MAR14-2014-021052

Abstract for an Invited Paper
for the MAR14 Meeting of
the American Physical Society

Positioning of Microtubule organizing centers (MTOC) in 3D confinement

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Important functions of eucaryotic cells, like motility or division, depend sensitively on cytoskeletal mechanics and organization. In particular, microtubules (MTs) are dynamic polymers that can move and position organelles such as their MTOC by pushing, pulling or sliding [1]. How the shape and size of cells, as well as the presence of pushing and/or pulling forces influence this positioning is in many cases still unclear. To assess the influence of confinement on MTOC positioning, we reconstruct a dynamic microtubule cytoskeleton in vitro, inside 3D water in oil emulsion droplets. We study the positioning of centrosomes, from which microtubules are nucleated, that exert pushing and/or dynein- mediated-pulling forces when reaching the cortex. We show that the central position of one centrosome confined in a spherical droplet is drastically destabilized by pushing forces, while pulling forces tend to center the centrosome. Interestingly, when two centrosomes are present, pushing forces will lead the centrosomes to take a stable position at opposite sides of the droplet. When both pushing and pulling forces are present, two centrosomes adopt an equilibrium position balancing the centering effect of the cortical pulling forces and the repulsion effect of the two centrosomes. Summarizing, we show that very simple systems, involving only microtubule dynamics, confinement, pushing and pulling forces can lead to self-organized patterns that are biologically relevant. In particular, we reproduce a mitotic spindle like organization with just these components. This sets the stage for a better understanding of the function of additional components of natural mitotic spindles such as mitotic motors and crosslinkers that we plan to add to our system.

[1] Tolic-Norrelykke I, **2008** Push-me-pull-you: how microtubules organize the cell interior *Eur Biophys J* 37: 1271-1278

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