closure are themselves sensed by the channel gate, using parallel kinetic and electrophysiological analysis of mutants that affect the individual docking steps. Either way, the processes observed by Jayaraman and colleagues are likely to have important consequences for our understanding of the kinetics of binding and gating interactions in this physiologically important family of ion channels.

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Molecular motors: rocking and rolling

Linda A Amos

Kinesins are the molecular motors responsible for movement of vesicles inside cells. Evidence is now presented for how kinesin moves forward, as well as side to side.

Kinesin is a molecular machine that pulls vesicles around in cells by walking along microtubules. Although much is known about how kinesin moves, a number of questions remain, including details of how the driving force is applied and what determines its forward directionality. The molecular mechanism can now be modeled in great detail using information from mechano-optical experiments that are reported in a pair of papers in this issue, one from Yanagida and coworkers characterizing entropy as the driving force for propelling kinesin in the forward direction[1], and one from Yajimi and Cross visualizing a rotational motion that accompanies forward movement of kinesins[2].

Much has been learned about how kinesin 'walks' using two major visualization methods[3,4]. If microtubules are made to slide over a carpet of motor proteins attached to a flat surface, their movements can be followed in detail by light microscopy. Alternatively, microscopic beads attached to individual motor molecules can be observed traveling along a fixed microtubule. Then, trapping of the bead by optical tweezers allows the researcher to measure and control the effective load on the motor molecule. Optical trapping allows one to investigate the behavior of individual motor molecules and is particularly good for those that produce fairly large steps. Sliding on a glass surface provides an easier way of studying the combined effects of a large number of molecules, whose individual displacements may be small.

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Past experiments using the optical tweezer technique have shown that kinesin moves by treating the tubulin subunits as a regular (8-nm spaced) series of stepping stones. Each dimeric molecule walks processively; that is, its two motor domains attach one at a time to the microtubule and thus are able to travel for long distances without detaching. To explain the mechanism of movement, two different kinds of model have been proposed. In 'power stroke' models, energy derived from ATP hydrolyzed in the motor domains drives a series of conformational changes that produce the walking motion. In thermal ratchet models, each motor domain is brought into contact with a new binding site along the track by random Brownian movements. Here, the binding and splitting of ATP provides signals to control the sequence of interactions with the microtubule (**Fig. 1**).

A fundamental difference between these two types of model is that in the former case, a power stroke is directly responsible for a large advance along the track, whereas in the latter, the move to a new place on the track and the exertion of force after arrival are separate processes. In the first case, a long lever arm (such as is found in myosins[5]) amplifies a small but powerful change in the motor protein; in the other, it enables a wider search for a new binding site. For kinesin it was already known that the energy released by binding the neck linker could not provide the full force of the movement[6], leaving open the question for how forward motion is propelled.

An intriguing aspect of kinesin walking is that occasionally, the molecules will take one or more backward steps. Carter and Cross have recently shown that backward steps resemble forward steps in being able to occur processively, as well as needing ATP[7]. Walking backwards happens when forward steps are completely inhibited by the load imposed by the optical trap. The molecule may also detach under this load, known as stall force. Yanagida and coworkers have carried out optical trapping at a series of different temperatures and counted the number and frequency of forward steps, backward steps and detachments against increasing loads, up to and including stall force. By analyzing the time intervals between 8-nm steps to see how the kinetic rate constants vary with temperature and load, they have demonstrated that the mechanisms of forward and backward stepping are similar: a rapid initial process that does not vary with load is followed by a slower load-dependent phase in both cases. Using this same data, the authors analyzed the energetics of forward and backward walking. They found that the enthalpic barrier was the same in the forward and reverse directions, whereas the entropy was much greater for a forward step, leading to the proposal that kinesin directionality is entropically controlled. In their model, the currently bound head holds the tension while the second head rocks around until it fits a neighboring site on the microtubule. This docking of the second head and its conversion to strong binding mode owing to loss of ADP provides the strong pull required to substantiate the advance along the microtubule. It occurs much more readily for a step in the forward direction—unless there is a large force pulling from behind—because of the way the neck linker and neck are attached to the head[6] (**Fig. 1**). Thus neck-linker docking

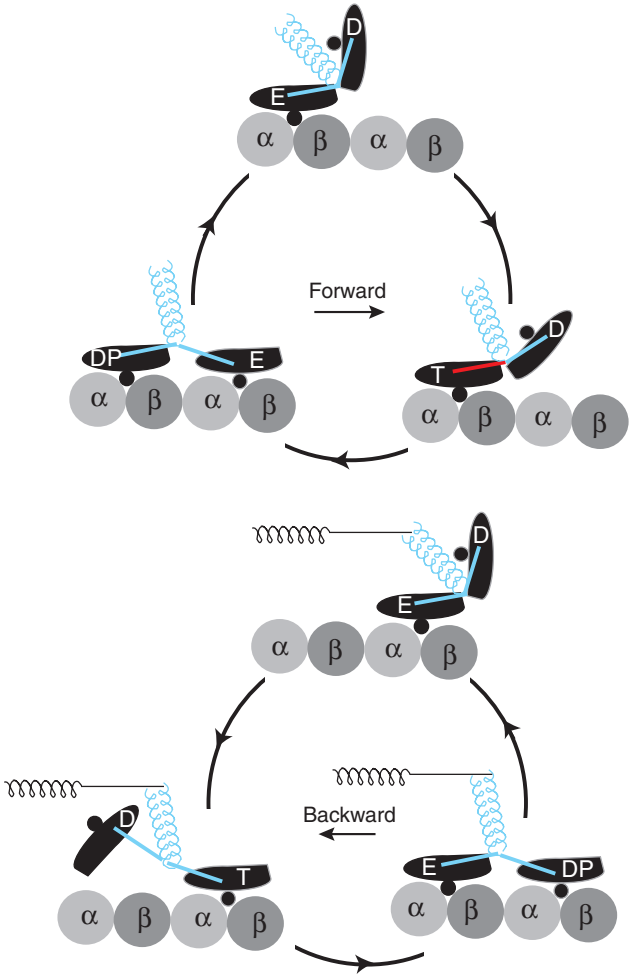

Figure 1 A schematic diagram of the ATPase cycle in kinesin dimers (black) stepping on tubulin αβ-heterodimers (gray). T represents bound ATP, D is ADP and P is the cleaved phosphate; E represents an empty binding site. The kinesin monomers dimerize via the coiled coil neck (blue helices), and there may also be head-head interactions[10], except when both heads are attached to tubulin. In the model shown, binding of ATP to one head frees its ADP-bound partner to search for a binding site; contact with tubulin then releases ADP and, after binding of fresh ATP, stimulates its hydrolysis. This happens whether the new binding site is forwards or backwards. Binding of the neck linker to a specific site (red) on the side of the attached motor domain during forward but not backward movement is an important factor in ensuring that kinesin normally moves forwards. Yanagida and colleagues[1] now suggest that the orientation of the free head facilitates forward binding (see their Figure 6) and may account for the remaining difference in entropy between forward and backward stepping. Interactions between the heads could also contribute to the bias. A large load (represented by the black spring) can inhibit forward movement. The possibility of moving backwards increases kinesin's ability to remain attached in difficult circumstances.

is believed to be important, though not sufficient, for forward processivity.

Kinesins are generally believed to produce only a forward, axial movement while walking. Structural studies by X-ray crystallography and cryo-EM have shown that the bulk of the motor domain of kif1a, a monomeric kinesin from vertebrate brain, is able to rotate relative to helix α4, the primary contact with the microtubule[8]; however, this is a static picture, and the significance of this rotation for kinesin movement was not certain. Additionally, the *Drosophila melanogaster* motor, ncd produces a rotation during sliding, but this motor is not necessarily representative of conventional kinesin being a slow nonprocessive motor that habitually travels backwards[9]. Yajima and Cross looked at kinesin movement using microtubules decorated with a broken piece of microtubule extending at a angle from one end. Using light microscopy, they observed monomeric kinesin heads attached to glass being propelled along microtubules. The authors found that during kinesin forward stepping, the sideways extension made it apparent that there was an accompanying rotational movement. This is the first evidence that conventional kinesin produces a simultaneous rotational and axial force while walking. The authors also demonstrated that this rotational motion is directly coupled to ATP turnover. It is possible that the sliding and rolling force is the result of heads fixing themselves onto the neck linker, but it is more likely to be due to a conformational change within the motor domain. There may be detectable conformational changes either when ADP is released and strong binding is achieved, or when a fresh molecule of ATP enters the site and the neck linker is docked[6]. Kinesin interacts with tubulin over a large interface via several different polypeptide loops. The rocking and rolling motions that have been detected in these experiments may reflect changes in the strengths of these various interactions as kinesin goes through its active cycle[8].

Both groups explain their new data in terms of a thermal ratchet–biased binding model, although they leave open the possibility that a conformational change may contribute an impulse of directional force and motion. The clear message of both papers is that we now have to focus on (i) the detailed nature of the directional bias that causes preferential selection of a forward binding site, (ii) the conformational changes that take place and (iii) the means by which pairs of heads communicate. All of this will require a combination of single-molecule and structural techniques.

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