

Porters Versus Rowers: A Unified Stochastic Model of Motor Proteins

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Abstract. We present a general phenomenological theory for chemical to mechanical energy transduction by motor enzymes which is based on the classical “tight-coupling” mechanism. The associated minimal stochastic model takes explicitly into account both ATP hydrolysis and thermal noise effects. It provides expressions for the hydrolysis rate and the sliding velocity, as functions of the ATP concentration and the

number of motor enzymes. It explains in a unified way many results of recent in vitro motility assays. More importantly, the theory provides a natural classification scheme for the motors: it correlates the biochemical and mechanical differences between “porters” such as cellular kinesins or dyneins, and “rowers” such as muscular myosins or flagellar dyneins.

I. Introduction

RECENT progress in understanding the action of motor proteins has come from several directions. In addition to new structural data which provided us with a more detailed molecular picture of the particular motor enzymes, such as myosin (37), and of the associated protein fibers, such as f-actins (8), the intense effort in genetics and molecular biology has led to the discoveries of the kinesin and dynein superfamilies (33). These discoveries opened up new vistas: acto-myosin, which had been for many decades a prototype for the studies of the mechanisms of chemical to mechanical free energy transduction is now just one of many motor systems under study.

In addition, the progress in enhanced video microscopy allowed many research groups to observe directly the action of a few motors or even a single motor protein (12, 26). It has become possible to measure, for instance, the speed of the motors on the fiber or the force exerted by a single enzyme. It is thus not surprising that such direct and rather precise observations not only pointed out similarities between the action of myosins, kinesins, or dyneins but also demonstrated some important differences between them (20). In fact, variations of biochemical rate constants or the speeds of the movement of the motors can be quite important even among the members of the same family.

A natural question then arises: is there more than one mechanism of free energy transduction in these systems or can they be unified in a single picture? For instance, can one apply a model developed for myosins sliding on actins in a muscle also to describe kinesin motors moving neurotransmitter vesicles on a microtubule in a neuron? And, if yes,

what are the differences between the different motors within the framework of such a unified picture?

In this article, we would like to address some of the above issues from a theoretical point of view. (A short, preliminary description of the theory presented here has appeared in 18). In particular, we are interested in the following problem. The results of recent in vitro studies seem to indicate that there exist at least two very distinct classes of motor proteins: (a) enzymes which work in large ensembles such as muscle myosins or flagellar dyneins; (b) enzymes which work in small groups or alone, such as cytoplasmic kinesins or dyneins. At the same time these two classes differ not only in the number of coworking enzymes but also in the “mechanics” of their action. For instance, in vitro motility assays show that a few muscle myosin molecules, belonging to the first class of motors, can move an actin filament, provided it is kept close to the motors. If this is not the case, however, the fiber detaches rapidly and diffuses away, losing its contact with the motors. This means that a working muscle myosin spends a large fraction of its time detached from the fiber (26). On the other hand, this is not the case for kinesin molecules, which belong to the second class of motors. A single cytoplasmic kinesin can move along a microtubule without losing contact for quite a long time, which means that it spends a relatively small fraction of its time detached from the fiber (1). An important question which then arises is whether the distinction between the two classes of motors can indeed be made both on the basis of the number of motors working together and on the basis of the time spent in the detached (or the attached) “state” or whether this correlation is just a coincidence. In addition, one would like also to establish the connection with the existing differences among biochemical properties of the different motors (see for example 15), if possible on a semiquantitative basis.

In fact, correlations between different properties of the

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motor proteins belonging to the above-defined two classes seem quite natural from the point of view of their function. Muscular myosins or flagellar dyneins evolved as parts of specialized organs which have often to perform work at high loads (21). They thus have to work in large ensembles such as bundles. The important thing is to realize, however, that the action of the individual molecules in these ensembles are probably not correlated over long distances (although there could be some steric or geometric correlations between the nearest neighbors in a bundle). Therefore, the motors have to avoid working against one another, i.e., they have to minimize the "protein friction" (28) due to the motors that remain attached to the fiber after completing the working part of the cycle. A good way to do this is for a motor to detach from the fiber as soon as its work is done in each working cycle. On the other hand, such a strategy would be disastrous for the other type of motors such as cellular kinesins or dyneins. They are used in the cell as carriers of vesicles, chromosomes, or other organelles, and have to stay attached to the fibers (microtubules) most of the time in order to avoid diffusing away. The loads they are moving are relatively small, so they can function alone or in very small groups; the protein friction resulting from their secure attachment thus does not seriously impede their motion.

Although such statements seem quite intuitive, we would like nevertheless to make them more precise, e.g., by defining these two classes of motor proteins as limiting cases of the behavior of a single model. This in our opinion has several attractive consequences: one shows that the different classes of behavior can stem from a single underlying mechanism of the chemical to mechanical free energy transduction and the proteins under consideration can indeed use similar mechanisms for their action, as suggested by their structural or biochemical similarities. Secondly, the unifying model presented here defines the regimes in which one could expect different possible behaviors of the motor enzymes, thus pointing out relevant biochemical (kinetic) differences between the different classes of motors. Finally, such a unifying picture suggests the possibility of the existence of other classes of motors and introduces a simple classification scheme for these molecules.

II. Choice of the Model

The theoretical model which we construct here is in the class of tight-coupling mechanism of motor functioning (10, 11, 13, 21). It is constructed to be "minimal", i.e., to include the minimal number of states which cannot be reduced if one seeks agreement with established biochemical and mechanical data for actomyosin (see Appendix V). The results of recent *in vitro* motility assays introduce serious constraints on any theoretical model. In typical assays, motor proteins (or their fragments) are attached to a substrate and a fiber is sliding above (in another type of assay it is the fiber which is attached and a colloidal particle with motor proteins adsorbed on it is moving along the fiber); one can thus measure the speed of the movement, or even the statistical distribution of the speeds, as a function of the ATP concentration, the number of motors in action, and the load. Our aim is to build the simplest model which explains the results of such experiments for different motor systems. Note that this means in particular that we do not intend to explain *in vivo* experi-

ments performed on muscle fibers or flagella, where many other phenomena (such as regulation, interactions with other components, etc.) may be influencing the behavior.

Any realistic theoretical model constructed to describe the action of motor proteins and the phenomenon of chemical to mechanical free energy transduction should incorporate the process of ATP binding and hydrolysis (3), the source of free energy, and of binding (unbinding) of the motor proteins to (from) the fibers (10, 11, 13). The effects of thermal noise—which have a major influence on molecular processes—should also be properly taken into account. Indeed, motor proteins are stochastic rather than deterministic machines. One must model them in terms of probabilities of different chemical or conformational states and frequencies of transitions between these states. Stochastic equations for these probabilities which describe the action of motor proteins can be solved explicitly in the steady state case for the model described below: the solutions can then be compared with the quantities directly measured in the motility assays. We shall do this below and show that different limits, corresponding to distinct hierarchies between the time scales of the model, seem to describe the behavior of different classes of motor molecules.

Example of Phenomenological Modeling: Protein Friction

Before we present our model, let us first illustrate the role of phenomenological models by showing a simple example of how they can be useful in describing the behavior of motor proteins. The example involves motor enzymes whose ATPase activity has been inhibited by addition of an appropriate chemical (e.g., vanadate). It has recently been observed in a motility assay for vanadate-treated dyneins that a microtubule translocated by such "blocked" enzymes is effecting a simple Brownian motion (36). The longitudinal component of such a random walk can be measured and it appears to be characterized by a single diffusion constant, D_{\parallel} . The measured value of this constant is, however, one or two orders of magnitude smaller than what one would estimate from Einstein's relation: $D_{\parallel} = k_B T / \zeta$, where k_B is the Boltzmann constant, T temperature, and ζ is the hydrodynamic friction coefficient for a longitudinal movement of a microtubule in water. This apparent discrepancy has been quantitatively explained within the framework of a simple phenomenological model (28) which we summarize briefly now.

The blocked motor proteins, which cannot go through the whole cycle of ATP hydrolysis, alternate between two states: one state (D) in which the motor is detached from the fiber and an attached state (A) in which it is weakly bound to the fiber. Let us suppose that the binding/unbinding is an equilibrium process characterized by a rate $k_{AD} \equiv t_{AD}^{-1}$ for detachment and an equilibrium constant K_{DA} . For a fiber interacting with N motors and moving at a constant (average) speed v , the energy dissipated by the motors per unit of time is on one hand given by: $W = \text{frictional drag force} \times v \equiv \zeta_{\text{eff}} v^2$, where ζ_{eff} is the effective friction coefficient due to the motors;

$$W = N \times \text{frequency of detachments} \times \text{energy lost in a detachment} = N \frac{P_A}{t_{AD}} \Lambda (t_{AD} v)^2,$$

where Λ is the elastic constant of the attached motor/fiber

complex (in Hooke approximation), $P_A = K_{DA}/(K_{DA} + 1)$ is the fraction of time the motor spends in the weakly bound (A) state and t_{ADV} is the average distance traveled before detachment. By comparing these two formulae, one obtains a simple expression for $D_{||}$:

$$D_{||} = k_B T / \zeta_{\text{eff}} = k_B T / NP t_{AD} \Lambda$$

which, after substituting into it the typical values for t_{AD} , K_{DA} , and Λ , leads to diffusion constants of the same order of magnitude as those observed in experiments. The simple picture that emerges is thus one in which the friction is mainly due to the motor proteins constantly attaching and detaching from the fiber. This protein friction is much larger than the hydrodynamic friction from the surrounding fluid. A simple model, like the one summarized here, can quantitatively describe the protein friction effect on the phenomenological level. The microscopic details underlying the parameters t_{AD} , K_{DA} , and Λ do not need to be specified for this level of analysis. Thus, for example, the elastic constant Λ can represent bending, stretching, and/or conformational change of the motor protein, of the fiber and/or of their attachment. Such protein friction will play an important role in the model discussed below.

We would like to have a similar description for the motor proteins which can hydrolyse ATP. Therefore, we seek a phenomenological model in which the motors not only attach to and detach from the fiber and change the relative position on the fiber, but also undergo the biochemical transitions of ATP hydrolysis and the release of the hydrolysis products. Such a model is thus a synthesis of the purely mechanical models, in which ATP hydrolysis does not appear explicitly (10, 13), and the biochemical kinetic schemes (19, 23) which do not explicitly include the mechanical events (strain build up, motion, and strain release, etc.).

Four States of a Motor System

Our model regroups all chemical and conformational states of the motor/fiber/nucleotide complex into four "effective" states (Fig. 1). Assuming the existence of only four states is an obvious simplification; one should think rather about each of the states as consisting of an ensemble of substates, with stochastic transitions among them included. This simplification of course is based on the assumption that the transitions among the substates are not the time-limiting steps and do not play a dominant role in the mechanism of the motor action.

In Appendix I, we discuss the model rather generally without specifying which biochemical states are being treated. This allows one to consider various possible relationships between the biochemical and mechanical cycles. In the following, we choose one such relationship to examine (see Fig 1 *b*) what is consistent with the data for actomyosin. We leave the analysis of other possibilities for future studies; it has been recently suggested that the connection between the biochemical and mechanical cycles for kinesin on microtubules may differ from that for actomyosin (24, 29).

The model has one detached state, D, and three attached states, which we denote A1, A2, and A3. In the specific realization of the model which we will study here, the states are (a) D state, in which the motor protein is detached from the fiber, but it binds the nucleotide. This state may in fact in-

clude typically two substates: one with bound ATP and another with bound but hydrolyzed nucleotide ADP. P_i , each with a different conformation of the motor (e.g., "relaxed" and "cocked"); (b) A1 state, in which the motor, with the nucleotide still attached to it, is adsorbed on the fiber. For actomyosin this state is often called "weakly bound"; (c) A2 state, in which the motor remains attached to the fiber and part of hydrolysis products (typically P_i) is released from the complex. This state includes different conformational or elastically deformed substates of the enzyme. The famous "power stroke" of the tight-coupling mechanism happens primarily in this state, i.e., the enzyme does its work mainly due to motion taking place in this state. The motor in the A2 state cannot directly detach from the fiber; the motor must pass through state A3 before detaching; and (d) A3 state, in which the motor protein is in a "rigor" connection with the fiber. In this state all hydrolysis products (i.e., both P_i and ADP) are released, but a new ATP molecule has not yet been bound. This state can differ in conformation from all the conformational substates of state A2; or even it can include itself several distinct conformational substates. In fact, part of the power stroke could in principle be made in this state. (Remark: it is often assumed that the power stroke takes place during a transition between two states of the motor. Note however, that within our definition the A1 and A2 states differ only chemically and the transition between them is too rapid for an important amount of work to be performed. It is rather during the slower relaxation of elastic strains or conformational transitions included in the state A2 that most of the work is done. Of course, all this is a matter of definition—the whole idea of attributing a discrete number of states to the motor/fiber/nucleotide complex is a matter of convention and in fact could be an oversimplification [see below].) It is important to stress that our model does not, and cannot, give any definite predictions concerning detailed structural issues. In fact it can be applied to systems with rather different structures. The main point is that we assume that ATP hydrolysis and/or release of the hydrolysis products induces strains which are then transformed to mechanical work. The strains obviously take place near the motor/fiber interface but it is not crucial for our model (and its conclusions) whether they involve only motors or also the fiber proteins. We have assumed that the release of P_i is the triggering event for the release of strain and it is the presence of the bound ADP which makes the detachment of the motor from the fiber impossible in the A2 state. This need not be true for all motor systems; but these assignments are quite consistent with biochemical and motility assay results for actomyosin. We have also systematically searched for a simpler model of this type with only two or three states which could explain these results but there does not appear to be one (see Appendix V).

Transitions between the States

The transitions between different states are assumed to be stochastic. The origins of this randomness are thermal: for instance, the motor proteins, submerged in a solution at temperature T , are subject to Brownian movements (translations, rotations). Similarly, thermal noise can induce random deformations of the motor or fiber proteins or even collective excitations of fibers such as bending or torsion. This, in turn, affects the binding/unbinding transitions of motors to a fiber,

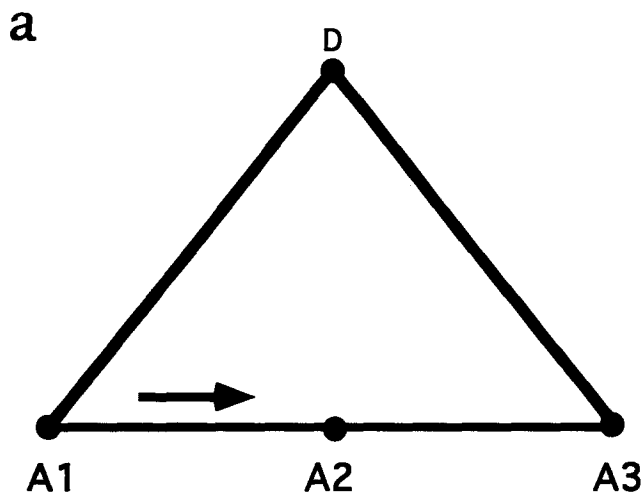


Figure 1. (a) Schematic diagram of the model. The dots represent the four states: detached (*D*) and three attached (*A1*, *A2*, and *A3*). The lines show the allowed transitions, while the arrow indicates the transition where the strain change(s) take place.

ATP hydrolysis, etc. Thus, thermal noise manifests itself also in "biochemical noise", i.e., in the stochastic nature of the biochemical processes involved in free energy transduction (7). Thus our model is for the temporal evolution of the probability distributions $P_j(x,t)$ of the system being in the four possible states (the subscript *j* can take thus one of four values *D*, 1, 2, or 3, the latter denoting *A1*, *A2*, and *A3*).

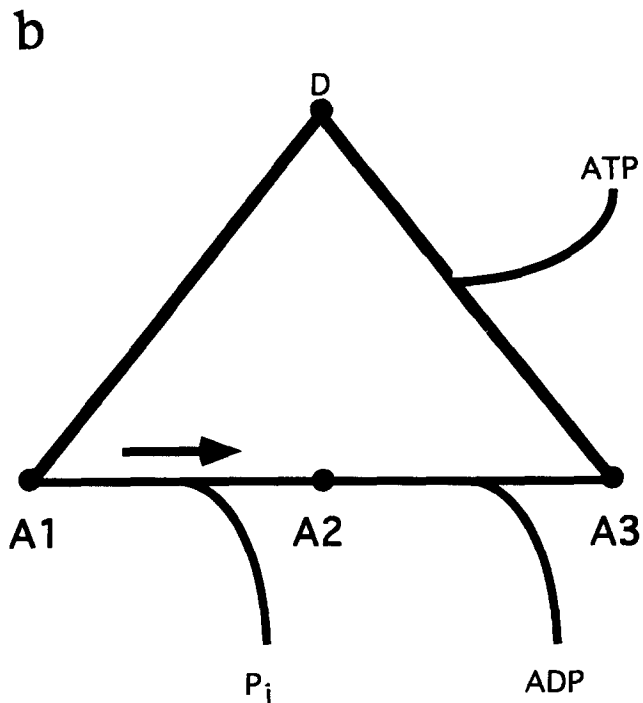


Figure 1. (b) A realization of the model represented in a. The explicit biochemistry is designed to be consistent with acto-myosin data. It is this realization which is chosen for explicit calculations in the present paper.

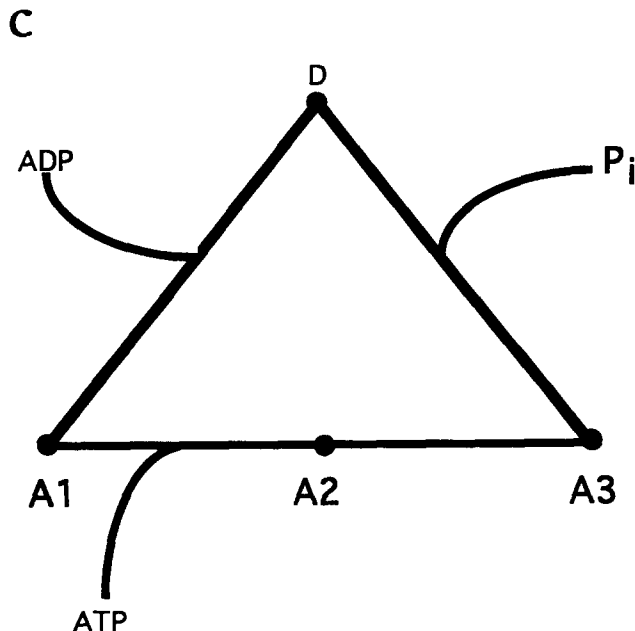


Figure 1. (c) The same formalism as presented here can be applied to models with different biochemical schemes. The diagram shows a scheme suggested by recent results for kinesin. The analysis of this scheme gives similar conclusions as the one presented in this paper (Holy T., S. Leibler, and D. A. Huse, manuscript in preparation).

Here, *x* denotes the strain in the motor/fiber complex at time *t*. The evolution of these probability distributions is governed by stochastic equations (presented in Appendix I) which take into account the motion of the motor and the transitions between the four states.

All the transitions are treated as instantaneous; indeed, the durations of the transitions themselves are much shorter than the characteristic times t_{ij} between the transitions (inverses of the rate constants, k_{ij}). It is also important to remember that the rate of thermal relaxation of a protein in a solution is also much faster than its biochemical rate constants, therefore the system operates at a uniform temperature, *T*. The temperature thus can influence the rates of transitions between different states, but cannot provide a "fuel" for molecular engines, as a difference of temperatures does in Feynman's classical example of a "thermal ratchet" (34). Another important and general statement about the stochastic transition between the different states is that the rates $k_{ij} = t_{ij}^{-1}$ at which they occur are independent of the speed of the motor movement, since the system operates at very low Reynolds numbers (For an example of a model in which the binding probabilities depend on the speed see reference 2). Although these transition rates can in fact depend on the strain *x*, in the simplest version of the model we assume that most of the rates are strain-independent (see section VI and Appendix V for more discussion of this point).

The stochastic transitions in our model are (see Appendix I for the detailed description): (a) detachment $A1 \rightarrow D$, characterized by the rate constant $k_{1D} \equiv t_{1D}^{-1}$. (b) weak attachment $D \rightarrow A1$, with a rate which is the product of k_{1D} , and the equilibrium constant K_{D1} which takes into account the free energy of binding, and a Boltzmann factor incor-

porating the free energy of the elastic deformation when the motor attaches to the fiber with a nonzero strain, x . (c) the transition $A1 \rightarrow A2$, with the rate constant $k_{12} \equiv t_{12}^{-1}$. The release of the phosphate in this transition makes possible the release of the strain accumulated during the hydrolysis of ATP. For simplicity, we assume that the rate of the reverse transition, $A2 \rightarrow A1$, is negligible, thus we take $k_{21} \equiv 0$. The analytic solution of the model by the method we use (see Appendix I) does not work for $k_{21} \neq 0$. Thus in the event that k_{21} is not negligible, one would probably have to resort to numerical solution of the model. (d) transition $A2 \rightarrow A3$ between two attached states, accompanied by the release of the second hydrolysis product, ADP, with the rate constant $k_{23} \equiv t_{23}^{-1}$. We assume that the strain, x , is unchanged by this transition. (e) reverse transition $A3 \rightarrow A2$, with the rate $k_{32} = k_{23}K_{32}$, where K_{32} accounts for the (conformational and chemical) free energy difference between the states $A3$ and $A2$ at fixed strain, x . (f) detachment $A3 \rightarrow D$, provoked by binding of ATP to the motor protein, with rate $k_{3D} \equiv \kappa[\text{ATP}]$. (g) strong attachment $D \rightarrow A3$, with a rate which is the product of k_{3D} , the equilibrium constant $K_{D3} \equiv K/[\text{ATP}]$ which takes into account the free energy of binding, and a Boltzmann factor incorporating the free energy of the elastic deformation when the motor attaches to the fiber with a nonzero strain, x .

III. Stochastic Equations and Steady-State Solutions

Having defined the states of the model and the transitions between these states, we can write a set of stochastic equations which govern the behavior of the motors/fiber system. For this we assume a simple geometry in which a long, stiff fiber can be translated by motors along its axis, thus the movement is purely one-dimensional. We suppose also for the moment that the number of motors, N , is large ($N \gg 1$). The probability distributions $P_j(x,t)$ are thus the averages over many different motor proteins. The strain variable x is supposed continuous; for the sake of simplicity we neglect the fact that the binding sites of the motor proteins to the fiber are discrete, and thus that one should describe them as a periodic rather than a constant binding potential.

The stochastic equations derived in detail in Appendix I describe time evolution of the probability distributions $P_j(x,t)$. They form a generalization of usual ordinary differential equations describing time evolution of the population of reacting chemical species. In fact, the four states of the motors/fiber system can be viewed as different chemical states (species). However, in the present case each state includes a distribution of possible strains, x . This has two important consequences for the stochastic equations: (a) as described above, the terms which describe transitions between different states include some strain-dependent rate constants, e.g., binding constants depending on x ; and (b) there are extra "convection-like" terms which correspond to changes in the strain x of the proteins without changes of their chemical state. The change of x is due to the relative movement of the fiber and thus the stochastic equations describe both the changes in the space of chemical states as well as those in real space.

We can search for steady-state solutions of the stochastic equations for which the probability distributions $P_j(x,t)$ do

not change in time and the fiber moves with a steady speed, v . This is in general a difficult problem which can only be solved with the help of numerical methods. However, if one seeks the integrated probabilities only, $P_j(t) \equiv \int dx P_j(x,t)$, then, when all the rates except the attachment rates are strain-independent, one can transform the equations to a purely algebraic form and obtain simple expressions for P_j as function of the ATP concentration, the rate constants, k_{ij} , and the equilibrium constants, K_{ij} . If, in addition, one integrates the steady-state equations over x dx , then one obtains an analogous set of algebraic equations for the first moments of the probability distributions. This set of equations then allows us also to calculate the average velocity of the fiber, v . We shall discuss now the main quantities obtained in this way from the solutions of the steady-state equations.

Probabilities of Different States

The probabilities P_i depend on the ATP concentration through a simple hyperbolic Michaelis-like law. For large $[\text{ATP}]$, the probability of the "rigor" state, P_3 , becomes arbitrarily small, while the others, P_D , P_1 , and P_2 , saturate at extremum values. Particularly interesting are the two limiting quantities for large $[\text{ATP}]$: P_D^{\max} which measures the fraction of time the motors spend detached from the fiber, and P_2^{\max} , often called the duty ratio, which measures the fraction of time spent by the motors in the working state, $A2$. We obtain the following simple formula for these quantities:

$$P_D^{\max} \equiv \frac{(t_{1D} + t_{12})}{K_{D1}(t_{23} + t_{12}) + t_{1D} + t_{12}} \quad (1)$$

$$P_2^{\max} \equiv \frac{t_{23}K_{D1}}{K_{D1}(t_{23} + t_{12}) + t_{1D} + t_{12}} \quad (2)$$

In the present model these quantities, as well as other properties at large $[\text{ATP}]$, depend only on three rate constants k_{1D} , k_{12} , and k_{23} , and only one equilibrium constant K_{D1} .

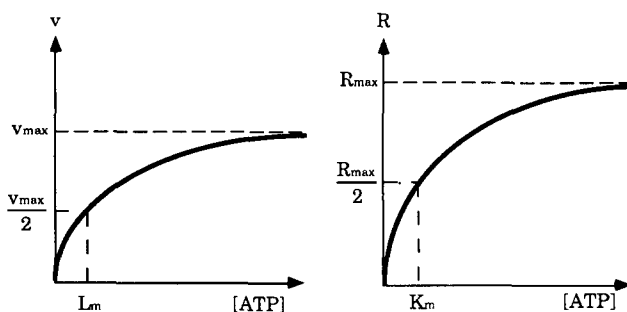


Figure 2. The predicted dependence of the velocity, v , and the ATPase activity, R , on the ATP concentration. The ATPase activity follows the Michaelis law (Eq. A22). The velocity obeys a generalized Michaelis-like law (Eqs. 4 and A19) with L_m playing the role analogous to K_m , the concentration for the half-maximal activity. Note: in general, the ratio

$$\omega \equiv L_m/K_m$$

does not need to be equal to 1.

Average ATPase Activity, R

The probabilities P_i allow us to calculate the rate, R , per motor, of ATP hydrolysis and product release. This quantity obeys a simple Michaelis law (Fig. 2) with the maximum rate for large ATP concentrations equal to:

$$R_{\max} = \frac{K_{D1}}{K_{D1}(t_{23} + t_{12}) + t_{1D} + t_{12}} \quad (3)$$

Average Velocity of the Fiber, v

In the absence of external load, the dependence of the fiber velocity, v , on the ATP concentration is not exactly of Michaelis form (Fig. 2), however, it still has a simple functional form (see Appendix II):

$$v = \delta \frac{[\text{ATP}] + a(\text{ATP})^2}{T_0 + T_1[\text{ATP}] + T_2[\text{ATP}]^2}, \quad (4)$$

which means that, just as with a Michaelis law, the velocity increases linearly for small [ATP] and saturates at a maximal value for large [ATP]:

$$v_{\max} = \frac{\delta a}{T_2} = \frac{\delta t_{23}}{t_{23}^2 + (t_{23} + t_{12})t_{1D}t_{12}/(t_{1D} + t_{12})}. \quad (5)$$

In fact, one can define a Michaelis-like constant, L_m , as the ATP concentration for which the velocity is half-maximal. This constant is analogous, e.g., to K_m for the ATP hydrolysis rate R .

The Ratio of Michaelis (-like) Constants, ω

It is important to notice that the ratio of Michaelis-like constants for the velocity and ATPase activity,

$$\omega \equiv \frac{L_m}{K_m}$$

does not need to be equal to 1 even if one supposes the tight-coupling mechanism for motor proteins. In the present model:

$$\omega \approx \frac{v_{\max}}{\delta R_{\max}} = \frac{t_{23}(t_{1D} + t_{12})[(t_{23} + t_{12}) + (t_{1D} + t_{12})/K_{D1}]}{[t_{23}^2(t_{1D} + t_{12}) + t_{1D}t_{12}(t_{23} + t_{12})]}. \quad (6)$$

If only one motor interacts with the fiber, the velocity curve will generally follow the hydrolysis curve, i.e., $\omega = 1$, however if many motors are present, the velocity v can saturate at much larger ATP concentrations than does the hydrolysis rate, R . Indeed, for large [ATP], the motors can spend a large part of their cycle time, $P_{\text{B}^{\text{ax}}}$, detached, during which the fiber can be moved by other motors which are in the working state A2.

Effective Step Size, Δ

One can define the average distance by which the fiber is moved between two hydrolysis events taking place on one motor. This effective step size,

$$\Delta \equiv \frac{v}{R}$$

is always equal to the molecular step size, δ , for small ATP concentrations, but at large [ATP], we obtain:

$$\Delta \approx \delta \omega, \quad (8)$$

and thus the effective step size can be bigger than the molecular one (2).

"Saturating" Number of Motors, N^*

It is straightforward to generalize the stochastic equations of the model (see Appendix III) to the case of a small number of motors interacting with the fiber. These equations describing for instance the steady state case can provide in particular the dependence of the velocity v on the number of motor proteins interacting with the fiber, N . For large [ATP], we expect that the velocity will increase linearly for small N but then it will saturate at some value $N = N^*$. Such a behavior has been indeed observed in numerical studies of our stochastic equations, but it is in general difficult to obtain analytical solutions to these equations. To estimate N^* , it is sufficient, however, to solve the equations only for $N = 1$, and then use the linear extrapolation towards v_{\max} (given by Eq. 5, for $N \gg 1$, Fig. 3). We then arrive at a surprisingly simple result (Appendix III):

$$N^* \approx \omega \quad (9)$$

This means that for large [ATP] and for a broad range of reasonable time and equilibrium constants, t_{ij} and K_{ij} , the number N^* should be close to the ratio of the two Michaelis (-like) constants!

IV. Porters and Rowers

We are now ready to analyze the results of our model and in particular to try to answer some questions addressed in the Introduction, such as what are the differences between motor proteins and whether one can describe different motors within a single phenomenological picture. A nice feature of the large [ATP] limit is the fact that the observable quantities such as the probabilities P_D and P_2 (Eqs. 1 and 2), the ATPase rate R (Eq. 3), the velocity v (Eq. 5), the ratio ω (Eq. 6), or the number of motors N^* , depend only on three time constants t_{1D} , t_{12} , and t_{23} (provided the value of K_{D1} is fixed). We can thus analyze the model relatively easily in various limiting cases. This is done in Appendix IV, and here we present the main results of such an analysis.

A2 \rightarrow A3 Is Rate Limiting: Porters

When the time constant t_{23} is much larger than t_{1D} and t_{12} , i.e., the rate-limiting step is the transition between the strongly bound state and the rigor state, we obtain from Eqs. 1 and 2:

$$(1 - P_2) \ll 1; P_D \ll 1. \quad (10)$$

This limit corresponds therefore to the case of motors which spend most of the time in the working state and a very small part of the time detached from the fiber. This is adequate for the motors such as cytoplasmic kinesin, which need to transport vesicles along microtubules for long distances, without losing the contact with the fiber. We can call such motor proteins, porters. Eqs. 6 and 9 show that for these proteins:

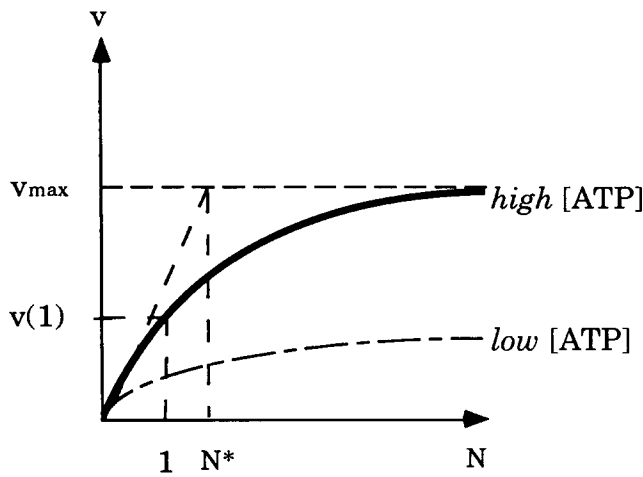


Figure 3. The velocity, v , as a function of the number of active motors interacting with the fiber. For large ATP concentrations the velocity saturates at N of the order of N^* , in general different from 1, whose value can be obtained by a linear extrapolation procedure.

$$N^* \approx \omega \cong 1. \quad (11)$$

This shows that motors which do not detach much from the fiber achieve their maximal speed already when working alone (or in very small groups). Indeed, more enzymes would add to the motor force but they would also increase the (protein) friction. The effective step size is here close to the microscopic one: the relative position of the fiber changes by δ , for each hydrolysis event per motor.

***D*→*A1* or *A1*→*A2* Are Rate Limiting: Two Types of Rowers**

When the time constant t_{1D} is much larger than t_{23} and t_{12} , i.e., the rate-limiting step is the binding to the fiber rather than the release of the hydrolysis products, Eqs. 1 and 2 show that:

$$(1 - P_D) \ll 1; P_2 \ll 1. \quad (12)$$

This limit corresponds thus to motors which spend most of the time detached from the fiber. Most of the attachments result then in the release of the hydrolysis products and the stroke. On the other hand, if it is the time constant t_{12} that is much larger than t_{23} and t_{1D} , i.e., the time-limiting step is the transition *A1*→*A2*, then:

$$(1 - P_1 - P_D) \ll 1; P_2 \ll 1. \quad (13)$$

The motor proteins while “waiting” for the stroke are thus cycling rapidly between the detached (*D*) and weakly attached (*A1*) states. Both situations, 12 and 13, are very favorable from the point of view of minimizing the protein friction in systems where many motors are interacting with the fiber. In fact, the only way large groups of uncorrelated motors can work together without disturbing one another is precisely by detaching often and/or for long periods of time from the fiber. This would be the way to row by a large crew boat without a coxswain—we thus call such motor proteins the rowers, but one should keep in mind that the “rowing” (strokes) takes place here at very low Reynolds numbers,

thus inertial effects are completely absent. Eqs. 6 and 9 show that in both limiting cases:

$$N^* \approx \omega \gg 1, \quad (14)$$

which is the direct result of low protein friction. Most importantly, Eq. 8 then implies that the effective step size, Δ , can be much larger than the microscopic one, δ . The rowers move the fiber for long distances per one hydrolysis event per motor; simply each motor spends much of its time being detached or cycling rapidly between *D* and *A1* states, thus between two strokes of a given motor there is enough time for other motors to move the fiber over distances much larger than δ .

V. Comparison with Motility Assay Results. Examples

Although a lot of progress has been made recently in in vitro motility measurements, one cannot yet make a full comparison of the results of the motility assays with the predictions of quantitative models. For instance, the [ATP] dependence of the speed of the fiber, v , and of the ATPase hydrolysis rate, R , have been measured simultaneously only in very few assays whose precision is too low to quantitatively check the validity of the Michaelis-like law for v (Eq. 4). Clearly, more experiments are needed, however, it seems that at the qualitative level both v and R , measured in the motility assays for myosins/actin and kinesins/microtubule systems, show the dependence on [ATP] consistent with the results of our model. In addition, recent experiments on actomyosin show that the speed v indeed saturates with the increasing number of motor proteins, N . These assays, as well as the assays made for kinesins give a rough estimate of the saturating number of motors, N^* .

Despite these shortcomings, the motility assays provide us with a rough check of the predicted relations between N^* , ω and Δ/δ (Eqs. 8 and 9) for large ATP concentrations, as well as their relations to the probabilities P_i . This is therefore a semiquantitative verification of the classification scheme presented in the last section.

Muscle Myosins on Actin—An Example of Rowers

For muscular myosins the motility assays show that L_m , the ATP concentration at half-maximal velocity, ranges from 50 to 150 μM (5, 16), while that for the ATPase activity, K_m , ranges from 2 to 6 μM . (5, 30). Thus we can estimate that ω is between 10 and 30. The value of N^* is believed to be: $N^* \geq 10$ (31), thus in agreement with Eq. 9. The “duty ratio” P_5 , on the other hand is known to be small, in qualitative agreement with Eqs. 12 and 13 (26). It is interesting to note that the present model predicts (Eq. 8) that for large [ATP] the effective step size for myosin should be 10δ or more, which could be as long as 50–100 nm (12).

Cellular Kinesins on Microtubules—an Example of Porters?

For cellular (bovine brain) kinesin motility assays estimate L_m as $\sim 20 \mu\text{M}$, (9) while K_m for small tubulin concentrations is estimated as 10 μM (4, 17). Thus ω appears to be roughly 2. This seems to be in agreement with Eq. 9, since recent experiments have shown that N^* is 1 or 2 (1). (Note

that these values can vary for different molecules from the kinesin family, e.g., $L_m \cong 40 \mu\text{M}$ for *Drosophila* kinesin and $L_m \cong 120 \mu\text{M}$ for sea urchin kinesin [25]). The probability of being detached, P_D , has been estimated for large [ATP] as $P_D \leq 0.05$ (1, 9) in excellent agreement with Eq. 10. Our model predicts also that the effective step size is of the order of δ , even for large [ATP] concentrations.

This apparent agreement between the results of our model and the motility assays for kinesins strongly suggest that cellular kinesins belong to the class of porters. This is particularly interesting in light of very recent experiments (24) which seem to indicate that the biochemical cycle for kinesins may be somehow different from the actomyosin cycle explicitly adopted in Appendices I and II. It would indicate that the general classification for motors into porters and rowers, as well as relations such as the Eqs. 8 and 9, are quite general and hold for other possible biochemical schemes. We are currently exploring in detail such schemes, in particular those suggested by the kinesin assays.

VI. Conclusions and Perspectives

Some Testable Predictions of the Model

We have achieved the main goal stated in the Introduction, namely, we have obtained a natural classification scheme in which the differences among various motor enzymes can be explained. In addition, this unifying picture correlates the biochemical differences among the motor proteins with the differences among their motility and mechanical properties. The two quoted examples of cellular kinesins and muscular actins show that the scheme proposed here may work even on a (semi-) quantitative level. Of course, there are many more motor proteins for which the predictions of the model could be tested. For instance, for flagellar dynein, it has been shown that L_m is of the order of $100 \mu\text{M}$ (15, 20, 35) while K_m is smaller than $1 \mu\text{M}$ (22), which leads to $\omega > 100$. By measuring the number of flagellar dyneins at which the velocity in the motility assay saturates one could check the general relation (9) predicted by the model: $N^* \approx \omega$. In addition, by measuring simultaneously v and the ATPase rate R one could check another general relation valid for large [ATP]: $\Delta \approx \delta\omega$. Note that $\omega \gg 1$ means that the flagellar dyneins are rowers and therefore $P_2 \ll 1$. This could be checked, at least qualitatively, in the motility assays. Some observations made by EM on flash-frozen preparations suggest indeed that these motors are rarely attached to the microtubules. Similarly, many of the general relations could be checked for other motor proteins. If, in addition, the biochemical constants such as t_{1D} , t_{12} , and t_{23} are known, then the Eqs. 1–14 provide a (highly overdetermined) set of relations which can be used to test the model.

An interesting question would arise if one finds that for a given type of motor some relations are not satisfied. This would mean that at least one of the assumptions of the model does not hold. For instance, we have made the strong simplifying assumption that the time constants t_{1D} , t_{12} , and t_{23} are strain independent. For some systems this assumption may be far from true. Some motor enzymes might in fact be "designed" to be strain sensors in addition to energy transducers, e.g., the kinetochore motors might be used not only to move the chromosomes but also to sense strains in order to position and synchronize the chromosome during the mi-

osis. One could speculate that, if this is the case, the biochemical constants are strain dependent, and then the relations predicted here should be modified. In fact, a simple way to check this is to measure the relation between the applied load, F , and the velocity, v , in the motility assays (e.g., in isotonic conditions). Such experiments could be done, e.g., through the use of optical tweezers. As is shown in Appendix II, our model with strain-independent rates predicts a simple linear dependence of F on v , instead of the nearly hyperbolic law measured in muscles by A. V. Hill (6). It would be very important to verify whether the nonlinear dependence of F on v in muscle originates from the strain dependence of the biochemical constants of actomyosin alone or is connected to the presence of other regulating mechanisms in muscle fibers.

A simple extension of our analysis is to consider mixtures of two different motors moving the same object. Thus one might have two types of kinesin or dynein moving the same microtubule along a substrate or the same bead along a microtubule. A very interesting case would be if the two different motors actually work in opposition, one inducing plus-end directed motion and the other minus-end directed motion. A small concentration of a high duty ratio (high P_2) motor (e.g., a kinesin or a cytoplasmic dynein) could then greatly hinder or even reverse the motion due to a much larger concentration of low duty ratio (low P_2) motors (e.g., flagellar dynein) because the low duty ratio motors spend most of their time in the detached state. Such assays could be quite useful in providing quantitative and direct comparisons between the properties of different motor proteins and can be readily analyzed within the present model.

Let us stress again that the model discussed here does not make microscopic or structural predictions. The model works on a phenomenological level where only the essential states and transition rates are kept explicitly and the microscopic and structural details are not specified. Thus, for example, the microscopic rate-limiting step underlying the transition from state A1 to state A2 may be the release of P_i , or it may be a conformational change in the motor and/or the fiber that may either precede or follow the release of P_i . Similarly, each state kept explicitly in the model may incorporate many microscopic conformational states of the interacting molecules that they visit at random as they are pushed around by the thermal fluctuations of their local environment.

Can One Observe Elementary Steps?

Recent progress in motility assays (14, 32) suggest that measurements of the motion due to one or more motor proteins of sufficiently high spatial and temporal resolution may possibly be able to directly detect the elementary step of an individual motor. However, this will not follow solely from increased resolution, since the steps will be superimposed on the random Brownian motion of the motor and the object (fiber or bead) being moved. Since the amount of work being done per step is not much larger than $k_B T$, the motion due to a single step of the motor is not much larger than those due to random thermal motion. This will make it difficult to separate the signal due to the steps from that due to the Brownian motion. We have numerically simulated the motion of a single motor within our model to see when the steps are readily detected (Maggs, A.C., D. A. Huse, and S. Leib-

ler, unpublished observations). The steps are not simply seen in a graph of position versus time for parameters similar to those expected to apply for myosin. It is only when we reduce the diffusion constant due to the Brownian motion by a factor of five or more from that at ambient temperature that the steps became apparent on such a graph. This suggests that experiments that hope to detect the elementary step should be carefully optimized to reduce the effects of Brownian motion. For example, the connection between the motor or fiber and the object (e.g., the bead) whose position is being measured should be as rigid as possible. The relative motion of interest is only the relative motion of the motor and the fiber. Any additional relative thermal motion between other parts of the assembly will only add to the noise obscuring the signal of interest. Similarly, there may be something to be gained by carefully modeling and optimizing the hydrodynamic properties of the objects that are being moved through the solution.

Appendix I

Deriving the Stochastic Equations

The stochastic transitions in our model are (a) detachment $A1 \rightarrow D$ and $A3 \rightarrow D$, at rates k_{1D} and k_{3D} , respectively. For simplicity we shall assume that the motors relax very quickly after detachment and attain their equilibrium (Boltzmann) strain distribution $P_D(x)$. This is one of the few additional simplifying assumptions which, without affecting the essential features of the physical picture of motor functioning, allow us to solve the equations analytically, rather than numerically, and obtain simple expressions for the measurable quantities such as the speed of the sliding movement. (b) Attachment $D \rightarrow A1$ and $D \rightarrow A3$, with the rates

$$k_{D1} \frac{1}{L} \exp\left(-\frac{\Lambda x^2}{2k_B T}\right) dx$$

and

$$k_{D3} \frac{1}{L} \exp\left(-\frac{\Lambda x^2}{2k_B T}\right) dx,$$

respectively, where x is the strain in the attached state (in dx). We suppose here a simple Hookean law for the energy of protein deformation with elastic constant Λ . The constant

$$L \equiv \sqrt{\frac{2\pi k_B T}{\Lambda}},$$

is the normalization factor for the Boltzmann distribution of strains,

$$\exp\left(-\frac{\Lambda x^2}{2k_B T}\right).$$

(c) Transitions among attached states $A_i \rightarrow A_j$ at rate k_{ij} , where i and j take on adjacent values in the sequence 1, 2, and 3. These transitions represent biochemical changes that may change the strain in the system. Thus we take the transition $A1 \rightarrow A2$ to be from state $A1$ with strain x to state $A2$ with strain $(x - \delta)$. Note that the zero-strain state $x = 0$ is the relaxed state. Thus this transition does not involve motion of the motor, instead its relaxed position has moved due to the biochemical change, and the motor subsequently tries to move towards that new relaxed position, while it resides in states $A2$ and $A3$. For simplicity, we assume that the transitions between states $A2$ and $A3$ do not involve strain changes. To allow the analytic solution of the model shown in the next Appendix the rates k_{ij} among the attached states must be strain independent. If the rate of the strain-changing transition $A1 \rightarrow A2$ is strain independent, then detailed balance would require that the rate of the reverse transition $A2 \rightarrow A1$ depend on strain. Thus to allow the analytic solution we make the additional assumption that this reverse transition does not occur: $k_{21} \equiv 0$. Of course the model can be analyzed for quite general strain-dependent rates and the stochastic equations derived in this Appendix are still valid when the rates are strain-dependent and $k_{21} \neq$

0; however, in that case one would have to resort to numerical methods to solve for the velocity, etc.

Note that at this point we have not specified which biochemical transitions (e.g., binding ATP, releasing hydrolysis products, etc.) are being discussed. This fairly general form of the model can be analyzed for various possible relationships between the mechanical and biochemical cycles. Below we analyze one specific such relationship, which is suggested by data for actomyosin. The essential features that allow an analytic solution of this model are the rapid relaxation of strain in the detached state and the strain independence of the rates for all the transitions between and out of attached states. If these conditions are relaxed (the latter is very likely an over simplification for some systems), then a numerical solution can be performed. This general form of model can, of course, be analyzed for more or fewer states. The reasons why we feel four states is the minimal number to model actomyosin are discussed below in Appendix V.

We are now ready to write the stochastic equations which govern the behavior of the system. We assume a simple geometry in which a long, stiff fiber can be translated by motors along its axis, thus the movement is purely one-dimensional. We suppose also for the moment that the number of motors, N , is large ($N \gg 1$). The probability distributions $P_j(x, t)$ are thus the averages over many different motor proteins. The strain variable x is supposed continuous; for the sake of simplicity we neglect the fact that the binding sites of the motor proteins to the fiber are discrete and thus that one should describe them as a periodic rather than a constant binding potential.

Let us consider for instance the protein motors in the attached state $A2$ with the strain x and let us write the equation for the rate of change of the probability $P_2(x, t)$ of being in state $A2$ with strain x . Within our model the motors may enter state $A2$ either through the transition $A1 \rightarrow A2$, or through the transition $A3 \rightarrow A2$. These two transitions contribute to the rate of change of $P_2(x, t)$ by $k_{12}P_1(x + \delta, t) + k_{32}P_3(x, t)$. Note that since the strain x is defined as the difference between the given conformation and the relaxed (unstrained) one, and since the relaxed conformations in the $A1$ and $A2$ states (towards which the motors tend to evolve) differ by δ , this microscopic "step size" δ appears explicitly in the first term. The motor, on the other hand, can leave the $A2$ state through the $A2 \rightarrow A1$ (if this transition is permitted) and $A2 \rightarrow A3$ transitions, which contributes to

$$\frac{\partial P_2(x, t)}{\partial t}$$

by $-(k_{21} + k_{23})P_2(x, t)$. Finally, the motors could stay in the $A2$ state but change their strain due to the fiber movement. The contribution of such elastic transformation is simply

$$-\frac{dx}{dt} \frac{\partial P_2(x, t)}{\partial x} = -v \frac{\partial P_2(x, t)}{\partial x}.$$

In a similar way, we can construct the full set of stochastic equations for other probability distributions $P_j(x, t)$:

$$\frac{dP_D}{dt} = \int dx (k_{1D}P_1(x, t) + k_{3D}P_3(x, t)) - (k_{D1} + k_{D3})P_D(t) \quad (A1)$$

$$\frac{\partial P_1(x, t)}{\partial t} = -v \frac{\partial P_1(x, t)}{\partial x} + k_{D1} \frac{1}{L} \exp\left(-\frac{\Lambda x^2}{2k_B T}\right) P_D(t) + k_{21}P_2(x - \delta, t) - (k_{12} + k_{1D})P_1(x, t) \quad (A2)$$

$$\frac{\partial P_2(x, t)}{\partial t} = -v \frac{\partial P_2(x, t)}{\partial x} + k_{12}P_1(x + \delta, t) + k_{32}P_3(x, t) - (k_{21} + k_{23})P_2(x, t) \quad (A3)$$

$$\frac{\partial P_3(x, t)}{\partial t} = -v \frac{\partial P_3(x, t)}{\partial x} + k_{23}P_2(x, t) + k_{D3} \frac{1}{L} \exp\left(-\frac{\Lambda x^2}{2k_B T}\right) P_D(t) - (k_{32} + k_{3D})P_3(x, t) \quad (A4)$$

Note that we have written the first equation directly for the integrated probability $P_D(t) \equiv \int dx P_D(x, t)$ since we assumed above that the motors, when detached, relax very quickly to the equilibrium Boltzmann distribution. The Boltzmann factor

$$\frac{1}{L} \exp\left(-\frac{\Lambda x^2}{2k_B T}\right)$$

appearing in the Eqs. A2 and A4 in front of $P_D(t)$ contains the Hooke energy of stretching of the motor protein, characterized by the elastic constant, Λ .

We have examined the steady-state solutions to A1–A4 for the particular case illustrated in Fig. 1 *b*. This biochemical scheme is suggested by data for actomyosin, including the dependence on the ATP concentration [ATP]. Thus we take $k_{D1} = k_{1D}K_{D1}$, where K_{D1} is the equilibrium constant taking into account the free energy of attachment; $k_{21} = 0$, so the strain-changing transition A1→A2 is irreversible; $k_{32} = k_{32}K_{32}$, where K_{32} is the equilibrium constant taking into account the difference in free energy between states A2 and A3 and the concentration of ADP in the solution; $k_{3D} = \kappa[\text{ATP}]$ so the rate-limiting step in the detachment from the fiber of a motor in state A3 is assumed to be the attachment of an ATP to the motor; and $k_{D3} = \kappa K$, so K is the ATP concentration for which $k_{D3} = k_{3D}$. Note we are treating the ATP concentration explicitly, but not the ADP or P_i concentrations; this is for simplicity only. The model can still be solved analytically, at the cost of more complicated expressions, in the same manner as described below with the transition A2→A1 allowed ($k_{21} > 0$) and explicit inclusion of the concentrations of the hydrolysis products.

Appendix II

Solving the Stochastic Equations

An interesting feature of the Eqs. A1–A4 is that one can solve them explicitly for many quantities of interests in the steady-state case (i.e., for the distributions $P_j(x,t)$ which do not change in time: $\partial P_j(x,t)/\partial t \equiv 0$). To do this, we integrate the steady-state equations over the strain variable x and in this way obtain four algebraic equations for the steady-state values of

$$P_j \equiv \int dx P_j(x,t) \quad (j = D, 1, 2, \text{ and } 3): \quad (A5)$$

$$0 = \frac{[\text{ATP}]}{\tau} P_3 + \frac{P_1}{t_{1D}} - \frac{K}{\tau} P_D - \frac{K_{D1}}{t_{1D}} P_D$$

$$0 = \frac{K_{D1}}{t_{1D}} P_D - \frac{P_1}{t_{1D}} - \frac{P_1}{t_{12}} \quad (A6)$$

$$0 = \frac{P_1}{t_{12}} + \frac{K_{32}}{t_{23}} P_3 - \frac{P_2}{t_{23}} \quad (A7)$$

$$0 = \frac{K}{\tau} P_D + \frac{P_2}{t_{23}} - \frac{K_{32}}{t_{23}} P_3 - \frac{[\text{ATP}]}{\tau} P_3, \quad (A8)$$

where we have taken the transition rates given at the end of Appendix I and define $\tau \equiv 1/\kappa$ and $t_{ij} = 1/k_{ij}$ for $i, j = 1, 2, 3$, or D .

Probabilities of Different States

These Eqs. A5–A8 allow us to calculate the steady-state integrated probabilities P_j . They are given by the following simple expressions (with $A \equiv (\tau K_{D1} + t_{1D}K + t_{12}K)$ and $B \equiv K_{D1}(t_{23} + t_{12}) + t_{1D} + t_{12}$):

$$P_D = \frac{(t_{1D} + t_{12})[\text{ATP}]}{A(K_{32} + 1) + B[\text{ATP}]} \quad (A9)$$

$$P_1 = \frac{t_{12}K_{D1}[\text{ATP}]}{A(K_{32} + 1) + B[\text{ATP}]} \quad (A10)$$

$$P_2 = \frac{AK_{32} + t_{23}K_{D1}[\text{ATP}]}{A(K_{32} + 1) + B[\text{ATP}]} \quad (A11)$$

$$P_3 = \frac{AK_{32} + t_{23}K_{D1}[\text{ATP}]}{A(K_{32} + 1) + B[\text{ATP}]} \quad (A12)$$

Note that for large concentrations of ATP the probability of the motor protein being detached from the fiber saturates at:

$$P_D^{\text{max}} \equiv \frac{(t_{1D} + t_{12})}{K_{D1}(t_{23} + t_{12}) + t_{1D} + t_{12}} \quad (A13)$$

while the probability of the motor being in the “working” state A2, saturates at:

$$P_2^{\text{max}} \equiv \frac{t_{23}K_{D1}}{K_{D1}(t_{23} + t_{12}) + t_{1D} + t_{12}} \quad (A14)$$

The probability P_2 of being in the working state is often called the “duty ratio” of motor proteins.

Average Velocity of the Fiber, v

We can also, in a similar way, integrate the steady-state Eqs. A2–A4 after having first multiplied them by x and in this way obtain three algebraic equations for the first moments of the distribution densities in the steady state:

$$P_j X_j \equiv \int dx x P_j(x,t):$$

$$0 = v P_1 - \frac{P_1 X_1}{t_{12}} - \frac{P_1 X_1}{t_{1D}} \quad (A15)$$

$$0 = v P_2 + \frac{P_1 X_1 - P_1 \delta}{t_{12}} + \frac{K_{32}}{t_{23}} P_3 X_3 - \frac{P_2 X_2}{t_{23}} \quad (A16)$$

$$0 = v P_3 + \frac{P_2 X_2}{t_{23}} - \frac{K_{32}}{t_{23}} P_3 X_3 - \frac{[\text{ATP}]}{\tau} P_3 X_3 \quad (A17)$$

For steady motion the total average force on the fiber must vanish. The force components are those coming from strained motors, from hydrodynamic friction and from the load, F , applied to the fiber; thus we have

$$0 = N\Lambda(P_1 X_1 + P_2 X_2 + P_3 X_3) + F + \zeta v, \quad (A18)$$

where ζ is the friction coefficient due to hydrodynamic drag on the fiber or any other object moved by the motors. These equations allow us to obtain not only the first moments of the probability distributions but also the expression for the average velocity of the fiber, v .

Since A15–A18 are linear equations in F and v , they do imply a linear dependence of the velocity, v , on the load, F .

For zero load and neglecting the hydrodynamic friction (compared with the protein friction as described in Section II), the general formula for v as a function of the ATP concentration is of the form:

$$v = \delta \frac{[\text{ATP}] + a[\text{ATP}]^2}{T_0 + T_1[\text{ATP}] + T_2[\text{ATP}]^2} \quad (A19)$$

with

$$a \equiv \frac{t_{23}}{\tau(K_{32} + 1)}, \quad T_0 \equiv \frac{A(K_{32} + 1)}{K_{D1}},$$

$$T_1 \equiv \frac{At_{23}K_{32}}{\tau K_{D1}} + \frac{t_{1D}t_{12}}{(t_{1D} + t_{12})K_{D1}} + t_{23},$$

and

$$T_2 \equiv \frac{1}{\tau(K_{32} + 1)} \left(t_{23}^2 + t_{1D}t_{12} \frac{t_{23} + t_{12}}{t_{1D} + t_{12}} \right).$$

For small concentrations of ATP one obtains a linear dependence:

$$v = \frac{\delta}{T_0} [\text{ATP}] \quad (\text{small ATP}) \quad (A20)$$

while for large ATP concentrations the velocity saturates at its maximum value:

$$v = v_{\text{max}} \equiv \frac{\delta}{T_2} a \quad (\text{large [ATP]}). \quad (A21)$$

Average ATPase Activity, R

The steady-state solutions allow us also to calculate the rate, R , per motor, at which the ATP molecules are hydrolyzed and products released (the ATPase activity):

$$R \equiv \frac{P_1}{t_{12}} = \frac{K_{D1}[ATP]}{A(K_{32} + 1) + B[ATP]} \quad (\text{A22})$$

where we have used Eq. A10. The rate, R , thus obeys a simple Michaelis law, which for small $[ATP]$ reduces to:

$$R = \frac{K_{D1}[ATP]}{A(K_{32} + 1)} = \frac{[ATP]}{T_0} \quad (\text{small } [ATP]), \quad (\text{A23})$$

while for large $[ATP]$ it saturates at the maximum value:

$$R_{\max} \equiv \frac{P_1^{\max}}{t_{12}} = \frac{K_{D1}}{K_{D1}(t_{23} + t_{12}) + t_{1D} + t_{12}} = \frac{P_2^{\max}}{t_{23}} \quad (\text{large } [ATP]). \quad (\text{A24})$$

The Ratio of Michaelis (-like) Constants, ω

For a hyperbolic law, as Eq. A22 for the ATPase rate, R , one usually defines the Michaelis constant, K_m , as the concentration at which R is equal to the half of the maximal rate, R_{\max} . One can similarly define a Michaelis-like constant, L_m , for the velocity v , as the concentration of ATP at which v is equal to the half of the maximal velocity, v_{\max} . One can then introduce a ratio of these two concentrations:

$$\omega \equiv \frac{L_m}{K_m}.$$

This quantity is approximately equal to Eqs. A21 and A24:

$$\omega \approx \frac{v_{\max}}{\delta R_{\max}} = \frac{t_{23}(t_{1D} + t_{12})[(t_{23} + t_{12}) + (t_{1D} + t_{12})/K_{D1}]}{[t_{23}^2(t_{1D} + t_{12}) + t_{1D}t_{12}(t_{23} + t_{12})]}. \quad (\text{A25})$$

Note, that ω , unlike the concentrations, K_m or L_m , does not depend on the binding constant K_{32} nor on τ or K .

Effective Step Size, Δ

In the discussion of the motility assays one often introduces a notion of an effective step size,

$$\Delta \equiv \frac{v}{R},$$

which measures the average distance which the fiber slides in the time corresponding to one ATP hydrolysis event per motor protein. According to Eqs. A19 and A22 for small $[ATP]$ the effective step size is simply δ ,

$$\Delta \approx \delta \quad (\text{small } [ATP]) \quad (\text{A26})$$

while for large ATP concentrations the effective step size saturates at:

$$\Delta \approx \Delta_{\max} = \delta\omega. \quad (\text{large } [ATP]) \quad (\text{A27})$$

Appendix III

A Small Number of Motors

One can generalize the stochastic equations A1–A4 to the case of a small number of motor enzymes, N , interacting with the fiber. In such a case one has to introduce the probabilities $P_N(\{\sigma_i\}, \{x_i\})$, $i = 1 \dots N$, that the i th motor is in the state σ_i (one of the four states $\sigma = D, A1, A2$, or $A3$) with strain x_i . This makes the number of coupled equations large and in general one can solve the set only by numerical methods. Instead of writing down the general stochastic equations let us write these formulae for the simplest case $N = 1$. The equations for $p(\sigma, x) \equiv P_1(\sigma, x)$ are analogous to A1–A4, the main differences being: (a) one cannot neglect the friction of the fluid surrounding the motor and the fiber, since the protein friction is totally absent for $N = 1$. Each stochastic equation will include therefore the friction term:

$$\frac{\Lambda}{\zeta_f} \frac{\partial (xp(\sigma, x))}{\partial x}$$

taking into account the relaxation of strain due to the friction. (b) For consistency one includes the diffusion term

$$D_f \frac{\partial^2 p}{\partial x^2},$$

which describes the thermal motion of the fiber in the fluid, with

$$D_f = \frac{k_B T}{\zeta_f}.$$

We shall calculate the velocity of the fiber in the steady state of zero load. The steady-state integrated probabilities (A9–A12) are independent of N for this model. The analogues of Eqs. A15–A18 here are

$$0 = P_1 X_1 + P_2 X_2 + P_3 X_3 + \frac{\zeta_f v(N=1)}{\Lambda} \quad (\text{A28})$$

$$0 = -\frac{\Lambda}{\zeta_f} P_1 X_1 - \frac{P_1 X_1}{t_{12}} - \frac{P_1 X_1}{t_{1D}} \quad (\text{A29})$$

$$0 = -\frac{\Lambda}{\zeta_f} P_2 X_2 + \frac{P_1 X_1 - P_1 \delta}{t_{12}} + \frac{K_{32}}{t_{23}} P_3 X_3 - \frac{P_2 X_2}{t_{23}} \quad (\text{A30})$$

$$0 = -\frac{\Lambda}{\zeta_f} P_3 X_3 + \frac{P_2 X_2}{t_{23}} - \frac{K_{32}}{t_{23}} P_3 X_3 - \frac{[ATP]}{\tau} P_3 X_3. \quad (\text{A31})$$

For large $[ATP]$ this set of equations leads to:

$$v(N=1) = \frac{\Lambda P_2 X_2}{\zeta_f} = \frac{\Lambda k_{12} P_1 \delta}{\zeta_f (k_{23} + \Lambda/\zeta_f)} \approx k_{12} P_1 \delta = R_{\max} \delta. \quad (\text{A32})$$

We have supposed that $\Lambda/\zeta_f \gg k_{23}$, which is the case for typical values of parameters for motor proteins (27, 28). (This assumption would not be valid, e.g., for very viscous fluids where the friction would stop motors from working well). We can now estimate N^* , by linearly extrapolating $v(N)$: $v(N=1)N^* = v_{\max}$, which leads to Eq. 9: $N^* \approx \omega$.

Appendix IV

Different Limits of the Model

Let us now look systematically at different limits of the model which correspond to different types of motor proteins. We shall consider the limit of large ATP concentration, for which P_3 is negligible. There are two main classes of motors: (1) high duty ratio motors ($P_2 \approx 1$; $P_2 \gg P_1, P_D$). These conditions can be rewritten through Eqs. 1 and 2 as:

$$t_{23} \gg t_{12}, \frac{t_{1D} + t_{12}}{K_{D1}}.$$

They imply that $N^* \approx \omega \gtrsim 1$. The motors are working alone or in small groups, so that there is no strong protein friction. They spend most of their time attached to the fiber and working against the viscous drag. These are porters. (2) small duty ratio motors ($P_2 \ll 1$). Here one can distinguish two main subclasses of such motors: (2a) mostly detached motors ($P_D \approx 1$; $P_D \gg P_2$). This condition signifies that

$$\frac{t_{1D} + t_{12}}{K_{D1}} \gg (t_{23} + t_{12}).$$

If, in addition, $t_{23} \gg t_{12}$, i.e., $P_2 \gg P_1$, then the motors will not detach from the fiber before completing the working period ("stroke"). For these motors, Eqs. 2 and 6 imply

$$N^* \approx \frac{1}{P_2} \gg 1.$$

These are therefore quite efficient rowers, which have low protein friction because they are detached most of the time from the fiber. (2b) mostly attaching/detaching motors ($P_1 \gtrsim P_D$; $P_1 \gg P_2$). These conditions mean that

$$t_{12} \gtrsim \frac{t_{1D} + t_{12}}{K_{D1}}$$

and $t_{23} \ll t_{12}$. If, in addition, $t_{23}^2 \ll t_{12} t_{1D}$, then the motors do complete their strokes,

$$N^* \cong \frac{1}{P_2} \gg 1,$$

and therefore the proteins are efficient rowers. They minimize the protein friction by frequently detaching from and reattaching to the fiber. If, on the other hand, $t_{23}^2 \ll t_{12}t_{1D}$, then the motors do not complete their strokes and are not very efficient.

Appendix V

On the "Minimal" Nature of the Four-State Model

The simple analytic solution of our model is possible when the transition rates out of each of the attached states are all strain-independent. We have examined all three-state models with this restriction and find only one that behaves in a way consistent with the results of motility assays. This three-state model has the ATP adsorbed out of the solution in the transition from the detached state (D) to the weakly-bound state (A1). The A1-A2 transition is as in our four-state model, but the strongly bound state (A2) can detach directly, and there is no A3 state. The behavior of this three-state model is qualitatively the same as our four-state model and in the limit of large ATP concentration is actually identical. However, it has the feature that the detached state does not have a bound nucleotide; the ATP is not acquired until when the motor first attaches to the fiber. This feature seems quite inconsistent with the accepted biochemistry of myosin on actin. If one builds in the feature that it is the acquisition of ATP that initiates detachment of the motor from the fiber after its work is done, then one needs four states within this class of models for the motor to function properly at large ATP concentrations.

If, on the other hand, one allows arbitrarily strain-dependent rates, then one can make models for motors that function with only two states, one attached and one detached. Unfortunately, such models are generally not analytically solvable and must be solved either approximately or numerically. Also, it is important to avoid the situation in which one fits/explains a few existing motility assay curves (such as $v[\text{ATP}]$ or $v[\text{N}]$) with as many adjustable functions, which represent the strain dependence of the rates or binding constants (21).

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