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Motor Proteins as Nanomachines: The Roles of Thermal Fluctuations in Generating Force and Motion

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Abstract. Motor proteins are enzymes that convert chemical energy derived from the hydrolysis of ATP into mechanical work used to power directed movement along cytoskeletal filaments inside cells. Motor proteins have essential biological functions such as driving the contraction of muscle, the beating of sperm and cilia, and the transport of intracellular cargoes. Motor proteins are also interesting from a physical point of view because they do what no man-made engines do: they convert or transduce chemical energy directly to mechanical work without using heat or electrical energy as an intermediate. A central issue in the mechanism of this chemomechanical transduction by motor proteins concerns the roles played by thermal fluctuations, diffusion and Brownian motion. In this lecture I discuss several molecular models for motor proteins, including so-called ratchet models, and compare predictions of these models to experimental results for the microtubule-based motor protein kinesin. I argue that kinesin, which has two motor domains or heads, walks using a hand-over-hand mechanism such that at least one head is bound to the microtubule. Diffusion likely plays an essential role by facilitating the search of the unbound head for the next binding site, a distance 8 nm away. During this diffusive phase, the bound head supports the load ensuring that forward motion can still take place even against loads up to several piconewtons.

The force-generation problem

The structures of both kinesin-1 (Kozielski et al., 1997), the first member of the kinesin family to be discovered, and the microtubule (Nogales et al., 1998; Nogales et al., 1999) have both been solved with atomic resolution (Figure 1). Using lower resolution electron microscopy images, the approximate location and orientation of the motor relative to the microtubule has been deduced (Hirose and Amos, 2007; Sindelar and Downing, 2007). In the absence of ATP, one of the motor domains, or heads, sits in the crevice between the α and β subunits of the tubulin dimer, the building block of the microtubule. The other motor domain may be free as shown in Figure 1 (see below). Kinesin-1, henceforth call kinesin, moves towards the fast-growing end of the microtubule, termed the plus end, which is capped by the β-subunit. Single-molecule techniques (e.g. Howard et al., 1989) have shown that kinesin-1 moves at 800 nm/s at high ATP concentration and in the absence of load. This corresponds to about one hundred 8-nm steps (from one tubulin dimer to the next) per second. On average, kinesin take about 100 steps along a microtubule

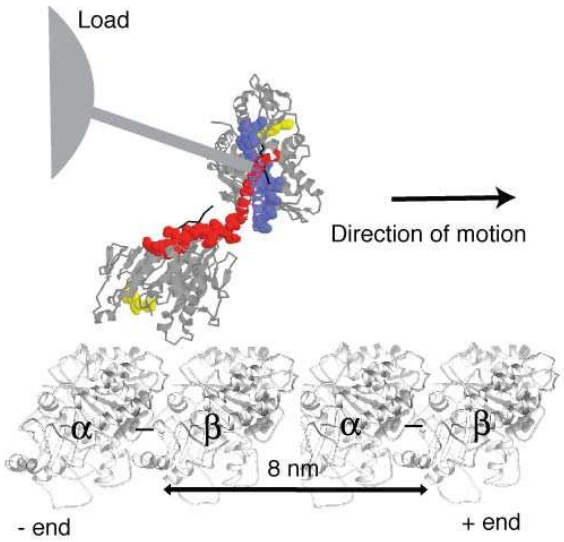

Figure 1: **Structure of kinesin-1 docked onto the surface of the microtubule.** The red and blue regions highlight the amino acids that form the region where the two motor domains come together to form the dimer. Cellular cargoes attach to the motor via additional amino acids not shown in the structure but denoted by the thick grey line. The yellow regions are where the ATP binds.

before dissociating; it is therefore said to be a processive motor.

A load force slows down the motion. Several techniques have been used to apply loads to a single motor: hydrodynamic loads by increasing the viscosity of the aqueous medium through which the motor moves, and elastic loads from a flexible fiber or an optical trap. The motor stops at a load of $\approx 7pN$ (reviewed in Howard, 2001). Larger loads cause motion in the opposite direction (Carter and Cross, 2005).

Despite 20 years of structural and single-molecule studies, how kinesin translated the 8 nm to the next tubulin dimer is not well understood. The force-generation problem is a difficult one.

Rectified-diffusion model

An early idea was that the motor diffuses along the microtubule surface. The nucleotide binding and hydrolysis would then somehow rectify the diffusion such that the motor would let go at the starting site and then bind again when it reached the next subunit (Braxton and Yount, 1989; Vale and Oosawa, 1990). Note that several kinesins have been shown to be held electrostatically to the surface of the microtubule and to diffuse along the surface in the absence of ATP (Okada and Hirokawa, 2000; Helenius et al., 2006).

If diffusion plays a primary role in translocating the motor to the next binding site, then the speed is expected to depend on the viscous damping from the solution. By solving the diffusion equation. The first-passage time for a particle released at a reflecting boundary to reach at absorbing boundary a distance d away is

$$t = d^2/2D = d^2\gamma/2kT$$

(Howard et al., 1989) where D is the diffusion coefficient, γ is the corresponding friction coefficient associated with the diffusion coefficient via the Einstein relation,

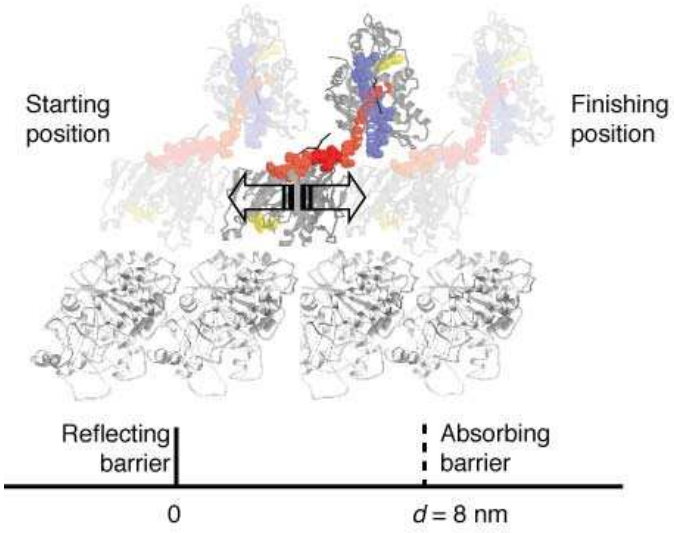

Figure 2: **The rectified-diffusion model.** The motor releases from its starting binding site, diffuses along the surface of the microtubule, and is then captured by the next binding site. The release and capture are coupled, in some unspecified way, to the hydrolysis of ATP and the release of products.

k is the Boltzmann constant and T the absolute temperature. The maximum force exerted against a viscous load is therefore

$$f_{\max} = \gamma v_{\max} = \gamma d/t = 2kT/d \approx 1pN$$

where $v_{\max}$ is the maximum velocity and we have taken $d = 8nm$ (Hunt et al., 1994).

The maximum force against a viscous load was measured by Hunt et al. (Hunt et al., 1994) using the assay shown in Figure 3a. In this upside-down assay, the motor is held at the surface of a chamber viewed under a microscope. When the motor attaches to and moves along the microtubule, the microtubule glides across the surface. The viscosity of the surrounding aqueous medium was increased by addition of solutes. Because the drag on a long microtubule is much greater than that on a single motor, drag forces of up to several piconewtons can be applied. Analysis of microtubule gliding speeds over a range of viscosities and microtubule lengths indicate that a single kinesin molecule can generate a force of up to $4pN$ against this viscous load (Figure 3b).

Because the measured force is much larger than the $1pN$ predicted from the first-passage-time analysis, the rectified diffusion model can be rejected.

Flashing-ratchet model

The flashing ratchet model shown in Figure 4. According to this model, the surface potential felt by the motor alternates between an asymmetric sawtooth potential profile (the ratchet) and a flat profile along which the motor can diffuse freely (Figure 4a). The switching from one profile to the other is controlled by ATP hydrolysis and product release. Again it is assumed the motor is constrained to the surface of the microtubule.

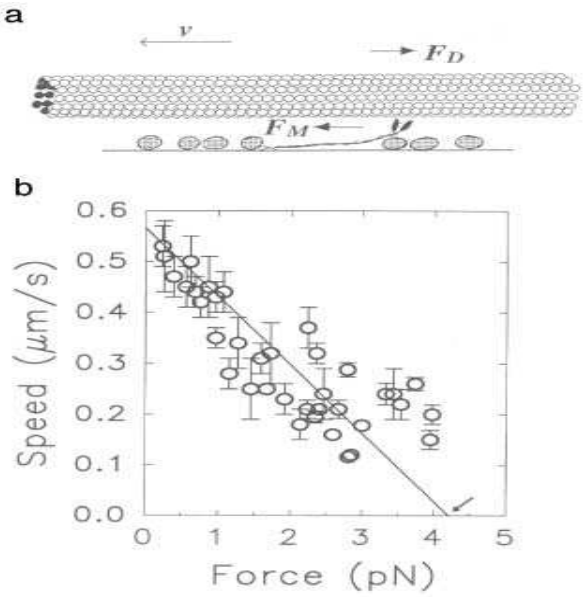

Figure 3: **Force exerted by kinesin-1 against a viscous load.** (a) In the up-side down assay, the kinesin is attached to the surface of the microscope slide and drives the gliding of the microtubule across the surface. The drag force increase with the viscosity of the aqueous medium and the length of the microtubule. (b) The force-velocity curve. The drag coefficient can be calculated from the hydrodynamic theory of slender rods near surfaces (Hunt et al., 1994). The drag force can then be calculated as the drag coefficient times the velocity.

When the profile is sawtoothed, the motors concentrate in the valleys where the energy is lowest (Figure 4b). When the profile switches to flat, the motor is equally likely to diffuse in either direction. When the sawtooth profile returns, the direction of movement of the motor depends on its current. If it diffuses only a small distance, then most of the time the motor will return to its initial position. However, if it diffuses past the position of the peak in the sawtooth, then it will move to a different valley. If and only if the potential is asymmetric, there will be a net movement in one direction.

This model can be ruled out definitively. A key prediction of the model is that on average the motor requires at least two switching processes per step (the greatest economy is when the sawtooth is most asymmetric). If the switching is driven by ATP hydrolysis, then this requires two ATP per step (on average). The stoichiometry of the movement of kinesin (steps per ATP) has been measured using a number of different approaches in different laboratories. For example, Coy et al. measured the speed of kinesin and the rate of ATP hydrolysis under identical conditions: dividing the speed by the ATPase gives 9 nm/ATP (Table 1). Similar results were obtained in another lab (Iwatani et al., 1999). Other measurements have shown that the step size is 8 nm (Ray et al., 1993; Svoboda et al., 1993; Carter and Cross, 2005). Thus there is 1 step per ATP. 1 step per 2 ATPs can be ruled out.

Table 1(Coy et al., 1999)

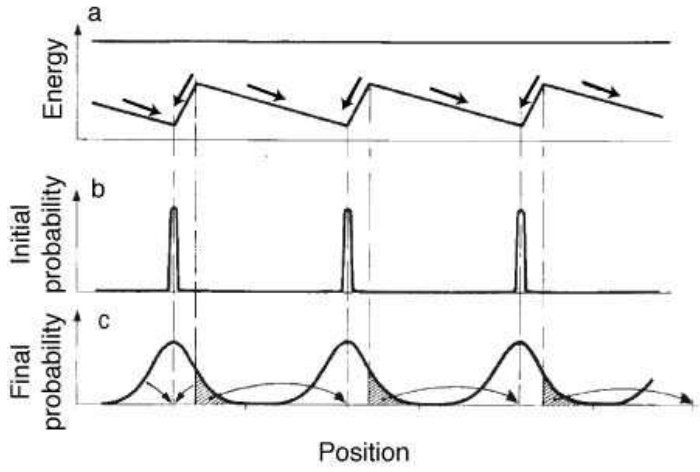

Figure 4: **Flashing-ratchet model.** After Ajdari and Prost, 1992; Magnasco, 1993; Rousselet et al., 1994; Astumian and Bier, 1994.

Speed ± SE (nm/s)	ATPase ± SE (s-1)	Distance/ATP (nm/ATP)	Stoichiometry (steps/ATP)
772 ± 29	88 ± 6	8.7 ± 0.7	1.08 ± 0.09

Huxley 1957 and powerstroke models

Two models that have been extensively discussed in the muscle literature. According to the Huxley 1957 model (Figure 5a) (Huxley, 1957), the motor domain contains an elastic element that undergoes thermal fluctuations. The motor domain can only bind to its site on the filament when the spring is strained. The force due to the strain in the spring then drives motion. Following relief of the strain, the motor domain unbinds. The binding and unbinding are position dependent and are coupled to the hydrolysis of ATP. This model has also been called a thermal ratchet model (Cordova et al., 1992) after Feynmans pawl and ratchet (Feynman et al., 1963).

A criticism of this model was that it would take too long for the strain to be built up through a thermal fluctuation alone (Eisenberg and Hill, 1978). However, this problem can be solved using the Kramers theory (Kramers, 1940) and it can be shown (Hunt et al., 1994) that for the dimension of the motor domain and the viscosity of the aqueous solution in which it is bathed, the time is not prohibitive (at least at low loads). Nevertheless, this criticism led to the development of powerstroke models (Figure 5b) (and also Huxley and Simmons, 1971). The key difference to the Huxley model is that rather than a thermal fluctuation leading to strain, the strain is developed by a conformational change in the motor domain (shown in Figure 5b as a lengthening, though a rotation is now favored given the structure of the myosin head, see Howard, 2001). The conformational change is coupled to the ATP hydrolysis cycle. Detailed powerstroke models have been developed (Eisenberg et al., 1980). In addition, the flashing ratchet model can be converted to a powerstroke model by replacing the flat profile with another ratchet, that is phase shifted relative to the original sawtooth landscape. Alternation of these profiles will lead to directed motion (Parmeggiani et al., 1999).

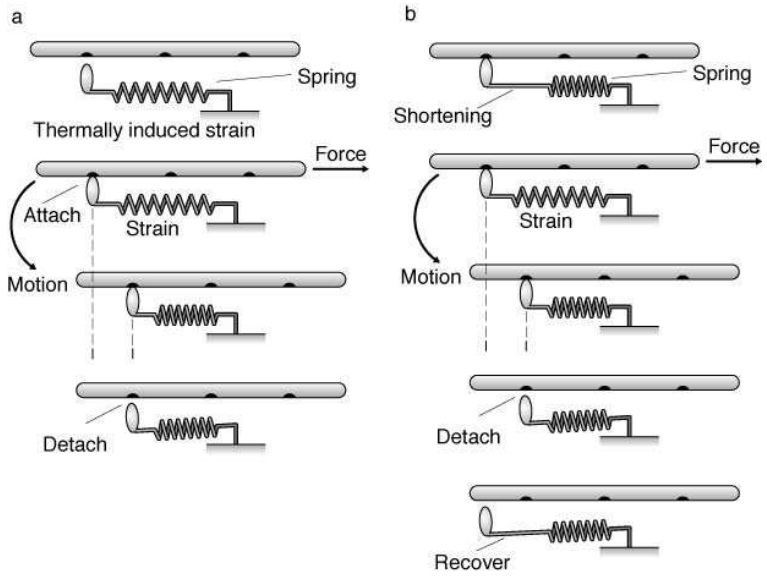

Figure 5: **Huxley 1957 (left) and powerstroke models.** These models were initially developed for myosin, with the thermal ratchet model following Huxley (1957) and the powerstroke model following Eisenberg and Hill. See text for details.

The Huxley and powerstroke models are actually closely related (Howard, 2006). Both can be understood in terms of a transition-state model (Figure 6). The difference between them comes down to the location of the transition state along the **reaction coordinate**. For a motor protein, the reaction coordinate is defined as the position of the load carried by the motor (e.g. a bead held in an optical trap) projected onto the axis of the filament.

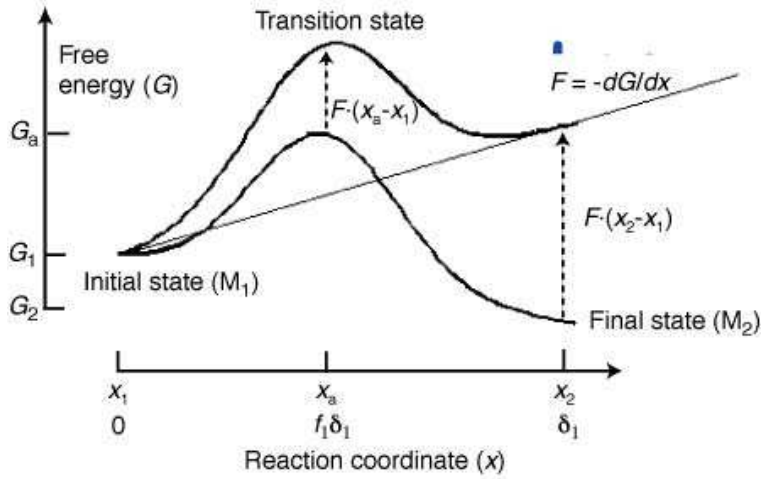

Figure 6: **Transition-state model**

In the Huxley model, the transition state is near the final state so that the motor domain must diffuse through a large distance with respect to the step size. In the powerstroke model, the transition state is close to the initial state, so that

thermal motion of the motor domain is not required to reach it. However, it is important to realize that even in the powerstroke mechanism energy is still required to reach the transition state and the energy must come from thermal fluctuations; in the powerstroke mechanism the fluctuations occur along a reaction pathway that is orthogonal to the axis of the microtubule and therefore requires no physical motion of the load in the direction of motion.

The two models behave quite differently in the presence of a load force. A load force tilts the energy diagram as shown in Figure 6 and changes the height of the activation barrier. If the transition state is close to the final state (Huxley model), then the activation barrier is increased considerably compared to the case where the transition state in near the initial state (powerstroke). This suggests that the models can be distinguished based on the load-dependence of the motor speed. For a single activation barrier, the forward transition rate is expected to depend exponentially on the load force F according to

$$k_+ = k_0 \exp\left(-\frac{Fx_a}{kT}\right)$$

where k_0 is the transition rate in the absence of load, and x_a is the distance to the transition state. For kinesin, the velocity (which is proportional to the transition rate), decreases e-fold per $\approx 4pN$ (Figure 7). This implies that the distance to the transition state is only $\approx 1nm(kT/4pN)$. Comparing this distance to the $8nm$ step size, we conclude that the transition state lies close to the initial state. Thus the powerstroke-type models are more compatible with the experimental data. Another way to view this is that Huxley-type models in which a load-bearing element has to undergo considerable diffusion along the microtubule axis will result in a very strong force dependence that is not compatible with the comparatively high forces that motors can generate.

Hand-over-hand models

Does the rejection of the Huxley 1957 model for kinesin rule out a role for diffusion in the motor mechanism? Absolutely not! The reason is that processive motors like kinesin-1 (and also myosin-5) have two motor domains. The two motor domains alternate their binding to the microtubule: they walk hand-over-hand (Hackney, 1994; Hancock and Howard, 1998; Hancock and Howard, 1999; Asbury et al., 2003; Kaseda et al., 2003; Yildiz et al., 2003; Warshaw et al., 2005; Schief et al., 2004). Thus while one motor domain can support the load, the other is free to diffusively search for and bind to the next binding site (Figure 8). After binding, the load can be transfered to the forward head. In this way the kinetic slowing of the motor by the load force is reduced.

There are several lines of evidence that the free head does indeed use a diffusive search for the next binding site. The most convincing data are from myosin-5. (i) The powerstroke is 25 nm, leaving a shortfall of $\sim$ 11 nm (the step size in this case is 36 nm) (Veigel et al., 2002). (ii) In an ingenious chop-sticks experiment Shiroguchi and Kinosita, 2007 attached microtubules to the motor domains of myosin-5 and observed large-scale diffusive motions. For kinesin-1, the evidence is less clear. According to a popular model, the forward motion of the free head is driven by a

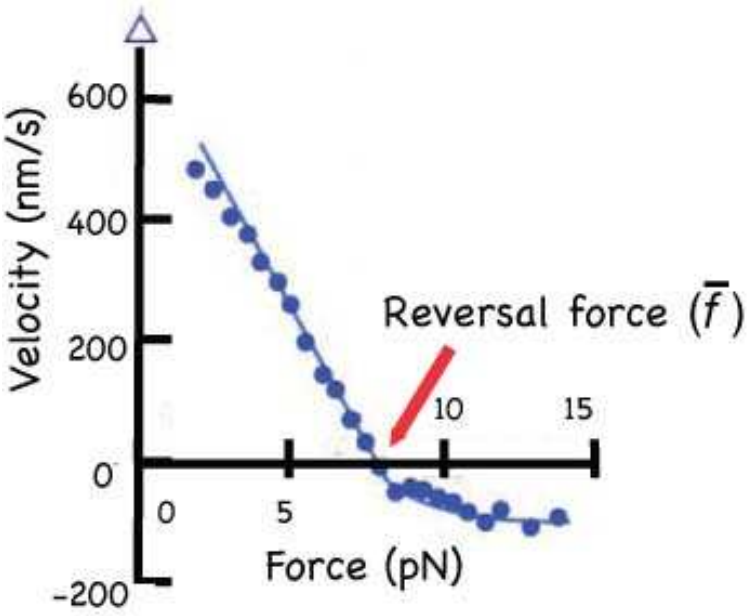

Figure 7: **Force-velocity curve for kinesin measured using an optical trap** (after Carter and Cross 2005).

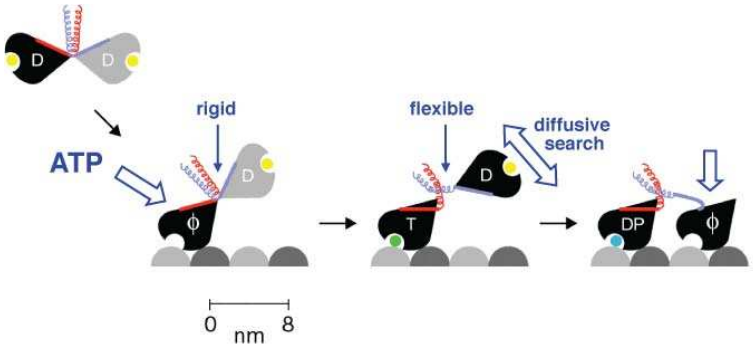

Figure 8: **Hand-over-hand model** (after Schief and Howard, 2001).

powerstroke-like conformational change involving the docking of the neck-linker domain (Rice et al., 1999). However, several lines of evidence suggest that the linking region between the two heads is highly flexible. For example, at high loads, kinesin-1 can move backwards or forwards, suggests that there is considerable degrees of flexibility (Carter and Cross, 2005; Yildiz et al., 2008). In addition early experiments showed that increasing the viscosity of the medium slowed down the motion in a way consistent with reducing the diffusive mobility of the free head (Hunt et al., 1994).

Open questions

The force-generation problem has proven to be a very challenging one. The simple question how do motor proteins work? does not have a simple answer. Several key questions are still open:

(i) Are both motor domains bound to the filament in the waiting state (when the motor is yet to bind ATP)? Figure 8 shows the waiting state (the first bound state) to have one of the heads free, but is that right?

(ii) What is the nature of the communication between the motor domains? It is clear that their activities are highly coordinated - e.g. the binding of one motor domain greatly accelerates the unbinding of the other (Hancock and Howard, 1998; Hancock and Howard, 1999), but how can strain between the heads modulate the binding?

(iii) To what extent do macroscopic concepts such as friction help us to understand the performance of molecular engines?

(iv) What structural changes occur during translocation, and in particular how are structural changes in the nucleotide-binding pocket, the motor-filament interface, and the region between the motor domains coupled?

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