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Molecular Motors: A Theorist's Perspective

Anatoly B. Kolomeisky[1] and Michael E. Fisher[2]

[1]Department of Chemistry and Chemical and Biomolecular Engineering, Rice University, Houston, Texas 77005; email: tolya@rice.edu

[2]Institute for Physical Science and Technology, University of Maryland, College Park, Maryland 20742; email: xpectnil@umd.edu

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Abstract

Individual molecular motors, or motor proteins, are enzymatic molecules that convert chemical energy, typically obtained from the hydrolysis of ATP (adenosine triphosphate), into mechanical work and motion. Processive motor proteins, such as kinesin, dynein, and certain myosins, step unidirectionally along linear tracks, specifically microtubules and actin filaments, and play a crucial role in cellular transport processes, organization, and function. In this review some theoretical aspects of motor-protein dynamics are presented in the light of current experimental methods that enable the measurement of the biochemical and biomechanical properties on a single-molecule basis. After a brief discussion of continuum ratchet concepts, we focus on discrete kinetic and stochastic models that yield predictions for the mean velocity, $V(\boldsymbol{F}, [\mathrm{ATP}], \ldots)$, and other observables as a function of an imposed load force $\boldsymbol{F}$, the ATP concentration, and other variables. The combination of appropriate theory with single-molecule observations should help uncover the mechanisms underlying motor-protein function.

INTRODUCTION

ATP: adenosine triphosphate

Biological cells are complex heterogeneous systems that undergo many dynamic biochemical processes, such as gene replication, transcription and translation, transport of vesicles and organelles between different locations, and segregation of chromosomes during mitosis (i.e., cell division) (1–3). A cell's ability to sustain these processes in a fast and effective way relies heavily on a class of protein molecules generally called motor proteins or molecular motors (1–6).

Although many types of motor proteins are currently known (such as myosins, kinesins, dyneins, DNA and RNA polymerases, and helicases), and new motor species are constantly discovered, it is widely believed that all function by converting chemical energy into mechanical motion. The most common source of chemical energy for motor proteins is, first, the hydrolysis of ATP (adenosine triphosphate) or related compounds, and, second, the polymerization of nucleic acids and proteins such as tubulins. These transformations of chemical energy into mechanical work typically involve a complex network of biochemical reactions and physical processes. They often take place on millisecond or shorter timescales with a high thermodynamic efficiency (4). However, the microscopic details of the mechanochemical couplings in motor proteins remain largely unknown (1–6). Understanding these mechanisms is one of the more challenging problems that require concerted efforts by chemists, physicists, and biologists.

From the mechanical point of view, motor proteins can be considered as submicroscopic nanometer-size motors (4) that consume fuel (via chemical processes) to produce mechanical work. However, in contrast to macroscopic engines, molecular motors operate mainly at the single-molecule level in nonequilibrium but isothermal conditions. The state of the local molecular environment and thermal fluctuations are critically important. A successful theoretical description of motor-protein mechanisms should recognize their multiple conformational transitions, account for the complex mechanochemical processes involved, and explain their efficiency.

The past decade has seen great progress in experimental studies of motor proteins [see the monograph by Howard (4) and References 7–47]. It is now possible to monitor and control the motion of a single motor-protein molecule under a variety of external conditions and measured loads with high spatial and time resolution. These investigations have revealed many previously unknown microscopic details, and their quantitative results have stimulated various theoretical discussions of the mechanisms underlying the dynamics of molecular motors (48–83).

In this brief review we summarize recent experimental advances and selected theoretical developments in the field of motor proteins. Because the information gained from experiments is growing rapidly, we focus only on the principal biochemical and biophysical features. There are important classes of molecular motors that rotate (4, 7, 14, 18, 36, 42, 43, 51, 58), in particular, bacterial flagella motors and F_0F_1-ATPase, which generates ATP in mitochondria. However, we consider here only motor proteins that transform chemical energy into linear translational motion: they might be called translocases. This is not inappropriate because many of the experimental and theoretical approaches to rotary and linear motor proteins are essentially the same (4).

Similarly, we focus theoretically on discrete stochastic or biochemical-kinetic models because at this stage in the subject, they seem the most appropriate for concrete quantitative understanding.

Motor Proteins

The variety of biological functions that molecular motors must perform in cells determines their complex multidomain structure (see **Figure 1**, which depicts three important motor proteins) (1, 3, 4, 11, 22, 84). For these motors, on which we focus attention, the most crucial parts are the motor domains, often called "heads," where the enzymatic activity takes place and which bind strongly to specific molecular tracks, such as microtubules and actin filaments (or, in other cases, to DNA and RNA molecules). The catalytic activity of a motor domain is strongly diminished when it unbinds from its linear filament. For most motor proteins, there is only one active site for enzymatic transformation per motor domain, as in kinesins and myosins (3, 4). However, the motor domains of cytoplasmic dyneins have at least four binding sites for ATP (39, 46); the existence of additional potentially active sites might be related to the regulation of motor activity.

As seen in **Figure 1**, the motor domains are connected by tethers or stalks (often of coiled-coil structure) to tail domains that also play a role in the activity of the motor (1, 3, 4, 84). These connect to cellular cargo, such as vesicles and organelles, and in the absence of a suitable load, the tail domains may bind to the motor domains and thereby cut off the enzymatic activity (4).

Several classes of motor proteins function as single independent entities as does a locomotive: they move on their tracks by repeatedly hydrolyzing ATP molecules

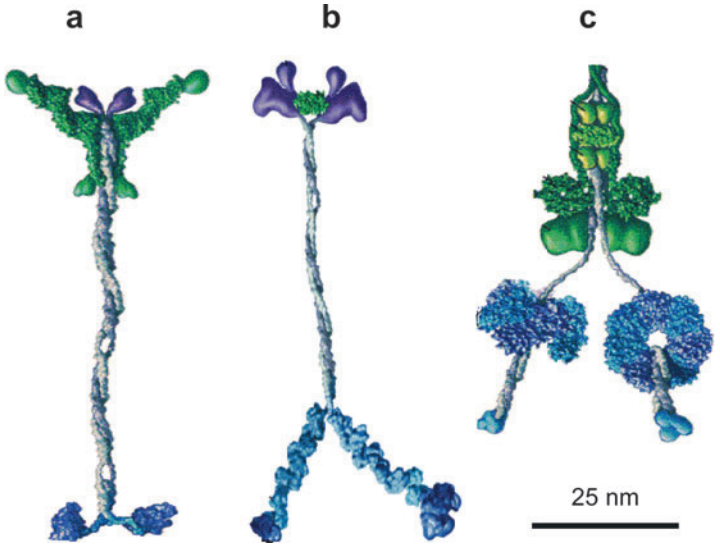


Figure 1

Domain structures of (*a*) conventional kinesin, (*b*) myosin V, and (*c*) cytoplasmic dynein. The tail domains are at the top, and the motor domains or heads are at the bottom. The (approximate) scale bar indicates 25 nm. Figure extracted from a review by Vale (6) with permission from Elsevier.

(at rates of order one per 10 ms), taking hundreds of discrete, close-to-equisized, nanoscale steps before finally dissociating. Among such processive motor proteins are conventional kinesin, cytoplasmic dynein, and myosins V and VI. The first pair walk on microtubules, kinesin towards the plus (or fast-growing) end, whereas dynein is minus-end directed. Myosins, however, bind to actin filaments, myosin V moving towards the barbed or plus end, whereas myosin VI moves oppositely towards the pointed/minus end.

Most single-molecule experiments have been performed on these enzymes. Many motor proteins, however, most notably the muscle myosins (4), function biologically only in large groups, although the details of the cooperative mechanism are largely unresolved (3, 4). Such nonprocessive motors normally complete only one or a few steps, or strokes, before completely detaching from their filaments. It is widely believed that the specific processivity of a motor protein is closely related to its particular structural features (85, 86). Nonprocessive motors are often monomers, whereas processive motor proteins exist in dimeric or even oligomeric forms (3, 4). This latter observation explains why processive motor proteins can stay attached to their filaments for long times: thus while one motor domain or head moves forward (presumably in an unbound or weakly bound state), the other head (or heads) can remain bound and carry the load imposed by the cargo (4, 48).

EXPERIMENTS

Structural information about motor proteins (such as seen in **Figure 1**) results principally from diffraction-based techniques and cryomicroscopy (4). Although such data are vitally important and can also lead to insights concerning intermediate structural states in a motor, our present understanding of the dynamics of molecular motors has largely rested on two classes of in vitro investigations. On the one hand, bulk solution observations of ensembles of motor molecules principally determine the chemical-kinetic properties of the various biochemical processes they undergo. On the other hand, single-molecule experiments uncover the fluctuations and mechanochemical responses of individual molecules. The approaches are complementary, and both are important for elucidating the mechanisms of motility (4, 22, 87). Furthermore, both can be enhanced by the study of mutated versions of the motor, which can reveal the roles of specific structural domains and their interactions.

Studies of motor proteins in bulk solutions constitute a convenient approach because the well-developed chemical-kinetic methods (such as stopped flow, isotope exchange, fluorescent labeling, and temperature quenching) can be applied to determine equilibrium and nonequilibrium properties of motor enzymes (15, 20, 88–92). The results of such experiments demonstrate that the functioning of a motor protein may include multiple states and conformations coupled in a complex biochemical network. For many motor proteins, however, one or a few biochemical pathways prove dominant and control the overall dynamics. Thus for conventional kinesins and for myosins V and VI (15, 20, 88–90, 92), the dominating biochemical pathway always includes a sequence of at least four states of ATP hydrolysis.

Single-Molecule Observations

The most informative data concerning the dynamics of motor proteins have recently come from single-molecule experiments, which include optical-trap spectrometry, magnetic tweezers, Förster resonance energy transfer (FRET), dynamic force microscopy, fluorescent imaging, and many other techniques (7, 8, 10–14, 16–19, 21–37, 40–44, 46). The ability to passively monitor and actively influence the dynamics of individual single molecules (in particular by imposing forces and torques) provides a powerful tool for uncovering motor mechanisms.

FRET: Förster resonance energy transfer

One of the most successful and widely used methods is optical-trap spectrometry (11, 12, 17, 21, 23–27, 32, 34, 35, 39, 41, 46). In this approach a single motor protein is chemically attached to a micron-sized or smaller bead that is captured by an external laser beam. The bead follows the motion of the motor molecule as it binds to its track and proceeds to move (**Figure 2**). Because the external electromagnetic field is nonuniform, the bead is trapped close to the focal point at which the light is most intense. Any nanometer-scale displacement of the bead from the focal point produces a restoring force, of the order of pico-Newtons, that is almost proportional to the displacement that in turn can be measured by differential outputs from a quadrant photodiode (10, 12, 17). Thus optical tweezers generate a harmonic potential well that can be calibrated with high precision.

Figure 3 shows the striking results of such a force-displacement-time experiment for kinesin moving on a microtubule. The binding of the motor to its track is detected by the sharp drop at time $t \simeq 1.5$ s in motor-to-bead stiffness (as monitored dynamically) (**Figure 3*b***). Immediately, in the presence of ample ATP (typically at

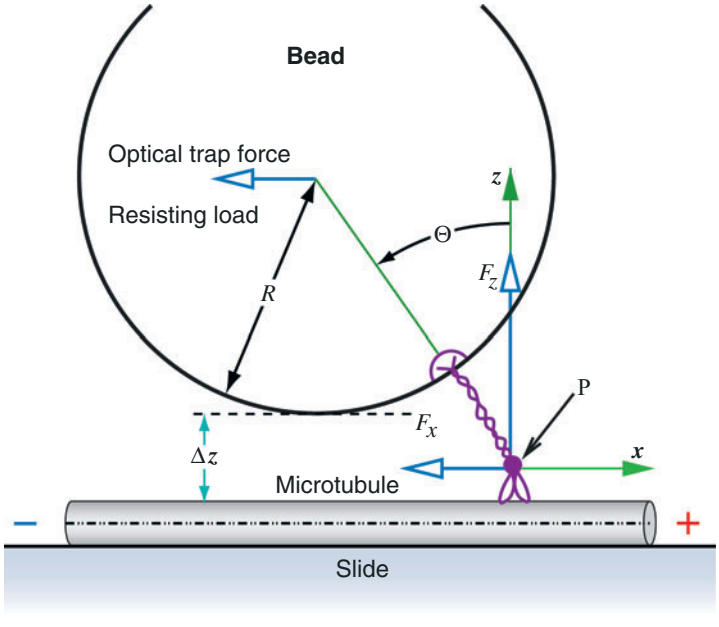
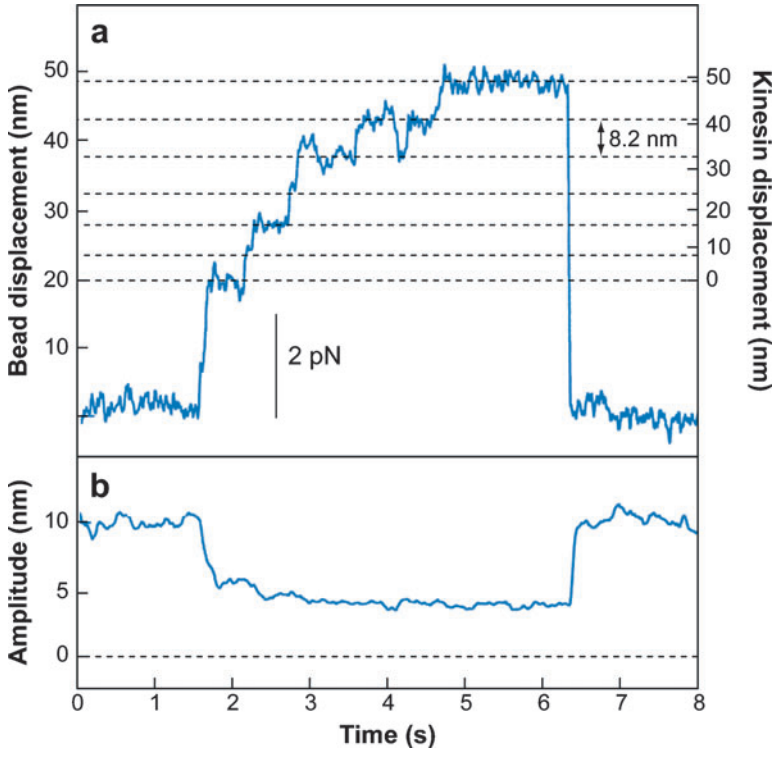


Figure 2

Schematic diagram of a kinesin/microtubule/bead complex in an optical-trap experiment. The microtubule is fixed to a glass slide that can be moved relative to the (fixed) optical trap. The force F_x exerted on the bead by the optical trap is transmitted by the tether to the point of attachment P on the motor at which the two heads are joined (see **Figure 1*a***). The mean offset $\Delta z \simeq 5$ nm is a result of thermal fluctuations; to scale, Δz should be much smaller, whereas the bead diameter, $R \simeq 250$ nm, should be twice as large. Figure adapted from Reference 69.

Figure 3

Optical-trap measurements of the motion of a single kinesin molecule at 20 μM ATP (10). (*a*) Bead displacement as a function of time. Note this is proportional to the load force (see the 2-pN scale bar). (*b*) Measurements of time-dependent kinesin-to-bead stiffness. Figure taken from Yanagida and coworkers (10).



millimolar levels), the motor starts to drag the bead out of the trap not continuously but rather by taking a series of plus-end-directed discrete steps. The step length, d, proves close to 8.2 nm which is the periodicity of a microtubule protofilament, in other words, the (α, β) tubulin one-dimensional lattice spacing (3, 6). For myosin V moving on an actin filament, the mean step length $d \simeq 36 \pm 3$ nm is, similarly, close to the half-period of the actin double helix (3, 6, 22). Furthermore, other experiments demonstrate (93–95) that each forward step (at low loads) corresponds to the hydrolysis of a single ATP molecule; this basic observation is referred to as tight coupling. When the bead is drawn further out of the trap, the resisting force (F_x in **Figure 2**) increases, and the motor slows down, reaching stall conditions (i.e., a zero mean velocity, V) at loads of 7 to 8 pN. As evident in **Figure 3**, reverse or back steps may then occur until, after some time, the motor detaches from the track (at $t \simeq 6.3$ s). Repeating such an experiment many times with the same identical molecule reveals the intrinsic stochastic fluctuations and yields, for example, the mean velocity, $V(F_x, [\mathrm{ATP}])$, as a function of load and fuel supply.

By incorporating feedback controls, a force clamp can be imposed, enabling processive runs of tens to hundreds of steps to be observed under steady, controlled loads. Then one can measure further statistical parameters such as

$$r(F_x, [\mathrm{ATP}]) = 2D/Vd \approx \langle [\Delta x(t)]^2 \rangle / d\langle x(t) \rangle, \qquad (1)$$

which has been called the randomness (8, 17). Here $x(t)$ is the displacement of the motor along the track as a function of time; the angular brackets, $\langle\cdot\rangle$, denote averages over many runs; and $\Delta x(t) = x(t) - \langle x(t)\rangle$ so that $D \approx \frac{1}{2}\langle[\Delta x(t)]^2\rangle/t$ measures the diffusivity or dispersion (8, 96).

FIONA: fluorescent imaging with one-nanometer accuracy

More recently the force-clamp set-up has been extended to allow the observation of single-protein dynamics under controlled vectorial forces $\boldsymbol{F} = (F_x, F_y, F_z)$, assisting as well as resisting, and sideways at an arbitrary angle (25, 26). As yet, however, simultaneous control of F_z (see **Figure 2**) has not been implemented although it is desirable (9, 69, 70). In addition to high spatial resolution (of order 1 nm), time resolutions of order 10 μs or better can be achieved (23, 41).

Closely related to the optical-trapping technique is magnetic tweezers spectroscopy (19, 29, 36). One end of a motor protein is again fastened chemically to a magnetic bead while the other end is fixed to a surface. The motor is maintained under tension by an imposed magnetic field gradient normal to the surface. The distance, z, of the bead from the surface and the observed magnitude of the transverse fluctuations of the bead, $\langle\delta x^2\rangle$, yield (via the equipartition theorem) the force exerted as $F_z = k_B T z/\langle\delta x^2\rangle$. Controlled torques can also be exerted. Magnetic tweezer experiments are especially suitable for studying motors such as topoisomerases and helicases (19, 29) that serve to unwind, untangle, and remove supercoiling in double-stranded DNA. Although magnetic tweezers are simpler to construct and use than optical traps, they are currently less sensitive and of lower resolution.

Selvin and coworkers (28, 37, 38) have developed another experimental approach of particular value, which they dubbed FIONA, standing for fluorescent imaging with one-nanometer accuracy. This method enables one to track the position of a single dye molecule attached to a specific location on a motor-protein molecule with nanometer accuracy at subsecond resolution. Although the fluorescent image has a diffraction-limited spot-size of several hundred nanometers, the brightest point, which corresponds to the desired position of the dye molecule, can be determined with a precision down to 1 nm, provided sufficiently many photons can be collected. With the aid of this technique it has been proved unambiguously that individual double-headed motors, such as kinesins and myosins V and VI, step in a so-called hand-over-hand fashion, meaning that the two heads exchange leading and trailing positions as the motor walks along its track (28, 37, 38). Recent evidence (46, 47) suggests that cytoplasmic dynein moves in a similar fashion by alternately shuffling its relatively large motor domains past one another.

THEORETICAL ASPECTS

The significant quantitative data resulting from single-molecule experiments have stimulated notable efforts in theory (48–73, 81). A framework for describing motor-protein dynamics should respect the basic laws of physics and chemistry and recognize the symmetries of the system such as periodicity, polarity, and chirality. A fully successful theory should not only provide a minimal consistent and reasonably quantitative description but should also yield mechanistic insights and experimentally testable predictions.

The central task of theoretical models for molecular motors is to connect biochemical processes to directed mechanical motion. It is fundamental that all biochemical transitions are reversible, even when available data may not provide direct evidence. The reverse transitions might be slow, but they should not be neglected in a comprehensive analysis as that may lead to unphysical conclusions (41, 54, 69). This observation implies that under some conditions motor proteins that hydrolyze ATP when they step forward can resynthesize ATP when they step backward. This fact is well established for the rotary motor F_0F_1-ATP synthase (36, 43). For typical processive motor proteins, the situation is open experimentally, but recent observations (92, 97) suggest that ATP can similarly be synthesized by such motors.

We can divide current theoretical approaches into two main groups: continuum ratchet models (49–52, 57, 63–65, 72, 73, 82) and discrete stochastic (or chemical-kinetic) descriptions (48, 53–55, 59–62, 66–71, 81, 82, 83).

Continuum Ratchets

In this physics-oriented approach, a motor protein at point $\boldsymbol{r} = (x, y, z)$ close to its track is viewed as diffusing on two or more spatially parallel, periodic but in general asymmetric coarse-grained free-energy surfaces (see **Figure 4**) (49–52, 57, 63–65, 72, 73, 82). The corresponding potentials, say $\Phi_j(\mathbf{r})$, describe distinct biochemical states of the motor. The ratchet-like character of the potentials depicted in **Figure 4** cannot itself induce directed motion in an isothermal environment. Under the input of chemical energy, however, the motor switches stochastically between different potentials. Then, as illustrated in **Figure 4**, the system evolves according to a set of coupled Fokker-Planck equations (4) so that, in general, a biased diffusion ensues. This might well be called processive Brownian motion; but sustaining such directed, albeit thermally fluctuating, movement demands the continued overall positive supply of chemical energy.

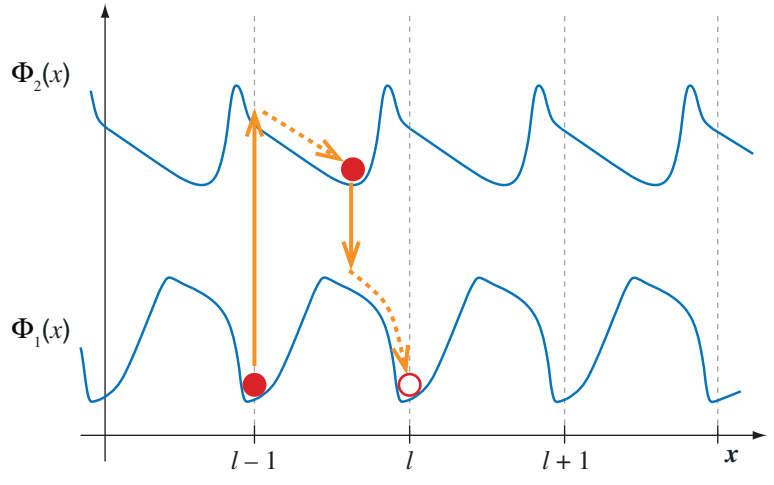


Figure 4

Illustration of the dynamics of a motor protein in the simplest two-potential periodic continuum ratchet model in the absence of a load. The vertical arrow represents the input of chemical energy, for example, via the hydrolysis of an ATP molecule; this is followed by diffusion, a drop to the lower potential surface, and further diffusion.

Such chemically driven ratchets (50, 57, 65), which might more descriptively be termed Markov-Fokker-Planck models (72, 74), provide a physically appealing, rather concrete picture of motor-protein dynamics that can be handled by established mathematical tools. But there are several troublesome aspects. With the exception of a few oversimplified and mostly unrealistic potentials, general analytical results cannot be found. One can, of course, resort it to a full numerical approach, but because the necessary computations are relatively demanding and many functional parameters are entailed, determining the range and uniqueness of fits to real data is a nontrivial task. Furthermore, in light of currently available knowledge of the relevant protein structures and their motions, deriving appropriate realistic potential functions, $\Phi_j(\mathbf{r})$, that are meaningfully detailed presents significant challenges. [Indeed, corresponding transition-rate functions $k_{ij}(\mathbf{r})$ (72, 74) are also required.] As a consequence, although successful fits to experimental dynamical data have been obtained [notably for the F_0F_1-ATPase system and the bacterial flagella motor (51, 72, 74)], it is hard to judge the reliability and instructiveness of the resulting implications for real motor proteins. Thus, for the present, we believe such continuum models can most profitably be utilized to describe various qualitative rather than quantitative features of motor dynamics. Nonetheless, as experiments reveal further structural and dynamical information at a molecular level, the continuum aspects of motor-protein motion seem likely to demand modeling at the more intrinsically mechanical levels that ratchet models enable.

Discrete Stochastic Models

A rather different approach adapts the discrete stochastic models of traditional chemical kinetics (48, 53–55, 59–62, 66–71, 78–82). The simplest model supposes that during each enzymatic cycle [processing a single fuel molecule, typically ATP (93–95)] the motor steps from a binding site l to the next one, $l + 1$, at distance d along the track and passes through a sequence of N intermediate biochemical states (see **Figure 5**). These states should, in general, be associated with distinct spatial locations, as

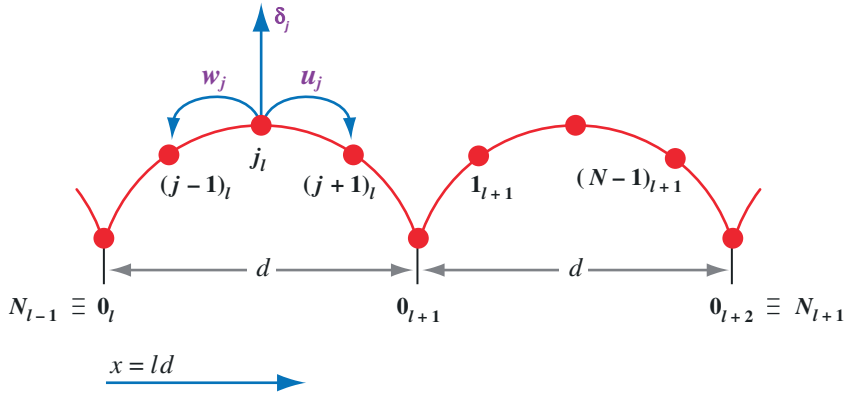


Figure 5

Kinetic scheme for the simplest N-state periodic stochastic model. A motor in state j_l can move forward at a rate u_j, backward at a rate w_j, or can dissociate irreversibly from the track at a rate δ_j.

P_i: inorganic phosphate

ADP: adenosine diphosphate

suggested in **Figure 5**. A motor in the mechanochemical state j_l ($j = 0, 1, 2, \cdots, N-1$) moves forward to state $(j+1)_l$ at a rate u_j or backward to state $(j-1)_l$ at rate w_j. (Detachment from the track at rate δ_j may also be recognized.) We take 0_l to specify the long-lived state in which the motor is strongly bound to its track, awaiting the arrival and binding of a fuel molecule. Note that reverse transitions are taken into account in accordance with the observations of back steps (see **Figure 3** and References 23, 26, and 41).

The dynamics of discrete kinetic models are governed by linear master equations that specify the net gain/loss, $dP_j(l, t)/dt$, where $P_j(l, t)$ is the probability that the motor is in state j_l at time t. Mathematically, this simple sequential model (with $\delta_j = 0$, all j) describes a particle that hops randomly on a one-dimensional periodic lattice of sites (of period N). One may thus use (and extend as needed) the powerful theoretical formalism constructed by Derrida in 1983 (54, 55, 59–61, 81, 98). This yields exact and explicit expressions for the asymptotic mean velocity and the dispersion for all N in terms of the rates u_j and w_j (53–55). For example, the velocity and dispersion for $N = 2$ are given by

$$V = d\frac{u_0u_1 - w_0w_1}{u_0 + w_0 + u_1 + w_1}, \quad D = \frac{d^2}{2}\frac{(u_0u_1 + w_0w_1) - 2(V/d)^2}{u_0 + w_0 + u_1 + w_1}. \qquad (2)$$

Randomness and number of states. It is valuable to know that the randomness, $r = 2D/Vd$, an observable measure of the dynamical fluctuations of a motor (see Equation 1), obeys the inequality $r \geq 1/N$ (96). Indeed, if reverse rates are neglected (setting $w_j \equiv 0$), one finds $r = 1/N$ when all the forward rates are equal (i.e., $u_j = u$). As an aside, if one also accepts $N = 1$ as a first-level motor model, the step size can be estimated experimentally, via $d = 2D/V$, by measuring D and V (99). More informatively, $r \simeq 0.39 \leq \frac{1}{2}$ has been observed for kinesin (12) at so-called saturating ATP levels (beyond which V does not increase). This means that $N \geq 3$ [ATP]-independent intermediate transitions contribute to the motor dynamics, with, indeed, comparable weight. This conclusion is in accord with the basic (N=4)-state biochemical view of ATP hydrolysis, namely for, say, kinesin, K, bound to a microtubule, M:

$$\mathrm{M\cdot K + ATP} \underset{w_1}{\overset{u_0}{\rightleftharpoons}} \mathrm{M\cdot K\cdot ATP} \underset{w_2}{\overset{u_1}{\rightleftharpoons}} \mathrm{M\cdot K\cdot ADP\cdot P_i} \underset{w_3}{\overset{u_2}{\rightleftharpoons}} \mathrm{M\cdot K\cdot ADP} \underset{w_0}{\overset{u_3}{\rightleftharpoons}} \mathrm{M\cdot K}, \qquad (3)$$

where ADP denotes adenosine diphosphate, and P_i represents inorganic phosphate. Note that neither the water of hydrolysis nor the released P_i and ADP are shown in Equation 3. Most of the rates exhibited here can be determined in bulk biochemical experiments (87–90). Furthermore, the first forward rate may be written $u_0 = k_0$[ATP] because it must depend on the ATP concentration.

Detachment and processivity. A major advantage of the discrete stochastic models is that they can readily handle more complex biochemical reactions than the linear sequence in Equation 4 or **Figure 5**. As a basic example, note that an active motor cannot stay forever bound to its track: as seen in **Figure 3** it will eventually dissociate—say, with state-dependent rates δ_j as in **Figure 5**. In single-molecule

experiments such detachments may be regarded as irreversible (because the motor rarely reattaches rapidly). The analysis for V and D under stationary conditions can then be extended by mapping onto a renormalized model with no detachments (59, 62). The effects on velocity and dispersion are relatively small, but the processivity, as measured by mean run lengths or in other ways (4, 17), is strongly affected (17, 62). Amusingly, allowance for detachments can, in principle, yield enhanced velocities if, at slower speeds, the motor spends more time in states with higher rates of detachment.

Parallel pathways. Beyond simple detachments, biochemical observations indicate that motor proteins need not follow one simple, sequential reaction path in stepping from a state 0_l to 0_{l+1}. Specifically, experiments on single-headed kinesins (100) and RNA polymerases (101) demonstrate the possibility of parallel biochemical pathways, whereas studies of myosin V (32) in which many but not all 36-nm steps exhibit distinctive substeps seem to demand some branched parallelism. The single-pathway analysis (54, 55, 62) can again be generalized to provide exact results for models with general branches (59) and for parallel paths (61). Simple, direct site-to-site diffusive or weakly bound parallel transitions correspond to what might be regarded as slipping: the increase in randomness seen for kinesin at low loads ($|F_x| \lesssim 1$ pN) may demand such a mechanism (4, 62).

Waiting-time distributions. A central tenet of traditional chemical kinetics implies that once a motor reaches a state j, its subsequent stochastic motion is independent of how it arrived. Accordingly, the time a motor spends in a state j represents a Poisson process. More concretely, suppose $\psi_j^+(t)dt$ is the probability of jumping forward to state $j+1$ in the time interval t to $t + dt$ after arriving in state j at $t = 0$, and $\psi_j^-(t)dt$ is the corresponding probability of a reverse transition to $j-1$. Then these two waiting-time distributions are pure exponents with a common decay rate; i.e., $\psi_j^{\pm}(t) \propto e^{-(u_j+w_j)t}$. In principle such distributions are directly observable in single-molecule studies, but in practice, identifying the arrival and subsequent departure of a motor in a particular mechanochemical state is seldom possible. Then if various intervening states are missed, the distributions $\psi_j^{\pm}(t)$ will be nonexponential. Similarly, in a continuum ratchet model, the diffusive motions entailed in a transition between two relatively long-lived substates, such as the potential wells in **Figure 4**, again mean that the associated waiting-time distributions will be nonexponential. It is thus of interest to extend the exact analysis to arbitrary waiting-time distributions $\psi_j^+(t)$ and $\psi_j^-(t)$, etc. This can be achieved (60) by appealing to the theory of generalized master equations in which the relaxation kernels or memory functions are directly related to the waiting-time distributions (102). It transpires that nonexponential distributions still yield the same rate-dependent relations for mean velocities; the dispersion expressions are, however, significantly changed. The departures from simple chemical-type processes are conveniently quantified by defining mechanicities, M_j^+, etc., which can provide a more economic description (with fewer parameters) of randomness observations (60, 62, 69).

Substeps and Load Dependence

A unique feature of single-molecule experiments is the ability to impose a measured force, $\boldsymbol{F} \equiv (F_x, F_y, F_z)$, directly on a single motor protein and to observe changes in velocity, V, and other properties. But what might this teach us? The point of application of the load, say, $\boldsymbol{r} \equiv (x, y, z)$ (see P in **Figure 2**), may be identified as the dynamic position, $\boldsymbol{r}(t)$, of the motor as it moves. However, because the motor shifts from, for example, $\boldsymbol{r}_0$ in state 0 to $\boldsymbol{r}_0 + d\hat{\boldsymbol{x}}$ (where $\hat{\boldsymbol{x}}$ is a unit vector parallel to the track) as one enzymatic cycle is completed, $\boldsymbol{r}(t)$ may also be regarded as a mechanochemical reaction coordinate. As the reaction progresses, $\boldsymbol{r}$ passes through positions, $\boldsymbol{r}_j$, that physically locate the intermediate mechanochemical states $j = 1, 2, \cdots$. The successive differences $\boldsymbol{d}_0 = \boldsymbol{r}_1 - \boldsymbol{r}_0$, $\boldsymbol{d}_1 = \boldsymbol{r}_2 - \boldsymbol{r}_1$, $\cdots$ then represent substeps characterizing the motor mechanism. En route from state j to $j+1$, the path should pass through a transition state, say, at $\boldsymbol{r}_j^+ \equiv \boldsymbol{r}_{j+1}^-$. If substeps are sufficiently large (greater than 1 nm or so), they should be detectable experimentally.

Now, as customary, one may also visualize the state point $\boldsymbol{r}$ as exploring a free-energy landscape with a potential $\Phi(\boldsymbol{r})$ and a valley (or potential well) at each $\boldsymbol{r}_j$ and a col (or saddle point) for a transition state at $\boldsymbol{r}_j^+$ (4, 57, 69, 70). One may then view the imposition of a load $\boldsymbol{F}$ most simply as adding a term $-\boldsymbol{F}\cdot\boldsymbol{r}$ to $\Phi(\boldsymbol{r})$, which tilts the landscape in the direction of the force.[1] This, in turn, changes the relative heights of the transition-state barriers that separate successive mechanochemical states; thereby the corresponding rates u_j and w_{j+1} become force dependent. The standard reaction-rate theories (see, e.g., Reference 4) then lead to (70)

$$u_j(\boldsymbol{F}) = u_j(0)\exp(\boldsymbol{\theta}_j^+\cdot\boldsymbol{F}d/k_BT), \tag{4}$$

$$w_j(\boldsymbol{F}) = w_j(0)\exp(-\boldsymbol{\theta}_j^-\cdot\boldsymbol{F}d/k_BT), \tag{5}$$

in which the dimensionless load-distribution vectors, θ_j^+ and θ_j^-, describe how the work $\boldsymbol{F}\cdot\boldsymbol{d}$ performed by the external force is apportioned between the various forward and reverse transitions. Furthermore, the vectors $\boldsymbol{\theta}_j^\pm$ relate simply to the substeps via $\boldsymbol{d}_j = (\boldsymbol{\theta}_j^+ + \boldsymbol{\theta}_{j+1}^-)d$, while the transition-state displacements satisfy $\boldsymbol{d}_j^\pm = \boldsymbol{\theta}_j^\pm d$. In Equations 4 and 5, terms of order F^2 have been neglected in the exponent (see also References 1, 54, 69, and 70). More elaborate treatments yield $\boldsymbol{F}$-dependent prefactors as well. However, the linear exponential factors normally dominate strongly, so that, unless $\boldsymbol{\theta}_j^\pm\cdot\boldsymbol{F}$ vanishes for some j (69), there is little merit in employing more elaborate but inevitably approximate expressions.

The strategy is now clear. Using Equations 4 and 5 in the expressions for V and other parameters (see Equation 2), one may attempt to fit force-velocity-[ATP] and other dynamical data (12, 17, 25, 26, 32, 40, 41). Even for the simplest $N = 2$ models in which $F_y = 0$ and one neglects F_z and possible z excursions of $\boldsymbol{r}(t)$ (53, 54, 55, 62, 67), there are seven parameters: specifically, the rates at zero-load $u_0^0 = k_0^0[\mathrm{ATP}]$, u_1^0, w_1^0, and w_0^0 [which requires special consideration (62, 66, 70)] and the

[1] Following References 69 and 70, we take $F_x > 0$ to specify an assisting load on a motor, whereas $F_x < 0$ represents a normal resisting load (see **Figure 2**).

(now-scalar) load-distribution factors θ_0^{x+}, θ_1^{x+}, and θ_1^{x-} [since $\sum_j(\theta_j^{x+} + \theta_j^{x-}) = 1$ is required]. However, to a large degree the separate regimes of high and low loads ($|F_x| \simeq 1$ pN to 10 pN) and limiting and saturating [ATP] ($= \mu$M to mM) are dominated by distinct parameter sets. Thus the stall force, F_S, at which V vanishes is given by

$$F_S = (k_B T/d) \ln \prod_{j=0}^{N-1} \left(u_j^0 / w_j^0\right). \tag{6}$$

Furthermore, some rates can be checked against bulk biochemical observations.

Some lessons and predictions. A first conclusion that may be rather generally valid for processive motors is that the observed decrease of V under resisting loads ($F_x < 0$) is governed primarily by the force dependence of the reverse rates, w_j, rather than the relatively insensitive forward rates. This conflicts with traditional views and ad hoc models that typically neglect backward rates and assume all the force dependence resides in a single, forward power stroke.

The original analysis of the extensive kinesin data of Block and coworkers (12, 17) neglected the F_z dependence and predicted an initial substep, on binding ATP, of $d_0 = 1.8$–2.1 nm (62), seemingly supported by structural studies (13). Subsequent observations at 1-nm resolution ruled this out (23, 41); however, the discrepancy was resolved by allowing for F_z dependence and incorporating later data (26) that also encompassed assisting (and sideways) loads (69). The new treatment predicts that on binding ATP, the motor might be said to crouch so that the initial movement, $\boldsymbol{d}_0$, is directed downward towards the track with $d_0^z \simeq -(0.5$–$0.7)$ nm (while $|d_0^x| \leq 0.2$ nm) (69). Also predicted and as yet unverified is a strong sensitivity to bead size under assisting loads. At the same time, the analysis uncovered a mechanism that, by increasing F_z by approximately 2 pN, opposes assisting and leftward loads. On the other hand, extending the fitted (V, F_x) plots to superstall loads ($F_x \lesssim -7$ pN) predicts surprisingly small negative velocities that exhibit shallow minima 30–40% below F_S. In fact, Carter & Cross (41) have now observed such behavior; it can be linked theoretically to substep geometry (104).

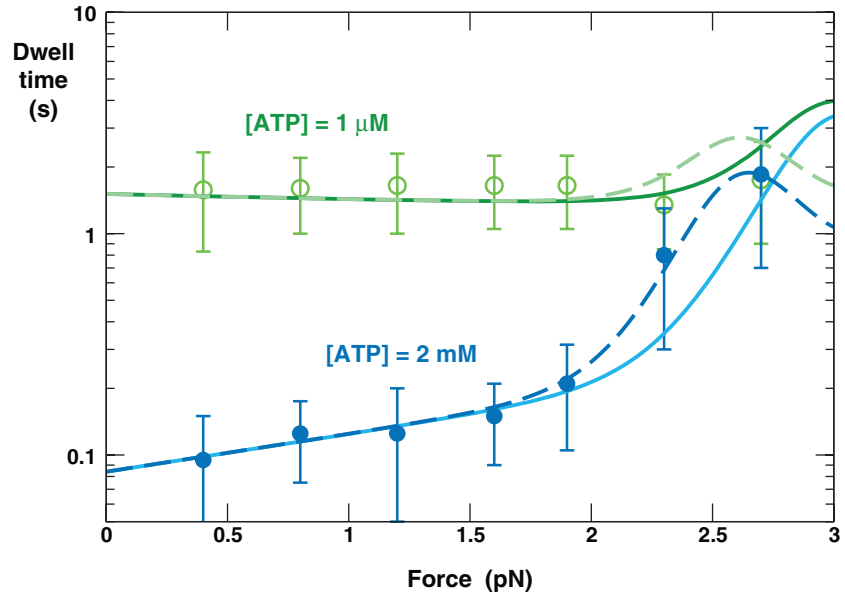
Experiments on myosin V presented in **Figure 6** (66) reported mean dwell times measured before forward steps (16, 22). These were analyzed using $N = 2$ expressions for the mean forward cycle time (66, 67, 105), which involves a random-walk first-passage calculation (103), namely,

$$\tau_+^0 = (u_0 + u_1 + w_0 + w_1)/(u_0 w_0 + u_1 w_1). \tag{7}$$

Furthermore, as illustrated by the fits in **Figure 6**, one could allow for an observed distribution in step sizes (66). A striking prediction (66) was the presence of a substep of magnitude $d_0^x = 13$–14 nm. Recent experiments (32) have indeed unambiguously revealed such a substep although of slightly smaller size, $d_0^x \simeq 11$ nm. The difference might indicate the need to allow for vertical displacements (69, 70) of the point of

Figure 6

Mean dwell times of myosin V as a function of external load at different ATP concentrations. The symbols correspond to the experimental data of Mehta et al. (16). The solid lines are theoretical predictions from a discrete stochastic model with a fixed step length, whereas the dashed curves allow for variable step size. Figure adapted from Reference 66.



attachment.[2] Even more intriguing, the data appear to demand a branching reaction because not all full steps of mean size $d \simeq 36$ nm display the substep (32, 33).

Available work and efficiency. The free energy, ΔG, available to a motor to do work via the hydrolysis of ATP (or other nucleotides) may be found from biochemical studies. Under the physiological conditions normally used for in vitro studies, Howard (4) concluded that $|\Delta G_{\rm ATP}| \lesssim 25\ k_B T$. The maximum force a motor taking a step d can exert is $F_{\rm max} = \Delta G/d$, which, because $k_B T \simeq 4.1$ pN nm, yields approximately 2.8 pN for actin-based motors. For myosin V this corresponds closely to the observed stall force, F_S. From this perspective, therefore, myosin V operates at close to 100% efficiency. The same holds for the rotary F_0F_1-ATPase motor (18). For microtubule tracks, however, one finds $F_{\rm max} \lesssim 10$–13 pN, which significantly exceeds the observed stall forces of 7–8.5 pN for kinesin and dynein (41, 46). Understanding this 30% loss of efficiency at a molecular level is a major unsolved problem.

Back steps and dwell times. In the absence of back steps, or when their fractional occurrence, say, $\pi_- = 1 - \pi_+$, is negligible, as at low loads and high [ATP], the full cycle dwell time τ_+^0 given in Equation 7 is essentially the reciprocal of the velocity (66, 67). However, when stall is approached, the ratio π_+/π_- must fall rapidly; at stall, back steps balance forward steps, so $\pi_+/\pi_- = 1$. Then for F_x beyond F_S, as observed by Carter & Cross (41), the ratio falls rapidly to zero. Furthermore, the theory shows,

[2] In connection with myosin V, attention should be drawn to more ambitious mechanoelastic structural models recently advanced (75–77).

perhaps surprisingly, that the full-cycle dwell times, τ_+^0 and τ_-^0, prior to forward and backward steps are equal (notwithstanding that the probabilities of moving forward, backward, or dissociating are quite distinct) (67). Appropriate observations confirm this fairly well (23, 41).[3]

However, an important subtlety emerges when the observed back-step fraction is nonnegligible (105). Specifically, although a principal step typically of magnitude close to d is readily seen, small mechanochemical substeps (e.g., less than 1 nm) escape detection. Then, for example, a motor may execute a major forward step (e.g., between states 2_l and 3_l in an $N = 4$ description) but fail to complete a full cycle (say, to $0_{l+1} \equiv 4_l$) before making a back step. When one allows for such hidden substeps (105), one finds, in particular, that in the near-stall exponential fit

$$\pi_+/\pi_- \approx \exp[(F_x - F_S)d^*/k_BT], \tag{8}$$

valid for $(F_x - F_S)$ small (41, 104, 105); the putative effective step size, d^*, is significantly smaller—by a factor of $\sim\frac{1}{2}$ for kinesin (41)—than d, which is the full-cycle prediction. Developing the appropriate first-passage theory (104, 105) further demonstrates the value of recording and analyzing more detailed statistics such as π_{++} and π_{--}, the fraction of forward steps following a forward step and vice versa, and, correspondingly, the conditional dwell times τ_{++} and τ_{+-}, etc. (105).

Multimotor complexes. In considering dimeric processive motors, one may regard the cooperative coupling of the two heads as a lead problem in the general issue of interacting molecular motors (68, 76, 77, 79, 82), and in mitosis, homotetrameric kinesin plays a role (3, 84). Again, in living cells more than one motor may bind to a single vesicle and cooperate or, as in the case of a kinesin and a dynein on the same microtubule, even compete. Other motor proteins, such as RecBCD helicases, consist of distinct domains that individually have enzymatic activity (1, 2, 30). Indeed, separated RecB and RecD domains consume ATP and unwind DNA (30); but single-molecule experiments find that the RecBCD cluster moves faster than the separate components (30). A simple, discrete stochastic model provides insight into these facts by invoking a coupling energy of order $6k_BT$; it predicts that as the velocity is increased, the fluctuations are reduced (68). Groups of motor and multimotor complexes also produce many interesting phenomena of collective behavior, such as flagellar beating, and chromosome and spindle oscillations, that are important for biological systems (80).

A Concluding Remark

As illustrated by the issues raised above, such as the inefficient stall forces of kinesin and dynein, and yet others unmentioned, such as the mechanism of the cylindrical viral capsid-packing motors (21), there are many open problems concerning motor

[3] As a result, interpretations suggesting that ATP is, hence, hydrolyzed on back steps, or not synthesized, and so on, are unconvincing (67, 69).

proteins and their dynamics in which theory may provide predictions and conceptual insights. Furthermore, the field is constantly growing, with the discovery of new biological nanomachines, the refinement of established techniques, and the development of novel experimental approaches. One may anticipate exciting progress on all fronts.

SUMMARY POINTS

1. Motor proteins are special enzyme molecules that transform chemical energy into mechanical work. They exist in many forms.
2. Current experimental techniques enable one to study the dynamics of a single molecular motor with high spatial and time resolution.
3. The modeling of motor-protein dynamics has utilized two approaches: continuum ratchet pictures and discrete stochastic or chemical-kinetic descriptions.
4. Discrete stochastic models can account for available experimental observations, such as the load and [ATP] dependence of mean motor velocities, and they provide a flexible theoretical framework for understanding motor-protein mechanisms.

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