Ben-Gurion University of the Negev
אוניברסיטת בן-גוריון בנגב

Graduate Students Seminar

Department of Chemistry

Sunday, May 28th, 2023
Time 14:30
Bldg. 43 Room 015

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Mechanisms by which bi-directional kinesin-5 motor performs its multiple mitotic roles

The *S. cerevisiae* Cin8 is a mitotic kinesin-5 motor protein that crosslinks microtubules (MTs) and converts chemical energy from ATP hydrolysis into a mechanical force and movement along MTs. Cin8 performs essential functions during mitosis, in spindle assembly, maintenance of bipolar spindle structure and spindle elongation during anaphase. It is not clear how Cin8 performs its different roles during mitosis. Previously it was demonstrated in our lab that Cin8 is a bi-directional motor, moving in different directions under different experimental conditions. For instance, at the single molecule level, Cin8 moves towards the minus-end of the MTs. The mechanisms of this phenomenon are yet to be established.

In this study, we aim to characterize the motor functions of Cin8 on different types of in vitro polymerized MTs, in order to shed light on the mechanism of bi-directional stepping. We also aim to understand how mutations in the motor domain affect the ability of Cin8 to perform its multiple mitotic roles, and how the intracellular phenotypes of these mutants correlate with the motile properties of Cin8 *in vitro*.

We first analyzed the motility of wild-type Cin8 on MTs polymerized and stabilized by different methods: in the presence of GMPCPP, resulting in GTP-like MTs; and in the presence of GTP/Taxol resulting in GDT/Taxol MTs. These two methods result in conformational differences in MT lattice, which may directly affect the affinity of motor proteins to the MTs and alter their motile properties. We thus used single-molecule *in vitro* motility assay, to examine the effect of different types of MT polarization on the motility of bi-directional kinesin-5 Cin8, tagged with GFP.

Our results demonstrate differences in Cin8 motor behavior as a response to changes in MT conformation, which are opposite to previous reports on the plus-end directed kinesin-1 motors. These results indicate that the bi-directional and plus-end directed kinesin motors interact differently with the MT lattice. We propose that the bi-directional kinesin motors contain non-canonical binding site(s) to MTs that enable stepping in two directions and is essential for bi-directional motility.

To understand what motor functions of bi-directional kinesin motors are important for mitosis, we examined the *in vivo* and *in vitro* motile properties of Cin8 variants with mutations in the motor domain (Cin8-R196K and Cin8-F647A), which were previously shown to exhibit different defects in motor functions *in vitro*, such as reducing the ability of Cin8 to move and bind MTs. Since motor functions of these mutants was previously characterized only partially, we have analyzed motility and MT binding of these mutants on the single-molecule level, and their ability to facilitate MT gliding and antiparallel MT sliding *in vitro*. We found that both Cin8-F647A and Cin8-R196K exhibit processive motion in the minus end direction at single molecule level but defected in their ability to crosslink the MTs and to produce directional and processive MT sliding.

Based on these results, we conclude that both microtubule binding and motility by Cin8 are crucial for the proper formation of the bipolar spindle structure.