Research Summary

Kinesin: molecular nano machines – function and regulation

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Molecular motors from the Kinesin superfamily are nanometric machines that move objects along microtubule (MT) filaments. While it has been demonstrated that their function is essential for intracellular vesicle trafficking, cell locomotion and cell division, the mechanism and regulation of their activity have not as yet been established. My research focuses on the study of Kinesin-5 motor proteins, whose function is essential for chromosome segregation during mitotic cell division. Since these proteins perform their functions by producing force and motion on a nanometric scale, our objective is to study their activity and regulation by applying cell-biology, biophysical and biochemical approaches.

To address the open issues in the Kinesin-5 field, over the recent years, we focused on the study of Cin8 and Kip1, two Kinesin-5 homologues in the genetically-tractable eukaryote *Saccharomyces cerevisiae*. We examined spindle morphogenesis [1,2] and MT dynamics [3] during mitosis in *S. cerevisiae* cells, using high resolution fluorescence microscopy and fluorescence-recovery after photobleaching (FRAP) techniques.

To study the effect of Kinesin-5 motors on MT dynamics, we used cells expressing a fluorescent version of tubulin (tubulin-GFP), the building proteins of MTs. We photobleached a considerable portion of the mitotic spindles, and followed the recovery at the plus-ends of MTs. We found that at the final stages of mitosis, MTs maintain high dynamics at their plus-ends, with growth and shrinkage rates of 1.3 and 7.3 μm/min, respectively. Fluorescence recovery of anaphase spindles followed first-order kinetics. We observed that in cells lacking the spindle-stabilizing and organizing protein Ase1, the MTs are highly dynamic, demonstrating the importance of proper spindle organization in controlling spindle MT dynamics. Finally, deletion of the Kinesin-5 Cin8, reduced the rate of MT recovery kinetics in intermediate anaphase spindles, indicating that Kinsin-5 motors are involved in MT destabilization.

In addition, to study the regulation of Kinesin-5 motors, we examined how phsophorylation in the catalytic motor domains of Cin8 and Kip1 affects their localization to the spindles [3,4]. We found that dephosphorylation increases, while phosphorylation decreases attachment to the spindles MTs of Cin8 and Kip1.

To examine how phosphorylation affects the *in vitro* motor functions of Kinesin-5 proteins, we are currently studying motile properties of Cin8 and Kip1 in multi-motor MT-sliding and single-molecule fluorescence motility assays. For these biophysical assays, we are using high temporal and spatial resolution fluorescence live-imaging techniques such as spinning-disc confocal and TIRF microcopies [5].

In the future, we plan to undertake a technology-oriented project and develop a kinesin-MT-based device to directionally transport ~50 nm particles on a patterned surface. Since we expect that the *in vitro* biophysical nano-approaches will become more dominant in our research during the upcoming years, I am applying to become a member of the Ilse Katz Institute for Nanoscale Science and Technology. Such membership will

enlarge my group's interaction with researchers who are interested in similar topics and are applying similar methodologies in their research.

References

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