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TWO DYNAMIC BEHAVIOURS OF THE MICROTUBULES AT THE CELL CORTEX REVEAL THE PULLING AND PUSHING FORCES THAT POSITION THE MITOTIC SPINDLE IN C. ELEGANS EMBRYOS.

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In the *Caenorhabditis elegans* zygote, the mitotic spindle assembles in the cell-centre and is maintained there during metaphase by the so-called centring forces. Then, after the anaphase onset, cortical pulling forces displace the spindle posteriorly. It is achieved by astral microtubules likely pushing against and being pulled from the periphery, respectively. These forces are essential to position the mitotic spindle, and in turn to properly distribute daughter cell fate determinants.

We developed an assay to measure the spatial distribution of the residence times of the microtubules at the cortex. We viewed labelled microtubules by spinning disk fluorescence microscopy along the mitosis, denoised the images and tracked the spots corresponding to the microtubule contacts. We computed the per–embryo histogram of the track durations (residence times) and performed a maximum likelihood fit over all embryos, sharing model parameters. By a Bayesian analysis comparing models, we found that a double exponential offered the best fit, suggesting the presence of two dynamic behaviours. Because the two corresponding characteristic times are close, typically 0.5 s and 1.5 s, and because fitting a mixture of exponentials is a known difficult problem, we validated our image analysis pipeline by processing *in-silico*-fabricated images. We also used different algorithms for denoising and tracking. We challenged the statistical analysis through the generation of *in-silico*-track duration. Overall, it suggests that our approach is robust and precise and enabled us to highlight the minimal number of tracks and embryos to ensure reliable results.

Modelling of anaphase oscillations suggested that single cortical pulling event lasted less than 1 s [1], and dynein was found to reside at the cortex for about 0.6 s [2]. Both durations are consistent with the one of the short-lived population. To support that this population reflects the pulling force events, we observed that it is polarised and that its distribution depends on the proteins involved in generating pulling forces and their regulators. Conversely, we genetically depleted proteins targeting microtubule dynamics and altered mostly the long-lived population, indicating that this latter corresponded to microtubules growing against the cortex. Overall, we suggest that different functions of microtubules at the cell periphery correlate with distinct dynamics, which in turn offers a readout of the distribution of the forces regulating spindle positioning in space and time. It opens a novel avenue to explore the pushing- and pulling-force mechanisms and how they co-exist over the course of the mitosis.

[1] J. Pécréaux, et al. *Spindle oscillations during asymmetric cell division require a threshold number of active cortical force generators*, Curr. Biol., 16:2111-2122, 2006.

[2] R. Rodriguez Garcia, et al., *Dynein dynamics at the microtubule plus-ends and cortex during division in the C. elegans zygote*, bioRxiv:118604, 2017.