

# XMAP215 and $\gamma$ -tubulin additively promote microtubule nucleation in purified solutions

Brianna King, Michelle Moritz, Haein Kim, David Agard, Charles Asbury, and Trisha Davis

*Corresponding author(s): Trisha Davis, University of Washington*

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*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 #E20-02-0160

TITLE: XMAP215 and  $\gamma$ -tubulin additively promote microtubule nucleation in purified solutions

Dear Dr. Davis:

The reviews of your manuscript have been received. Both reviewers found your data on the contribution of  $\gamma$ -tubulin and XMAP215 to microtubule nucleation interesting and important. You will see that Reviewers #1 and 2 suggest a list of revisions that you should incorporate in the revised version of your manuscript. In particular, the need for more detail in the methods is raised by both reviewers, as well as data presentation and robustness. In light of the current situation, the additional experiments suggested by Reviewer 1 in point 5 are not needed; however, a more detailed discussion of this aspect should be included in the manuscript. A clearer description of the structure of the  $\gamma$ -tubulin arrays would also strengthen the manuscript.

I hope you are all safe and healthy.

Sincerely,

Antonina Roll-Mecak  
Monitoring Editor  
Molecular Biology of the Cell

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Dear Dr. Davis,

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.

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](mailto: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 at [mboc@ascb.org](mailto:mboc@ascb.org).

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if it is deemed necessary to render a completely informed decision.

In preparing your revised manuscript, please follow the instruction in the Information for Authors ([www.molbiolcell.org/info-for-authors](http://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](mailto: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](mailto:mbc@ascb.org)

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Reviewer #1 (Remarks to the Author):

The authors study the effects of gamma-tubulin and the microtubule polymerase XMAP215 on microtubule nucleation in turbidity experiments with purified proteins. They find that gamma-tubulin promotes microtubule nucleation and attribute this to the formation of "gamma-tubulin arrays" whose cryo-EM structure is presented. They report that XMAP215 promotes nucleation as shown previously, and that both proteins together promote nucleation additively, but non-synergistically. Using different, XMAP215 constructs, the authors find that the effect of XMAP215 on nucleation correlates with its effect on growth speed. This work is interesting in the context of other previous studies that showed a synergistic effect of XMAP215/chTOG and gamma-tubulin ring complex (gTuRC) on microtubule nucleation, which has been ascribed to XMAP215 binding to gamma-tubulin. The current study appears to question the latter result, although the authors are not explicit about this. Overall this study is interesting, but could be made more complete by the addition of missing experiments and a clearer presentation of some of the data/analysis.

Concerns/questions:

1. Figure 1A, B. The authors could provide a clearer description of the structure of the gamma-tubulin arrays and of the arrangement of gamma-tubulins in these arrays compared to their arrangement in a gTuRC complex. The images show a cylindrical structure with a diameter of 25nm, which may be understood as being rather similar to a microtubule structure, but it seems that gamma-tubulins are rotated by 90 degrees compared to the orientation of alpha/beta-tubulins in a microtubule - and compared to the expected orientation of gamma-tubulins at the base of a microtubule. How do microtubules nucleate from these arrays? Do the authors see examples in their electron microscopy images? It seems that starting to grow a tube from such arrays is very different from starting to grow a microtubule from a gTuRC. Detail: The authors mention a five-fold symmetry. Does this mean that 10 rows of gamma-tubulins form a tube in these arrays?

2. The authors state that the stimulating effect of gamma-tubulin on microtubule nucleation depends on gamma-tubulin array formation, but do not demonstrate this. They show the concentration dependence of the effect on nucleation, but not the concentration dependence of array formation.
3. Analysis of turbidity curves. The authors fit their data, but show only fits to a very small part of the curves (in some cases only to 2 points). Can fits be shown for larger parts of the curves and can the fit parameters be reported and interpreted? A reference for the used method should be provided.
4. The authors provide a formula used for their fit to Fig. 3F, but do not give any further explanation/interpretation for/of the particular values of the prefactor and exponent. Why has the formula been chosen and what do the obtained parameter values mean?
5. The key message of this manuscript is that XMAP215's effect on nucleation correlates with its effect on growth speed and that no synergy between gamma-tubulin and XMAP215 in terms of promoting nucleation is observed. This implies that XMAP215 does not bind to gamma-tubulin, contradicting a previous report from the Petry lab. The authors do however not test whether XMAP215 binds to gamma-tubulin or to gamma tubulin arrays under their conditions to clarify this, although they have the reagents to do so. This is a weakness of the manuscript, because the answer could provide support/an explanation for the finding that the nucleation stimulating effect of XMAP215 depends on its microtubule growth accelerating activity and does not synergise with stimulation of nucleation by gamma-tubulin arrays. It may also have implications for the mechanism by which XMAP215 stimulates gTuRC-mediated microtubule nucleation, as the authors have already started to discuss.
6. Title: It maybe be worth considering to replace 'gamma-tubulin' by 'gamma-tubulin arrays'.
7. Statistics. Have repeats for the experiments shown in figures 2, 3E, 4A been performed, or does one curve represent an individual experiment?

Reviewer #2 (Remarks to the Author):

Gamma-tubulin ring complexes (gamma-turcs) are notoriously bad microtubule nucleators. They have been hypothesized to undergo conformational changes to activate and/or function in synergy with microtubule associated proteins to aid nucleation from these templates.

Recently XMAP215 and the gamma-turc were reported to function synergistically (Chang, Surrey, Petry labs) i.e. that the presence of XMAP215 is promoting nucleation from gamma-turcs. One study (Thawani et al. 2018) reported a direct interaction of gamma tubulin with the C-terminus of XMAP215.

The authors of the current manuscript interpret these results as an argument for direct synergy between XMAP215 and gamma-tubulin and motivate the manuscript with the aim to study this potential effect.

In my mind this is a weak set-up for a manuscript that ultimately provides substantial evidence for the presence of at least two distinctive steps that are rate-limiting during microtubule nucleation. Those findings are compellingly discussed and could be the main focus?

Comments:

1) Light scattering assay: The manuscript depends to a great degree on the reliability of the light scattering assay.

Why are only representative turbidity traces shown throughout the manuscript?

Is the data not reproducible?

If the effect size is reproducible but absolute numbers are not?

Is it necessary to normalize all the data?

Some of the measured differences seem rather small (fig 2A). Why do the authors believe that those effects are not within the noise of the experiment?

Where are the error bars for the nucleation lag in fig 3F coming from if the data cannot be averaged?

2) The method section needs more detail. All experiments are hinging on the quality of reagents and precision and reproducibility of the assays used:

- A SDS-page protein gel showing purified proteins

- "Experimental reactions" used in the spontaneous tubulin assembly assay section remain undefined in terms of buffers and composition

- Is this composition identical among experiments?

- "scaling performed by hand" - does this refer to the normalization procedure? What exactly does that mean?

3) Is there a hypothesis why gamma-tubulin is forming arrays that are detected by EM but do not produce any light scattering in the turbidity assay (fig S1)?

Are the conditions between those experiments identical?

4) The authors show a correlation between the polymerase activity of XMAP215 and the spontaneous nucleation activity.

-How does the "nucleation" from seeds compare? The authors should have that information in their growth data and might contribute to the ongoing discussion if growth from seeds is comparable to nucleation or elongation.

-A strong prediction for this nucleation from templates would be that there is zero additive effect from the addition of gamma tubulin?

5) The Discussion justifies the use of the light scattering assay due to the difficulties with fluorophore-tagged tubulin. The authors advertise light scattering as the method of choice because of the use of unlabeled tubulin.

Yet, the authors use DIC for their microscopy assays without fluorophores.

6) Furthermore the authors discuss light scattering as the method of choice to "monitor formation of small nucleation intermediates". The authors directly contradict themselves by reporting that gamma-tubulin assemblies cannot be detected in their assay. Aren't those equivalent in size to nucleation intermediates?





July 2, 2020

Antonina Roll-Mecak  
Monitoring Editor  
Molecular Biology of the Cell

RE: Manuscript #E20-02-0160

TITLE: XMAP215 and  $\gamma$ -tubulin additively promote microtubule nucleation in purified solutions

Dear Antonina:

Thank you very much for the positive reviews of our manuscript. We appreciate the flexibility of MBoC in not requiring additional experiments during this pandemic. Our responses to each of the reviewers' comments are given below. The reviewer comment is shown in blue and our response is shown in black. We believe our manuscript is now ready for publication.

Sincerely,

Trisha N. Davis  
Professor and Chair  
Earl W. Davie/Zymogenetics Endowed Chair

## Reviewer #1:

1. Figure 1A, B. The authors could provide a clearer description of the structure of the gamma-tubulin arrays and of the arrangement of gamma-tubulins in these arrays compared to their arrangement in a gTuRC complex. The images show a cylindrical structure with a diameter of 25nm, which may be understood as being rather similar to a microtubule structure, but it seems that gamma-tubulins are rotated by 90 degrees compared to the orientation of alpha/beta-tubulins in a microtubule - and compared to the expected orientation of gamma-tubulins at the base of a microtubule. How do microtubules nucleate from these arrays? Do the authors see examples in their electron microscopy images? It seems that starting to grow a tube from such arrays is very different from starting to grow a microtubule from a gTuRC. Detail: The authors mention a five-fold symmetry. Does this mean that 10 rows of gamma-tubulins form a tube in these arrays?

-Thanks to the reviewer for pointing out that we did not sufficiently describe  $\gamma$ -tubulin oligomers for the reader's understanding. To help clarify their structure and properties, we have edited the manuscript (lines 89-105), and included a cross-section of the reconstruction and a cartoon representation of how alpha beta-tubulin dimers would theoretically interact with the gamma-tubulin arrays in Figure 1.

2. The authors state that the stimulating effect of gamma-tubulin on microtubule nucleation depends on gamma-tubulin array formation, but do not demonstrate this. They show the concentration dependence of the effect on nucleation, but not the concentration dependence of array formation.

-We have added additional data to Supplementary Figure 1 showing that gamma-tubulin forms arrays at 300 nM and 1.1  $\mu$ M, but does not form arrays at 80 nM.

3. Analysis of turbidity curves. The authors fit their data, but show only fits to a very small part of the curves (in some cases only to 2 points). Can fits be shown for larger parts of the curves and can the fit parameters be reported and interpreted? A reference for the used method should be provided.

-We are grateful to the reviewer for this comment and for the opportunity to more clearly explain the data acquisition and analysis. The lines in between points shown in the graphs of turbidity data are not fits to the data, they are only shown to connect data points from a single experiment. Each turbidity data set shown is a representative time course. We have addressed the confusion by revising the figure legends (lines 559-561, 565-568, 586-587, 604-605) and also by showing experimental replicates in Supplementary Figures 1 and 2. (Please see our response to comment 7 for explanation of data acquisition and analysis.)

4. The authors provide a formula used for their fit to Fig. 3F, but do not give any further explanation/interpretation for/of the particular values of the prefactor and exponent. Why has the formula been chosen and what do the obtained parameter values mean?

-We appreciate the opportunity to clarify the relationship between XMAP215 elongation activity and nucleation activity. We have revised Figure 3F and the manuscript text. From lines 154-165, "To fit the data, a power curve was chosen because it fits the theoretical relationship between microtubule elongation and nucleation. As microtubule growth rate approaches zero, the



nucleation lag should increase and approach infinity. In contrast, as microtubule growth rate approaches infinity, the nucleation lag should decrease and approach zero. The curve fit was constrained to pass through (1,1) because elongation and nucleation are defined as 1 in the absence of XMAP215 constructs. The resulting power curve fit is  $f(x) = x^{-0.33}$ . The exponent is negative. Therefore, as elongation rate increases, the nucleation lag decreases. The magnitude of the exponent is less than 1, which indicates that active XMAP215 constructs increase elongation rates at growing microtubule tips more potently than they increase addition of tubulin dimers to nucleation intermediates.”

5. The key message of this manuscript is that XMAP215's effect on nucleation correlates with its effect on growth speed and that no synergy between gamma-tubulin and XMAP215 in terms of promoting nucleation is observed. This implies that XMAP215 does not bind to gamma-tubulin, contradicting a previous report from the Petry lab. The authors do however not test whether XMAP215 binds to gamma-tubulin or to gamma tubulin arrays under their conditions to clarify this, although they have the reagents to do so. This is a weakness of the manuscript, because the answer could provide support/an explanation for the finding that the nucleation stimulating effect of XMAP215 depends on its microtubule growth accelerating activity and does not synergise with stimulation of nucleation by gamma-tubulin arrays. It may also have implications for the mechanism by which XMAP215 stimulates gTuRC-mediated microtubule nucleation, as the authors have already started to discuss.

-Because of the current pandemic, we are unable to do this experiment. We appreciate the flexibility of MBoC. We have also slightly adjusted the focus of this manuscript away from the synergy with gamma-tubulin as suggested by reviewer 2.

6. Title: It maybe be worth considering to replace 'gamma-tubulin' by 'gamma-tubulin arrays'.

-We appreciate this suggestion. We carefully describe the arrays in the manuscript in Figure 1 and do not believe the longer title is helpful.

7. Statistics. Have repeats for the experiments shown in figures 2, 3E, 4A been performed, or does one curve represent an individual experiment?

-For Figures 2, 3E, and 4A, each curve is an individual experiment and was chosen as an accurate representative of the group of experiments. Each experimental reaction was run simultaneously with a tubulin alone control using the same freshly thawed and cleared tubulin aliquot. Nucleation lag was quantified by comparing time to reach 0.1 maximum polymer, or 10% maximum polymer. Each sample was normalized to its corresponding tubulin alone control. Error bars represent the standard error of the mean for the normalized data.

We have addressed this comment by adding additional explanations in the Materials and Methods section (lines 271-276) and clarifying the figure legends. We have also added two supplementary figures, which show all the raw data for XMAP215 and gamma-tubulin stimulated nucleation (Supplementary Figures 1 and 2).

## Reviewer #2 (Remarks to the Author):

Gamma-tubulin ring complexes (gamma-turcs) are notoriously bad microtubule nucleators. They have been hypothesized to undergo conformational changes to activate and/or function in synergy with microtubule associated proteins to aid nucleation from these templates.

Recently XMAP215 and the gamma-turc were reported to function synergistically (Chang, Surrey, Petry labs) i.e. that the presence of XMAP215 is promoting nucleation from gamma-turcs. One study (Thawani et al. 2018) reported a direct interaction of gamma tubulin with the C-terminus of XMAP215.

The authors of the current manuscript interpret these results as an argument for direct synergy between XMAP215 and gamma-tubulin and motivate the manuscript with the aim to study this potential effect.

In my mind this is a weak set-up for a manuscript that ultimately provides substantial evidence for the presence of at least two distinctive steps that are rate-limiting during microtubule nucleation. Those findings are compellingly discussed and could be the main focus?

-We thank the reviewer for this perspective. We agree that the presence of at least two distinctive steps, promoted in non-overlapping ways by XMAP215 and gamma-tubulin, is an equally if not more compelling narrative. Changes to the abstract and to the introduction (lines 64-74) reflect this revision.

## Comments:

1) Light scattering assay: The manuscript depends to a great degree on the reliability of the light scattering assay.

Why are only representative turbidity traces shown throughout the manuscript?

Is the data not reproducible?

If the effect size is reproducible but absolute numbers are not?

Is it necessary to normalize all the data?

Some of the measured differences seem rather small (fig 2A). Why do the authors believe that those effects are not within the noise of the experiment?

Where are the error bars for the nucleation lag in fig 3F coming from if the data cannot be averaged?

-We thank the reviewer for these insightful questions. Please see response to comment #3 and #7 from Reviewer #1.

2) The method section needs more detail. All experiments are hinging on the quality of reagents and precision and reproducibility of the assays used:

- A SDS-page protein gel showing purified proteins

- "Experimental reactions" used in the spontaneous tubulin assembly assay section remain undefined in terms of buffers and composition

- Is this composition identical among experiments?

- "scaling performed by hand" - does this refer to the normalization procedure? What exactly does that mean?

-Thank you for these suggestions. In response, first, we have added a supplementary figure (Supplementary Figure 2A) showing an SDS-PAGE of the purified proteins. Second, we have revised Materials and Methods to include the composition of the experimental reactions (lines 257-261). For an explanation of how data was analyzed, we have revised Materials and Methods (lines 271-276) and included two additional supplementary figures, which show raw data for Figure 2 (Supplementary Figures 1 and 2). Briefly, the experimental protocol included a

control reaction with tubulin alone for each experimental reaction. Light scattering was normalized to the maximum plateau value of the experiment during the first forty minutes of the experiment. Then experimental data was quantified by comparison to the tubulin alone reaction run at the same time.

3) Is there a hypothesis why gamma-tubulin is forming arrays that are detected by EM but do not produce any light scattering in the turbidity assay (fig S1)?  
Are the conditions between those experiments identical?

-The conditions in the two light scattering experiments (Supplementary Figure 1) are identical. We have added more explanation of this in the text (lines 98-105 and Supplementary Figure 1). It is likely that even under the conditions shown in the light scattering assay of Supplementary Figure 1, the concentration of gamma-tubulin arrays is too low to be detected by light scattering.

4) The authors show a correlation between the polymerase activity of XMAP215 and the spontaneous nucleation activity.

-How does the "nucleation" from seeds compare? The authors should have that information in their growth data and might contribute to the ongoing discussion if growth from seeds is comparable to nucleation or elongation.

-A strong prediction for this nucleation from templates would be that there is zero additive effect from the addition of gamma tubulin?

-Thank you for this suggestion. Unfortunately, the data as previously collected cannot be used to answer these questions. The videos used to quantify growth rates are not sufficiently long to give an accurate representation of how often seeds fire.

5) The Discussion justifies the use of the light scattering assay due to the difficulties with fluorophore-tagged tubulin. The authors advertise light scattering as the method of choice because of the use of unlabeled tubulin.

Yet, the authors use DIC for their microscopy assays without fluorophores.

-This is a good point, that DIC could be used to monitor the fraction of seeds growing microtubules, and it might be possible to use DIC to measure the number of microtubules formed in a given time. We have not seen DIC used in this way. We don't believe this changes our main point that the prior assays that used fluorescent tubulin to study nucleation might have had altered results because fluorescent tubulin does not assemble as well as non-labeled tubulin.

6) Furthermore the authors discuss light scattering as the method of choice to "monitor formation of small nucleation intermediates". The authors directly contradict themselves by reporting that gamma-tubulin assemblies cannot be detected in their assay. Aren't those equivalent in size to nucleation intermediates?

-Thank you for the comment. We agree that our wording was misleading as to what the bulk assay measures. A bulk assembly of nucleation intermediates must reach sufficient length in order to be detectable. We have revised the manuscript to say, "Light scattering, on the other hand, uses unlabeled  $\alpha\beta$ -tubulin heterodimers to monitor nucleation" (lines 84-85).

RE: Manuscript #E20-02-0160R

TITLE: "XMAP215 and  $\gamma$ -tubulin additively promote microtubule nucleation in purified solutions"

Dear Dr. Davis:

I hope this email finds you and your team as well as is possible during these trying times. I am happy to let you know that both reviewers are enthusiastic about your manuscript. Thus, I am pleased to accept your manuscript for publication in Molecular Biology of the Cell. I look forward to seeing this work out and thank you for submitting your interesting work to MBoC.

Sincerely,  
Antonina Roll-Mecak  
Monitoring Editor  
Molecular Biology of the Cell

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Dear Dr. Davis:

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](http://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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We are pleased that you chose to publish your work in MBoC.

Sincerely,

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

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Reviewer #1 (Remarks to the Author):

The authors have satisfactorily addressed the comments that can be addressed without additional experiments. The only reservation of this reviewer is that leaving out the "arrays" from the title can be misleading, as they behave differently from soluble gamma-tubulin as the authors show. But it's up to the authors to decide how clear their title should be.

Reviewer #2 (Remarks to the Author):

The response to reviews is thoughtful and comprehensive.  
The fact that I have many more questions about the science provoked by the manuscript is probably a good sign that it is ready to be published.