LIS1 Clamps Dynein to the Microtubule

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Cytoplasmic dynein is a motor essential for numerous mechanical processes in eukaryotic cells. How its activity is regulated is largely unknown. By using a combination of approaches including single-molecule biophysics and electron microscopy, Huang et al. in this issue uncover the regulatory mechanism by which LIS1 controls the activity of cytoplasmic dynein.

Cytoplasmic dynein is a microtubule motor that carries out the majority of tasks depending on minus-end directed motility in the cytoplasm of most eukaryotic cells (Allan, 2011). Several accessory proteins modulate dynein’s properties and functions. Prominent examples are the dynactin complex, LIS1 and NudE (Kardon and Vale, 2009). How these cofactors regulate dynein’s cellular activities is still poorly understood. In this issue of Cell, Huang et al. (2012) unravel the molecular mechanism by which LIS1 regulates the motility of cytoplasmic dynein from *Saccharomyces cerevisiae*.

Cytoplasmic dynein is a fascinating enzyme. It is a large complex consisting of two dynein heavy chains and several smaller subunits. The smaller subunits associate with the N-terminal part of the heavy chains, forming the cargo binding region. The C-terminal part of the heavy chain forms the motor domain. Each motor domain consists of: (1) A hexameric AAA+ (ATPase associated with various cellular activities) ring with the major ATP hydrolysis site located in the AAA1 domain (Figure 1A); (2) The microtubule binding domain (MTBD) located at the end of an elongated anti-parallel coiled coil (~15 nm) called the stalk that protrudes from AAA4 domain (Figure 1A); (3) The linker connecting AAA1 with the N-terminal sequence of the heavy chain. This linker represents the major mobile mechanical element responsible for force generation and directional movement of this motor (Cho and Vale, 2012).

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As for filament-dependent motors in general, directional force production requires intramolecular coupling of three processes: ATP hydrolysis, movement of a mechanical element, and cycling of the filament binding site through states with high and low affinity (Figure 1A). This allows transformation of the energy released by ATP hydrolysis into mechanical work precisely when the motor is attached to its filament. Therefore, motors typically make one step per one hydrolyzed ATP. Remarkably, in dynein the major ATP hydrolysis site and the microtubule binding site are separated by a distance of ~25 nm (Cho and Vale, 2012). Therefore, information must be transmitted over this distance through conformational changes that enable correct intramolecular coupling between the biochemical and mechanical cycles. Such conformational communication is not unusual for AAA+ proteins, some of which are DNA helicases or protein folding chaperones (Erzberger and Berger, 2006). For dynein, this communication gains a level of complexity as information also has to be transmitted through the coiled coil to the MTBD, which is likely achieved by relative sliding of its individual helices (Cho and Vale, 2012). Huang et al. (2012) now report a mechanism by which a regulator, LIS1, modulates dynein motility by interfering selectively with the usual coupling of the ATP hydrolysis and microtubule binding affinity cycles.

LIS1, first identified as being involved in lissencephaly, a severe human brain disorder (Reiner et al., 1993), is now recognized to be a regulator that is required for a variety of dynein-dependent processes. They include organelle and mRNA transport, nuclear and centrosomal positioning, mitotic spindle orientation, and kinetochore activity. In contrast to other known proteins interacting with dynein, LIS1 binds to dynein’s motor domain (Allan, 2011; Kardon and Vale, 2009). By using electron microscopy and mutational analyses, Huang et al. (2012) show in vitro and in yeast cells that LIS1 binds the AAA3/4 interface of the dynein motor domain (Figure 1B), in contrast to previous proposals. Remarkably, this interaction uncouples the ATP hydrolysis cycle from microtubule binding affinity changes at dynein’s MTBD, as demonstrated by a combination of biochemical analysis and fluorescence imaging. This uncoupling effect is somewhat similar to a clutch in a car that can mechanically uncouple the engine from the wheels. With LIS1 bound, dynein is arrested in a strongly microtubule-bound state, although ATP hydrolysis can still go on. The authors provide evidence that a conserved structural element at the AAA3/4 interface of dynein—that is also critical for other AAA+ protein activities (Erzberger and Berger, 2006)—is most likely involved in mediating the conformational transmission of information from the ATP hydrolysis site to the MTBD. This brings us one step closer to understanding the molecular mechanism of intramolecular communication in the dynein ring. LIS1 binding disrupts this communication.

What could this type of regulation be useful for? In budding yeast, as in several other species, LIS1 and dynein accumulate at growing microtubule ends. LIS1-mediated induction of a strongly bound state might help dynein to track microtubule ends by decreasing its dissociation rate (Huang et al., 2012). LIS1 usually acts in combination with the nuclear distribution protein E (NudE) or its paralog NudE-like (Nudel) (Kardon and Vale, 2009). Huang et al. (2012) show that the only known budding yeast ortholog Nudel supports LIS1’s clutch activity, in agreement with observations in living yeast cells (Li et al., 2005). An attractive possibility is that binding of dynactin or other interaction partners could unlock the LIS1/NudE-arrested state of dynein and trigger dynein motility once it is attached to its cargo or at the cell cortex. In budding yeast, dynein’s major functional role is to pull on astral microtubules once it is anchored at the cortex to position the nucleus with its spindle into the bud-neck during mitosis. When LIS1 is present at concentrations at which it is expected to occasionally dissociate from the motor domain, budding yeast cytoplasmic dynein walks along the microtubule but with reduced speed. Similar pausing and slowing down of motility was observed previously.

Figure 1. LIS1 Uncouples Communication between the Primary ATPase and Microtubule Binding Domains of Cytoplasmic Dynein

(A) Schematic of a dynein heavy chain. The motor domain consists of six AAA+ domains (numbered) forming a ring (light blue), the stalk with the microtubule binding domain (MTBD) at its tip (dark blue), and a mechanical element, the linker (magenta), connecting AAA1 and the tail (dynein cargo-binding region). Regular motor stepping requires coordination of (i) ATP hydrolysis in AAA1, (ii) movement of the mechanical element, and (iii) alternation of the MTBD between strongly and weakly microtubule (MT)-bound states. Note: Only one monomer of the two heavy chains is shown here.

(B) LIS1 (red) binds at the AAA3/4 interface of the dynein motor domain, uncoupling the communication between the ATPase and MTBD. With LIS1 bound, ATP hydrolysis can occur, whereas the MTBD stays “locked” in a strongly microtubule bound state. How the movements of the linker are affected by LIS1 binding, remains to be investigated in the future. Nudel (green) is a regulatory protein that interacts with both dynein and LIS1.
also for mammalian dynein adsorbed to microspheres (McKenney et al., 2010). LIS1 was also reported to help mammalian dynein to work against an external load, something that has not yet been explored for yeast dynein. This finding suggests that the clutch effect of LIS1 binding might itself be load-dependent. In this context it will be important to understand how linker movements are affected by LIS1 binding, both in the presence and absence of load. Other open questions concern the action of NudE and Nudel. Although they recruit LIS1 to mammalian dynein, NudE/Nudel have been shown to strongly reduce the LIS1-induced effects on mammalian dynein (McKenney et al., 2010; Torisawa et al., 2011; Yamada et al., 2008), in striking contrast to the situation in budding yeast (Li et al., 2005; Markus et al., 2009; Huang et al., 2012).

In the future, it will be interesting to investigate to what extent dyneins from different species evolved varying regulatory control mechanisms, possibly reflecting different tasks they perform in these species. The availability of recombinant dynein also from other organisms, including mammals, will be crucial for dissecting the molecular mechanism of dynein’s regulation, as the elegant work presented by Huang et al. (2012) demonstrates.

REFERENCES

A Cornucopia of Candidates for Deafness

Many genes involved in deafness are yet to be discovered. Here, Senthilan et al. focus on the Drosophila Johnston’s organ to uncover a wide variety of genes, including several unexpected candidates as well as those already known to underlie deafness in mice and humans.

Gene discovery is a persistent challenge. New genes that are found by analyzing interesting phenotypes are often not those that would have been predicted, whereas genes that might be expected to be important may prove upon creation of a null allele to be nothing of the sort. Deafness, the most common sensory deficit in the human population, is a prime example of such a problematic phenotype. Many genes are known to contribute to deafness, but there are undoubtedly many more that have not yet been found (http://hereditaryhearingloss.org/). In this issue, Senthilan and colleagues make good use of Drosophila, which up until now had only 24 genes associated with “sensory perception of sound,” to successfully screen for many more candidates (Senthilan et al., 2012).

The organ of hearing in fruit flies is Johnston’s organ (Figure 1), an array of chordotonal sensilla in the third antennal segment, which has a feathery arista that serves as the sound receiver. The sensilla of the Johnston’s organ consist of mechanosensory neurons accompanied by scopalpale, cap, and ligament cells, which are all supporting cells (reviewed in Bechstedt and Howard, 2008). Mechanosensory and supporting cells are specified by the basic helix-loop-helix protein Atonal (Ato) (Jarman et al., 1993), the ortholog of which (Atoh1 or Math1) serves the same purpose in specifying hair cells in mammalian inner ears (Bermingham et al., 1999). In addition to Ato, flies and mammals also share Myosin 7a, Prestin, and several TRP channels, and the mechanical principles behind sensing of sound waves are very similar in flies and vertebrates (reviewed in Boekhoff-Falk, 2005).

Senthilan et al. use a specific atonal null mutant, which lacks Johnston’s organ, to carry out microarray experiments. Several careful approaches are taken to ensure that the data are robust, including cluster analyses and scatter plots comparing .