Title

Connecting Motors and Membranes: A Quantitative Investigation of Dynein Pathway Components and in vitro Characterization of the Num1 Coiled Coil Domain

Authors

Bryan J. St. Germain, *University of Massachusetts Amherst* Follow

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Abstract

In the budding yeast, *Saccharomyces Cerevisiae,* dynein, a minus-end directed motor, is involved in nuclear migration and proper orientation of the mitotic spindle during mitosis. Our lab has developed a model that involves the loading of cytoplasmic dynein onto the plus-end of astral microtubules through interactions with Pac1/LIS1 and Bik1/CLIP-170. Dynein is then delivered to the cell cortex and anchored through a cortical receptor protein, Num1. Num1 is a 313KDa protein that localizes to the cell cortex and is an essential component of dynein mediated nuclear migration.

Using quantitative fluorescence techniques I was able to create a molecular inventory of various dynein pathway components. Our results revealed Dyn1, dynein heavy chain, and Pac1/LIS1 associate at the plus end in a 1:1 ratio. Additionally we found that dynein and dynactin associate in a 3:1 ratio at the plus ends and a 2:1 ratio at the cortex. Interestingly, we found that over expression of Pac1/LIS1 augments cortical dynein activity while maintaining the dynein to dynactin ratio and this activity is separate from loss of She1, a negative regulator of dynein-dynactin interaction, which results in a 1:1 ratio of dynein-dynactin at the plus-ends, as well as, the cortex. Our results uncover molecular ratios that enable us to create more defined and detailed model of the dynein pathway.

To elucidate how Num1 attaches dynein to the cortex we created truncations of the Num1 protein. We were able to determine that two coiled-coil (CC) domains in the N-terminus of Num1 are responsible for bright foci formation on the cortex. Cells without these bright foci exhibit a binucleate phenotype similar to that of *dyn1*Δ implicating that these bright foci are required for the proper function of Num1 in the dynein pathway.

To test the hypothesis that the CC is capable of mediated bright patch assembly through self-association I purified a recombinant CC domain and performed gel filtration analysis, as well as, equilibrium sedimentation. I was able to determine that the CC domain exists as a dimer in solution. However, the mechanism of CC self-assembly may involve a requisite of targeting the CC to the cortex first.

First Advisor

Wei-Lih Lee

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