COURSE 1

MECHANICS OF MOTOR PROTEINS

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1 Introduction

Motor proteins are molecular machines that convert the chemical energy derived from the hydrolysis of ATP into mechanical work used to power cellular motility. In addition to specialized motile cells like muscle fibers and cellular processes like cilia, all eukaryotic cells contain motor proteins (Fig. 1). The reason is that eukaryotic cells are large and their cytosols are crowded with filaments and organelles; as a result, diffusion is too slow to efficiently move material from one part of a cell to another (Luby-Phelps *et al.* 1987). Instead, the intracellular transport of organelles such as vesicles, mitochondria, and chromosomes is mediated by motor proteins. These proteins include myosins and dyneins that are relatives of the proteins found in the specialized muscle and ciliated cells, as well as members of a third family of motor proteins, the kinesins, which are distantly related to the myosin family.

The focus of this chapter is on how motor proteins work. How do they move? How much fuel do they consume, and with what efficiency? How do chemical reactions generate force? What is the role of thermal fluctuations? These questions are especially fascinating because motor proteins are unusual machines that do what no manmade machines do–they convert chemical energy to mechanical energy directly, rather than *via* an intermediate such as heat or electrical energy. Tremendous insight into this chemomechanical energy transduction process has come from technical developments over the last ten years that allow single protein molecules to be detected and manipulated. The goal of this review is to provide a framework within which to understand these new observations: how do mechanical, thermal, and chemical forces converge as a molecular motor moves along its filamentous track. For background, the reader is directed to *Molecular Biology of the Cell* (Alberts *et al.* 2002) for an introduction to the biology of cells and molecules, to *Cell Movements* (Bray 2000) for a broad review of cell motility, and to *Mechanics of Motor Proteins and the Cytoplasm* (Howard 2001) for more detailed discussion of the mechanics of molecular motors and the cytoskeleton.

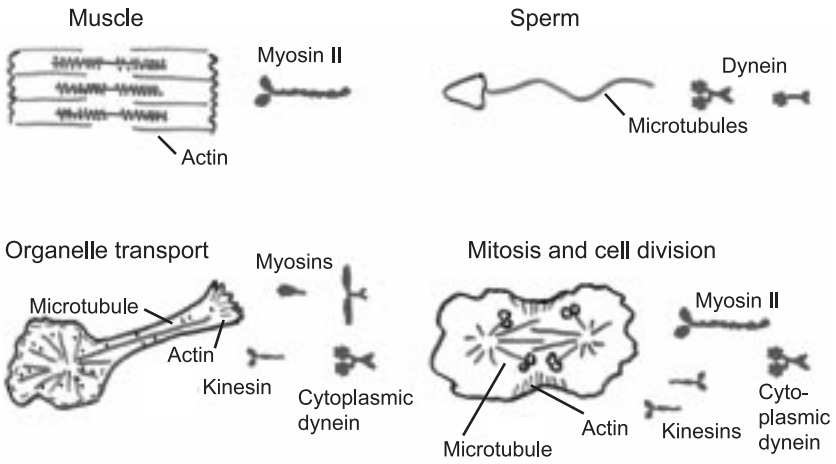

Fig. 1. Motor proteins and cellular motility.

2 Cell motility and motor proteins

The study of motor proteins begins with myosin, which drives the contraction of muscle. Myosin was first isolated as a complex with actin filaments by Kühne (Kühne *et al.* 1864), though it was not until the 1940s that the complex was dissociated into the separate proteins, myosin and actin (Straub 1941-2; Szent-Gyorgyi 1941-2). The discovery of the myosin crossbridges by H.E. Huxley in 1957 (Huxley 1957b, Fig. 2A) provided a molecular basis for the contraction of muscle: the bending or rotation of these crossbridges causes the actin-containing thin filaments to slide relative to the myosin-containing thick filaments, and the sliding of these filaments, in turn, leads to the shortening of the muscle, as had been demonstrated a few years earlier (Huxley & Hanson 1954; Huxley & Niedergerke 1954).

Since its initial discovery, the crossbridge (also called a head) has proven to be central to the mechanism of cell motility. Dynein, which drives the beating of cilia, was identified in the 1963 (Gibbons 1963). The dynein crossbridges cause the adjacent doublet microtubules to slide with respect to each other (Fig. 2B). Because shear between the microtubules at the base of the cilium (*e.g.* near the head of the sperm) is prevented by strong linkages, the sliding is converted into bending of the microtubules along the length of the cilium. In this way the sperm undergoes its snake-like propulsion through solution. Kinesin, which moves organelles along microtubules, was purified in 1985 (Brady 1985; Vale *et al.* 1985). Attached to the cargo at one end, the crossbridges at the other end of the kinesin molecule walk along the surface of the microtubule (Fig. 2C).

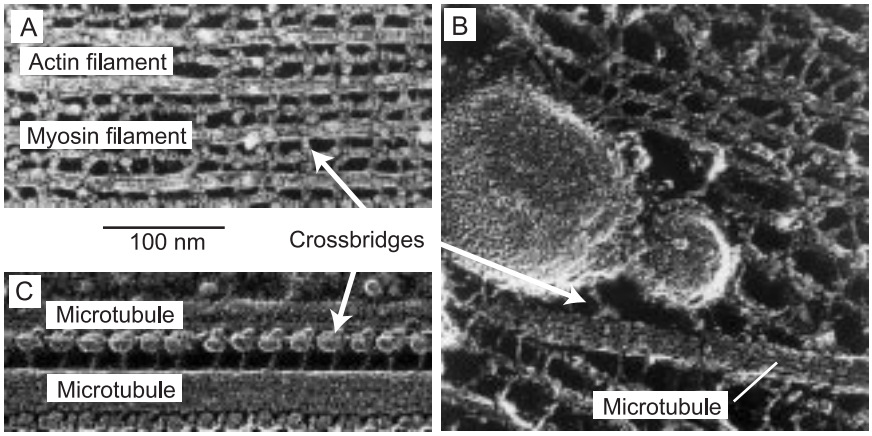

Fig. 2. Crossbridges formed by the motor domains of motor proteins drive motion along cytoskeletal filaments. A. Muscle. Myosin crossbridges protruding from the thick, myosin-containing filament drive the sliding of the thin, actin-containing filaments. B. Sperm. Dynein crossbridges cause the sliding of adjacent microtubules. C. Kinesin crossbridges walk along microtubules carrying organelles.

In all cases studied in detail, the motion of a motor protein is directed. Actin filaments and microtubules are polar structures made of asymmetric protein subunits, and a given motor always moves towards a particular end of the filament. The myosin, dynein and kinesin families have a large number of members. For example, humans have 33 genes which code for proteins of similar amino acid sequence to the heavy chain of muscle myosin (`http://www.gene.ucl.ac.uk/nomenclature/`, `http://www.mrc-lmb.cam.ac.uk/myosin/myosin.html`), and they have 21 dynein heavy-chain genes and 45 kinesin genes (Miki *et al.* 2001; `http://www.blocks.fhcrc.org/ kinesin/index.html`). Interestingly, different myosins go in different directions along actin filaments and different kinesins go in different directions along microtubules. This is important because the orientation of actin filaments and microtubules in cells is tightly controlled: thus, by using differently directed motors cells are able to move cargoes from one part of the cell to another (and back) in order to organize the cells internal structure.

3 Motility assays

The study of motor proteins was revolutionized by the development of *in vitro* motility assays in which the motility of purified motor proteins along purified cytoskeletal filaments is reconstituted in cell-free conditions.

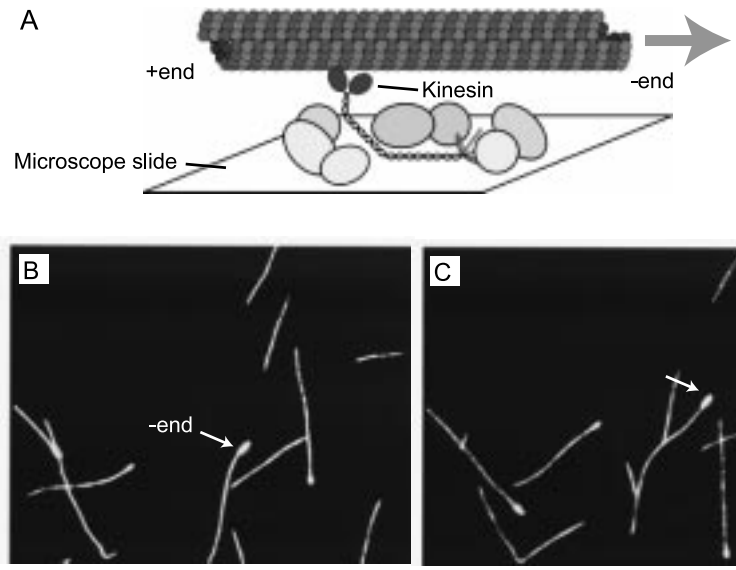

Fig. 3. A Motility assay in which the motor is bound to a surface and the filament is observed to glide over the surface in the presence of ATP. B, C. Fluorescently labeled microtubules gliding across a kinesin-coated surface (15 s between frames). The arrowed microtubule has a bright mark on its slowly growing end (called the minus end): this end leads, showing that kinesin is a minus-end-directed motor.

An important milestone in this development was the visualization of fluorescent beads coated with purified myosin moving along actin cables in the cytoplasm of the alga *Nitella* (Sheetz & Spudich 1983). This was quickly followed by the first completely reconstituted assay in which motor-coated beads were shown to move along oriented filaments made from purified actin that had been bound to the surface of a microscope slide (Spudich *et al.* 1985). Though "threads" of actin and myosin had been known to contract in the presence of ATP (Szent-Gyorgyi 1941), this contraction was very slow. The significance of the new findings was that they proved that myosin (together with actin) was sufficient to produce movement at rates consistent with the speeds of muscle contraction and cell motility.

There are two geometries used in *in vitro* motility assays: the gliding assay and the bead assay. In the gliding assay, the motors themselves are fixed to the substrate, and the filaments are observed under a light microscope as they diffuse down from solution, attach to, and glide along the motor-coated surface (Fig. 3) in the presence of ATP (Fig. 4). In the bead assay, filaments are fixed to a substrate, such as a microscope slide, and

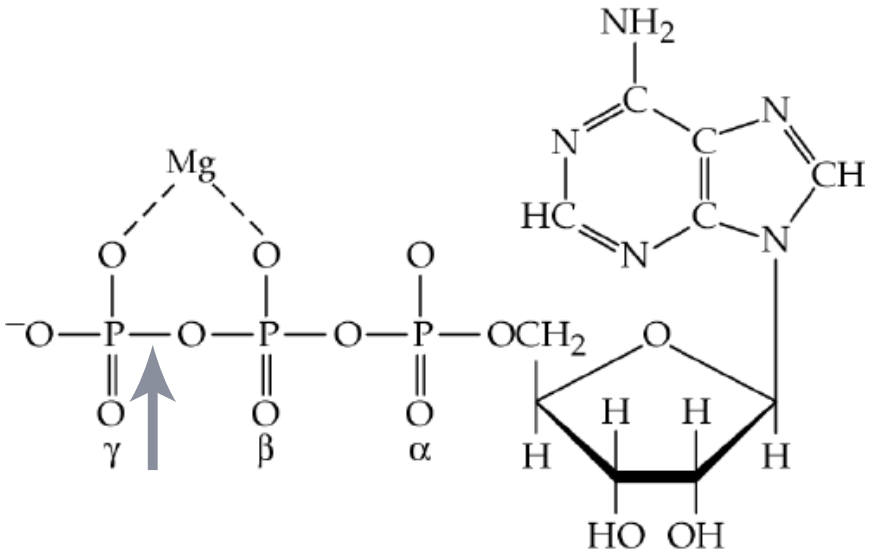

Fig. 4. The hydrolysis of the gamma-phosphate bond (arrowed) of ATP can be summarized by the following reaction: ATP $\leftrightarrow$ ADP + Pi

$$\Delta G = \Delta G_0 - kT \ln \frac{[\mathrm{ATP}]_c}{[\mathrm{ADP}]_c[\mathrm{P_i}]_c} \qquad \Delta G_0 = \ln \left[\frac{[\mathrm{ATP}]_{eq}}{[\mathrm{ADP}]_{eq}[\mathrm{P_i}]_{eq}} \right] = -54 \times 10^{-21} \text{ J}$$

where the subscript c refers to cellular concentrations and the subscript eq refers to equilibrium concentrations. In cells, the reaction is very far from equilibrium with typical concentrations [ATP] = 1 mM, [ADP] = 0.01 mM and [Pi] = 1 mM; this makes the free energy very large and negative $\Delta G \cong -100 \times 10^{-21}$ J.

motors are attached to small plastic or glass beads with typical diameters of 1 μm. The motions can be recorded and the speed measured by tracking the centroid of the bead or the leading edge of the filament (see Scholey 1993 for detailed methods). There is good overall agreement between the speed of a motor protein *in vitro* and the speed of the cellular motion that is attributed to the motor (Table 1).

4 Single-molecules assays

The progress of research on motor proteins has gone hand-in-hand with increases in the sensitivity of light microscope techniques. Single protein molecules can now be observed and manipulated. A crucial development was the visualization of individual actin filaments by darkfield microscopy (Nagashima & Asakura 1980). This was followed by visualization of micro-

Table 1. Motor speeds *in vivo* and *in vitro*.

Motor	Speed[a] *in vivo* (nm/s)	Speed[b] *in vitro* (nm/s)	ATPase[c] (s^{-1})	Function
Myosins				
1. Myosin IB	?	200	6	Amoeboid motility, hair cell adaptation
2. Myosin II	6000	8000	20	Fast skeletal muscle contraction
3. Myosin II	200	250	1.2	Smooth muscle contraction
4. Myosin V	200	350	5	Vesicle transport
5. Myosin VI	ND	–58	0.8	Vesicle transport?
6. Myosin XI	60 000	60 000	ND	Cytoplasmic streaming
Dyneins				
7. Axonemal	–7000	–4500	10	Sperm and cilial motility
8. Cytoplasmic	–1100	–1250	2	Retrograde axonal transport, mitosis, transport in flagella
Kinesins				
9. Conventional	1800	840	44	Anterograde axonal transport
10. Nkin	800	1800	78	Transport of secretory vesicles
11. Unc104/KIF	690	1200	110	Transport of synaptic vesicle precursors and mitochondria
12. Fla10/KinII	2000	400	ND	Transport in flagella, axons, melanocytes
13. BimC/Eg5	18	60	2	Mitosis and meiosis
14. Ncd	ND	–90	1	Meiosis and mitosis

tubules by differential interference contrast microscopy (Allen *et al.* 1981) and actin filaments by fluorescence microscopy (Yanagida *et al.* 1984). Further refinement of the motility assays led to detection of movement by single motor molecules (Howard *et al.* 1989). With improved fluorescence sensitivity, it was even possible to image individual fluorescently labeled motors (Funatsu *et al.* 1995) (rather than the much larger filaments), and to watch the motors individually while they move along filaments (Vale *et al.* 1996; Yajima *et al.* 2002). The combination of these assays with increasingly sophisticated optical and mechanical techniques such as optical tweezers (Svoboda *et al.* 1993) has allowed measurement of the stepwise movement of motors along their filaments (Fig. 5) and the measurement of the force generated by a single motor protein (Fig. 11).

Single-molecule mechanical and optical techniques are now being applied to many biochemical processes mediated by other molecular machines; these

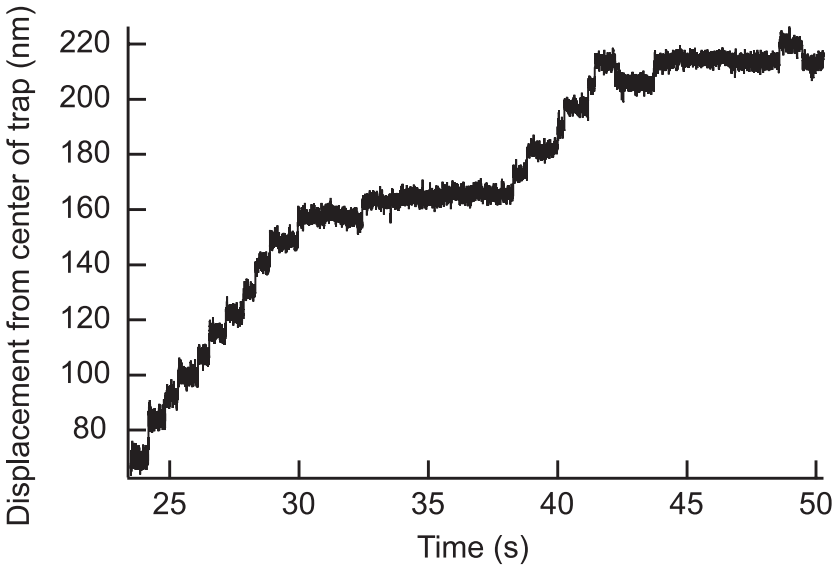

Fig. 5. A rat kinesin molecule taking 8 nm steps along a microtubule at low ATP concentration. (Courtesy of Nick Carter and Rob Cross).

include ATP synthesis (Noji *et al.* 1997), DNA transcription (Wang *et al.* 1998), DNA replication (Wuite *et al.* 2000), The folding of individual proteins (Deniz *et al.* 2000) and RNA (Liphardt *et al.* 2001) can also be followed. The techniques can even be used to record from molecules on the surfaces of intact cells (Sako *et al.* 2000; Benoit *et al.* 2000; Schutz *et al.* 2000) and recordings deep inside cells should soon be possible. Thus single-molecule techniques are becoming to cell biology what the patch clamp technique and single ion-channel recordings are to neurobiology (Neher & Sakmann 1976; Sakmann & Neher 1995).

5 Atomic structures

The structural and physical basis for motility has been placed on a firm foundation by the solution of the atomic structures of actin (Kabsch *et al.* 1990) and myosin (Rayment *et al.* 1993) and of tubulin (Lowe and Amos, 1998; Nogales *et al.* 1998) and kinesin (Kull *et al.* 1996). By fitting the atomic structures into electron micrographs, atomic models of the actin filament (Holmes *et al.* 1990) and the microtubule (Nogales *et al.* 1999) have been built. There are also reasonable guesses for how the motors dock to these filaments (*e.g.* Fig. 6).

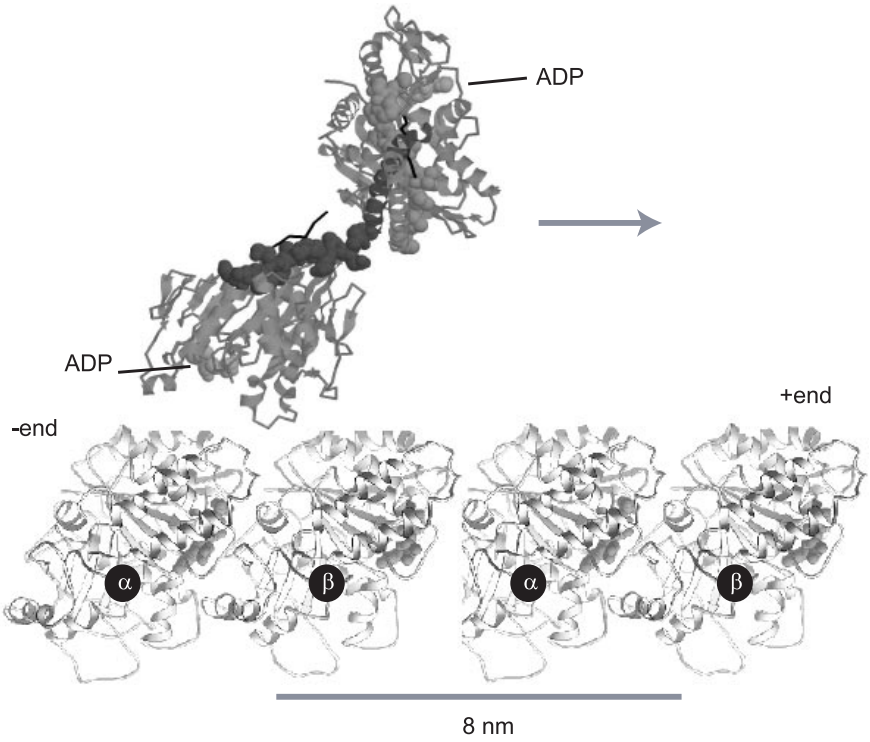

Fig. 6. Kinesin docked to the microtubule. Kinesin has two identical heads joined by the dimerization domain (dark). Each head binds nucleotide, which was ADP in these crystallization conditions. The microtubule is composed of dimers of the closely-related α and β tubulins. The dimers associated "head-to-tail" to form a polar structure that has the β-subunit at the plus end.

The atomic structures have brought many key questions into focus. For example, how do small changes associated with the hydrolysis of ATP (on the order of a few Angströms) lead to protein conformational changes on the order of several nanometers? What determines the directionality of a motor protein? The detailed answers to these problems will require many additional atomic structures: the motors complexed with their filaments, and the motors with different nucleotides bound to them (*e.g.* ATP, ADP and P_i, ADP and no nucleotide). But this will be very difficult, and even when solved, these structures will provide only static pictures, with no kinetic or energetic information: photographing an internal combustion engine at top dead center and at the bottom of the down stroke does not explain how it works. In the following sections I will address some of physical and ther-

modynamic questions which are essential to answer in order to understand how conformational changes driven by ATP hydrolysis might generate force and produce directed motion.

6 Proteins as machines

The structural and single-molecule results reinforce the concept of proteins as machines (Alberts 1998). According to this view, a molecular motor is an assembly of mechanical parts–springs, levers, swivels and latches–that move in a coordinated fashion as ATP is hydrolyzed. How does such a molecular machine move in response to a internal and external forces? The answer depends on the Reynolds number, which determines the pattern of fluid flow around a moving object. The Reynolds number is equal to

$$Re = \frac{\rho L \nu}{\eta} \tag{1}$$

where ρ is the density of the liquid, L is the characteristic length of the object (in the direction of the flow), ν is the speed of the movement relative to the fluid, and η is the viscosity. Note that the Reynolds number is dimensionless, and its physical meaning is that it is the ratio of the inertial and the viscous forces. For proteins in aqueous solution ρ 10^3 kg/m^3, L 10 nm, ν 1 m/s (corresponding to 1 nm per ns, which is on the order of the fastest global conformational changes of proteins) and η 10^{-3} Pa.s. This makes Re 10^{-2} (and even less for slower motions). A Reynolds number much less than one means that the inertial forces can be neglected and that the motion is highly overdamped: when subject to a force, a protein will creep into a new conformation rather than undergo oscillations. The time constant of the motion is γ/κ, where γ is the drag coefficient ($\sim \eta L$ according to Stokes law) and κ is the stiffness of the protein.

To get a feeling for how proteins move, imagine that the size of a protein were increased by a factor of 10^7, so that a 10-nm-diameter protein became a mechanical device of diameter 100 mm, fitting nicely in the palm of one's hand (Fig. 7). Now the density and rigidity (Young's modulus) of protein is similar to that of plastic or Plexiglas, so that we can consider that our device is built of plastic (Howard 2001). If the viscosity of the fluid bathing the device is increased by the same factor of 10^7 (by putting it in honey, for example), then the ratio of the inertial to the viscous forces will the same for both the protein and the macroscopic device. The Reynolds number will be unchanged (see Howard 2001 for the detailed argument) and the pattern of fluid flow will be preserved, just scaled in size. However, to deform the plastic device to the same relative extent as the protein will require a much larger force because the device has a much greater cross-sectional area: whereas a force of only 1 pN might be needed to induce a

Fig. 7. Comparison of scales between a motor protein (left) and a toy car (right).

Quantity	Motor protein	Macroscopic device
Dimension	10 nm	100 mm
Material (Youngs modulus)	Amino acids (2 GPa)	Plastic (2 GPa)
Solution (viscosity)	Aqueous (1 mPas)	Honey (10 kPas)
Speed	1 m/s	1 m/s
Reynolds number	0.01	0.01
Time constant	100 ns	1 s
Force	1 pN	100 N
Energy	$1{-}100 \times 10^{-21}$ J	1–100 J

protein conformational change of 1 nm (corresponding to a strain of 10%), a force of 100 N, corresponding to a weight of 10 kg, would be required to produce the same strain in the plastic device. In response to the respective forces, the protein and the mechanical device will move at the same initial speed, but because the protein conformational change is so much smaller, the relaxation of the protein will be complete in much less time: a relaxation that took an almost imperceptible 100 ns for the protein will take a leisurely 1 s for the device. The work done scales with the volume, so the free energy corresponding to the hydrolysis of one molecule of ATP scales to ~ 100 J.

7 Chemical forces

In addition to mechanical forces, proteins are subject to chemical forces. By chemical forces, we mean the forces that arise from the formation or breakage of intermolecular bonds. For example, consider what happens when a protein first comes in contact with another molecule: as energetically favorable contacts are made, the protein may become stretched or distorted from its equilibrium conformation. Chemical forces could also arise from changes in bound ligands: the cleavage of ATP (Fig. 4) will relieve stresses within the protein that had been built up when the ATP bound. If a protein can adopt two different structures, the binding of a ligand or the change in

a bound ligand could preferentially stabilize one of these structures and therefore change the chemical equilibrium between the structures. In this way, we imagine that a chemical change produces a local distortion that in turn pushes the protein into a new low-energy conformation.

To understand how protein machines work, it is essential to understand how proteins move in response to these chemical forces. Just as a chemical force might cause a protein to move in one direction, an external mechanical force might cause the protein to move in the opposite direction. For example, the binding of a ligand might stabilize the closure of a cleft, whereas an external tensile force might stabilize the opening of the cleft; as a result, the mechanical force is expected to oppose the binding of the ligand. Thus mechanical forces can oppose chemical reactions and conversely, chemical reactions can oppose mechanical ones. If the chemical force is strong enough, the chemical reaction will proceed even in the presence of a mechanical load: in this case we say that the reaction generates force.

8 Effect of force on chemical equilibria

The influence of a mechanical force on the chemical equilibrium between two (or more) structural states of a protein can be calculated with the aid of Boltzmanns law. If the difference between two structural states is purely translational–*i.e.* if state M_2 corresponds to a movement through a distance Δx with respect to state M_1 as occurs when a motor moves along a filament against a constant force–then the difference in free energy is $\Delta G = -F \cdot \Delta x$, where F is the magnitude of the force *in the direction* of the translation. If the length of a molecule changes by a distance Δx as a result of a conformational change, then the difference in free energy is

$$\Delta G \cong \Delta G^0 - F\Delta x \tag{2}$$

where F is the tension across the molecule and ΔG^0 is the free energy difference in the absence of tension. The equality is exact if the molecule is composed of rigid domains that undergo relative translation, or if the two structural states have equal stiffness. Application of Boltzmanns law shows that at equilibrium

$$\frac{[M_2]}{[M_1]} = \exp\left[-\frac{\Delta G}{kT}\right] \cong \exp\left[-\frac{\Delta G^0 - F\Delta x}{kT}\right] = k^0_{\rm eq} \exp\left[\frac{F\Delta x}{kT}\right] \tag{3}$$

where $K^0_{\rm eq}$ is the equilibrium constant in the absence of the force. *The crucial point is that an external force will couple to a structural change if the structural change is associated with a length change in the direction of the force.* If the change in length of a molecule is 4 nm, then a force of

1 pN will change the free energy by 4 pN.nm $\cong kT$, the unit of thermal energy where k is Boltzmanns constant and T is absolute temperate. According to equation (3), this will lead to an e-fold change in the ratio of the concentrations. Because protein conformational changes are measured in nanometers, and energies range from 1 kT to 25 kT (ATP hydrolysis) (Fig. 4 legend), it is expected that relevant biological forces will be on the scale of piconewtons (10^{-12} N).

An expression analogous to equation (2) holds for the effect of voltage on membrane proteins (Hille 1992). If a structural change of a membrane protein such as an ion channel is associated with the movement of a charge, Δq, across the electric field of the membrane, then the energy difference between the open and closed states will include a term $V\Delta q$, and this makes the opening sensitive to the voltage, V, across the membrane (Fig. 8). The openings of the voltage-dependent Na and K channels that underlie the action potentials in neurons are strongly voltage dependent: classic experiments by Hodgkin and Huxley showed that the ratio of open probability to closed probability increased approximately e-fold per 4 mV (Hodgkin 1964). This indicates that the opening of each channel is associated with the movement of about six electronic charges across the membrane ($\Delta q = kT/V \cong 6e$, where e is the charge on the electron). The predicted movement of these electronic charges has been directly measured as a nonlinear capacitance of the membrane (Armstrong & Bezanilla 1974). Protein conformational changes are sensitive to many other "generalized" forces including membrane tension, osmotic pressure, hydrostatic pressure, and temperature. Sensitivity to these forces requires that conjugate structural changes occur in the protein, respectively area, solute accessible volume, water accessible volume and entropy (Howard 2001).

The chemical analogy to force and voltage is chemical potential, a measure of the free energy change associated with a molecular reaction. For example, if a ligand at concentration L preferentially binds to one state of a protein over another, then the difference in free energy between the two states is equal to $kT \ln L \cdot \Delta n$, where $kT \ln L$ is the chemical potential of the ligand and Δn is the number of ligand-binding sites on the protein. If a protein is subject to a combination of mechanical forces, electrical forces and chemical forces, then the free energies will add, allowing one to calculate how physical and chemical forces trade off against each other under equilibrium conditions.

9 Effect of force on the rates of chemical reactions

To understand how force might influence the kinetics of protein reactions is more difficult. Proteins are very complex structures, and a full description

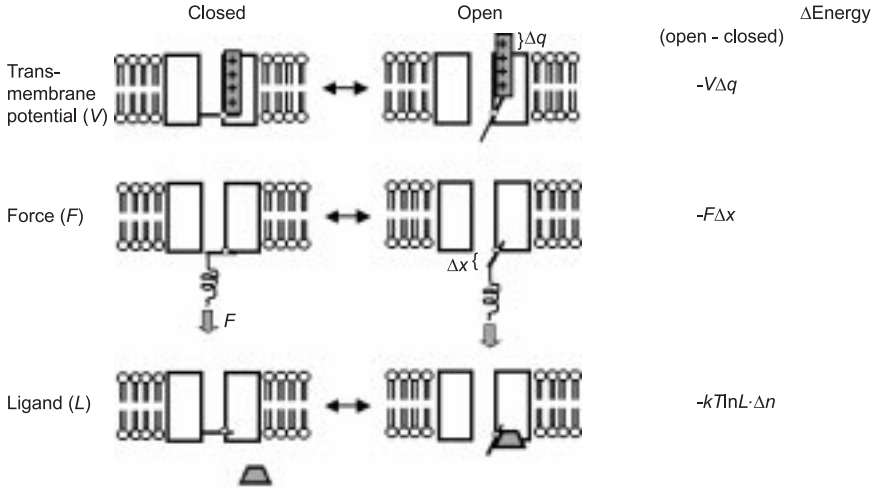

Fig. 8. Effects of generalized forces on protein conformations. In this example an ion channel sitting in a membrane can be either open or closed depending on the position of the gate. If the gate is coupled to a movement of charge (top), then the channels probability of being open will depend on the membrane potential. If it is coupled to a vertical movement (middle), then the probability will depend on a vertical force. And if the opening of the gate is coupled to the binding of a small molecule (bottom), then the probability will depend on that molecules concentration ($\ln L$) and the number of binding sites (Δn).

of the transition from one structure to another would require following the trajectories of each of the amino acids. The problem is even more difficult because it expected that there are a huge number of different pathways from one structure to another, and so a full description would require the enumeration of all the different pathways, together with their probabilities. This is simply not possible at present given that we do not even understand how a protein folds into even one structure. Thus we need a simple model for the kinetics of protein reactions.

The simplest model for a chemical change between two reactants M_1 and M_2 is a first order process:

$$M_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} M_2 \qquad \frac{\mathrm{d}[\mathrm{M}_1]}{\mathrm{d}t} = -k_1[\mathrm{M}_1] + k_{-1}[\mathrm{M}_2]. \tag{4}$$

This reaction is said to obey first-order kinetics because the rate of change depends linearly on the concentration of the species. The constants of proportionality, k_1 and k_{-1} are called rate constants and they have units of s^{-1}.

Many protein reactions have successfully been described by one first order processes (*e.g.* McManus *et al.* 1988), though some reactions may not be describable in this way (Austin *et al.* 1975).

Almost inherent within the concept of a first order reaction is that it occurs *via* a high-energy intermediate, called a transition or activated state. If the transition state has a free energy, $G_{\rm a}$, then the rate constant is for the transition is equal to

$$k_1 = A \exp\left[-\frac{\Delta G_{\rm a1}}{kT}\right] \qquad \Delta G_{\rm a1} = G_{\rm a} - G_1 \tag{5}$$

where A is a constant called the frequency factor. A similar expression holds for the reverse reaction so that the ratio,

$$\frac{k_1}{k_{-1}} = \exp\left[-\frac{\Delta G}{kT}\right] = \frac{[{\rm M}_2]}{[{\rm M}_1]} \tag{6}$$

accords with Boltzmanns law at equilibrium.

The activated-state concept makes specific predictions of how rate constants depend on external force. If the protein structures are very rigid and the transitions ${\rm M}_1 \rightarrow {\rm M}_{\rm a} \rightarrow {\rm M}_2$ are associated with displacements x_1, x_a, and x_2 *in the direction of the force*, F, then the energies of states will be decreased by Fx_1, $Fx_{\rm a}$ and Fx_2 respectively. This implies that

$$k_1 \equiv A \exp\left[-\frac{\Delta G_{\rm a1} - F\Delta x_{\rm a1}}{kT}\right] = k_1^0 \exp\left[\frac{F\Delta x_{\rm a1}}{kT}\right]$$

$$\Delta G_{\rm a1} = G_{\rm a} - G_1 \qquad \Delta x_{\rm a1} = x_{\rm a} - x_1. \tag{7}$$

An analogous expression holds for k_{-1}. Note that the ratio of the forward and reverse rate constants must give the correct force dependence for the equilibrium (Eq. (3)).

A useful way of thinking about the effect of force on the reaction rates is that it tilts the free energy diagram of the reaction (Fig. 9). If the displacement of the activated state is intermediate between the initial and final states ($x_1 < x_{\rm a} < x_2$), then a negative external force (a load) will slow the reaction, whereas a positive external force (a push) will accelerate the reaction. However, if $x_{\rm a} = x_1$–*i.e.* if the transition state is reactant-like–then force will have has little effect on the forward rate constant. On the other hand, if $x_{\rm a} = x_2$–*i.e.* if the transition state is product-like–then the force will have little effect on the reverse rate constant. If the displacement of the activated state is not intermediate between the initial and final states, it is even possible that a load could increase the forward rate constant (if $x_{\rm a} < x_1$), though in this case the backward rate would be increased even more.

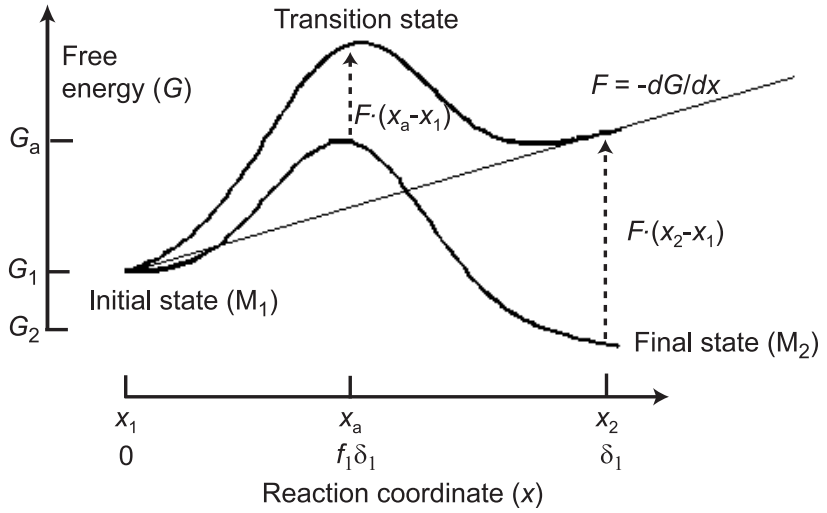

Fig. 9. Transition -state concept. A high energy barrier limits the rate of the transitions between the initial and final states of a chemical reaction.

10 Absolute rate theories

To predict the absolute rate of a biochemical reaction, a more detailed theory is needed in order to estimate the frequency factor A. Two such detailed theories are the Eyring rate theory and the Kramers rate theory. Both require that the reaction coordinate, the parameter that measures the progression of the reaction, be specified. If a protein changes overall length as a result of the $M_1 \rightarrow M_2$ transition, then we could make length the reaction coordinate, though, many other reaction coordinates are possible; indeed, the distance between any two atoms that move relative to one another during the reaction could be used as a reaction coordinate. If the protein is subject to a force, then the natural reaction coordinate is the length of the protein in the direction of the force.

The Eyring rate theory was originally introduced in the 1930s to describe reactions between small molecules such as the bimolecular reaction $2ClO \leftrightarrow Cl_2 + O_2$. The theory assumes that the reaction occurs *via* a transition state which is in equilibrium with the reactants. The transition state breaks down to the products when one of its molecular vibrations becomes a translation (Eyring & Eyring 1963; Moore 1972; Atkins 1986). The rate is

$$k_{\mathrm{E}} = \varepsilon \frac{kT}{h} \exp\left[-\frac{\Delta G_{\mathrm{a1}}}{kT}\right] \qquad \Delta G_{\mathrm{a1}} = G_{\mathrm{a}} - G_1 \tag{8}$$

where $\varepsilon \sim 1$ is an efficiency factor (equal to the probability of making the transition when in the transition state), and the frequency factor, kT/h, is equal to $\sim 6 \times 10^{12}$ s^{-1}, where h is the Planck constant, and $G_{\rm a}$ is the free energy of the activated state (ignoring with the vibrational degree of freedom that breaks down). According to the Eyring equation, the absolute rate is the frequency factor reduced by the exponential term: assuming that the efficiency is unity, a reaction with a rate constant of 10^3 s^{-1} would have an activation free energy of 22 kT. The Eyring theory proved to be a better model than collision theory to describe the rates of small-molecule reactions in the gas phase. It might also apply to covalent changes of proteins and their ligands. But there is no reason to think that it would apply to global conformational changes of proteins in aqueous solution where there are many degrees of freedom and the motions are expected to be highly overdamped.

Kramers had a different view of the passage through the transition state (Kramers 1940). He assumed that the transition corresponded to a diffusive motion of a particle out of a potential well. This might correspond to the thermal fluctuation of two protein domains held together by a flexible region. Let the (reduced) mass be m, the damping equal γ, and the spring constant of the well be κ. If the motion is underdamped, then the Kramers expression for the rate is

$$k = \frac{\omega_0}{2\pi} \exp\left(-\frac{\Delta G_{\rm a1}}{kT}\right) \tag{9}$$

where $\omega_0 = \sqrt{\kappa/m}$ is the resonance frequency associated with the potential well. This is a generalization of the Eyring expression which holds for quantum mechanical and non-quantum cases (Haenggi *et al.* 1990). If the motion is overdamped, the rate is

$$k = \frac{1}{2\pi\tau^*} \exp\left(-\frac{\Delta G_{\rm a1}}{kT}\right) \tag{10}$$

where τ^* is the geometric mean of the damping time constant in the well (γ/κ) and the damping time constant at the top of the barrier (γ/κ^* where κ^* is the negative of the second derivative at the top of the energy barrier). Note that in the overdamped case, the pre-exponential factor depends on the shape of the energy barrier because the efficiency of the reaction (probability of making the transition) depends on the shape. If the peak is precipitous–if there is an absorbing boundary at the top of a harmonic well–then the rate constant in the overdamped case is

$$k_{\rm K} = \frac{1}{\pi\tau}\sqrt{\frac{\Delta G_{\rm a1}}{kT}} \exp\left[-\frac{\Delta G_{\rm a1}}{kT}\right] \cdot \tag{11}$$

Because global conformational changes of proteins are expected to be overdamped (Howard 2001), the overdamped equations are more appropriate, and it is for the overdamped result that the Kramers paper is most widely appreciated (Haenggi *et al.* 1990). According to the overdamped Kramers rate theory, the frequency factor is approximately equal to the inverse of the relaxation time, $\tau = \kappa/\gamma$ where κ is the stiffness of the protein and γ is the damping from the fluid (the other terms are close to unity). This makes intuitive sense: we can think of the protein sampling a different energy level every τ seconds, because τ is the time over which the protein's shape becomes statistically uncorrelated. The protein can react only when it attains the energy of the transition state, and the probability of this occurring is proportional to $\exp(-\Delta G_{\mathrm{a1}}/kT)$.

The Eyring and Kramers rate theories represent two extreme views of the mechanism of global conformational changes of proteins. In the Eyring model, the transition state is like the initial state. A sudden, local chemical change (such as the binding of a ligand or the chemical change in a bound ligand) creates a highly strained protein which subsequently relaxes into a new stable conformation. In the overdamped Kramers model, the transition state lies towards the final state on the reaction coordinate: after the protein has diffused into the transition state, the reaction proceeds and locks the conformational change in. The Kramers model is a "foot-in-door" mechanism, in the which the foot plays the role of the chemical change that prevents the opening from being reversed. Obviously these are two extreme cases, and in general the transition state could be anywhere in between the initial and final states (or even outside). The important point of all this is that the various mechanisms can be distinguished: *in the Eyring model the forward rate is independent of force whereas in the Kramers model the forward rate (and perhaps the reverse rate as well) will depend on force.* Thus force dependence offers a way to tell whether a transition is chemical in the sense that it is reached by localized fluctuations, or whether the transition is physical in the sense that it is reached by global spatial fluctuations. The latter, Kramers-type mechanisms have been termed a thermal ratchet mechanism (see below).

11 Role of thermal fluctuations in motor reactions

From a physical viewpoint, we expect thermal fluctuations to play crucial roles in the motor reaction because thermal forces at the molecular scale are large compared to the average directed forces that motor proteins generate. For example, an unconstrained protein will diffuse through a distance equal to its own size, $\sim$ 10 nm, in tens of microseconds; this is three orders of magnitude faster than the duration of the ATP hydrolysis cycle, which is

typically 10 to 100 ms (motors typically hydrolyze 10–100 ATP per second while they are moving, Table 1). Indeed, it is their noisy, diffusive environment that distinguishes molecular machines from the man-made machines of our everyday world. From a chemical viewpoint, we also expect thermal fluctuations to play crucial roles: all chemical reactions require thermal energy in order for a molecule to enter the transition state (see last section), and the chemistry of the hydrolysis reaction is no exception. The challenge is to merge these two views.

An extreme model is that movement of a motor to its next binding site on the filament is purely diffusive, and the role of ATP hydrolysis is to somehow rectify diffusion so that motion in the wrong direction is blocked (Braxton 1988; Braxton & Yount 1989; Vale & Oosawa 1990). This is reminiscent of the pawl and ratchet discussed by Feynman (Feynman *et al.* 1963): if a pawl (the motor) and ratchet (filament) are at different temperatures then they can do work without violating the second law of thermodynamics. But motor proteins cannot literally be heat engines because the diffusion of heat is so rapid over molecular dimensions that thermal gradients will dissipate within picoseconds, much faster than the timescale of the biochemical reactions (Howard 2001). Instead of being driven by temperature differences like heat engines, motors are driven by chemical reactions (ATP hydrolysis) that are out of equilibrium.

These ratchet ideas have inspired a number of papers in the physics literature that explore the fundamental requirements of directed motility (Ajdari & Prost 1992; Magnasco 1993; Rousselet *et al.* 1994; Astumian & Bier 1994; Zhou & Chen 1996). Some of the physical models are very specific, and fail to explain how real motor proteins move. For example, Astumian and Bier proposed a mechanism in which a motor alternates between times when it diffuses along a filament and times when it is subject to an asymmetric energy profile (a ratchet). However, such a purely diffusive, thermal ratchet model fails in two respects. First, the maximum force that it can generate against viscous loads is small, only 2 kT/δ, where δ is the distance between binding sites (Hunt *et al.* 1994). But for kinesin, which has $\delta = 8$ nm, this force is only 1 pN, much less than the measured value of 4 to 5 pN (Hunt *et al.* 1994). And second, because the motor diffuses in the right direction only half the time, it is expected that two molecules of ATP would be hydrolyzed for each forward step. But for kinesin, there is one step per ATP hydrolyzed (Coy *et al.* 1999; Iwatani *et al.* 1999). While they may fail in some specific cases, the physical models have never-the-less been very important because they have clearly defined the two conditions that are necessary for directed motion: spatial asymmetry (a ratchet), which for motor proteins arises from their stereospecific binding to polar filaments; and temporal asymmetry which arises from an out-of-equilibrium chemical

reaction (*e.g.* ATP hydrolysis).

A less extreme model is that only part of the motor diffuses to the next binding site, rather than the whole motor. In other words, the motor is elastic, like a spring, and picks up mechanical energy *via* thermal fluctuations; once it is "cocked" it can then bind to the filament and generate force. This was the picture in the original A.F. Huxley model of muscle contraction (Huxley 1957a), and it forms the basis of other ratchet models (Cordova *et al.* 1992). If the cocking of the spring requires a global conformation change, then the diffusion time will be limited by the stiffness of the spring and the damping from the fluid (as well as possible internal damping within the protein). This a Kramers-type mechanism. The Huxley model was abandoned in the 1970s by (Huxley & Simmons 1971; Eisenberg & Hill 1978; Eisenberg *et al.* 1980) in favor of a mechanism in which a local conformational change in the nucleotide-binding pocket drives the subsequent global conformational change. This is an Eyring-type mechanism. The Kramers-type mechanism was abandoned because it was argued that it would take too long for myosin to pick up an appropriate amount of mechanical energy by diffusion; but if a more reasonable mechanical efficiency of muscle is assumed ($\sim$ 50%) then the diffusion time is not prohibitive (Hunt *et al.* 1994).

Thermal energy always plays a crucial role in chemical reactions. In the local, Eyring-type model, thermal fluctuations are still needed to get the molecule into the transition state for the localized structural change. The advantage of the local mechanism is that it is faster: because a short lever is stiffer than a longer one and because the damping on a small domain is less than the damping on a large domain, high-energy states can be reached much more quickly through localized conformational changes. For example, if the lever ratio is 10, then a local conformational change occurs 1000-times faster than a global change (Howard 2001). The extent to which force-generating protein conformational changes are due to diffusive global conformational changes that are locked in by chemical changes, or to localized chemical changes that drive global changes must be determined experimentally by measuring the dependence of the rates on force.

12 A Mechanochemical model for kinesin

To illustrate how these concepts apply to motor proteins, I present a model for the chemomechanical cycle of kinesin. Structural, biochemical and biophysical experiments suggest that the two heads of kinesin alternate in their binding to the microtubule, first one head leading and then the other (Fig. 10, Schief & Howard 2001, but see Hua *et al.* 2002). For simplicity, we assume that the ADP and P_i concentrations are zero (as is approximately

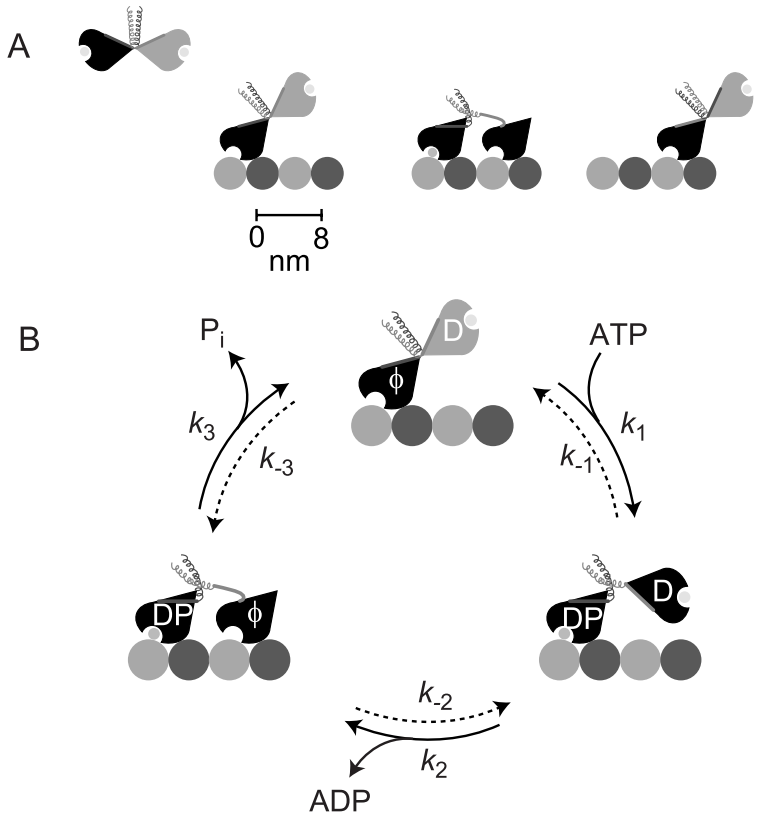

Fig. 10. Hand-over-hand model for kinesin. T = ATP, D = ADP, Pi = phosphate, ϕ = no nucleotide.

the case in the *in vitro* assays). This gives the following kinetic equation

$$\mathrm{M} \overset{\overset{k_1 T}{\leftarrow}}{\underset{k_{-1}}{\rightarrow}} \mathrm{M \cdot D \cdot P} \xrightarrow{k_2} \mathrm{M \cdot P} \xrightarrow{k_3} \mathrm{M} \tag{12}$$

where M symbolizes the motor, T the ATP, D the ADP, and P the phosphate ion.

The solution to this equation is

$$\nu = \delta \cdot k_{\mathrm{ATP}\alpha\sigma\varepsilon} = \delta \cdot k_{\mathrm{cat}} \frac{[\mathrm{T}]}{K_{\mathrm{M}} + [\mathrm{T}]} \quad k_{\mathrm{cat}} = \frac{k_2 k_3}{k_2 + k_3} \quad K_{\mathrm{M}} = \frac{k_3(k_2 + k_{-1})}{k_1(k_2 + k_3)} \tag{13}$$

where ν is the average speed of movement, k_{ATPase} is the ATPase rate, and δ is the distance per ATP (8 nm for kinesin).

In accordance with our discussion above, we model the force-dependence by assuming that the transitions between states are associated with structural changes through distances $\delta_i = x_{i+1} - x_i$, so that the rates depend on a *load*, F, in the opposite direction as δ_i, according to

$$k_{+i} = k^0_{+i} \exp\left[-f_i \frac{F\delta_i}{kT}\right]$$
$$k_{-i} = k^0_{-i} \exp\left[(1-f_i)\frac{F\delta_i}{kT}\right] \qquad (14)$$

where f_i is the location of the transition state as a fraction of the distance between the two states E_i and E_{i+1}, and k^0_{+i} and k^0_{-i} are the rate constants in the absence of load (Fig. 10). Note that $\delta_1 + \delta_2 + \delta_3 = 8$ nm, the step size. Normally we think that f_i lies between 0 and 1, but this is not an absolute requirement. For the hand-over-hand model, we make the simple assumption that all $f_i = 1$, meaning that the transition state is displaced all the way towards the final state. This puts all the force sensitivity in the forward step, like a Kramers-type mechanism. The solid curves in Figure 11 are generated with $\delta_1 = 1$ nm, $\delta_2 = 1$ nm, $\delta_3 = 6$ nm; $k^0_1 = 100\ \mu\mathrm{M}^{-1}.\mathrm{s}^{-1}$, $k^0_{-i} = 3000\ \mathrm{s}^{-1}$, $k^0_2 = 105\ \mathrm{s}^{-1}$, and $k^0_3 = 5000\ \mathrm{s}^{-1}$.

13 Conclusions and outlook

The agreement between the model curve and the experimental data in Figure 11 shows that the ideas presented in this review are plausible: the motor reaction can be described as a small number of first order chemical reactions in which force effect the rate constants through a Boltzmann-type exponential prefactor. However, the picture is very incomplete. What is the reaction coordinate for a molecular motor? How does diffusion and thermal agitation drive a protein's moving parts during the transition from one structural state to another? There are clearly many conceptual questions to be addressed, in addition to the practical ones. It is likely that ideas from protein folding will prove useful, and that experiments using single-molecule techniques will become increasingly useful for probing the motor reaction.

Study of the mechanics of motor proteins has given us a deeper understanding of biological force generation, and in particular how mechanical, chemical, and thermal forces act on proteins. Because conformational changes of proteins, RNA, and DNA are driven by the mutual action of forces, it is likely that the principles learned from the interactions of motors with their filaments should have wide application to other macromolecular machines.

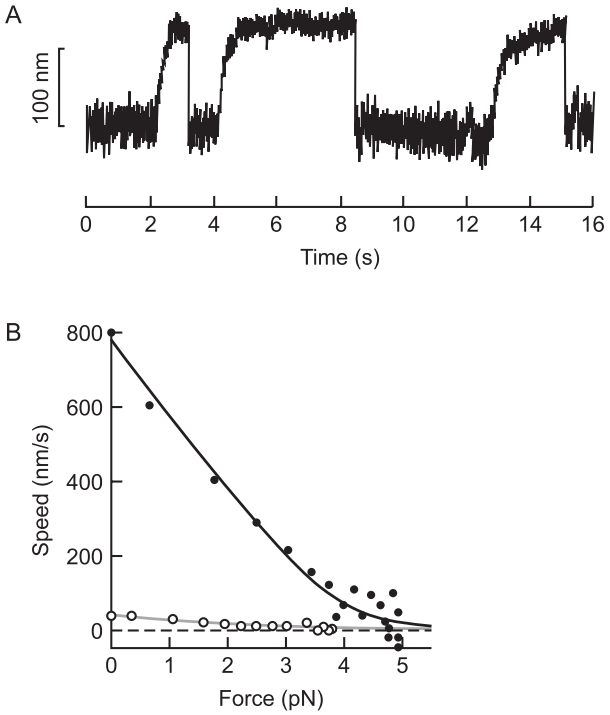

Fig. 11. A. Three examples of a kinesin molecule walking along a microtubule at a high ATP concentration. The molecule is pulling against a flexible glass fiber and stalls when a maximum force of ~ 5 pN is reached. B. The speed as a function of the force at high (filled circles) and low (open circles) ATP concentration. The curves correspond to the model in the text.

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