

LIS1 at the microtubule plus end and its role in dynein-mediated nuclear migration

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The cytoplasmic dynein complex and its accessory dynactin complex are involved in many cellular activities including nuclear migration in fungi (for review see Karki and Holzbaur, 1999). LIS1, the product of a causal gene for human lissencephaly (smooth brain), has also been implicated in dynein function based on studies in fungi and more recent studies in higher eukaryotic systems (for review see Gupta et al., 2002). Exactly how LIS1 may regulate the behavior of cytoplasmic dynein in various organisms is a fascinating question. In this issue, Lee et al. (2003) describe important new findings in *Saccharomyces cerevisiae* regarding the role of LIS1 (Pac1) in dynein-mediated nuclear migration.

In *S. cerevisiae*, the mitotic spindle forms and elongates within the nuclear envelope, and proper nuclear migration toward the bud is important for segregating the genetic materials to both the mother and daughter cell. Nuclear migration (or spindle orientation) in *S. cerevisiae* occurs in two steps using two partially overlapping pathways. During the first step, the nucleus moves closer to the bud neck using a Kar9-dependent microtubule (MT)*-capturing and -shrinking mechanism. During the second step, the spindle moves into the neck, with the pulling force generated by dynein/dynactin-dependent MT sliding along the bud cortex (Adames and Cooper 2000). In this current paper, Lee et al. (2003) report that this MT-sliding behavior is absent in cells lacking Pac1. Both Pac1 and cytoplasmic dynein heavy chain Dyn1 localize to the plus ends of cytoplasmic MTs. Although Pac1's plus end localization is not Dyn1 dependent, lack of Pac1 results in the loss of Dyn1's MT plus end localization. These results suggest that Pac1 plays a role in targeting cytoplasmic dynein to the plus ends, and that such a role is important for dynein-mediated MT sliding along the bud cortex and the subsequent spindle movement into the bud neck.

How the plus end-localized dynein and LIS1/Pac1 function during nuclear migration is a question of significant interest. For MTs to slide along the cortex and pull the nucleus,

dynein would need to be anchored at the cortex and walk along the MTs toward the minus end (the spindle pole body) (Carminati and Stearns, 1997). Although dynein at the bud cortex was not observed in this current study, physical interaction has previously been detected between a putative dynein subunit and the cortical protein Num1 (Farkasovsky and Kuntzel, 2001). Num1 is a pleckstrin homology domain-containing protein (Farkasovsky and Kuntzel, 2001) known to be important for MT sliding along the bud cortex (Heil-Chapdelaine et al., 2000). In this current paper, the authors propose an interesting model suggesting that the interaction between the MT plus ends and cortical Num1 helps to offload dynein and Pac1 from the MT ends to the bud cortex. The anchored cortical dynein is then activated and walks toward the MT minus end at the spindle pole body. Consistent with this model, the authors have found that the plus end accumulation of both Dyn1 and Pac1 is increased in cells lacking Num1, supporting a role of Num1 in dynein and Pac1 offloading. In addition, enhancement of the dynein and Pac1 signals at MT ends has also been observed in cells lacking dynactin, suggesting that dynactin may also be involved in this offloading process. Whether Num1, dynactin, and Pac1 are also involved in activating the dynein motor at the cortex is another important question. In a recent independent study, similar results on the effects of Pac1, Num1, and dynactin mutations on dynein's plus end localization have also been obtained (Sheeman et al., 2003). Because the loss of dynactin or Num1 caused not only an increase in dynein accumulation at the plus end, but also a decrease in dynein accumulation at the minus end (spindle pole body), the authors suggest that Num1 may activate the dynein motor or enhance motor processivity, possibly by clustering the motor onto lipid microdomains (Sheeman et al., 2003).

Although details of the proposed MT plus end-to-cortex delivery of dynein and the subsequent mechanism of motor activation need to be examined in the future, it is reasonable to propose that the dynamic plus ends might allow bound dynein to interact with the cortex. Plus end-localized dynein may also be involved in regulating MT dynamics and MT-cortex interactions important for the initial capturing of dynein by cortical proteins. Consistent with this view, earlier studies have found that dynein mutants alter MT dynamics and MT-cortex interactions, both of which are important for nuclear migration in *S. cerevisiae* (Carminati and Stearns, 1997; Shaw et al., 1997; Cottingham et al., 1999).

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*Abbreviation used in this paper: MT, microtubule.

The mechanism by which dynein is targeted to the MT plus end in vivo is another important issue. Although these results from the budding yeast clearly demonstrate a role of Pac1 in the plus end localization of dynein, this is in sharp contrast to results obtained in the filamentous fungus *Aspergillus nidulans*. In *A. nidulans*, GFP-labeled cytoplasmic dynein and NUDF (LIS1) form comet-like structures that accumulate at the dynamic plus ends of cytoplasmic MTs (Han et al., 2001). Both the dynein heavy chain and intermediate chain comets are not only present, but are even more prominent, in cells lacking NUDF (Zhang et al., 2003). Similarly, prominent dynein comets have also been observed in cells lacking a NUDF interacting protein NUDE (RO11 of *Neurospora crassa*) (Efimov, 2003). Differences have also been found regarding how dynein and LIS1 are targeted to MT plus ends. In *A. nidulans*, the dynactin complex is important for dynein's plus end accumulation (Zhang et al., 2003), whereas in *S. cerevisiae*, dynactin is more likely to be involved in offloading dynein to the cortex.

These results suggest that different mechanisms may be used to target dynein to the MT plus end in these two organisms. The reason behind this can only be speculated at this point. Cells of filamentous fungi are very long compared with budding yeast. In a manner similar to higher eukaryotic organisms, filamentous fungi use MT and their motors not only for spindle functioning and nuclear migration, but also for vesicle transport and positioning of other organelles (Seiler et al., 1999). Previous studies in mammalian cells have also found MT plus end localization of dynactin, dynein, and LIS1 (Coquelle et al., 2002; for review see Schroer, 2001). Since dynein is a minus end-directed motor, dynactin at the dynamic plus end has been proposed to facilitate cargo capturing. Evidence in support of this view has come from a recent observation that plus end dynactin interacts transiently with cargo just before cargo transport (Vaughan et al., 2002). Since LIS1 physically interacts with both the cargo-binding region and the first AAA (ATPases associated with cellular activities) repeat of the dynein heavy chain, LIS1 may be involved in the coordination between motor activity and cargo binding (Tai et al., 2002). In *A. nidulans*, lack of NUDF may cause dynein to be kept at its inactive state and inhibit its transport of bound cargo, resulting in an over-accumulation at the plus end. Another recent finding in *A. nidulans* is that a conventional kinesin KINA is important for the plus end accumulation of dynein and dynactin (Zhang et al., 2003). Interestingly, budding yeast does not have such conventional kinesins. Therefore, it is likely that due to differences in cell size and the mechanisms used for general intracellular transportation, these two organisms may use different mechanisms for dynein's plus end targeting.

Dynein has been found at the cell cortex in higher eukaryotic organisms, and the mechanism by which cortically anchored dynein pulls on astral MTs to move the nucleus or to reorientate the spindle is likely to be conserved during evolution (for review see Dujardin and Vallee, 2002). The idea that plus end-localized dynein and LIS1 may be delivered from the MT plus end to the cortex is extremely interesting, and deserves to be further tested in various systems. Whether this mechanism is also used for reorientating the MT-organizing center and moving the nucleus during directional cell

movement is another interesting question to be addressed in the future. It is possible that the neuronal migration defect observed in lissencephaly patients is caused by a nuclear migration defect in the neuron (for review see Morris et al., 1998). Thus, our understanding of the disease mechanism for human lissencephaly will clearly benefit from these basic studies using different organisms and cell types.

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