ESCargo: a regulatable fluorescent secretory cargo for diverse model organisms

Jason Casler, Allison Zajac, Fernando Valbuena, Daniela Sparvoli, Okunola Jeyifous, Aaron Turkewitz, Sally Horne-Badovinac, William Green, and Benjamin Glick

Corresponding author(s): Benjamin Glick, The University of Chicago

Review Timeline:

Submission Date:	2020-09-17
Editorial Decision:	2020-10-16
Revision Received:	2020-10-19
Accepted:	2020-10-23

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 #E20-09-0591
TITLE: "ESCargo: a regulatable fluorescent secretory cargo for diverse model organisms"

Dear Ben,

Your manuscript "ESCargo: a regulatable fluorescent secretory cargo for diverse model organisms" has now been reviewed by two experts in the field and their comments are included below. As you will see both reviewers were very positive about this work and thought the development and description of this new tool for an extended set of model organisms was an important advance in the field and that the work was well executed and will likely appeal to the broad readership of MBoC. Both reviewers raised a few points which they believe will further improve the manuscript. In particular reviewer #2 would like you to include a more extended description/comparison of competing methods for visualizing secretory cargoes than is present in the current version of this manuscript. Overall, the two reviewers' suggestions appear to be straightforward and will likely not require additional experimentation.

I look forward to receiving a revised version of this manuscript. Thank you for submitting this interesting work to MBoC.

Sincerely,

Patrick Brennwald
Associate Editor
Molecular Biology of the Cell

Dear Dr. Glick,

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):

The manuscript by Casler et al describes the development of powerful tools to image and measure a wave of secretory cargo as it transits the secretory pathway. The authors use a fluorescently-tagged FKBP fusion that forms aggregates when targeted to the ER but can be disaggregated by addition of an FKBP ligand for regulated ER exit. Advances reported here exploit use of an ER export signal to compare receptor-mediated vs bulk flow transport rates and expand the range of experimental models to include yeast, Drosophila, mammalian and Tetrahymena cells. These findings demonstrate the advantages and generality of this system to monitor synchronized transport of cargo through the secretory pathway. The ESCargo technology will be a valuable resource for the cell biology community. I have only minor suggestions to strengthen this important study.

1. As standard for the field, suggest adding molecular weight markers to the immunoblot in Fig. 1F. For the blots shown in Fig. 1F and 5B, these are presumably representative examples, which could be mentioned in the methods or figure legends.

2. The results and methods are well described although suggest including the percentage of cells that display the characteristic secretory wave for each cell type. For example, in the mammalian cell line experiments it is reported that nearly all of the cells contain bright fluorescent round aggregates that dissolved upon SLF addition to fill the ER. This sounds like a high percentage. In yeast cells, the experiments focus on cells containing moderate amounts of aggregated ESCargo, which is fine, but it would help to include the percentage of this cell population. There was less information on these percentages in the Drosophila and Tertrahymena cell experiments. This information could help other investigators plan and design their experiments.

Reviewer #2 (Remarks to the Author):

The manuscript by Casler et al. describes an experimental system to visualize trafficking of soluble secretory cargo, which is applicable to yeast, mammalian, and even to Drosophila and Tetrahymena cells. It is based on the design that an artificial cargo, DsRed tetramer hooked with dimeric FKBP, is first trapped in the ER as large aggregations and then gets exported upon addition of SLF, a compound to dissolve the aggregates. The original method was developed for yeast cells with elaborate tricks, including the addition of an Erv29-dependent ER export signal, and was already published. What's new in the present work is its application to other organisms. With some modifications, the authors have successfully expressed the cassette in mammalian cells to mobilize the cargo in an export-signal-dependent or -independent manner and shown that an efficient secretory wave can be created in the former case. It is nice that basically the same design works even in Drosophila cells. The data of Tetrahymena cells did not look very convincing to me, but I agree it may offer applicability to many different organisms.

Imaging of secretory cargo movement in live cells is one of the most important tools to understand mechanisms of membrane traffic. Many methods have been proposed and tested. The system the authors developed is one of them and has been successfully applied to visualize cargo trafficking during Golgi cisternal maturation in yeast. It should be remembered, however, that Nakano's group has developed a different method for cargo visualization, which takes advantage of temperature-dependent accumulation and release of natural cargo proteins. It is designed as 1) heat-shock-promoter-dependent pulse synthesis of an authentic cargo and its simultaneous accumulation in the ER in a temperature-sensitive sec mutant at high temperature, and 2) subsequent chase of the cargo by lowering temperature (Kurokawa et al., Nat.Comm. 2014; JCB 2019). By this way, Axl2, a natural transmembrane cargo destined to the plasma membrane, can be visually tracked from the ER to the PM, which was used to analyze cargo behavior during Golgi cisternal maturation in the paper published back to back with the authors' group (JCB 2019). It is not fair to ignore it in the present manuscript. This method has also been successfully utilized by Nakatogawa's group to assess the contribution of COPII to autophagosome formation (Shima et al., JCB 2019).

The RUSH system that Perez's group developed is also very popular in this field and has been successfully used in many applications. I agree it is not almighty and may have some limitations, but the great advantage of RUSH is its flexibility in choosing cargo among many natural secretory and membrane proteins. Indeed, RUSH has proven quite powerful in showing different behaviors of different types of cargo in the secretory pathway, especially in the post-Golgi events. The totally artificial cargo system that the authors devised has pros and cons: it can easily incorporate a defined pair of an export signal and its receptor but cannot be used for unknown mechanisms. Nevertheless, developing different systems for different objectives should be helpful, and so I support publication of this work, provided the above concern is cleared.

Responses to Reviewers

Reviewer #1 (Remarks to the Author):

The manuscript by Casler et al describes the development of powerful tools to image and measure a wave of secretory cargo as it transits the secretory pathway. The authors use a fluorescently-tagged FKBP fusion that forms aggregates when targeted to the ER but can be disaggregated by addition of an FKBP ligand for regulated ER exit. Advances reported here exploit use of an ER export signal to compare receptor-mediated vs bulk flow transport rates and expand the range of experimental models to include yeast, Drosophila, mammalian and Tetrahymena cells. These findings demonstrate the advantages and generality of this system to monitor synchronized transport of cargo through the secretory pathway. The ESCargo technology will be a valuable resource for the cell biology community. I have only minor suggestions to strengthen this important study.

Thanks to the reviewer for the positive comments.

1. As standard for the field, suggest adding molecular weight markers to the immunoblot in Fig. 1F. For the blots shown in Fig. 1F and 5B, these are presumably representative examples, which could be mentioned in the methods or figure legends.

The original blot in Fig. 1F included molecular weight markers. Instead of marking the positions of those markers on a very small figure element, we modified the figure legend to indicate that the displayed band had an apparent MW of ~37 kDa, which is close to the predicted MW of 38.3 kDa.

The blots in Figs. 1F and 5B are indeed representative examples. This point is now indicated in the figure legends.

2. The results and methods are well described although suggest including the percentage of cells that display the characteristic secretory wave for each cell type. For example, in the mammalian cell line experiments it is reported that nearly all of the cells contain bright fluorescent round aggregates that dissolved upon SLF addition to fill the ER. This sounds like a high percentage. In yeast cells, the experiments focus on cells containing moderate amounts of aggregated ESCargo, which is fine, but it would help to include the percentage of this cell population. There was less information on these percentages in the Drosophila and Tertrahymena cell experiments. This information could help other investigators plan and design their experiments.

As suggested, the percentages are now indicated in the text for all of the tested cell types. About 75% of the yeast cells contained moderate amounts of aggregated ESCargo. For the other cell types, nearly all of the cells responded in the same way as the representative examples that are shown.

Reviewer #2 (Remarks to the Author):

The manuscript by Casler et al. describes an experimental system to visualize trafficking of soluble secretory cargo, which is applicable to yeast, mammalian, and even to Drosophila and Tetrahymena cells. It is based on the design that an artificial cargo, DsRed tetramer hooked with dimeric FKBP, is first trapped in the ER as large aggregations and then gets exported upon addition of SLF, a compound to dissolve the aggregates. The original method was developed for yeast cells with elaborate tricks, including the addition of an Erv29-dependent ER export signal, and was already published. What's new in the present work is its application to other organisms. With some modifications, the authors have successfully expressed the cassette in mammalian cells to mobilize the cargo in an export-signal-dependent or -independent manner and shown that an efficient secretory wave can be created in the former case. It is nice that basically the same design works even in Drosophila cells. The data of Tetrahymena cells did not look very convincing to me, but I agree it may offer applicability to many different organisms.

We appreciate the acknowledgment that ESCargo will likely have broad utility.

Imaging of secretory cargo movement in live cells is one of the most important tools to understand mechanisms of membrane traffic. Many methods have been proposed and tested. The system the authors developed is one of them and has been successfully applied to visualize cargo trafficking during Golgi cisternal maturation in yeast. It should be remembered, however, that Nakano's group has developed a different method for cargo visualization, which takes advantage of temperature-dependent accumulation and release of natural cargo proteins. It is designed as 1) heat-shock-promoter-dependent pulse synthesis of an authentic cargo and its simultaneous accumulation in the ER in a temperature-sensitive sec mutant at high temperature, and 2) subsequent chase of the cargo by lowering temperature (Kurokawa et al., Nat.Comm. 2014; JCB 2019). By this way, Axl2, a natural transmembrane cargo destined to the plasma membrane, can be visually tracked from the ER to the PM, which was used to analyze cargo behavior during Golgi cisternal maturation in the paper published back to back with the authors' group (JCB 2019). It is not fair to ignore it in the present manuscript. This method has also been successfully utilized by Nakatogawa's group to assess the contribution of COPII to autophagosome formation (Shima et al., JCB 2019).

The reviewer's point is well taken. We have enhanced the Introduction to include a paragraph describing the use of thermosensitive mutants to trigger ER exit in yeast.

The RUSH system that Perez's group developed is also very popular in this field and has been successfully used in many applications. I agree it is not almighty and may have some limitations, but the great advantage of RUSH is its flexibility in choosing cargo among many natural secretory and membrane proteins. Indeed, RUSH has proven quite powerful in showing different behaviors of different types of cargo in the secretory pathway, especially in the post-Golgi events. The totally artificial cargo system that the authors devised has pros and cons: it can easily incorporate a defined pair of an export signal and its receptor but cannot be used for unknown mechanisms. Nevertheless,

developing different systems for different objectives should be helpful, and so I support publication of this work, provided the above concern is cleared.

The RUSH method is powerful and is gaining in popularity. We modified the Introduction to emphasize these points.

But as indicated in the text, despite a concerted effort, we were unable to get RUSH to work with yeast.

RE: Manuscript #E20-09-0591R
TITLE: "ESCargo: a regulatable fluorescent secretory cargo for diverse model organisms"

Dear Ben,

I have looked over your revised manuscript and I think you have done a commendable job of addressing the concerns of the two reviewers. I am happy to recommend that the revised manuscript be accepted for publication in Molecular Biology of the Cell

Congratulations!

Patrick Brennwald
Associate Editor
Molecular Biology of the Cell

Dear Dr. Glick:

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
