THE EMBO JOURNAL

Structural basis of TRAPPIII-mediated Rab1 activation

Aaron Joiner, Ben Phillips, Kumar Yugandhar, Ethan Sanford, Marcus Smolka, Haiyuan Yu, Elizabeth Miller, and J. Christopher Fromme
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Editor: Elisabetta Argenzio

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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 9th Feb 2021

Thank you for submitting your manuscript entitled "Structure and mechanism of TRAPPIII-mediated Rab1 activation" (EMBOJ-2020- 107607) to The EMBO Journal. Your study has now been assessed by three reviewers, whose reports are enclosed below for your information.

As you can see, the referees find your work interesting, but also suggest that you address a few points in order to strengthen the main conclusions.

Given the overall interest of your study, we have decided to invite you to submit a new version of the manuscript revised according to the referees' requests. I should add that it is The EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in the revised version.

We generally grant three months as standard revision time. As we are aware that many laboratories cannot function at full capacity owing to the COVID-19 pandemic, we may relax this deadline. Also, we have decided to apply our 'scooping protection policy' to the time span required for you to fully revise your manuscript and address the experimental issues highlighted herein. Nevertheless, please inform us as soon as a paper with related content is published elsewhere.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File and will therefore be made available online. For more details on our Transparent Editorial Process, please visit our website:
http://emboj.embopress.org/about#Transparent_Process

Before submitting your revised manuscript, deposit any primary datasets and computer code produced in this study in an appropriate public database (see http://msb.embopress.org/authorguide#dataavailability). Please remember to provide a reviewer password, in case such datasets are not yet public. The accession numbers and database names should be listed in a formal "Data Availability" section (placed after Materials & Method). Provide a "Data availability" section even if there are no primary datasets produced in the study.

Feel free to contact me if you have any questions about the submission of the revised manuscript to The EMBO Journal. I thank you again for the opportunity to consider this work for publication and look forward to your revision.

Referee #1:

The authors present a well-written, comprehensive, and multidisciplinary analysis of the TRAPPIII complex (a GEF) and its interactions with Rab1/Ypt1 (a key regulatory GTPase in the secretory and autophagic pathways) and with membranes. While the linchpin of the story is the cryoEM structure of a TRAPPIII-Ypt1 complex (a noteworthy achievement in its own right), the impact of this structure is enhanced by an expansive array of complementary biochemical and in vivo experiments. Together, these reveal the binding site for the key juxtamembrane 'hypervariable'

region of the Rab which, in turn, suggests the orientation of the entire TRAPPIII complex on membranes. This hypothesis leads the authors to propose, and then experimentally confirm, that a disordered region of the TRAPPIII-specific subunit Trs85 forms a membrane-inserting amphipathic alpha-helix. The Trs85 amphipathic helix, by helping anchor the TRAPPIII complex to membranes in the proposed orientation, plays an essential role in Rab1 activation.

Taken together, these findings represent a major advance in our understanding of Rab1 biology. The story is mechanistically interesting and biologically important. Overall, while there are always further experiments one could propose, I think the manuscript in its current form is suitable in completeness and impact to merit publication without substantial revision.

Referee #2:

Using a cryo-EM based approach the authors report the structure of the yeast TRAPPIII complex bound to its cognate GTPase Ypt1. TRAPPIII (is one of two yeast TRAPP complexes - the other being TRAPPII) functions on at least three distinct membrane populations (COPII vesicles, the Golgi, and on autophagosomal membranes). The TRAPPs function as Ypt / Rab GEFs and share the same "core" components whilst containing compositionally distinct subunits that presumably mediate Ypt/Rab-specific interactions as well as membrane associations. In the case of TRAPPIII the distinct component is termed Trs85p (in yeast).

This is detailed and compressive study in which not only novel structural insight is provided, but also importantly, functional insight in cells. Through their structural studies the authors identify how Ypt1 associates with TRAPPIII and in so doing define the orientation in which TRAPPIII bindings to membranes. In addition, the authors also establish that an evolutionarily conversed amphipathic helix in Trs85 is instrumental in mediated TRAPPIII (and Trs85) association with anionic lipid containing liposomes and membranes in cells

I am not an expert in structural studies but nevertheless I would expect this study to be of significant interest to cell biologists in general, and to those who studying membrane trafficking mechanisms in particular (including autophagy).

The manuscript is well written and laid out in a manner which guides the reader.

Minor points to be addressed:

Figure 4 Panel B - the protein(s) migrating between 75 and 95kDa are labeled as non-specific but are of a similar MW to Trs85 and the Trs85 variant shown in panels A and C (respectively). So, presumably these non-specific proteins are also present in panels A and C. This should be mentioned in the main text (or expanded upon in the corresponding figure legend) to avoid confusion when future readers are scrutinizing the figure.

Page 9. Would the authors please describe more completely (in genetic terms) what they mean by "sensitized background" with reference to Bet3.

Although it is becoming increasing common in the literature to see references to amino acid substitutions as mutated amino acids - this does not (in this reviewer's opinion) make sense - but is

rather lab parlance / jargon. The authors should be encouraged to make changes to the main text to rectify this. For example, on the top of page 14.

Referee #3:

This paper takes a cryo-EM structural approach to examine yeast TRAPPIII, the GEF that activates the Rab1 homolog Ypt1 to regulate the early secretory pathway as well as autophagy. The structure reveals the binding site for the Rab1/Ypt1 hypervariable domain. Trs85 is the subunit that defines the yeast TRAPPIII version of the TRAPP complex, and the authors report that Trs85 apparently has a conserved amphipathic helix that anchors TRAPPIII in the membrane. This work extends previous structural and functional analysis of the core TRAPP complex, and it sheds light on mutations that compromise the function of TRAPPIII.

At 3.7 Å, the resolution of this structure is modest. The authors explain how the orientation bias of the complex precluded a higher resolution analysis, and how they used tilted grids to partially overcome this limitation. The core subunit structures are known from crystallography, while the Trs85 subunit structure was predicted with fairly high confidence and shows a reasonable fit to the cryo-EM data. Cross-linking mass spectrometry was used to validate the structure, so overall the results seem to be solid.

The authors push the system to argue for a specific interaction of the Rab1/Ypt1 hypervariable domain with the TRAPPIII complex, and for the presence in Trs85 of an amphipathic helix that mediates membrane association. Neither conclusion is supported directly by high-resolution structural data, but the combined evidence is strong and the interpretations are convincing. The end result is a picture of how TRAPPIII associates with membranes and with Rab1/Ypt1 to catalyze nucleotide exchange.

Overall this is an impressive, thorough, interesting study that significantly advances our understanding of the centrally important TRAPPIII complex. I have only a couple of minor comments:

1. Although the quantification in Figure 1E looks persuasive at first glance, the primary data in Figure 1D are much less compelling. All four panels in Figure 1D look similar. The issue seems to be that the signal is present over a high background, explaining why the statistical significance of the effect is borderline. Yet the text reads: "Consistent with our hypothesis, the intact TRAPPIII complex exhibited robust binding to synthetic liposomes, and the Trs85 subunit was required for stable membrane association (Figure 1E)." This statement needs to be qualified to acknowledge that the method is not actually so robust.

2. In Figure 4, what does "EV" stand for? I assume that "WCE" means whole-cell extract, but that's just a guess. In general, the authors should be careful to define the terms in their figures.

We offer our gratitude to the reviewers and to the editor for their support of our work and for their constructive criticism. We have revised the text based on the concerns presented below and believe that our manuscript has been strengthened accordingly. Please find our point-by-point responses in bold below.

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These non-specific proteins are E. coli contaminants that become more prominent in purifications with lower expression/yield, as is the case for this construct. If they are present in the other constructs, they are present at much lower levels.

To address this point, we have added another sentence (underlined below) to the legend of Figure 4, panel B. It now reads: *"B. As in A, using a Trs85 mutant containing only the final 198 amino acids. NOTE: Trs85[501-698] is ~25kD in size and now migrates at the same size as some of the core subunits. The species migrating near 85kD are contaminants present due to the lower expression level of this mutant construct."*

Page 9. Would the authors please describe more completely (in genetic terms) what they mean by "sensitized background" with reference to Bet3.

Thank you for this suggestion. For clarity, we have modified the text to read as follows: *"Because loss of Trs85 is synthetically lethal with C-terminal tagging of Bet3 (Sacher et al., 2001), we created a sensitized strain background for trs85Δ complementation tests in which the core Bet3 subunit is tagged at its C-terminus."* The genotype of the strain is also listed in the strain table.

Although it is becoming increasing common in the literature to see references to amino acid substitutions as mutated amino acids - this does not (in this reviewer's opinion)

make sense - but is rather lab parlance / jargon. The authors should be encouraged to make changes to the main text to rectify this. For example, on the top of page 14.

We agree with this sentiment and where appropriate we have modified all instances of “mutation” to “substitution” or “truncation” and from “mutant” to “substitution mutants”.

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This paper takes a cryo-EM structural approach to examine yeast TRAPPIII, the GEF that activates the Rab1 homolog Ypt1 to regulate the early secretory pathway as well as autophagy. The structure reveals the binding site for the Rab1/Ypt1 hypervariable domain. Trs85 is the subunit that defines the yeast TRAPPIII version of the TRAPP complex, and the authors report that Trs85 apparently has a conserved amphipathic helix that anchors TRAPPIII in the membrane. This work extends previous structural and functional analysis of the core TRAPP complex, and it sheds light on mutations that compromise the function of TRAPPIII.

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Thank you for the positive comments. We appreciate your critiques and support of the manuscript.

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1E)." This statement needs to be qualified to acknowledge that the method is not actually so robust.

While we agree that there is some background level of pelleting (both core TRAPP and the TRAPPIII complex do pellet somewhat in the absence of membranes), these experiments have been repeated dozens of times in various forms and the result is always the same (and similar results are presented in Figure 6).

We are puzzled by the reviewer's statement that all the panels in Figure 1D look the same. Panels 3 and 4 in Figure 1D look quite different: in panel 3, most of the complex is in the supernatant whereas in panel 4 most of the complex in the pellet.

Nevertheless, we have modified the text to be less assertive by removing the word "robust" from the description. We also removed the word "strongly" from the corresponding figure legend.

2. In Figure 4, what does "EV" stand for? I assume that "WCE" means whole-cell extract, but that's just a guess. In general, the authors should be careful to define the terms in their figures.

Thank you for catching those omissions. We have included definitions for the abbreviations within the corresponding figure legends.

1st Revision - Editorial Decision 23rd Mar 2021

Thank you for submitting your revised study. I have now checked your manuscript and the point-by-point rebuttal letter and find that the referees' points have been sufficiently addressed.

However, there are few editorial issues concerning the text and the figures that I need you to address before we can officially accept your manuscript.

2nd Authors' Response to Reviewers 7th Apr 2021

Thank you for submitting your revised study. I have now checked your manuscript and the point-by-point rebuttal letter, and find that the referees' points have been sufficiently addressed.

However, there are few editorial issues concerning the text and the figures that I need you to address before we can officially accept your manuscript.

We are grateful for the positive news and we have made the requested changes. See below for the details, our responses in bold.

2nd Revision - Editorial Decision 11th Apr 2021

I am pleased to inform you that your manuscript has been accepted for publication in The EMBO Journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: J. Chris Fromme
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2020- 107607

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	No statistical approaches were used to determine sample size.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	n/a
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	n/a
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	n/a
For animal studies, include a statement about randomization even if no randomization was used.	n/a
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	During analysis of the microscopy, the images were coded so as to make the identity of the sample hidden to the investigator throughout cell picking, colocalization analysis, and statistical analysis.
4.b. For animal studies, include a statement about blinding even if no blinding was done	n/a
5. For every figure, are statistical tests justified as appropriate?	Statistical tests are described in the Figure Legends, we used standard comparisons.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes. Equal variance for ANOVA analysis was assessed using the Brown-Forsythe test. For the unpaired t-tests the Welch t test was used, which assumes that both groups of data are sampled from Gaussian populations, but does not assume those two populations have the same standard deviation.

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http://www.selectagents.gov/

Is there an estimate of variation within each group of data?	Yes, as described in the Figure Legends.
Is the variance similar between the groups that are being statistically compared?	Yes. Equal variance for ANOVA analysis was assessed using the Brown-Forsythe test.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	anti-HA: mouse monoclonal 12CA5 (Roche #11583816001 ; anti-FLAG: mouse monoclonal M2 (Sigma #F1804); anti-GFP: mouse monoclonal (Sigma #11814460001); HRP-linked sheep anti-mouse IgG: (Cytiva #NXA931) ; HRP-linked donkey anti-rabbit IgG: (Cytiva #NA934)
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	n/a

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	n/a
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	n/a
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	n/a

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	n/a
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	n/a
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	n/a
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	n/a
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	n/a
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	n/a
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	n/a

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	RCSB PDB: 7MKT, EMDB: EMD-22928
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	n/a
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	n/a
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	n/a

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	n/a