

Molecular Motors: Dynein's Gearbox

Dispatch

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A new optical trapping study shows that the stepsize of cytoplasmic dynein varies according to the applied force, suggesting that this motor can change gear. Complementary biochemical kinetic work on yeast dynein mutants hints at the allosteric mechanisms involved.

Cytoplasmic dynein is a BIG molecule. Each of its twin pseudoglobular motor domains is about 15 nm in diameter, more than half the width of a microtubule. Dynein's role in the cell is to transport cargo towards the minus-ends of microtubules, at high speed (5–10 μm per second) and throughout the cell cycle. Cells use cytoplasmic dynein to move vesicular traffic towards the microtubule organising centre during interphase, to bundle microtubule minus ends together and focus them into the spindle poles at mitotic entry, to tense astral microtubules against the cell membrane, and to move chromosomes towards the spindle poles during mitosis. Dynein is thus pivotal to intracellular transport, yet until recently the mechanism of its movement has been something of a black box. Given the unwieldy size of the molecule and the difficulty of expressing and purifying mutants, studies of dynein's molecular mechanism have lagged behind those of myosin and kinesin. But the problem is fiercely attractive and a spate of recent studies have at last begun to elucidate mechanochemical detail at the single molecule level.

Dynein motor domains, or heads, are related to the AAA (triple-A) family of proteins, comprising six or possibly seven structurally related subdomains arranged in a ring. Most triple-A proteins assemble into hexamers. Dynein heads are a special case, in that their six subdomains are fused into a single heavy chain [1]. Domains AAA1–AAA4 are more strongly conserved than the remainder, and contain so-called Walker A (GXXXXGKT, the P-loop) and Walker B (DEXX) motifs that are predictive of nucleotide binding. Consistent with this, dynein heavy chains bind four molecules of ATP [2]. Only one subdomain, AAA1, shows powerful microtubule-activated ATPase activity. Microtubule binding accelerates the P_i and ADP release steps of the ATPase, and these steps, particularly ADP release, are thought to be coupled to the mechanical power stroke of the motor [3]. Nucleotide in the remaining sites may modulate the activity of the AAA1 site. An approximately 10–15 nm coiled-coil stalk with a positively charged microtubule binding site at its tip projects between AAA4 and AAA5.

The full cytoplasmic dynein molecule has two heavy chains, each of which comprises a head domain joined to a rod-like stem to which light intermediate chains

bind. The tail in turn connects to a substantial cargo-binding assembly called the dynactin complex, which can itself bind to microtubules. A new study using the 'optical-trap' technique [4] — in which molecules are physically manipulated using the force exerted by light — was carried out using dynactin-free purified dynein, passively adsorbed at low density to plastic beads. These bead–dynein complexes were held in a single beam infra-red optical trap, positioned against an immobilised microtubule so as to allow the dynein to run along the microtubule, and their movement was tracked. The geometry is the same as that pioneered by Block and colleagues [5] in their work on kinesin.

In this geometry, the motor pulls the bead out of the fixed optical trap, and in doing so tenses the linkage that attaches it to the bead. Pulling out the bead–motor link in this way stiffens it and helps to ensure that bead movements faithfully report motor movements. With this arrangement, kinesin stalls at 6–8 pN, but cytoplasmic dynein stalls at only about 1.1 pN, which means that, for dynein, the bead–motor link is relatively slack and the traces are noisy. Nonetheless, steps can be discerned and their amplitude can rigorously be studied using pairwise distance–function analysis, which extracts steps from noisy records.

Using such analyses, Mallik *et al.* [4] found that, for cytoplasmic dynein, the stepsize decreases with increasing load. At high loads, cytoplasmic dynein takes predominantly 8 nm steps, corresponding to the axial distance between adjacent tubulin heterodimer subunits of the microtubule. At lower loads, the steps are longer; and at zero load, the modal stepsize is 24 nm, with some steps as long as 32 nm. Under such zero load conditions, the authors found that the distribution of 'residence times' — the durations of processive runs — was single exponential, characteristic of a system in which one step corresponds to the binding of one ATP molecule. They accordingly suggest that, as at low load cytoplasmic dynein uses one ATP per 24–32 nm step and at high load it steps 8 nm at a time, it is likely that at high load the molecule steps 8 nm per ATP. This would imply, remarkably, that single dynein molecules can change gear.

Recent electron microscopic studies [6] of single molecules of dynein C, a flagellar inner arm dynein, have revealed a conformational change involving a tightening-together of the ring of AAA domains with a coupled shift in the angle between stalk and stem (Figure 1). This shift takes place between the dynein.ADP.V_i state (a stable analogue of the dynein.ADP.P_i state [7] with phosphate replaced by vanadate) and the apo or empty state. Clearly, one possible way of changing gear — changing the stepping distance per ATP — would be to modulate the degree of opening and closing of this ring, perhaps by varying the occupancy of the other ATP-binding sites in the molecule. Earlier work on flagellar dyneins has suggested that allosteric coupling exists between the supplementary nucleotide binding sites and the main ATPase site [8].

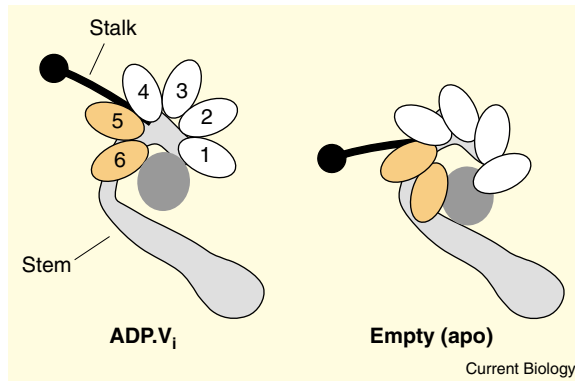


Figure 1. A model [6] of the force-producing conformational change in dynein C, a monomeric flagellar dynein related to cytoplasmic dynein.

The six subunit dynein head domains are about 15 nm in diameter. The conformational change corresponds to the transition from ADP.V_i to apo states of the active site, and opens the central pore whilst generating a compact configuration, possibly by concerted tilting of the subdomains. The change reduces the angle between the stem domain, which connects to cargo, and the stalk domain, which binds microtubules at the tip. Binding of microtubules at another site or sites is not excluded. Although the two heads of cytoplasmic dynein might move processively by binding alternately, processivity is also observed in single flagellar dynein heads [11]. One possibility is that a single head might bind alternately at the stalk tip and at some other region of the molecule.

Another way entirely in which a molecular motor might change gear is to vary the contribution of the diffusional-scanning and power-stroke components of the step. In molecular motors in general, each step consists of the sum of a passive scanning process, in which the tethered motor searches for its next binding site, and a subsequent power stroke which is coupled to product release. In a team of motors, for example in a myosin filament, the contribution of the passive component varies according to the applied force. Perhaps dynein can do this at the single molecule level? Microtubules bound to a surface coated with dynein.ADP.V_i oscillate back and forth in one-dimensional diffusion [9], suggesting that the pre-power-stroke dynein.ADP.P_i conformation is indeed capable of diffusional scanning. In the case of a bead-coupled motor, this diffusional component of the step will vary according to the size of the diffusing bead-motor complex and the trapping force. Applied force will rapidly reduce the amplitude of the scanning excursions, leaving just the power stroke component as important at high force.

The gear-change behaviour indicated by Mallik *et al.* [4] might relate to either, or both, of these mechanisms. Resolution is likely to come from careful mechanochemical studies of mutant dyneins in which the contribution of the other ATP binding sites in dynein is turned on and off by mutagenesis. Reck-Peterson and Vale [10] have recently reported exciting work in this direction. They mutated the non-essential *DYN1* gene of budding yeast, which is involved in, though not essential for, nuclear segregation. By systematically mutating all the nucleotide binding sites in this dynein, they were able to show that AAA2, AAA3 and AAA4 can be made

hydrolysis-incompetent without affecting function. Most interestingly, however, mutation of AAA3 to block nucleotide binding (as opposed to just hydrolysis) abrogated the protein's function *in vivo* and blocked ATP-induced release of dynein from microtubules *in vitro*, indicating allosteric effects. Mutations of AAA4 also had some subtle effects.

The importance of these two new studies [4,10] is that they open the way to single molecule mechanochemistry experiments that will elucidate the mechanisms of allosteric regulation in dynein. Perhaps we may ultimately learn precisely why it is so BIG.

References

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