

# Dynein Swings into Action

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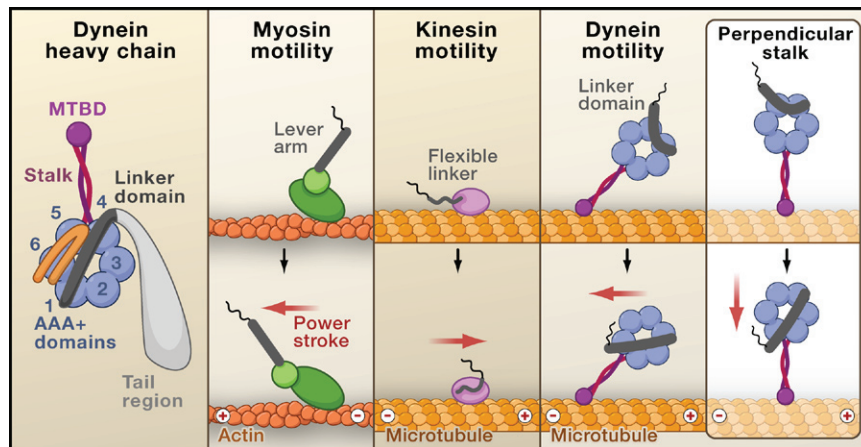
**Motor proteins, such as dynein, use chemical energy from ATP hydrolysis to move along the cytoskeleton. Roberts et al. (2009) now describe the arrangement of subdomains in the motor domain of dynein and propose a model for how these regions function together in force generation.**

Myosin, kinesin, and dynein comprise the three families of motor proteins that associate with the eukaryotic cell cytoskeleton to perform essential functions in processes such as cell motility, cell division, and the transport of molecules. Myosins move on actin filaments, whereas kinesins and dyneins are microtubule-associated motors. All three types of motor proteins convert the energy stored in ATP into movement by coupling the cycle of ATP hydrolysis to structural changes in the motor protein. During the cycle, the binding of the motor protein to the cytoskeletal track induces conformational changes that result in the displacement of a motor protein domain in the direction of travel (power stroke). This is followed by the dissociation of the motor from the track and reversal of the conformational change (recovery stroke). Although the structural changes for myosin and kinesin during motor displacement along a track are well characterized (Vale and Milligan, 2000), the understanding of how the much larger dynein motor functions remains rudimentary. In this issue of *Cell*, Roberts et al. (2009) use electron microscopy to map the subdomain organization within the motor domain of the *Dictyostelium discoideum* cytoplasmic dynein and to investigate the mechanism of dynein motility.

The dynein motor family is composed of several axonemal forms that drive the beating of cilia and flagella and two cytoplasmic forms that are responsible for a range of cellular functions such as cargo transport, mitosis, and cell polarization. All dyneins share a conserved motor domain, the structure of which has been modeled on the

basis of sequence analysis and electron microscopy but has not been solved at atomic resolution (Samsó and Koonce, 2004; Burgess et al., 2003). The core of the dynein motor domain consists of six tandem AAA+ ATPase domains. The first AAA+ domain (AAA1) is the primary site of ATP hydrolysis, whereas the other five domains (AAA2–6) have regulatory or structural functions. The microtubule binding domain of dynein, the atomic structures of which have recently been solved (Carter et al., 2008; Y.S. Kato et al., personal communication), is found

at the tip of a long antiparallel coiled-coil stalk (Gee et al., 1997) that emerges between AAA4 and AAA5. The N terminus of the motor domain is a  $\alpha$ -helix-rich region referred to as the linker domain and is required for dynein motor activity. Previous studies have suggested that this linker domain undergoes ATP-dependent movement (Burgess et al., 2003) and may function to transmit force during motor conformational changes. Using negative-stain electron microscopy to visualize the structure of the dynein motor domain harboring green



**Figure 1. The Structure and Motility of Dynein**

The dynein motor (heavy chain) is composed of a tail region (light gray), an N-terminal linker region (dark gray), six AAA+ domains that form a hexameric ring (blue), a C-terminal domain (orange), and a stalk region topped by the microtubule-binding domain (MTBD, purple). Motor proteins undergo structural changes that generate force for movement along cytoskeletal tracks (power stroke). The plus-end myosin motor converter subdomain (light green) and lever arm (gray) swing as a rigid structure in the power stroke as the motor transitions from a nucleotide bound or primed form (top) to a nucleotide-free or unprimed form (bottom). The kinesin motor uses the docking and undocking of a flexible linker (gray), regulated by the nucleotide-binding state of the motor domain, to generate force for motor movement along the microtubule track. Roberts et al. (2009) propose that for dynein, the swing of the N-terminal linker domain between the primed and unprimed motor states observed by electron microscopy produces force for movement along the microtubule. Upon the power stroke, the stalk remains bound to the microtubule at an angle close to 45° in the direction of movement. (White panels) If dynein docks with an orientation of the stalk that is perpendicular to the microtubule track, the swinging motion of the N-terminal linker domain would not be expected to be productive in driving movement along the track.

fluorescent protein (GFP) or blue fluorescent protein (BFP) tags in different regions, Roberts et al. (2009) obtain an improved understanding of the subdomain organization in the motor domain (Figure 1). The authors find that the core of the dynein motor domain consists of a hexameric ring structure formed by the six AAA+ domains, rather than a heptameric complex composed of both the AAA+ domains and the C terminus of the motor domain as previously thought. They also map the position of this C terminus region to an area spanning AAA6 and the base of the stalk region.

Important for the understanding of the operation of any motor is the nature of the conformational change that generates motility. In myosin, small movements in the motor domain resulting from the ATP hydrolysis cycle are transmitted by a converter subdomain to a relatively rigid lever arm. The lever arm amplifies the movements, resulting in a displacement of its position in the direction of travel along the actin filament (Figure 1). The equivalent power stroke step in kinesin is thought to involve transition of a flexible peptide linker from an unbound to a docked position (Figure 1). For dynein, it has been proposed that movement of the N terminus linker domain within its motor domain could generate a power stroke. To investigate the structural changes that the dynein motor domain undergoes during ATP hydrolysis, Roberts et al. compared the position of the N terminus linker domain in an ATP-bound (primed) dynein motor domain with that in a nucleotide-free (unprimed) dynein motor domain. The unprimed dynein motor domain structure shows close agreement with that previously determined for an unprimed axonemal dynein (Burgess et al., 2003). The linker domain exits the ring at an AAA+ domain close to the base of the stalk, which Roberts et al. identify as AAA4. The primed dynein motor domain structure shows a marked change in linker conformation in which the linker domain has swung across the face of the AAA+ domain ring, exiting around AAA2 on the opposite side of the hexa-

meric structure (Figure 1). Notably, this movement is considerably greater than that observed for the axonemal dynein and is likely to provide the necessary force for motor movement.

How does the linker domain move across the face of the AAA+ ring to generate force? It is possible that similar to the converter subdomain and lever arm in plus-end myosins (Figure 1), the dynein linker domain acts as a relatively rigid lever that amplifies small conformational changes in the AAA+ domains upon ATP hydrolysis. The linker domain could also undergo a conformational change itself, similar to minus-end myosin converters (Ménétrety et al., 2007) resulting in a shift in its position relative to the AAA+ ring. Another attractive model is that the linker domain generates force by transitioning between two or more docking sites on the AAA+ ring, a mechanism more like that of kinesin. It is clear that high-resolution structural and functional studies will be necessary to identify the critical interactions between the linker domain and the ring structure that control linker domain movements during the ATPase cycle.

Whereas the motor domains of kinesin and myosin bind directly to their tracks, the motor domain of dynein is separated from the microtubule by the stalk (Figure 1). This raises the question of how movement of the dynein linker within the motor domain is translated into directional movement along the microtubule. An insight into how dynein may achieve directional movement comes from the observation by Roberts and colleagues that linker domain movement during ATP hydrolysis occurs almost entirely parallel to the axis of the stalk. Taking this linker domain positioning into account, if the power stroke (linker domain movement) occurs with the motor docked such that the stalk axis is perpendicular to the microtubule, the force generated would be directed downwards and would not be productive in inducing motor movement along the microtubule (Figure 1, white panels). However, if dynein binds at an angle to the microtubule such that the stalk is pointed in the direction

of movement (Figure 1), the movement of the linker domain in the power stroke could generate motor movement toward the minus end of the microtubule. This model is in agreement with data from a recent study showing that the stalk in the dynein motor most likely remains pointed toward the microtubule minus end (the direction of dynein movement) both before and after the power stroke (Ueno et al., 2008). Results from a study examining the structure and function of the dynein stalk and microtubule-binding domain also came to similar conclusions (Carter et al., 2008). Thus, it is likely that the orientation of the stalk during linker domain movement plays an important role in determining the direction of motor movement along the microtubule. Whether the stalk can also change orientation during the motor cycle (Gennerich et al. 2007; Roberts et al. 2009) remains an important open question. The work of Roberts and colleagues, along with other recent contributions to the field, will now allow the elaboration of interesting and testable hypotheses regarding the mechanism of motility for dynein, the largest and most complex of the cytoskeleton motors.

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