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The Proline-rich Domain Promotes Tau Liquid Liquid Phase Separation in Cells

Xuemei Zhang, Michael Vigers, James McCarty, Jennifer Rauch, Glenn Fredrickson, Maxwell Wilson, Joan-Emma Shea, Songi Han, and Kenneth Kosik

Corresponding Author(s): Kenneth Kosik, University of California, Santa Barbara

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July 29, 2020

RE: JCB Manuscript #202006054

Dr. Kenneth S Kosik
UC Santa Barbara
Ocean Drive
Santa Barbara, California 93110

Dear Dr. Kosik:

Thank you for submitting your manuscript entitled "The Proline-rich Domain Promotes Tau Liquid Liquid Phase Separation in Cells". As you will see, both reviewers are very positive regarding your study and recommend publication following minor text edits and clarifications. We would therefore be happy to publish your paper in JCB pending addressing these reviewer points and final revisions necessary to meet our formatting guidelines (see details below).

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

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14) A separate author contribution section following the Acknowledgments. All authors should be mentioned and designated by their full names. We encourage use of the CRediT nomenclature.

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Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Eva Nogales
Monitoring Editor

Andrea L. Marat
Senior Scientific Editor

Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

In their manuscript, Zhang et al. investigate the liquid-liquid phase separation (LLPS) of the microtubule-associated protein, tau, in cells. Although tau has been shown to undergo phase transitions in vitro both on and off of the microtubule, this has not been shown in living cells to date. Zhang et al. examine tau condensation in cells and dissect the roles of each domain of tau (1-441) in driving condensate formation. They find that the polyproline rich domain (PRD), corresponding to aa 151-254, and phospho-state are responsible for driving tau LLPS. One of the nicest experiments in this study is shown in Figure 4, where the PRD condensates that lack the 4 microtubule-binding repeats, associate with microtubules. In addition, the inclusion of the N-terminus (1-254) inhibits tau condensation, highlighting the complex conformation changes that occur within this molecule to modulate its multiple modes of self-association and microtubule binding. The authors perform a number of elegant ex vivo studies to characterize tau LLPS, as well as simulations that reveal the importance of charged residues within the PRD for predicting phase separation. In a final set of interesting experiments, the authors implicate PRD condensation as an important basis for tau's interaction with EB1, which was previously described by the Arnal lab. PRD condensates even recruit EB1 to the microtubule lattice in cells. This is a very nice manuscript that should be of interest to the general JCB audience. In the light of the pandemic, I only have a few minor comments to improve the paper.

1) This appears to be 2N4R tau. Could the authors indicate the isoform in the manuscript?
2) Labels would be useful in Figure 5 and Figure S3 (on the figures themselves) to indicate which panel is which protein in the greyscale images.
3) Could the authors quantify the localization of EB1 in Figure S3 in the absence or presence of the PRD condensation? Punctate vs. Microtubule-Bound vs. Diffuse?

Reviewed by: Kassandra Ori-McKenney

Reviewer #2 (Comments to the Authors (Required)):

In this work, Kosik lab further investigated tau LLPS. The authors have shown that the microtubule-associated tau protein has the potential to form an LLPS in vivo. They identified the proline-rich domain of tau as a phosphorylation-dependent regulator of condensate formation.
It is a very nice work, well done and original.
I have only minor comments
The authors should also discuss their data regarding physiological tau concentrations. Overexpression of tau constructs does not reflect in vivo tau concentrations. It is a common criticism in LLPS experiments (Alberti S et al., 2019)
Some authors such as Lippens NMR group suggest that tau may bind to tubulin heterodimers. Is it relevant to the current observation?
Tau is also found aggregated in tauopathies. How these observations may be of interest in the pathological process?
Additional experiments with 3Rtau would have been of interest

Eva Nogales
Monitoring Editor

Andrea L. Marat
Senior Scientific Editor

Journal of Cell Biology

Dear Drs. Nogales and Marat:

Thank-you for sharing with us the reviews of our paper entitled, "The Proline-rich Domain Promotes Tau Liquid Liquid Phase Separation in Cells". We apologize for returning the manuscript five days beyond the deadline you set; but as you noted, extenuating circumstances related to the pandemic delayed the re-submission.

Below we detail our responses to each comment made by the reviewers

Reviewer #1 Dr. Kassandra Ori-McKenney:

In their manuscript, Zhang et al. investigate the liquid-liquid phase separation (LLPS) of the microtubule-associated protein, tau, in cells. Although tau has been shown to undergo phase transitions in vitro both on and off of the microtubule, this has not been shown in living cells to date. Zhang et al. examine tau condensation in cells and dissect the roles of each domain of tau (1-441) in driving condensate formation. They find that the polyproline rich domain (PRD), corresponding to aa 151-254, and phospho-state are responsible for driving tau LLPS. One of the nicest experiments in this study is shown in Figure 4, where the PRD condensates that lack the 4 microtubule-binding repeats, associate with microtubules. In addition, the inclusion of the N-terminus (1-254) inhibits tau condensation, highlighting the complex conformation changes that occur within this molecule to modulate its multiple modes of self-association and microtubule binding. The authors perform a number of elegant ex vivo studies to characterize tau LLPS, as well as simulations that reveal the importance of charged residues within the PRD for predicting phase separation. In a final set of interesting experiments, the authors implicate PRD condensation as an important basis for tau's interaction with EB1, which was previously described by the Arnal lab. PRD condensates even recruit EB1 to the microtubule lattice in cells. This is a very nice manuscript that should be of interest to the general JCB audience. In the light of the pandemic, I only have a few minor comments to improve the paper.

Reviewer: 1) This appears to be 2N4R tau. Could the authors indicate the isoform in the manuscript?

Authors: Yes, our study is based on 2N4R. We have now indicated this in the manuscript.

2) Labels would be useful in Figure 5 and Figure S3 (on the figures themselves) to indicate which panel is which protein in the greyscale images.

Authors: Thanks for pointing this out. We have now added the labels in Figure 5 and Figure S3.

3) Could the authors quantify the localization of EB1 in Figure S3 in the absence or presence of the PRD condensation? Punctate vs. Microtubule-Bound vs. Diffuse?

Authors:

Thank you for asking this intriguing question.

To quantify the EB1 Punctate vs Microtubule-Bound vs. Diffuse, we did image analysis with an additional preprocessing segmentation step to partition a digital image into multiple segments. To define the diffuse and non-diffuse population of EB1, each cell was outlined and auto-thresholded into a 2D binary image using the imageJ embedded Yen method. Next, we applied a particle analysis method to separate bundles from puncta according to segment size and shape. With this method, we analyzed EB1 (n=4), and EB1-CWT 151-254 (n=10), at 0, 2, 4, 24, 67, 380 seconds after light activation. Based on integrated density of identified segments, we found that EB1-GFP distribution differ significantly in the presence of CWT 151-254. Bundles were enhanced overtime upon activation in presence of CWT 151-254, while no apparent change occurred in the diffuse signal.

Although CWT 151-254 significantly enhanced EB1 clustering, it did not vary the ratio of EB1 on or off MT. The EB1 distribution on MT in cells is influenced by many factors, especially by other +TIPS proteins such as CLIP 170, CLIP 115 and Dynactin, etc, acting in concert to enhance EB1 on microtubule tip localization.

We added this conclusion together with quantification (Supplementary Figure 3), the corresponding image analysis and statistic method in the manuscript.

	Time (sec)	0	2	4	24	67	380	max
EB1 (n = 4)	**Diffuse**	0.897±0.056	0.758±0.204	0.796±0.253	0.815±0.095	0.943±0.044	0.733±0.176	0.943
	Bundles	0.013±0.025	0.002±0.004	0.012±0.024	0.055±0.081	0.007±0.009	0.005±0.006	0.055
EB1 with CWT 151-254 (n = 10)	**Diffuse**	0.874±0.102	0.906±0.072	0.88±0.085	0.876±0.083	0.887±0.103	0.835±0.144	0.906
	Bundles	0.05±0.077	0.032±0.051	0.046±0.073	0.051±0.07	0.056±0.099	0.118±0.138	0.118

The EB1-GFP signal per cell was quantified as bundles or diffuse (obtained from the whole cell minus puncta and bundles), in the absence or presence of CWT 151-254 light activation at the times indicated. Mean ± StDev is shown. EB1-GFP signal differences were found to be statistically significant (Roy's two way MANOVA: absence or presence of CWT 151-254, $p = 5.63 \times 10^{-6}$; time, $p = 0.396$ (n.s.) with a significant interaction $p = 5.642 \times 10^{-3}$).

Reviewer #2:

In this work, Kosik lab further investigated tau LLPS. The authors have shown that the microtubule-associated tau protein has the potential to form an LLPS in vivo. They identified the proline-rich domain of tau as a phosphorylation-dependent regulator of condensate formation.
It is a very nice work, well done and original.
I have only minor comments
The authors should also discuss their data regarding physiological tau concentrations. Overexpression of tau constructs does not reflect in vivo tau concentrations. It is a common criticism in LLPS experiments (Alberti S et al., 2019)

Authors:

As noted by the reviewer and Dr. Alberti et al. we recognize that observations of LLPS through protein overexpression in live cells does not reflect *in vivo* tau concentrations. We point out this potential shortcoming in the text. In route to achieving an authentic in vivo understanding of tau LLPS we believe that over-expression studies can serve as a heuristic model, particularly in the analysis of protein domains. Over-expression studies can reveal specific domain functions that are obscured in more in vivo settings due to competition with other domains in the same protein as is the case here with regard to the PRD and the amino terminal domain. We fully acknowledge (and make it clear in the revised text) that conclusions from such methods will require novel approaches to more closely approximate the in vivo setting.

Reviewer #2: Some authors such as Lippens NMR group suggest that tau may bind to tubulin heterodimers. Is it relevant to the current observation?

Authors: The question is certainly relevant but somewhat beyond the scope of our study. Elegant tau studies by Rhoades and Nogales as well as Lippens all of which were cited in our discussion were mostly based on alpha-beta tubulin heterodimers. We have attempted to extend their insights to full-length microtubules as observed in living cells with the above caveat concerning over-expression.

Reviewer #2: Tau is also found aggregated in tauopathies. How these observations may be of interest in the pathological process?

Authors: Linking these observation to tau aggregation is the subject of our current studies using tau mutations.

Reviewer #2: Additional experiments with 3Rtau would have been of interest

Authors: Agree that 3Rtau would have been of interest and we would like to address this question as well as the subject of tau mutations from the perspective of LLPS in our next study.