Forces Required of Kinesin during Processive Transport through Cytoplasm

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ABSTRACT   The purpose of this paper is to deduce whether the maximum force, steplike movement, and rate of ATP consumption of kinesin, as measured in buffer, are sufficient for the task of fast transport of vesicles in cells. Our results show that moving a 200-nm vesicle in viscoelastic COS7 cytoplasm, with the same steps as observed for kinesin-driven beads in buffer, required a maximum force of 16 pN and work per step of 1 ± 0.7 ATP, if the drag force was assumed to decrease to zero between steps. In buffer, kinesin can develop a force of 6–7 pN while consuming 1 ATP/step, comparable to the required values. As an alternative to assuming that the force vanishes between steps, the measured COS7 viscoelasticity was extrapolated to zero frequency by a numerical fit. The force required to move the bead then exceeded 75 pN at all times and peaked briefly to 92 pN, well beyond the measured capabilities of a single kinesin in buffer. The work per step increased to 7 ± 5 ATP, greatly exceeding the energy available to a single motor.

INTRODUCTION

The motor protein kinesin transports organelles within cells. Especially within neurons, where transport distances along axons can be large, it has long been conjectured that the work of transport presents a significant energy cost to the cell. Although considerable progress has recently been made in understanding the force, velocity, and energy coupling as kinesin drags a latex bead along microtubules in solution, no quantitative connections have been made to the forces and work of fast transport in cells.

A single kinesin molecule in buffer can generate a steady force of no more than 7.5 pN while dragging an attached bead up the potential well of an optical trap (Svoboda and Block, 1994; Kojima et al., 1997; Visscher et al., 1999). When the constraining force is less than the stall force, a single kinesin can drag the bead at an average velocity of 800 nm/s in buffer. If the position of the latex bead is measured more carefully, it is found that kinesin moves along the microtubule in abrupt 8-nm steps, with each step coupled to the hydrolysis of 1 ATP. To achieve the observed time-averaged bead velocity of 800 nm/s, a single kinesin must carry out 100 such steps per second. Each step takes a mere 50 μs in buffer (Nishiyama et al., 2001). Thus, a kinesin motor and its vesicle load in buffer are stationary 99.5% of the time, but when they move, their instantaneous velocity briefly exceeds 100,000 nm/s. Work is done only during the brief intervals when the bead moves.

In cells, the time-averaged velocity of fast axonal transport is 800–4500 nm/s (Howard 2001). However, kinesin molecules pulling a vesicle in a cell face a very different load. In the buffer-filled trap, the load is almost purely elastic, whereas in a cell, the load is almost purely viscous. The effect of viscous load has been explored in gliding assays (Hunt et al., 1994). The mobility decreases from 1.2 μm/s in buffer to 0.2–0.5 μm/s in an increasingly viscous mixture of dextran, Ficoll, and trypsin inhibitor. The limiting average value of the force generated by kinesin in the viscous medium is 4.0–5.2 pN. Because the position of the microtubule was determined at intervals of 0.1–1.0 s, the individual steps were not resolved.

Recently, high-resolution optical tracking of the Brownian motion of intracellular particles has enabled researchers to measure, for the first time, the complex viscoelastic modulus $G^*(\omega)$ within a living cell over a broad frequency range (Yamada et al., 2000). The modulus was determined for $0.5 \leq \omega \leq 30,000$ rad/s in a kidney epithelial cell line, COS7; the tracked particles were endogenous lipid droplets with radius 130–250 nm. The method used to determine G^* from Brownian motion has been extensively tested (Mason et al., 2000).

The purpose of this paper is to calculate the drag force and work required to move a spherical vesicle within a cell from the measurements of G^* in COS7 and the measurements of kinesin motion in an optical trap. It is assumed that kinesin moves in cytoplasm with the same quick steps as in an optical trap.

FORCE AND WORK IN A NEWTONIAN FLUID

In a Newtonian fluid at low Reynolds number, the drag force on a sphere moving with steady velocity is given by the well-known Stokes' formula, $F = 6\pi a \eta v$, where a is the radius of the sphere and η is the viscosity of the fluid. The
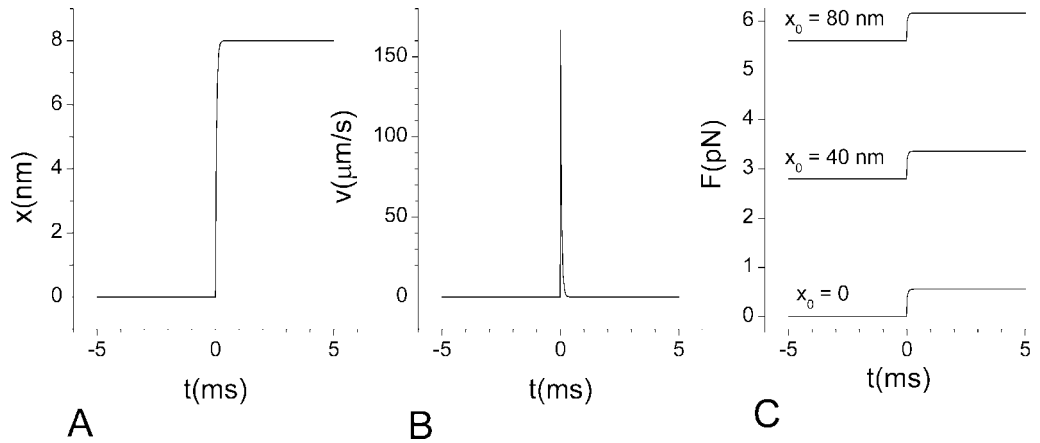
FIGURE 1 Dynamics of kinesin during a single step in an optical trap, according to the experimental results of Nishiyama et al. (2001). (*A*) *x*(*t*) according to Eq. 2. (*B*) *v*(*t*). (*C*) The force that kinesin must exert on the bead for offset $x_0 = 0$, 40, and 80 nm from the center of the trap.

work done by an external force in moving the sphere a distance *L* is then given by

$$W = \int_0^L F \, dx = \int_0^T Fv \, dt = 6\pi\eta a \int_0^T v^2 \, dt, \quad (1)$$

where *T* is the time required to travel distance *L*. For a sphere with $a = 100$ nm moving at a constant 800 nm/s in water ($\eta = 0.001$ Pa·s) the work per 8-nm displacement is 0.012 pN·nm. In vitro, kinesin consumes 1 ATP per step (Schnitzer and Block, 1997; Coy et al., 1999). Because the hydrolysis of 1 ATP releases approximately 100 pN·nm of energy (Howard, 2001), only a negligible fraction of the available energy is required to drag the sphere at constant velocity in buffer. To understand the drag force and work required to move organelles within cells, we need to modify these results, because η in cells is many orders of magnitude larger than in water. In addition, cytoplasm is shear thinning and viscoelastic.

TIME-DEPENDENT VELOCITY AND FORCE FOR KINESIN IN AN OPTICAL TRAP

Latex spheres in buffer moved by single kinesin motors advance with quick, 8-nm jumps rather than steadily (Svoboda et al., 1993; Coppin et al., 1996; Visscher et al., 1999; Nishiyama et al., 2001). The individual steps follow the relation

$$x(t) = x_0 + L(1 - e^{-t/\tau}), \quad (2)$$

with $\tau = 47$ μs and $L = 8.7 \pm 0.7$ nm for cargo spheres of radius $a = 100$ nm (Nishiyama et al., 2001). The velocity corresponding to this displacement is

$$v(t) = 0 \qquad \text{for} \quad t < 0$$
$$v(t) = v_0 e^{-t/\tau} = (L/\tau)e^{-t/\tau} \quad \text{for} \quad t \geq 0. \quad (3)$$

Graphs of *x*(*t*) and *v*(*t*) from Eqs. 2 and 3, and the corresponding force for different values of x_0, are shown in Fig. 1, A–C.

The peak velocity v_0 is 1.7×10^5 nm/s, about 200 times the average speed; nevertheless, the Reynolds number is less than 10^{-6}. In a Newtonian fluid, *W* is proportional to v^2 (Eq. 1), so quick steps are expected to require more energy than a steady pull over the same distance and same time interval. Indeed, for the same bead in water, the work done against viscous drag by a quick step is 1.25 pN·nm. This is about 100 times the work required to move the same sphere with steady *v*, but still less than 2% of the energy available from 1 ATP.

Nishiyama et al. (2001) observed that the 8-nm step consists of two sequential steps of 4 nm, with time constants of <25 and 40 μs, in quick succession. However, the time interval between the two substeps could not be determined with certainty. We therefore choose to use in our modeling their better-defined parameters for a single 8-nm step.

The calculation of the drag force requires values for $\tilde{v}(\omega)$, the Fourier transform of the bead velocity *v*(*t*). To eliminate the infinite acceleration of the sphere at $t = 0$ in Eq. 3, we replaced the leading edge discontinuity with a linear velocity ramp over 12.5 μs, a time interval consistent with the finite bandwidth of Yanagida's measurements. The resultant $\tilde{v}(\omega)$ extends from $\omega = 0$ to $\omega = 4 \times 10^5$ s^{-1} (Fig. 2). Viscoelastic data are needed over the same range.

VISCOELASTICITY OF CYTOPLASM

Stokes' Law and Eq. 1 are inappropriate for the analysis of the drag force and work required to move vesicles in cells because cytoplasm is shear-thinning and elastic, so energy can be stored and recovered from the medium during each step. For small stresses and strains, the time-dependent
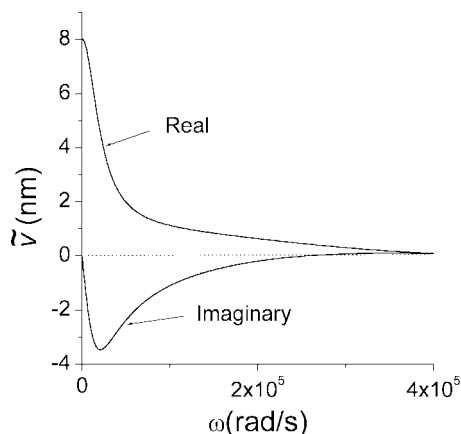
FIGURE 2 The real and imaginary parts of $\tilde{v}(\omega)$ for the softened Yanagida velocity function (Eq. A1).

stress $\sigma(t)$ is a convolution of the shear modulus $G(t)$ with the rate of strain $\dot{\varepsilon}(t)$ (Tschoegl, 1989)

$$\sigma(t) = \int_0^t G(t - u) \frac{d\varepsilon}{du}\, du. \tag{4}$$

If the fluid is subjected to periodic strain excitation, $\varepsilon = \varepsilon_0 e^{i\omega t}$, the stress is also periodic: $\sigma(t) \equiv \sigma(\omega) = G^*(\omega)\varepsilon(\omega)$, where $G^*(\omega)$ is the complex modulus. It is customary to split G^* into real and imaginary parts: $G^* = G' + iG''$.

Alternatively, it is often useful to relate the stress to the rate of strain rather than the strain, so that

$$\sigma(\omega) = \eta^*(\omega)\dot{\varepsilon}(\omega) = i\omega\eta^*(\omega)\varepsilon(\omega), \tag{5}$$

where $\eta^*(\omega)$ is the complex viscosity (Tschoegl, 1989). The complex viscosity can also be written in terms of a viscous part η' and an elastic part η'': $\eta^* = \eta' - i\eta''$. Absorption of energy occurs through G'' and η'; storage of energy occurs through G' and η''.

It can be shown that $\eta^*(\omega)$ is the Fourier transform of the shear modulus (Tschoegl, 1989),

$$\eta^*(\omega) = \tilde{G}(\omega) = \frac{G^*(\omega)}{i\omega} = \int_{-\infty}^{\infty} G(t)e^{-i\omega t}\, dt. \tag{6}$$

This leads to the relations $\eta' = G''/\omega$ and $\eta'' = G'/\omega$.

Recent determinations of G' and G'' by laser tracking microrheology are particularly promising for the current problem because the frequency range of the measurements is large, the probe is endogenous, and the probe is comparable in size to many vesicles. Laser tracking microrheology has been used to track spherical lipid storage granules of radius 130–250 nm in a kidney epithelial cell line, COS7 (Yamada et al., 2000). Figure 3 shows the real and imaginary parts of G^* over the frequency range 0.5–3×10^4 radians/s in the lamellar region of the COS7 cells (Yamada
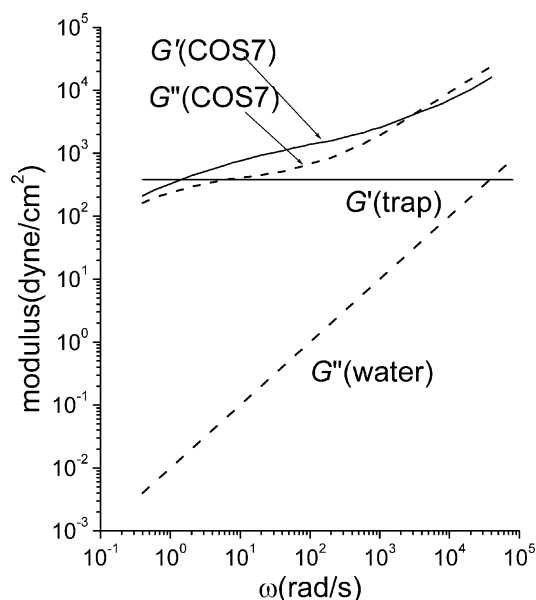

FIGURE 3 The storage modulus G' and loss modulus G'' for COS7 cells and for a buffer-filled optical trap. The COS7 data were measured in the lamellar region of the cells, using laser tracking microrheology (Yamada et al., 2000). The trap is characterized as a harmonic potential with spring constant $k = 0.071$ dyne/cm (Nishiyama et al., 2001), which results in a frequency-independent storage modulus. Buffer in the trap provides a frequency-dependent absorption modulus with a slope of 1.0.

et al., 2000). The figure shows that G' and G'' are comparable in magnitude to one another, a common behavior in concentrated high-polymer solutions. Although G' and G'' increase with ω, the rate of increase is sublinear, so that both $\eta' = G''/\omega$ and $\eta'' = G'/\omega$ decrease markedly at high frequencies. This shear-thinning aspect of G'' reduces the drag work for short, quick pulses; this effect on W is opposite from that caused by the v^2 factor in Eq. 1.

EFFECTIVE VISCOELASTIC PARAMETERS FOR AN OPTICAL TRAP

The load faced by kinesin in a buffer-filled optical trap differs from the load in a viscoelastic medium. By evaluating the forces on a bead executing harmonic oscillation in a water-filled trap with potential energy $V = \frac{1}{2}kx^2$, one finds that the trap alone can be represented by $G' = -(k/6\pi a)$, $G'' = 0$. There is no way to include the nonoscillatory force arising from offset of the oscillation midpoint from $x = 0$ in this formulation.

The values of G' for a 200-nm-diameter sphere in Yanagida's optical trap ($k = 0.071$ dyne/cm (Nishiyama et al., 2001)) and G'' for water ($\eta = 0.001$ Pa·s) are shown in Fig. 3 for comparison to the viscoelastic moduli for COS7 cells. Note that the absorptive modulus G'' in the buffer is many orders of magnitude smaller than the absorptive modulus of cytoplasm at low frequencies. Also, the elastic

FIGURE 4 The forces required to drag a 100-nm-radius sphere in COS7 cytoplasm, with case I extrapolation of viscosity to $\omega = 0$. The sphere is assumed to move with the softened Yanagida function (Eq. A1). (A) Elastic and viscous forces. (B) Total force.
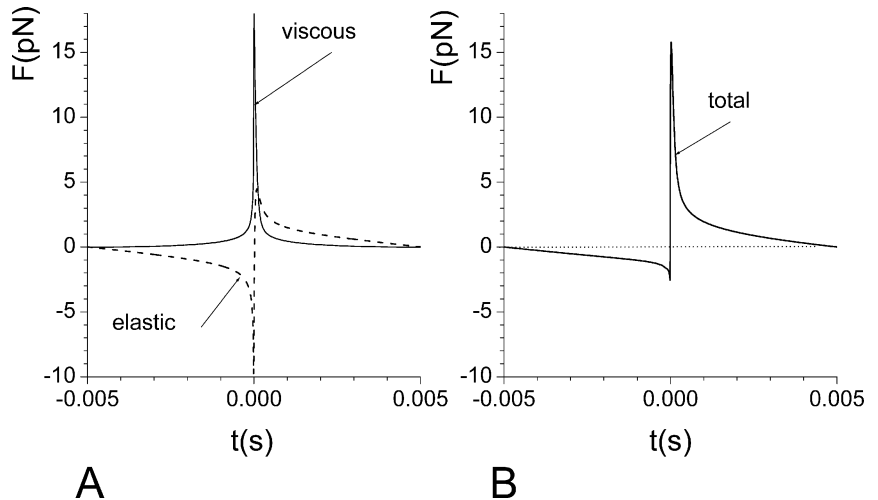
modulus G' for the trap is less than the elastic modulus of COS7 cytoplasm for all $\omega \geq 10$ rad/s.

FORCE IN A VISCOELASTIC FLUID

Starting from a very general model for the linear stress–strain relations in a viscoelastic fluid, Thomas and Walters (1965) used Laplace transform methods to obtain an expression for the drag force on a sphere moving in a viscoelastic medium. Their analysis was developed to describe the motions of a sphere falling under the influence of gravity, as in a falling-ball viscometer. Hwang et al. (1969) reformulated the Thomas–Walters solution, using Fourier transform methods, to analyze periodic motion in their magnetic rheometer, in which a spherical bead immersed in mucus is driven by a time-dependent sinusoidal force. Their analysis shows that $\tilde{F}(\omega)$, the Fourier transform of $F(t)$, is given by a Stokes-like relation,

$$\tilde{F}(\omega) = 6\pi a \tilde{G}(\omega) \tilde{v}(\omega). \qquad (7)$$

Because one frequently knows $G^*(\omega)$ rather than $\tilde{G}(\omega)$, it is helpful to substitute Eq. 6 into Eq. 7 to obtain

$$\tilde{F}(\omega) = 6\pi a \eta^*(\omega) \tilde{v}(\omega) = 6\pi a \frac{G^*(\omega)}{i\omega} \tilde{v}(\omega). \qquad (8)$$

Eq. 8 specifies how to obtain $\tilde{F}(\omega)$ from measurable quantities.

EVALUATION OF $\tilde{F}(\omega)$ FOR A SPHERE IN CYTOPLASM

$G^*(\omega)$ is known for $0.5 < \omega < 30,000$ rad/s for the cytoplasm of COS7 cells (Fig. 3). We assumed that kinesin moves in cytoplasm with the same quick 8-nm steps as in the optical trap, and that these steps repeat every 0.01 s. This repetition time ensures that the average velocity is $8/0.01 =$

800 nm/s, which is typical of fast axonal transport. The force scales linearly with the radius a of the sphere being dragged. We assume $a = 100$ nm, the radius in Yanagida's experiments. Many endogenous vesicles are of comparable size.

The specification of $\tilde{F}(\omega = 0)$ also poses a problem because $G^*(\omega)/\omega$ has not been measured at $\omega = 0$. This region is important because any net processive movement requires a nonzero $\tilde{v}(\omega)$ at $\omega = 0$. The problem is not unique to the current situation, and methods for dealing with it are known (Mason, 2000). If cytoplasm behaves like most viscoelastic fluids, and lacks a yield point, the contribution to W could be small compared to the contributions by other frequencies.

For our purposes, we have calculated force versus time curves under two conditions. Case I extrapolates G^*/ω to $\omega = 0$ from $\omega_1 = 628$ and $\omega_2 = 1256$ rad/s, the two smallest nonzero values in our 4096-point ω array. This gives $\eta' = 2.8 \times 10^{-7}$ pN·s/nm^2 at $\omega = 0$. With this assumption, the force on the sphere becomes negligible between pulses. Case II extrapolates measured values of G'/ω and G''/ω from $0.5 \leq \omega \leq 1.0$ rad/s to $\omega = 0$. This gives $\eta' = 5.1 \times 10^{-5}$ pN·s/nm^2 at $\omega = 0$. As will be shown below, this adds a "DC offset" to $F(t)$ at all times during processive movement of the sphere.

CALCULATION OF *F(t)*

The force $F(t)$ was calculated by numerical Fourier inversion of $\tilde{F}(\omega)$. The elastic and viscous components of the force and the total force are shown in Fig. 4 for the case in which G''/ω was extended to zero by method I. The viscous component was always positive and showed a roughly Lorentzian shape (Fig. 4 A), with a peak of 18 pN and a full width at half maximum of 70 μs, about twice the full width at half maximum of the Yanagida velocity function (Fig. 1).

FIGURE 5   The forces required to drag a 100-nm-radius sphere in COS7 cytoplasm, with case II extrapolation to a much larger value of $\eta'(\omega = 0)$. The sphere is assumed to move with the softened Yanagida function. (*A*) Elastic and viscous components of the force. (*B*) The total force.
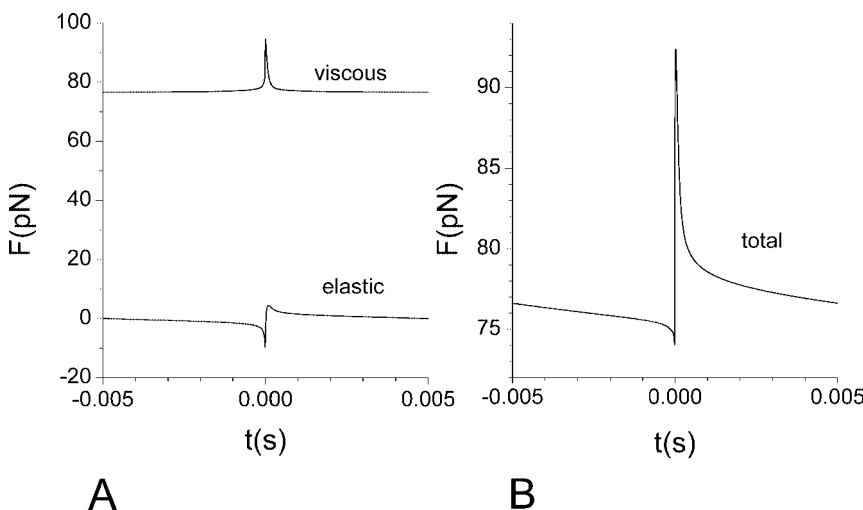
The elastic component of the drag force was negative for $t < 0$ and positive for $t > 0$, with peak magnitude roughly 20% of the absorptive peak. For a continuous series of such velocity pulses, as in processive motion, the elastic energy stored in the cytoplasm at the end of one step was returned to the sphere at the beginning of the next pulse. The resultant total force was initially negative, rose briefly to 16 pN, then dropped to zero after 5 ms (Fig. 4 *B*). The cycle then repeated itself.

The force was also calculated by method II, which assigns a much greater value to $\eta'(\omega = 0)$. The larger value of $\eta'(\omega = 0)$ caused a DC offset of 77 pN in the viscous part of $F(t)$ and increased the maximum total force to 93 pN (Fig. 5).

In single-molecule studies of kinesin in buffer, in which kinesin drags a sphere against the force of an optical trap, kinesin becomes detached from the microtubule if the optical force exceeds 6–7.5 pN (Svoboda and Block, 1994; Meyhoefer and Howard, 1995; Kojima et al., 1997; Crevel et al., 1999; Visscher et al., 1999).

WORK IN CYTOPLASM

If we assumed that the velocity of the sphere was the same in buffer as in cytoplasm and that $\eta'(\omega = 0)$ was small (case I), the work of dragging a sphere 8 nm evaluated to $W = 100 \pm 70$ pN·nm. The stated uncertainty arises from uncertainties in the measured values of G'' in COS7 cells (Yamada et al., 2000). An energy of 100 pN·nm is released during the hydrolysis of one ATP under typical cellular conditions (Howard, 2001). This suggests that kinesin could function in the cell with the same pulse-like steps that it exhibits in buffer. However, when the larger case II value of $\eta'(\omega = 0)$ was used, the work increased to 700 ± 500 pN·nm, or 7 ± 5 ATPs.

In all of the above calculations, the time interval between steps was fixed at 0.01 s. The results did not change significantly if the time intervals obeyed the distribution expected for a random process, as observed for single kinesin molecules in a trap (Schnitzer and Block, 1997).

DISCUSSION

Our calculations show that the pulsed forces and velocity that kinesin exhibits while pulling a bead up the potential gradient of an optical trap are similar in magnitude to the forces required to move a small spherical vesicle with similar pulse-like velocity in cytoplasm, if F goes to zero between pulses (case I). Although the maximum force required in the case I extrapolation scenario for $\eta'(0)$ is 16 pN in cytoplasm, 2–3 times the maximum steady force that kinesin can exert in a trap, this force is needed only 1% of the time in cytoplasm. In case II, the force required exceeds the maximum available force generated by 1 kinesin in buffer by a factor of 10 at all times. More than one kinesin would thus be required to move 200-nm vesicles in cells if case II is appropriate.

The chemical energy available from the hydrolysis of 1 ATP in a typical cellular environment is given by (Alberty and Goldberg, 1992; Howard, 2001)

$$\Delta G = -\Delta G_0 + kT \ln \frac{[\text{ADP}][\text{P}_\text{i}]}{[\text{ATP}]}$$

$$= -101 \text{ pN·}nm.$$

Because ΔG_0 depends on pH, magnesium concentration, and temperature, and the concentrations of ADP, P_i, and ATP can vary from cell to cell, ΔG is uncertain by at least $\pm 20\%$ for vertebrate cells. It is encouraging that, at least for the case I extrapolation of cytoplasmic viscosity, the work required to move a sphere one step, 100 ± 70 pN·nm, was comparable to the available chemical energy. For larger spheres, the work increases linearly with radius.

There are a number of assumptions that increase the uncertainty of the calculated values of $F(t)$ and W:

1. The cargo particle was assumed to be a rigid 100-nm-radius sphere, but in cells, vesicles are deformable and have a range of radii and shapes. For example, in hippocampal neurons, cargo is packaged in deformable cylinders of length 10 μm and diameter $<$0.5 μm (Kaether et al., 2000).

2. During single kinesin experiments in an optical trap, and in our calculations, the microtubules are stationary. However, microtubules in cells may move or deform when subjected to forces by bound kinesin.

3. The values of G' and G'' vary among different types of cells, and within different regions or times within the same cell (Sato et al., 1984; Valberg and Feldman, 1987; Bausch et al., 1999; Yamada et al., 2000). Moreover, optical and electron microscopy show that actin fibers and microtubules are not randomly oriented, so G' and G'' should really be tensors rather than scalars. Movement parallel to the fibers will take less force than perpendicular travel. Similarly, the existence of a nearby stationary cell wall will increase the drag force (Jones et al., 1994).

4. It was assumed that only one kinesin moves the model vesicle.

Experiments are needed to reduce these uncertainties and to understand how motor proteins couple their internal machinery to intracellular vesicle transport. Optical microscopic methods using a quad cell have the spatial and temporal resolution to determine whether kinesin-driven vesicles move with the same quick steps as beads in buffer. Fast-tracking of green fluorescent protein-labeled organelles (Kaether et al., 2000) in cells with a microscope and line-scan camera may also be able to reveal the presence of individual transport steps. The load-dependence of kinesin stepping could be studied microscopically in an optical trap if the buffer normally surrounding the trapped bead were replaced by a viscoelastic polymer solution. It may be possible to determine the drag forces on vesicles in cells with optical or magnetic tweezers. Motion of microtubules under load might be observable if the microtubules are fluorescently tagged or are attached to an atomic force microscope tip. Finally, it may be feasible to count the number of kinesin molecules pulling a vesicle by labeling the kinesin with green fluorescent protein.

Our model can provide additional insight into the effect of slowing down individual steps. This could occur because of load dependence within the kinesin motor or because of elasticity within the kinesin molecule (Svoboda and Block, 1994; Kojima et al., 1997). We modeled the possible overall effect of this on $F(t)$ and W by increasing all time constants in the assumed velocity of the sphere by a factor of 10, while keeping step size, step rate, and viscoelasticity unchanged. Using case I extrapolation to $\omega = 0$, the maximum

force on the bead decreased from 16 pN (Fig. 4 B) to 3 pN, about half the maximum force kinesin produces while working against the constant force of an optical trap. The drag work generated by the slower steps also decreased dramatically, from 100 to 20 pN·nm per step. If the viscosity were extrapolated to $\omega = 0$ by the case II method, lengthening the velocity pulse had only minor effects on the maximum force, and the work decreased from 700 to 600 pN·nm.

It has been shown experimentally that the average velocity of kinesin decreases approximately linearly with the magnitude of opposing force from an optical trap (Svoboda and Block, 1994; Coppin et al., 1997; Kojima et al., 1997; Visscher et al., 1999). These observations have been modeled by introducing force-dependent rate constants into a Michaelis–Menten description of the rate of ATP utilization (Schnitzer et al., 2000). The breaking of noncovalent bonds, such as those between kinesin and microtubules, probably depends critically on how fast the bond-breaking force is applied (Evans, 2001). Bonds under faster loading withstand larger forces but have shorter lifetimes.

The present study quantifies the drag force and work per step for intracellular transport by kinesin, assuming kinesin behaves within cells as it does within an optical trap. A single kinesin motor might be unable to move 200-nm vesicles through COS7 cytoplasm if it moves with the same quick jumps as in an optical trap. However, if each step occurred more slowly, the measured maximum force and energy from a single kinesin could be adequate.

APPENDIX

$\tilde{F}(\omega)$ was defined at 4096 points evenly spaced over the interval $0 \leq \omega \leq 2\pi N/T$, with $T = 0.01$ s. Doubling the number of data points did not significantly affect the results. $F(t)$ was computed from $\tilde{F}(\omega)$ by discrete Fourier inversion using commercial software. The softened Yanagida velocity function is

$$v = 0 \qquad \text{for} \quad t < -12.5 \times 10^{-6} \text{ s}$$

$$v = 8 \times 10^4 v_0 (t + 12.5 \times 10^{-6})$$

$$\text{for} \quad -12.5 \times 10^{-6} \leq t \leq 0 \text{ s}$$

$$v = v_0 e^{-t/\tau} \quad \text{for} \quad t \geq 0, \quad \text{with} \quad \tau = 47 \times 10^{-6} \text{ s.}$$

$$\text{(A1)}$$

The value of v_0 was chosen so that the distance traveled in one pulse remained 8 nm.

A comparison of Figs. 2 and 3 shows that the real part of $\tilde{v}(\omega)$ extends from 0 to 3×10^5 rad/s but G^* data are available only for 0.5–3×10^4 rad/s. To deal with the lack of experimental values for G^* at high frequencies, G' and G'' were linearly extrapolated on a log-log scale to 3×10^5 rad/s.

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