the problem of surviving by reducing the need for highly complex predator avoidance adaptations.

Twenty years of experimental and comparative studies support the prediction that longer lifespan will evolve in safer habitats. This latest study [2] provides yet further evidence for this pattern but theory suggests that the causal link between extrinsic mortality and innate lifespan is much more complex than previously thought. We have no doubt that the phenomenon is widespread. The challenge now is to figure out why.

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DOI: 10.1016/j.cub.2010.03.016

Motor Proteins: Kinesin Drives with an Underhead Cam

A high-resolution cryo-EM structure of kinesin bound to its microtubule track allows for near-atomistic visualization of nucleotide-dependent conformational changes in this motor protein.

Matthew J. Lang[1] and Wonmuk Hwang[2]

As the smallest known motor protein that can walk processively, kinesin has been an important model system for understanding translocating motor proteins [1,2]. While many experimental, computational, and theoretical efforts have provided piecemeal information about kinesin motility, a clear atomistic-level picture of the process underlying the motility cycle is lacking. A key question is how ATP binding, hydrolysis, and product release control the motor head conformation and thereby the motility cycle. Although dozens of X-ray structures of kinesin are now available, far less is known about the structure of kinesin bound to its microtubule track. Kinesin's ATPase activity is known to be heavily influenced by the microtubule, and there are only a limited number of cryo-EM structures of the complex available [3–5]. Sindelar and Downing's new high-resolution (8–9 Å) cryo-EM structures of Kinesin-1 bound to the microtubule [6], together with their earlier work [5], provide native-like snapshots of the complex in various nucleotide-bound states: ADP-bound, no nucleotide, and ATP-analog-bound. Their findings indicate that the microtubule-bound motor head conformations are similar to those of the X-ray structures obtained in the absence of the microtubule, with the exception of the microtubule-binding domain and the nucleotide pocket. Although these results are consistent with previous cryo-EM studies of other members of the kinesin family [3,4,7], the higher resolution density maps reveal a vivid picture of nucleotide-dependent conformational changes of Kinesin-1, the 'conventional' kinesin.

Some important kinesin components include, from the amino terminus to the carboxyl terminus: the cover strand (β0), switch I and switch II loops, the microtubule-binding loop L11, the switch II helix (α4), α6, and the neck linker (β9 and β10) (Figure 1). Domains surrounding the switch I, II, L11, and the amino-terminal end of α4 process ATP binding and hydrolysis, while α4 and α6 control the behavior of the neck linker. The cover strand and the neck linker, respectively protruding from the amino and the carboxyl termini of the conserved motor head core, are involved in force generation.

The new work reveals that the motor action appears to be controlled in part through an 'underhead cam' region of the motor–track complex that is directly coupled to the power stroke (or crank shaft) motion that is responsible for kinesin stepping. In the nucleotide-free state, the switch I loop of the kinesin motor head is in the 'nucleotide-ejecting' conformation (Figure 1A, axial view). Binding of ATP (i.e. the ATP analog ADP·Al·F_x) leads to opening of the nucleotide pocket as the switch I loop changes to a tube-like conformation that Sindelar and Downing call the 'phosphate tube'. The retracted switch loops are stabilized in part by the amino-terminal end of the switch II helix α4, which interfaces with the microtubule and extends underneath the ATP pocket by several helical turns in all nucleotide states, a feature not observed in X-ray structures of kinesin in the absence of the microtubule (Figure 1A,B, hashed red region): note that the switch II helix corresponds to the relay helix in myosin [8].

Interestingly, the nucleotide-dependent conformations control the docking of kinesin's neck linker region, located near the carboxy-terminal end of the switch II helix [9,10], through a seesaw-like motion of the head. The seesaw action is not along the direction

of stepping, but perpendicular to the axis of the microtubule (Figure 1). Orient yourself by looking along the microtubule in the direction of stepping: ATP binding occurs on the left side of the seesaw and the neck linker is on the rightward side. ADP- and ATP-bound conformations of the motor largely represent rightward and leftward tilting of the seesaw, respectively. Sindelar and Downing [6] identified specific fulcrum residues in kinesin — Y84, F82, L258 and L261 — that are associated with the seesaw action and lie halfway along the extended switch II helix (Figure 1, yellow).

The motility cycle begins with the seesaw tilted to the right in the nucleotide-free state of kinesin attached to the microtubule. In the rightward-tilting position, the carboxy-terminal end of α6 preceding the neck linker sterically collides with the switch II helix, favoring a shorter form of α6. The unwound portion of α6 elongates the neck linker, which is now detached from the motor head (Figure 1A). ATP binding leads to the series of conformational changes mentioned above that is stabilized by the left (amino-terminal) part of the extended switch II helix, causing kinesin to tack leftward, reducing collisions on the right side of the seesaw. The hybrid α6-neck linker residues, which can be found in α6 or the neck linker conformations, are winched in through the formation of additional α6 helix turns. This 'pulling-back' of the neck linker allows for its proper alignment with the cover strand that protrudes from the amino-terminal end of the conserved motor head core (Figure 1B). We have recently suggested that the formation of the β-sheet between the cover strand and neck linker, named the cover-neck bundle, is responsible for force generation and forward movement of the neck linker [11,12]. Sindelar and Downing's study [6] also explains why both ADP- and ATP-like states can be observed among crystal structures of ADP-bound kinesin in the absence of the microtubule track: in the absence of the switch II helix extension and associated stabilization of the rightward-tilted state of the seesaw, the motor can tilt leftward and assume an ATP-like state with a bound neck linker, even with a bound ADP [10]. Such quasi-equilibrium between pre- and post-stroke conformations

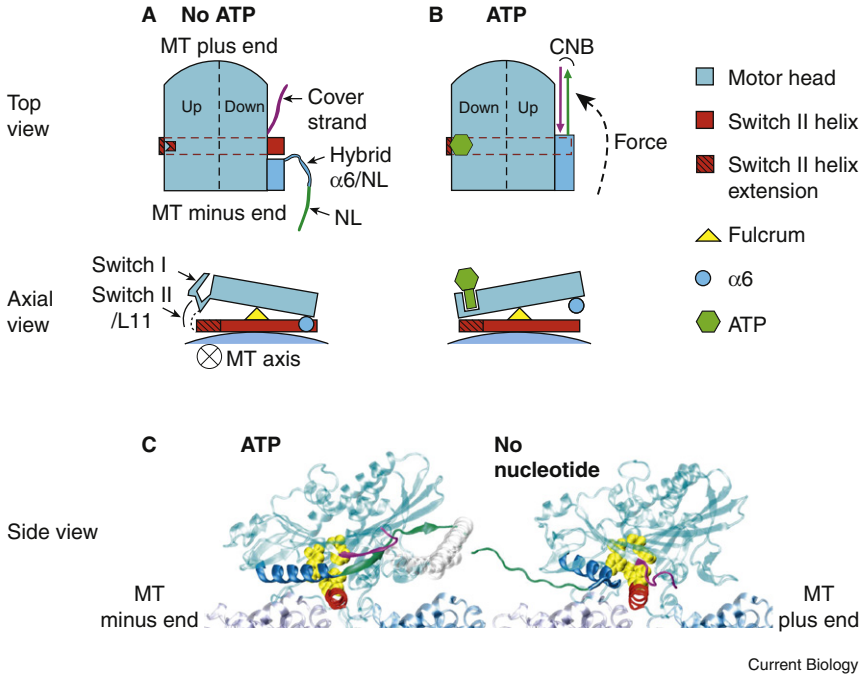

Figure 1. The seesaw movement of kinesin perpendicular to the microtubule axis leads to force generation.

(A,B) Schematic representation of the kinesin motor head bound to a microtubule in the presence and absence of ATP. CNB, cover-neck bundle; MT, microtubule; L11, loop 11. (C) A model of the kinesin dimer bound to the microtubule. Color codes are the same as in (A,B). The trailing head is in the ATP-bound state and the leading head is nucleotide-free. See text for full details.

in the absence of a bound track has also been observed in kinesin's ancestral relative, myosin [13].

When we consider that the motor consists of various mechanical parts [14], Sindelar and Downing's outstanding cryo-EM structures [6] reveal a detailed action of the fuel processor (the switch loops) and how it is transduced via the seesaw motion (perpendicular to the microtubule axis) to the force generator, i.e. formation of the cover-neck bundle (parallel to the microtubule axis). Analogous to our childhood seesaw, the absence of nucleotide on the left side results in the motor head tilting to the right, while ATP binding on the left side leads to the leftward tilt. In the latter state, a shorter neck linker is actually one that is involved with stepping, while the longer neck linker form, including the unwound portion of α6 in non-ATP-bound states, may allow for this region of kinesin to point rearward and reach the trailing motor head when both heads are bound to the microtubule (Figure 1C).

Clearer structural information allows us to ask deeper questions about how the motor works, in particular, the 'flow' of free energy [14]. For every kinesin cycle that involves the return to an identical state (a cycle involving two steps), the net change in free energy is equal to that released from the hydrolysis of two ATP molecules. However, kinesin is not a 'closed' system, because it exchanges free energy with the surroundings in different forms during its cycle. The binding energy of ATP may drive the conformational changes of the microtubule-bound kinesin that Sindelar and Downing report. Yet subsequent force generation and associated mechanical work is carried out through cover-neck bundle formation, a β-sheet folding process. The energy of ATP hydrolysis may actually be used to detach kinesin from the tight microtubule-bound state. Finally, energy of ADP release is associated with strong binding of the nucleotide-free motor head to the microtubule. Detailed elucidation of the energetics of different substeps will be crucial for understanding a diverse range of translocating motor proteins.

Unfortunately, despite the fact that kinesin is a *mechano*-chemical

amplifier, all available structures of kinesin, be they X-ray or cryo-EM structures, represent states in the absence of load, which is also the case for structures of all known motor proteins. As with the microtubule, mechanical force is an essential part of kinesin motility. For example, strain on the rearward-pointing neck linker of the leading head prevents ATP binding, which ensures that the ATPase cycles of the two motor heads are out of phase [15]. An atomistic resolution structure of a kinesin dimer bound to the microtubule is greatly needed to provide understanding of the mechanism of the mechanical allostery. It should be noted that, although high-resolution structures of a motor in various stages of its mechanochemical cycle are absolutely necessary, because the main feature of a translocating motor is its ability to move, we must also pay attention to what we do not see in the current 'static' structures. In the case of kinesin, the amino-terminal cover strand is invisible in most available structures, yet the force-generating element is the dynamically formed cover-neck bundle [11,12]. Here also, Sindelar and Downing's work [6] reveals the microtubule-dependent amino-terminal extension of the switch II helix. Similarly, in myosin, structures of all states are available except for the 'ephemeral' power stroke state [13]. As mentioned earlier, no single approach will reveal everything about a motor, and information gathered from many different studies should be cooperatively used to understand the motility. However, it is always a delight and surprise to see higher resolution structures of a motor in various states, as if a beautiful landscape is revealed after morning fog clears.

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DOI: 10.1016/j.cub.2010.03.015

Bidirectional Transport: Matchmaking for Motors

In bidirectional transport, opposing motors frequently require each other for full activity. A new study suggests that mechanical coupling between motors is the key to this reciprocal activation.

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Microtubule-based intracellular transport is fundamental for many cellular processes. Microtubules provide polarized tracks along which kinesin and dynein motors haul cargo to their cellular destinations. In bidirectional transport, plus- and minus-end directed motors work together on a single cargo, causing it to switch travel directions incessantly [1]. How such motors cooperate and avoid getting stuck in an unproductive tug-of-war has long been a mystery. Both modeling and experimental analysis suggest that motors avoid paralysis because when one set of motors is moving the cargo the opposing motors are temporarily inactive [2–4]. Whether reciprocal inactivation is triggered by a tug-of-war between motors or is mediated by dedicated coordinators is currently hotly debated [2,5,6]. A recent study by Ally *et al.* [7] now reveals that opposing motors can also activate each other. Activation requires more than the physical presence of the opposing motor since motility-defective plus-end motors compromise cargo motion in the minus-end direction, and vice versa. Apparently, these motors need their opposing partners to be functional. This yin-and-yang relationship may be a quality-control mechanism that ensures a balance of forces on a given cargo and thus robustness of transport [8].

That motors depend on their opposing partners is a well established but ill-understood phenomenon. For example, peroxisomes in cultured *Drosophila* cells undergo bidirectional transport, driven by the plus-end motor kinesin-1 and the minus-end motor cytoplasmic dynein. Depletion of either motor by RNA interference causes motion in both directions to cease [4]. Similar motor interdependence has been observed for mitochondria [9], axonal vesicles [10], neurofilaments [11], lipid droplets [8], ribonucleoprotein particles [12] and lysosomes [13]. Even more subtly, alteration of the number of active