A molecular motor finds its track

Jared C Cochran & F Jon Kull

The detailed mechanism by which the molecular motors kinesin and myosin travel along their respective protein tracks as they generate force during motile processes is still poorly understood. In a recent breakthrough, a crystal structure of kinesin in complex with tubulin illuminates the atomic-level details of a motor-track interaction, answering many questions yet leaving a number of mysteries unresolved.

The related motor proteins kinesin and myosin are essential for a wide variety of cellular processes, including vesicle transport, chromosomal segregation, cell motility, regulation of cytoskeletal dynamics and muscle contraction. Both use chemical energy stored in ATP to produce force along their protein filaments, microtubules and actin. Although much is known about their mechanochemistry, an atomic-resolution structure of a motor and its filament has been lacking, until now. In this issue, Gigant *et al.*[1] describe the structure of the motor domain of kinesin-1 in complex with an αβ-tubulin dimer, illuminating details of the microtubule-binding interface and clarifying the mechanism by which tubulin stimulates kinesin's ATPase activity.

Obtaining such a complex has been elusive, owing to difficulties in avoiding tubulin polymerization while obtaining crystals. To solve this problem, the authors used a designed ankyrin repeat protein (DARPin) to block polymerization[2,3] while allowing a tubulin dimer to form. Kinesin is free to bind this complex in a native-like manner. This clever solution will be useful to others studying kinesins and other microtubule-binding proteins. The complex (**Fig. 1**) is quite similar to existing structures of the kinesin[4–7] and tubulin[8,9] as well as to models of the complex derived from cryo-EM[10–12], with some notable differences. These differences raise multiple questions, as discussed below.

Does this structure represent a physiological state of kinesin bound to the tubulin heterodimer? To date, all high-resolution X-ray structures of αβ-tubulin in complex with a variety of associated proteins show tubulin in a curved conformation as opposed to a straight conformation as found in the microtubule lattice[8,13]. The tubulin dimer in this structure is also curved by about 9° with respect to the linear arrangement of αβ-tubulin; therefore, this structure probably does not reflect the conformation of kinesin bound to microtubules (described below). Many different proteins including several distinct kinesin-family members have been shown to regulate microtubule dynamics[14], presumably through the promotion of tubulin conformation changes at microtubule ends. Kinesin-13 is arguably the most well-studied microtubule depolymerase, yet recent moderate-resolution cryo-EM results suggest that both tubulin curvature and shearing result from kinesin-13 binding to tubulin[15]. Kinesin-1 is a transport kinesin that walks along the microtubule lattice, so its structure while bound to the curved tubulin heterodimer may be better interpreted as an intermediate structure for kinesins that regulate microtubule dynamics.

How does tubulin stimulate the kinesin ATPase cycle and promote force generation in the motor? The structure described in the paper was determined with ADP–AlF_4^- bound in the kinesin active site (which is composed of three functional motifs: the nucleotide-binding P loop and two γ-phosphate–sensing loops called switch I and switch II). The structure confirms what others have predicted on the basis of crystal structures and EM reconstructions concerning the dramatic switch I rearrangement. This motif moves

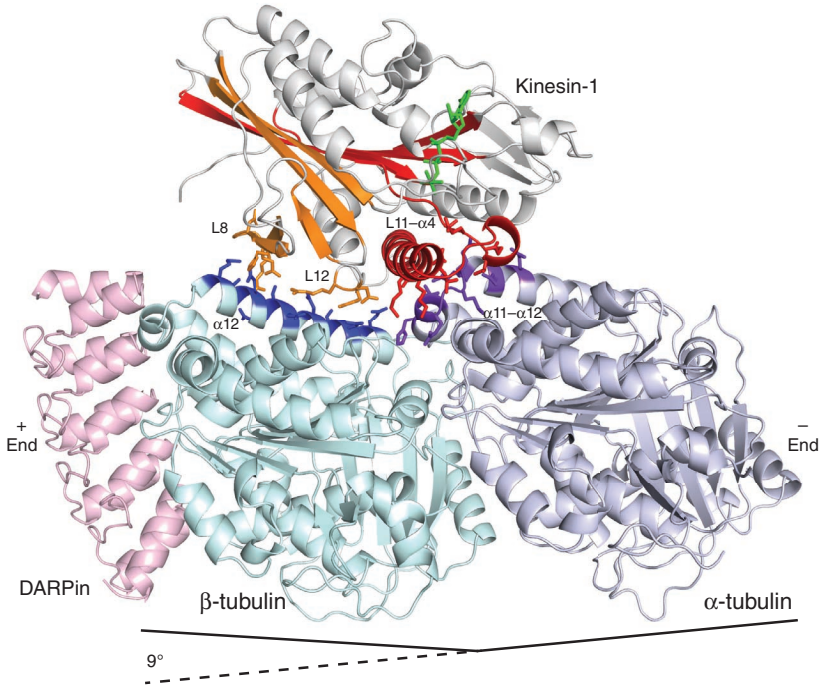

Figure 1 Structure of the kinesin-1 motor domain in complex with αβ-tubulin. Side chains involved in binding contacts (within 4 Å) are shown. The observed 9° curvature of tubulin subunits with respect to a linear microtubule is indicated. Side chains in the L11–α4 region of kinesin (red), which are structurally linked to switch II, interact with β-tubulin residues from the α11–α12 region (purple). Side chains in the L8 and L12 loops of kinesin (orange) are linked to switch I and interact with side chains from α12 of β-tubulin (blue). The core β-strands of kinesin are colored red on the P loop–switch II side and orange on the switch I side. In myosin, the twist in the core β-sheet in the nucleotide-free structure occurs between these regions. If such a twist occurs in kinesin, it would rotate the L8 and L12 region toward the microtubule with respect to the L11–α4 region, thus allowing for contacts with β-tubulin in a linear microtubule.

Jared C. Cochran is at the Department of Molecular and Cellular Biochemistry, Indiana University, Bloomington, Indiana, USA.
F. Jon Kull is at the Department of Chemistry, Dartmouth College, Hanover, New Hampshire, USA.
e-mail: f.jon.kull@dartmouth.edu

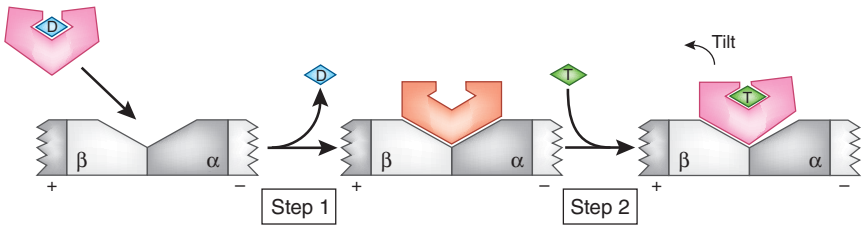

Figure 2 Model for the kinesin mechanochemical cycle. In step 1, kinesin-ADP binds the microtubule, and interactions with both α- and β-tubulin (indicated as α and β, respectively), result in a (hypothetical) twist of the kinesin's core β-sheet (orange), thus opening switch I and switch II and releasing ADP. In step 2, ATP binding induces closure of switch I and switch II, thus restoring the untwisted β-sheet (red) and resulting in a tilt of the motor domain toward β-tubulin and the plus end. Not shown is the ordering of the neck linker, which is also linked to ATP binding, resulting in movement of the unbound head of dimeric kinesin toward the plus end. D, ADP; T, ATP.

from the open conformation observed in the ADP-bound structure of kinesin-1 alone to a closed conformation nearly identical to that in kinesin-5 (ref. 6) and kinesin-4 (ref. 4) bound to AMP-PNP. With switch II also closed, catalytic side chains and waters are positioned for hydrolysis. Additionally, microtubule-dependent ordering of kinesin loop 11 (L11) into an extension of helix α4 enables microtubule binding to activate kinesin's ATPase cycle. Conformational changes induced by ATP binding have been shown to result in docking of kinesin's neck linker, and this biases the unbound head of dimeric kinesin toward the next microtubule-binding site. The structure reveals details of this conformational change analogous to the lever-arm swing observed in the myosin motor; microtubule binding results in a translation of α4–L12–α5 and the adjacent α6, thus opening up a binding cleft for the neck linker and resulting in kinesin's version of a lever-arm swing.

Two important questions arise from the work. How does microtubule binding result in ADP release from kinesin? What does the nucleotide-free kinesin structure look like when bound to tubulin or microtubules? To answer these questions, we can take advantage of recognized similarities between kinesin and myosin, including virtually identical active site chemistry, similar rearrangements of switch I and switch II during catalytic cycles and similar movements of helix α4 (which is called the 'relay helix' in myosin) during force generation. Together, these similarities suggest that kinesin and myosin use similar core motors to drive distinct mechanochemical engines. One feature of myosin that has not yet been observed in kinesin was visualized in a nucleotide-free structure of myosin in which the central β-sheet was twisted with respect to nucleotide-bound conformations[16]. This twist results in both switch I and switch II adopting the open conformation, thus removing interactions with Mg^{2+} and ADP. The possibility of a similar twist in kinesin seems very likely, and the structure from Gigant *et al.*[1] provides a clue as to how such a twist might occur. Given the extensive interactions between kinesin and α- and β-tubulin, it seems likely that these are the physiological interfaces. However, formation of linear αβ-tubulin dimers in microtubules would disrupt the interface between kinesin and α- and/or β-tubulin, unless the kinesin's conformation were to change upon microtubule binding. Modeling suggests that a twist in kinesin's β-sheet, similar to that observed in myosin, would enable kinesin to retain binding interactions with α- and β-tubulin and also cause switch I to leave the active site, thus resulting in Mg-ADP release (**Fig. 2**). Filling a huge gap in our understanding, this critical complex structure has laid the foundation for future studies to clarify the understanding of kinesin-based cellular motility.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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