Identification of defined structural elements within TOR2 kinase required for TOR Complex 2 assembly and function in S. cerevisiae

Jennifer Tsverov, Kristina Yegorov, and Ted Powers

Corresponding author(s): Ted Powers, UC Davis

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(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 #E21-12-0611
TITLE: Identification of defined structural elements within TOR2 kinase required for TOR Complex 2 assembly and function in S. cerevisiae

Dear Ted,

We've received comments about your manuscript from two reviewers. Both reviewers agree that the experiments are carefully done and provide new information about how distinct TOR complexes are assembled. Reviewer 1 did not have any major concerns and included a number of constructive suggestions for improvements. Reviewer 2 had concerns about the clarity of the logic behind the experiments and the interpretation of the results. Both reviewers had concerns about Figure 7. Reviewer 2 was also concerned that the manuscript does not present a substantial advance over previous studies and suggested that it would be helpful to include additional experiments that test hypotheses that arise from your analysis.

I read the manuscript and can see the points raised by both reviewers. I suggest that you revise the writing to address the concerns of both reviewers regarding organization and clarity. I agree with reviewer 2 that an additional experiment that tests hypotheses arising from your study would make the manuscript more complete and compelling, and would emphasize the future impact of your work. If you feel that this would be beyond the scope of your current work, perhaps you could include a discussion of next steps and future directions. Let me know if you have any questions or concerns.

Doug

Douglas Kellogg
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Powers,

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

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

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

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

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

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

Sincerely,

Eric Baker

Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

In this manuscript, the authors seek to define amino acid sequences within budding yeast TOR kinases that define assembly of distinct TORC1 and TORC2 complexes. The experiments build on the author's previous work in which they used chimeric TOR proteins to define a Major Assembly Specificity (MAS) domain that is required for specific assembly of TORC2 versus TORC1 complexes. Here, they use chimeric proteins to further map protein sequence determinants that are required for assembly and function of TORC2 complexes. A strength of the experiments is that they are carefully done and provide new information on sequences within TOR kinases that drive assembly of TORC2. This was particularly true for the analysis of the MAS B1 domain. However, the logic of the experiments and the interpretation of the results were not always clear. In addition, the experiments do not appear to provide a major advance over the author's previous studies or previous cryoEM structural studies of the TOR complexes from other labs. A revised paper that more clearly explains experimental logic and interpretations, and also tests hypotheses arising from the analysis of chimeric TOR proteins, would constitute a more complete and compelling study. A few specific issues are listed below:

In Figure 1, what is the logic for testing TOR chimeras in avo2Δ and avo3-ΔCT backgrounds? Why not first test the chimeras in a wild type background? The chimeras clearly showed genetic interactions with avo2Δ and avo3-ΔCT, but I had trouble interpreting the interactions and understanding their significance. Also, what is the logic for using the tor1-1 rapamycin resistant mutant allele for these experiments? Whatever the logic, the use of this allele should be introduced when first describing the rationale and experimental design for these experiments.

The authors interpret their results in the context of available cryoEM structures for TORC2, but I struggled to understand the relationship between their results and the TORC2 structure. The first two paragraphs of the Discussion and Figure 7 were particularly confusing. It was unclear how the author's results significantly extend the structural information beyond what is already known from the cryoEM structures.

In Figure 5D, the labels for the structures do not match the descriptions in the figure legend.

Reviewer #2 (Remarks to the Author):

In this work, Tsverov et al have investigated the molecular reasons underlying the specific incorporation of Tor1 into TORC1 and preferred incorporation of Tor2 into TORC2. This problem has intrigued the TOR field since the discovery of the two TOR complexes in S. cerevisiae. Addressing this problem is thus important, but, as illustrated in this work, not simple, as there appears to multiple TOR domains involved making the molecular dissection quite challenging.
The authors nicely exploit synthetic-lethal interactions as a sensitized genetic background to assess consequences of interchanging portions of the N-terminal HEAT repeats of TOR1/2 that determine TOR's ability to assemble into TORC2. Using this approach, the authors identify novel regions that are required for TORC2 assembly/function. A better understanding of these mutants awaits higher quality TORC2 structures wherein Avo2 and Avo3 are better resolved.

Globally the manuscript is very well written, the data well analyzed and the conclusions well supported. Although the take-home message is not a succinct one, the results presented will be of interested to a specialized audience.

Minor comments

i/ "For comparison, we also tested a plasmid that expressed WT TOR2 (pPL632)." Technically, this is not WT TOR2 as it contains a point mutation in the FRB domain. As the authors well know, they should not assume that this mutation is not without other phenotypic consequences beyond rapamycin resistance.

ii/ Perhaps use "Sub-minimal" instead of "Most Minimal"?

iii/ "By contrast, we observed that only full-length TOR2 is functional in combination with
the avo3-DCT allele, as well as captures all elements proximal to AVO3, including the MAS and
FRB domains (Figure 7B, left panel)." I don't understand this sentence on page 12.

iv/ Figure 7 is not particularly informative and could be improved. Protein chains are not properly labeled. Presenting different projections (left vs right) is not helpful.

RE: Manuscript #E21-12-0611R
TITLE: "Identification of defined structural elements within TOR2 kinase required for TOR Complex 2 assembly and function in S. cerevisiae"

Monitoring Editor (Remarks to Author):

Dear Ted,

Thanks for the revised version of your manuscript. I read your response to the reviewers and also did a quick read through the revised manuscript. It looks like you have addressed the reviewers concerns, so happy to accept the manuscript. I noticed a number of typos in the manuscript (examples below) so be sure to check carefully for typos before uploading a final version. Also, the genetic interactions will still be challenging for many readers so as you check for typos you might also be able to find more ways to make the logic as clear and simple as possible. Congratulations on your paper - looking forward to seeing the final version!

[Also, your figures are only 150 dpi, which is half the resolution we need. Please replace the current figures with improved ones. Requirements in the author instructions link. E.B.]

Doug

"Given that AVO3 is encoded by an essential gene and, therefore, we could not analyze a null-allele, used avo3- CT as a possible hypermorphic mutant.'

"Because that this arrangement of physical interactions is conserved between mTOR and Rictor, we predict interactions between monomers will also turn out to be crucial for mTORC2 assembly and/or stability."

"Remarkably, for one segment, TOR2 MAS-B1, were able to resolve this element to near amino acid resolution."

Dear Dr. Powers,

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).

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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):
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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

Department of Molecular and Cellular Biology
College of Biological Sciences
UC Davis

March 1, 2022

Douglas Kellogg
Monitoring Editor
Molecular Biology of the Cell

Dear Doug,

Thank you for your acceptance of our manuscript. We are thrilled to have this work published in MBC, where we think it will be of value to the wide readership of the journal. In response to your suggestions, we have increased the resolution of our figures to 300 dpi. We have also edited the paper for typos and clarity. We trust that our manuscript is now ready for publication.

Best regards,

-Ted

Ted Powers, PhD
Professor of Molecular and Cellular Biology
College of Biological Sciences
UC Davis

Response to Reviewers

Review 1

General comments:
We appreciate the positive comments by the reviewer about the significance of our study, that our experiments were carefully done and provided new information on sequences in the TOR kinases that drive assembly of TORC2. The reviewer had concerns, however, both about the logic of some experiments as well as the importance of our work in the absence of further experiments. We address the logic of individual experiments in the context of the specific comments (see below). In terms of overall significance, we believe that our findings demonstrate the importance of using functional approaches to examine the cryo-EM structures. In this regard, there are three points that we clarify in our revised manuscript:

(i) We predicted from our earlier work that we would be able to construct a more minimal version of the TOR2 MAS domain, based on known quaternary interactions from the published TORC2 structure from yeast. We were therefore quite surprised to find that we were unable to narrow this domain without causing impaired phenotypes. Our subsequent approach of using chimeras as a genetic system to identify specific functionally important regions throughout this domain provide unique insights into this domain that will guide further study. Our findings also highlight the fact that the published TORC2 cryo-EM models from both yeast and mammals are missing quite a bit of structural information for the partners (e.g. AVO3 and Rictor), which emphasizes the need for complimentary approaches to understand the assembly of these complexes.

(ii) We believe the pattern of synthetic interactions between our chimeras and mutations in AVO3, when viewed in the context of the cryo-EM model, are significant in that they suggest a functional basis for the dimeric assembly of TORC2. Because this aspect of TORC2 is conserved, we believe our findings extend to mTORC2 as well. While we argue testing the role of mTOR-Rictor interactions in mTORC2 assembly is beyond the scope of this study, we clarify in the revised manuscript that this is one of the important implications of our study.

(iii) The use of gene paralogs and synthetic lethality are two approaches that have seen a resurgence in recent years, including in the study of human cancers and in the analysis of multi-protein comlexes. Thus, our combined use of these tools is something we believe will be of value beyond the TOR field.

We have made efforts to clarify each of these points in our revised manuscript and hope we have convinced the reviewer of the significance of our study as it stands.

Response to specific comments (review comments are in *italics*):

In Figure 1, what is the logic for testing TOR chimeras in avo2Δ and avo3-ΔCT backgrounds? Why not first test the chimeras in a wild type background?

We thank the reviewer for pointing out we may have assumed a level of understanding of yeast genetics may not extend to the wide readership of the journal and, additionally, that we needed to clarify our approach and interpretation of our findings. We have expanded the experimental rationale in the revised manuscript and have provided a new Figure 1 to include an overview of our approach.

To the reviewer's questions, the requirement for a synthetic lethal approach is to combine two or more non-lethal mutations to observe the phenotype of the double mutant. Since AVO2 is encoded by a non-essential gene, we were able to conduct this analysis with the avo2Δ null allele. By contrast, because AVO3 is essential, we needed to use a non-null allele. In the course of our early studies, we discovered a synthetic phenotype when we combined the avo3-ΔCT mutant with one of our TOR chimeras, which led us to pursue using this allele for the present study.

In terms of investigating the chimeras in a wildtype background, we assume the reviewer means in a tor2Δ background, where both AVO2 and AVO3 are wild type. In the course of performing tetrad dissections, this combination of genes readily occurs, so we were able to determine that all of the chimera are functional in the absence of endogenous TOR2. We attempted to make this point more clear in our revised manuscript. To address directly the reviewer's concern, we also performed new tetrad dissections of the chimeras used in Figure 1 (Figure 2 in the revised manuscript) in the TOR2/tor2Δ heterozygous diploid strain. We include the results of this experiment at the end of this document for the reviewer's inspection. We are happy to include this as a supplemental figure if this would be considered helpful.

The chimeras clearly showed genetic interactions with avo2Δ and avo3-ΔCT, but I had trouble interpreting the interactions and understanding their significance.

While the interpretation of the results of synthetic interactions is complicated, especially when non-null alleles are involved, we were struct by the pattern of phenotypes displayed by the chimeras in the AVO2 versus AVO3 mutants. These differences became particularly informative when viewed within the context of the cryo-EM structure. This point is elaborated below when we address concerns over Figure 7.

Also, what is the logic for using the tor1-1 rapamycin resistant mutant allele for these experiments? Whatever the logic, the use of this allele should be introduced when first describing the rationale and experimental design for these experiments.

In terms of using the TOR1-1 allele, we use rapamycin resistance as a qualitative readout for TORC1 activity, because it is only if the TOR chimeras are functional when incorporated into TORC1 that cells will grow in the presence of the drug. We have clarified this point both in the text, as well as in the revised Figure 1, where we added a new schematic in Figure 1C.

The authors interpret their results in the context of available cryoEM structures for TORC2, but I struggled to understand the relationship between their results and the TORC2 structure. The

first two paragraphs of the Discussion and Figure 7 were particularly confusing. It was unclear how the author's results significantly extend the structural information beyond what is already known from the cryoEM structures.

We thank the reviewer for pointing out concerns with Figure 7, some of which were raised by Reviewer 2 as well. Much of the confusion is likely caused from where in the manuscript the modeling is discussed. To the reviewer's concern, by mapping our genetic results onto the structure for TORC2 we have gained important insight into how this complex is likely to be assembled and/or stably maintained. Thus, sequences surrounding AVO2 that result in synthetic lethal effects when switched to TOR1 are all contained within individual TORC2 monomers. By contrast, for AVO3, important elements in TOR2 are split among the two copies of AVO3. As this later arrangement is conserved in mTORC2 (for mTOR and Rictor), this has implications for mTORC2 assembly as well. To clarify these findings, we have reorganized discussion of the modeling and combined it with the genetic results, to create a revised Figure 2. We then highlight these findings and emphasize their significance in the Discussion. We hope the reviewer agrees that this change clarifies the relationship between structure and function revealed by our genetic analysis.

Reviewer 2

General Comments:
We thank the reviewer for their positive comments about our study and its implications for TORC2 assembly. We agree that the take home message may be complicated. However, as discussed above, we believe our approach should be broadly applicable to other systems as well.

Specific comments:

i/ For comparison, we also tested a plasmid that expressed WT TOR2 (pPL632). Technically, this is not WT TOR2 as it contains a point mutation in the FRB domain. As the authors well know, they should not assume that this mutation is not without other phenotypic consequences beyond rapamycin resistance.

The reviewer is completely correct that the RapR version of TOR2 is not equivalent to "wildtype". Indeed, elsewhere in the manuscript we distinguish between the WT TOR1 and the TOR1-1 allele in the case of control plasmids used for analysis. To avoid confusion, we have changed "WT" to "full-length" when describing the complete TOR2 gene that contains the TOR2-1 allele.

ii/ Perhaps use "Sub-minimal" instead of "Most Minimal"?

We thank the reviewer for this suggestion. We struggled with the question of the best nomenclature to use and agree this represents an improvement. We have changed the terminology in the text and in the revised figures.

iii/ "By contrast, we observed that only full-length TOR2 is functional in combination with the avo3-DCT allele, as well as captures all elements proximal to AVO3, including the MAS and FRB domains (Figure 7B, left panel)." I don't understand this sentence on page 12.

We agree this sentence is confusing and we have replaced it in the revised manuscript with the following statement (page 8 of the revised manuscript): “Intriguingly, contacts with AVO3 are partitioned with respect to elements in a single TOR2 protein. Thus, the N-terminal MAS domain is predicted to interact with one AVO3 monomer, whereas the C-terminus, including the FRB domain, is in proximity to a second AVO3 monomer (Figure 2F, left panel).” We hope this clarifies for the reviewer the point we are attempting to make regarding our findings.

iv/ Figure 7 is not particularly informative and could be improved. Protein chains are not properly labeled. Presenting different projections (left vs right) is not helpful.

As described above in our comments to Reviewer 1, we have modified both this figure as well as the text to clarify and underscore the significance of our findings. To this reviewer’s point, we have simplified the labeling of the figure. However, we feel there may also be some confusion about what the figure shows. Figure 7A (now Figure 2E in the revised manuscript) shows the same projection of yeast TORC2 and highlights the relationship between AVO2 and TOR2-specific sequences in three different chimeras. By contrast, Figure 7B (now Figure 2F in the revised manuscript) shows yeast TORC2 on the left and mammalian mTORC2 on the right. Thus, these are entirely different structures and not different projections of the same structure. The goal of this figure is to highlight the similarity between our findings and their relevance to mammalian mTORC2. We hope this clarifies the situation for this reviewer.

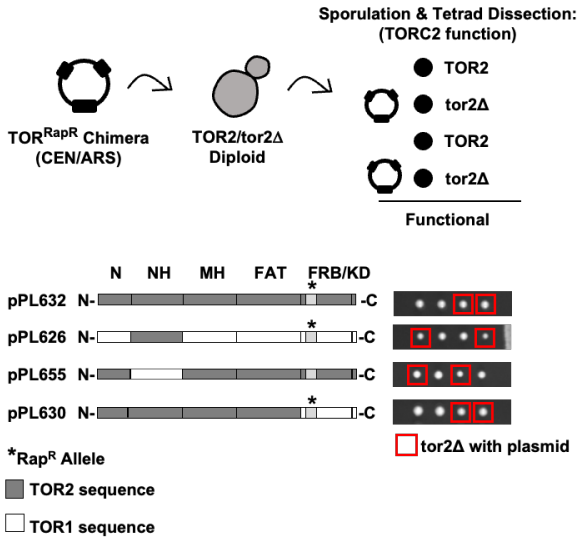

Figure 1: Demonstrating TOR1-TOR2 chimeras are functional in the absence of endogenous TOR2

RE: Manuscript #E21-12-0611RR
TITLE: "Identification of defined structural elements within TOR2 kinase required for TOR Complex 2 assembly and function in S. cerevisiae"

Dear Ted,

Thanks for the final version - looking forward to seeing it in MBoC!

Doug

Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Powers:

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.

Your paper is among those chosen by the Editorial Board for Highlights from MBoC. Hightlights from MBoC appears in the ASCB Newsletter and highlights the important articles from the most recent issue of MBoC.

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