

The cytoplasmic dynein motor complex at microtubule plus-ends and in long range motility of early endosomes, microtubule plus-end anchorage and processivity of cytoplasmic dynein

Submitted by Yvonne Roger

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Yvonne Roger

Abstract

Cytoplasmic dynein is a microtubule-dependent motor protein which participates in numerous cellular processes. The motor complex consists of two heavy chains, intermediate, light intermediate and 3 families of light chains. Dynein is able to bind to these accessory chains as well as to regulatory proteins which enables the motor protein to fulfil such a variety of cellular processes. The associated light chains participate in long-distance organelle and vesicle transport in interphase and in chromosome segregation during mitosis. However, how these light chains control the activity of the motor protein is still unknown.

In this study, I combine molecular genetics and live cell imaging to elucidate the role of the associated dynein light intermediate and light chains in dynein behaviour and early endosome (EE) motility in hyphal interphase cells as well as the anchorage of dynein to the microtubule (MT) plus-end in interphase and mitotic cells. I show that the dynein light intermediate chain (DLIC) as well as the light chain 2 (DLC2, Roadblock) are involved in dynein processivity and EE movement in interphase. The downregulation of either protein results in short hyphal growth which could be caused by a decreased runlength of EE and dynein. In addition, both proteins participate in dynein anchorage to the microtubule plus-end in interphase and mitosis as well as in spindle elongation during mitosis. Each protein causes a decrease of the motor protein dynein at MT plus-ends. Surprisingly, I found only minor or no defects in LC8 or Tctex mutants in the observed functions of dynein. LC8 seems to affect the dynein but not the EE runlength. In this case, dynein is still able to move into the bipolar MT array from where kinesin3 is able to take over EEs and move them towards the cell center. In contrast, Tctex has no effect on dynein or EE runlength or any other observed dynein function in hyphal cells. However, it causes a reduction in spindle

elongation. Taken together, DLIC and DLC2 are important for dynein behaviour in long distance transport as well as in spindle positioning and elongation during mitosis.

Furthermore, I studied the involvement of the dynein regulators Lis1 and NudE as well as the plus-end binding protein Clip1 (Clip-170 homologue) in the anchorage of dynein to the astral microtubule plus-ends during mitosis. The disruption of the anchorage complex at the astral MT plus-end causes a decrease in dynein number at this site and therefore slower spindle elongation in Anaphase B. Taken together, all three proteins are involved in anchorage of dynein to the astral microtubule tip and the subsequent spindle elongation. Furthermore, these findings also show that *Ustilago maydis* evolved two different mechanisms to anchor the motor protein to MT plus-ends in hyphal and mitotic cells. The plus-end binding protein Peb1 (EB1 homologue) and the dynein regulator dynactin mediate the dynein anchorage in hyphal cells whereas in mitotic cells the plus-ends binding protein Clip1 and the dynein regulators Lis1 and NudE anchor dynein to astral MT plus-ends.

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