be in strains with both Asi1 and Asi3? Is Asi2 always in excess and needs to be degraded? HA-Asi2 stabilisation in the Δ Asi1 Δ Doa10 strain would seem to indicate that Asi2 is both a component and a substrate of the Asi complex. Not an unheard-of scenario but perhaps the authors could provide additional context and detail regarding how this might work.

We have added additional information about Asi2 degradation to the manuscript. The reason for constitutive degradation of Asi2 remains somewhat unclear, but Boban et al., 2014 show that functional myc- and HA-tagged versions of the protein are constitutively degraded. Degradation kinetics are identical whether the tagged construct is expressed in an *asi2Δ* background or in addition to the genomic *ASI2*, suggesting, perhaps, that Asi2 is always in excess and therefore constitutively degraded.

SPECIFIC COMMENTS/QUESTIONS

Comment 1. In Supplementary Figure 1, there is no panel A, as is written on pg. 8 (Fig S1A)

Fixed

Comment 2. Pg. 10, line 7 should read Asi1-Asi2-Asi3

Fixed. Asi12- has been changed to Asi1

Comment 3. Pg 10, line 16, should read Asi3 and not Asi2 (in parentheses)

The text has been changed and omits this error.

Comment 4 Pg 21, lines 9 and 12, should be Figure 4C, not 4B

Fixed

Comment 5 Pg 22, last line, should be conformation, not confirmation

Fixed

Comment 6 Pg 23, line 4, should be affects, not effects

This is, to our knowledge, a circumstance where the verb is effects. X effects (brings about) Y whereas X affects (changes, alters) Y.

Comment 7 Figure 2B, missing the lines demarcations to identify the strains

Fixed

Comment 8 Pg 19, last line, retrotranslocation is misspelled

Fixed

Pantazopoulou, M., Boban, M., Foisner, R., & Ljungdahl, P. O. (2016). Cdc48 and Ubx1 participate in a pathway associated with the inner nuclear membrane that governs Asi1 degradation. *J Cell Sci*, 129(20), 3770–3780

Smoyer CJ, Katta SS, Gardner JM, Stoltz L, McCroskey S, Bradford WD, McClain M, Smith SE, Slaughter BD, Unruh JR, Jaspersen SL. Analysis of membrane proteins localizing to the inner nuclear envelope in living cells. *J Cell Biol*. 2016 Nov 21;215(4):575-590.

Xie Y, Rubenstein EM, Matt T, Hochstrasser M. (2010) SUMO-independent in vivo activity of a SUMO-targeted ubiquitin ligase toward a short-lived transcription factor. *Genes Dev*, May;24(9):893-903.

RE: Manuscript #E20-11-0720R

TITLE: "Inner-Nuclear-Membrane-Associated Degradation Employs Dfm1-Independent Retrotranslocation and Alleviates Misfolded Transmembrane-Protein Toxicity"

Dear Dr. Hampton:

Thank you for submitting your manuscript "Inner-Nuclear-Membrane-Associated Degradation Employs Dfm1-Independent Retrotranslocation and Alleviates Misfolded Transmembrane-Protein Toxicity" along with your point-by-point responses to the reviewers' comments. After reading your revised manuscript and rebuttal letter, I have concluded that the manuscript thoughtfully addresses the reviewers' concerns and provides important clarifications. The manuscript advances our understand of the mechanisms involved in inner-nuclear membrane-associated protein degradation and its crosstalk with ER-associated protein degradation to alleviate membrane-associated proteotoxicity. I am sure this manuscript will be of broad interest to cell biologists and I congratulate you on this interesting study!

I am pleased to report that your manuscript is now accepted for publication in Molecular Biology of the Cell without further modifications. Thank you for submitting this important research.

Sincerely,
James Olzmann
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Hampton:

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.

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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
