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Actin network architecture can ensure robust centering or sensitive decentering of the centrosome

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Thank you again for submitting your revised Review Commons manuscript for consideration by The EMBO Journal. In light of the positive original comments and the interest of the subject of the study, I decided to treat it similar to a regular revision, and sent it directly to one of the original referees to get their assessment of your responses to the various specific points raised during review of the preprint. Given their positive feedback (copied below for your information), we shall be happy to accept this study for EMBO Journal publication, as soon as the manuscript has been re-formatted and the following editorial points have been addressed:

REFEREE REPORTS

Referee #2:

The authors have satisfactorily addressed all of the concerns I expressed in my original review and would recommend publication of this important contribution to our understanding of MTOC positioning within cells.

Review Commons transferred Referee reports.

Review #1

Evidence, reproducibility and clarity

Yamamoto and colleagues have investigated the interplay between microtubules (MTs) and actin in positioning the MTOC at "the cell centre". They have developed a novel experimental setup akin to a synthetic cell to study this question. Essentially a cell-sized (15 μm) microwell that is coated in lipid and then tubulin/actin added and the positioning of a MTOC proxy is studied by microscopy. This is a well executed study. These complicated biochemical reconstitutions are the hallmark of Blanchoin and Théry's group, but even so, it's clear that the exact conditions (e.g. tubulin concentration) are fiddly and critical for these experiments to work. The data are clear, well analysed and presented. In brief, the conditions for centring a cytoskeletal network and decentring/polarising it are recapitulated. This is a short, straightforward paper and I found the results to be clear and the authors' interpretation to be well supported by the data.

Two questions occurred to me as I read the paper:

* While the setup is reminiscent of a cell, I suspect that the edge/wall of the microwell is much stiffer than the plasma membrane. So a MT that encounters the wall may behave differently in the cell. This would affect the non-actin conditions but possible also the conditions where an actin mesh is present. Maybe my intuition is not even correct, but I think this issue should be discussed in the paper as a potential limitation of the system.
* The graphs in 3C and 4G (lesser extent Fig 1) show nicely that the aMTOC position has apparently rested at a steady state. Some representative trajectories are shown in some figures, but not mentioned much in the text. How does the pathlength (cumulative distance) over time compare to the "distance to centre" measurement? Is there more or less travel under the different conditions? From the supplementary videos it looks like there is a difference. An apparent resting position may still represent significant motion, e.g. circling the centre. What does an analysis of tracklength tell us, if anything?

Very minor clerical point:
* the first two sentences of the abstract could be clearer. "The position of centrosome, the main microtubule-organizing center (MTOC), is instrumental in the definition of cell polarity. It is defined by the balance of tension and pressure forces in the network of microtubules (MTs)." In the second sentence, "it" and "defined" are confusing. Are you talking about the position of the centrosome or cell polarity?

Significance

As I see it, the main advance here is in novel experimental setup which has real potential in the field. Existing methods such as MTs inside lipid bubbles are limited, whereas as the microwell method with fabrication methods allows the shape of the "synthetic cell" to be carefully modulated. Tying the results together with cytosim simulations is also a powerful combination. There is a lot of interest in bottom-up reconstitution of cell biological phenomena, especially those that underlie specialised cell processes, e.g. polarity.

Review #2

Evidence, reproducibility and clarity

Summary:

This manuscript describes the use of an elegant in vitro reconstitution system to study the effect of variations in the organization of the actin network on the positioning of a microtubule organizing center (MTOC) within the cell. By using a reconstituted system the authors are able to specifically study the contribution of the "pushing" forces generated by microtubule (MT) growth, without the confounding influence of other factors, like pulling forces from MT motors. The authors find that a bulk actin networks at sufficient density can impair MTOC displacement, likely a result of the large viscous drag of the MTOC. Next they show that MTOC centering more resilient to changes in microtubule length. Finally they show that an asymmetric actin network can cause asymmetric positioning of the MTOC.

Major comments:

1) The model the authors put forth is that the growth of long MTs leads to decentering as a result of the MTs slipping along the well edge. The presence of a cortical actin mesh prevents this slipping. Their argument would be strengthened with and analysis of the MT behaviors in the various conditions. For example when discussing MTOC in well without actin...

"As they grew, they first ensured a proper centering but after an hour, MT elongation and slippage along microwell edges broke the network symmetry and MTs pushed aMTOC away from the center (Figure 1I, J and Supplementary Movie 2)"

In this movie I don't see evidence of MTs hitting the cortex and sliding on the "short" side of the well relative to the MTOC. An analysis of the behavior of MTs in various circumstances would help link the behavior of MTs to the movement of the MTOC for all of their conditions. What fraction of MTs hit the cortex and remain relatively motionless, what fraction slide, what fraction catastrophe, what fraction turn and follow the curve of the well? And how does this behavior change for microtubules that end up on the short side vs. the long side of the MTOC? This type of analysis would solidify their model for how centering/decentering occurs in the various conditions they test.

2) The authors use simulations to support their in vitro findings. However, their simulations have many more microtubules emanating from the MTOC than their experiment (Looks like about 50 in the cytosim and they state they are aiming for 15-20 in the aMTOCs). Do the simulations still reproduce the behavior of the in vitro system with a similar number of MTs?

3) When the actin networks are asymmetric, the authors see decentering of the MTOC towards the side with less actin. However there is still actin on the side where the MTOC will move to and in some of their images it looks pretty think. Is the actin on that side not dense enough to prevent MT sliding along the "cortex"? If so, can they generate less dense, but uniform actin networks on the "cortex", where MTs can slide. Again descriptions of MT behaviors would be useful in understanding what is happening.

Minor Comments:

1) Title - the current title implies that actin is balancing the forces generated by the MTs. I'm not sure this is a good description of what is shown in the paper.

2) The discussion would benefit from more explanation about how the results of this paper relate to the classic examples of MTOC positioning they cite. How do they envision the actin and MTs interacting in these systems and what new insight have we gained from the experiments in this manuscript.

Significance

Overall, this work is a significant advance in our understanding of the potential mechanisms of MTOC movement in cells via pushing by MT growth. The experimental system they have developed is powerful advance, allowing meaningful MTOC reconstitution experiments to be performed in chambers of approximately cellular size. This is an important contribution to understanding the interaction between microtubule pushing and the actin cortex.

Review #3

Evidence, reproducibility and clarity

Review of "The architecture of the actin network can balance the pushing forces produced by growing microtubules" by Yamamoto et al.

The means by which cells maintain their characteristic cytoskeletal architectures is not well understood. This is in part because there is considerable variation in such architectures with, for example, fibroblasts, neurons, and epithelial cells. It is also in part because the microtubule, actin and intermediate filaments engage in a wide range of mechanical and signaling crosstalk mediated by a wealth of proteins and signaling networks, which further complicates the picture.

In the current study, Yamamoto take the welcome step of developing a simplified system for assessing the mutual contributions of microtubules and F-actin for general cytoskeletal organization in vitro (specifically, in lipid-lined microwells). This allows them to define basic principles of microtubule-F-actin interactions in the absence of the various confounding factors alluded to above. Using their model, they show that artificial MTOCs (aMTOCs) alone will center but as a complex function of microtubule length (controlled by varying tubulin concentrations). That is, the aMTOCs are randomly positioned with short microtubules, stably centered with intermediate length microtubules, and randomly oriented with very long microtubules (following symmetry breaking).

They then assess the contributions of F-actin to the centering process. In low concentrations of "bulk" F-actin (ie F-actin distributed throughout the droplet) there is no effect on centering whereas at higher concentrations of bulk F-actin, centering is impaired as is the translocation of the aMTOCs. In the presence of uniform peripheral F-actin, in contrast, aMTOC centering is enhanced, and rendered less sensitive to variations in microtubule length. Finally, when the authors contrive a situation in which the peripheral F-actin is non-uniform (by lowering the

concentration of actin and adding alpha-actinin, which creates a peripheral ring of F-actin with (I think) relatively less F-actin within the ring), the aMTOCs position themselves within the ring.

Finally, the authors extend their results with simulations that indicate that the various behaviors can be explained by a combination of friction, pushing and slippage.

This study is fascinating and will be of general interest to anyone who seeks to understand the contributions of mechanical forces to cytoskeletal organization in a minimal system. I have only minor concerns; these are listed below.

1) Some of the terminology was a little confusing. The authors introduce the term "inner zone" (pg. 8) without defining it. From the context, it seems like they are talking about the approximate center of the ring of peripheral F-actin. If so, why not just do away with the term "inner zone" and refer to the ring center. If it isn't the ring center, then more explanation is needed as to what the inner zone actually is.

2) It is not clear from the text or the images if the region within the F-actin ring has less F-actin, more F-actin, or the same amount of F-actin as the region outside the F-actin ring. This point should be clarified, as it makes a big difference in the interpretation of the findings.

3) Ideally, the authors would include manipulations in which the high concentration of peripheral F-actin is combined with alpha-actinin because, as currently presented, the authors are drawing conclusions from changing two variables at once (ie going from a high concentration of peripheral F-actin to a lower concentration with added alpha-actinin). Thus, the authors cannot cleanly distinguish between effects that arise from F-actin asymmetry versus the presence of an F-actin crosslinker. Since the crosslinking is likely to change the mechanical properties of the peripheral F-actin network, this point should at least be addressed in the text, if not by experiments.

Significance

This is an elegant, well-designed study that provides a clear description of how basic mechanical forces can contribute to cytoskeletal organization in a simplified model system.

1st Authors' Response to Reviewers — 24th Jun 2022

The authors have made all requested editorial changes.

Accepted — 6th Jul 2022

Thank you for submitting your final revised manuscript for our consideration. I am pleased to inform you that we have now accepted it for publication in The EMBO Journal.

EMBO Press Author Checklist

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