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Force Production by Depolymerizing Microtubules: Load-Velocity Curves and Run-Pause Statistics

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ABSTRACT Experiments indicate that depolymerization of microtubules generates sufficient force to produce the minus-end-directed transport of chromosomes during mitosis (Koshland et al., 1988). In vitro, analogous transport of kinesin-coated microspheres exhibits a paradoxical effect. Minus-end-directed transport of the microspheres driven by depolymerization is enhanced by the presence of ATP, which fuels the motor action of kinesin driving the microspheres in the opposite direction, toward the plus end of the microtubule. Here we present a mathematical model to explain this behavior. We postulate that a microsphere at the plus end of the microtubule facilitates depolymerization and hence enhances minus-end-directed transport. The force-velocity curve of the model is derived; it has the peculiar feature that velocity is maximal at some positive load (opposing the motion) rather than at zero load. The model is used to simulate the stochastic process of microsphere-facilitated depolymerization-driven transport. Simulated trajectories at low load show distinctive runs and pauses, the statistics of which are calculated from the model. The statistics of the process provide sufficient information to determine all of the model's parameters.

INTRODUCTION

Recent experiments suggest that depolymerizaton of tubulin can drive the motion of chromosomes in the absence of nucleotide hydrolysis or of auxiliary proteins (Coue et al., 1991; Koshland, 1992; Koshland et al., 1988). In an analogous in vitro system it has been demonstrated that microspheres attached to a depolymerizing microtubule by a motor protein would move toward the minus end as the microtubule depolymerized (Coue et al., 1991; Lombillo et al., 1995). As nucleotide triphosphates were absent in this assay as well, the motion of the microspheres was driven somehow by the depolymerization process rather than active molecular motors.

The motion of microspheres during depolymerization also displayed certain unusual features. When attached by kinesin and by a chimeric protein fashioned from kinesin and NCD (a kinesin-like protein), the microspheres were successfully transported by depolymerization toward the minus end. However, although beads attached by flagellar dynein stayed on the microtubule, beads attached by cytoplasmic dynein simply fell off the depolymerizing ends (Lombillo et al., 1995). The situation controlling whether beads dissociate from the ends of microtubules is ambiguous. Active kinesin-coated beads will frequently run off the ends of microtubules and axonemes (Block et al., 1990), and unless $CaCl_2$ is added, kinesin-coated beads will stabilize depolymerizing microtubules (R. Stewart, personal communication, cited by anonymous referee). Strangely, the chimeric-attached beads moved to the minus end faster than the free depolymerization rate of the microtubules, as if the beads were somehow facilitating the loss of subunits from the plus end, whereas beads attached by kinesin did not exhibit such facilitation. Moreover, the motion of the beads was saltatory, consisting of runs and pauses, wherein the bead moved rapidly or remained almost stationary. The chimeric-coated beads were transported much more rapidly than the kinesin-coated beads. Most puzzling of all, when nucleotide was added to the kinesin bead assay so that the beads moved actively toward the plus ends of the microtubles, the depolymerization-driven velocity toward the minus end was enhanced.

In 1952, Inoue suggested that depolymerization could generate a force, and many workers have suggested that microtubule polymerization and depolymerization could generate forces (cf. Coue et al., 1991, and references therein). Hill (1985) constructed a model for how depolymerization could drive chromosome movements during mitosis, but his model does not exhibit the sorts of phenomena observed in the in vitro assays of Lombillo et al. (1995). Here we present a model that provides a possible explanation for the phenomenon of depolymerization-driven motion.

THE MODEL

The beads used in the assay mentioned above were ~1 μm in diameter, considerably larger than the microtubule diameter (~25 nm). The bead is coated with a protein that attaches it to the microtubule lattice. We shall assume that the surface density (number per unit area) of this protein is sufficiently high to permit the bead to roll on the microtubule lattice, so that we can treat the bead as if it were diffusively rolling along a one-dimensional track, as shown in Fig. 1.

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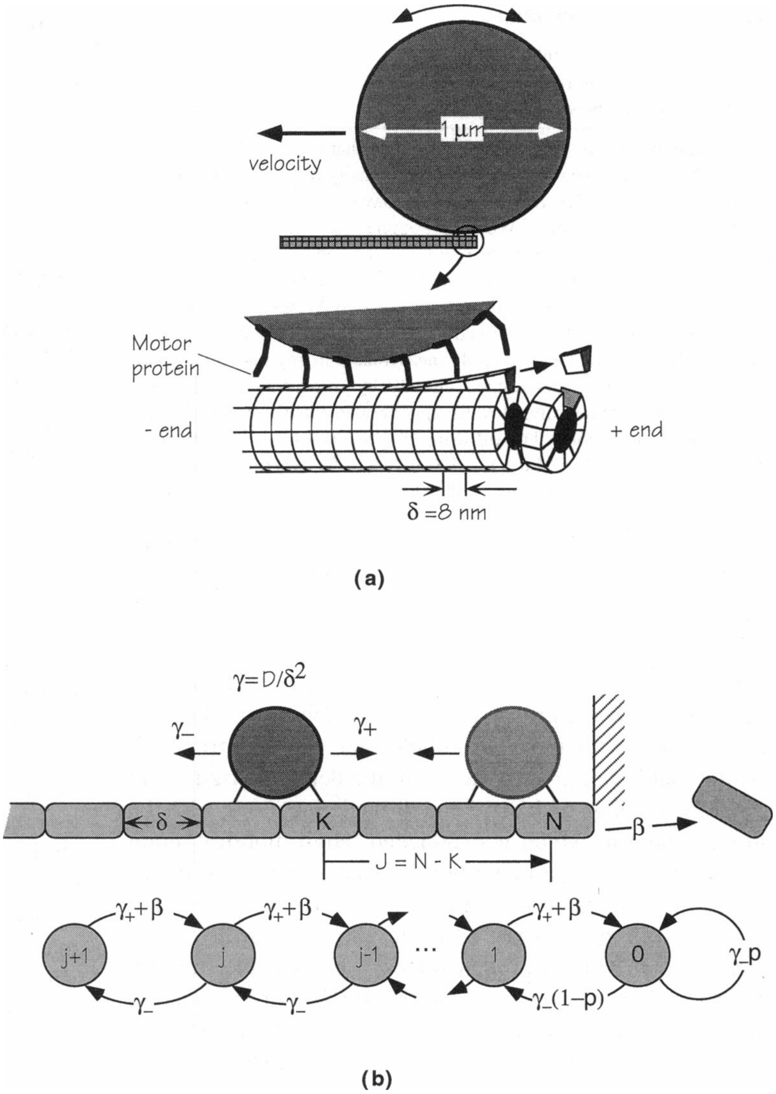

FIGURE 1 (*a*) An uncharged, latex microsphere coated with a motor protein rotationally diffuses along a protofilament. The bead is driven toward the minus end of the microtubule as it depolymerizes. The bead facilitates the depolymerization by helping tear a protofilament strip from the tubule plus end; the remainder of the ring of dimers then dissociates rapidly. (*b*) The model tubule is divided into N subunits of length $\delta = 8$ nm. The number of subunits between the right-most attachment of the bead and the plus end of the tubule is specified by the integer coordinate $J = 0, 1, 2 \ldots,$ N. The bead can jump between adjacent subunits with frequency γ_- to the left and γ_+ to the right, whereas the tubule depolymerizes from its right end at a rate β (except when the bead is attached to the terminal subunit of the tubule). Note that dissociation of a subunit has the effect of reducing J by 1; hence the rate constant for reduction of J in the transition diagram is $\gamma_+ + \beta$. The state $J = 0$ is special; when the bead rolls one subunit to the left starting from the right end of the tubule ($J = 0$), there are two possibilities. Either the terminal subunit dissociates (with probability p) and the bead finds itself at the new right end of the tubule ($J = 0$) or the terminal subunit fails to dissociate (with probability $1 - p$) and the bead finds itself one subunit away from the right end ($J = 1$).

Because the tubule consists of discrete subunits, we shall use a discrete formulation. Thus we divide the tubule into subunits of size $\delta = 8$ nm (the length of a tubulin dimer). Note that a subunit, thus defined, is a ring of 13 tubulin dimers. We adhere to the convention that the minus end of the tubule is fixed and that the plus end of the tubule is moving as a result of the depolymerization occurring there, so that depolymerization proceeds from right to left in Fig. 1. We allow the bead to jump parallel to the axis of the tubule between adjacent subunits with a frequency γ_+ for jumps toward the plus end (i.e., to the right with reference to Fig. 1) and γ_- for jumps toward the minus end (i.e., to the left in Fig. 1). These two frequencies will be equal unless there is an applied force (or the the action of a molecular motor) to bias the motion in one direction, as we shall discuss below. We denote by β the depolymerization rate of subunits from the plus end of the tubule when the bead is not on the terminal subunit. We obtain β from the observed depolymerization rate with no bead attached (Table 1).

When the bead is on the terminal subunit we assume that subunit cannot dissociate as it is held in place by bonds to the bead. However, when the bead rolls to the left away from the terminal subunit we assume that it has some definite probability, p, of dissociating the terminal subunit from the tubule. This assumption is motivated by the observation that a tubule with an attached bead depolymerizes faster than a tubule without a bead. Because the bead is much larger than the microtubule, it is safe to neglect the effect of pulling the terminal subunit on the bead's motion. To take this effect into account, one could use a different rate coefficient for the bead rolling away from the terminal subunit than for its other jumps to the left. This would introduce an additional parameter, and we avoid it here.

Some coupling molecules (e.g., cytoplasmic dynein) fall off the microtubule end as it depolymerizes and so do not exhibit the sort of motions we are modeling. Other coupling molecules (e.g., kinesin and NK350) do support depolymerization-associated motion because they seldom fall off the ends. That is the case that we consider, and so we simply impose the constraint that the bead cannot come off the end of the microtubule.

TABLE 1 Parameter values

δ = step size = size of tubulin monomer	8 nm[a]
β = depolymerization rate	48 steps/sec[b]
γ = transition rate between subunits	2000 s^{-1}[c]

[a] We assume that the density of proteins is such that a protein binds to each dimer. The actual value of δ depends on the density of proteins attached to the bead; at low densities, rolling diffusion may entail steps of greater than the 8 nm size of a dimer. We also assume that each terminal dimer destabilized by the bead causes the rapid loss of an entire ring of 13 dimers.

[b] Lombillo, et al. measured a shortening rate of 383 nm/s (Lombillo, et al., 1995), nearly the same as the 451 nm/s observed by Walker, et al. (Walker, et al., 1988). As we have defined it, β is related to the irreversible depolymerization rate, and is directly measureable in any particular assay.

[c] In general, γ can be determined directly by measuring the diffusion coefficient on stable microtubules. An upper bound can be estimated by the following considerations. The diffusion coefficient of a 1 μm diameter bead in water: $D_0 = k_B T/(6\pi\eta r) = 4.3 \times 10^5$ nm^2/s, where r is the radius of the sphere, η is the viscosity of the fluid, k_B is Boltzmann's constant, and T is the absolute temperature. Then, $\gamma = D/\delta^2 \leq D_0/\delta^2 = 4.3 \times 10^5/8^2 = 6700$/s. The rolling diffusion of the bead is reduced substantially by the necessity of breaking and making protein bonds attaching the bead to the microtubule.

The cases in which the bead does not fall off the end of the microtubule are probably those in which the bead makes many bonds with the microtubule and yet is sufficiently mobile that it can move away from the depolymerizing end rather than being stuck there and dissociating along with the protofilament to which it is attached. High mobility along the tubule is compatible with a large number of bonds because the bonds do not all have to be broken at the same time for rolling diffusion to occur. In such diffusion, on average, one bond is broken for each bond made. Indeed, when the density of motors on the surface of the bead is sufficiently high, the breaking of the old bond and the making of the new one can occur simultaneously, thus further lowering the energy barriers to rolling diffusion. If the bead attempts to roll off the end of the tubule, no new bonds are made to compensate for those that are lost. As pointed out by Hill (1985), this establishes an effective free energy gradient in the neighborhood of the end of the tubule that tends to keep the bead attached. Another mechanism that may contribute to keeping the bead attached to the tubule is the observed banana-peel conformation at the depolymerizing end as a protofilament peels off longitudinally (Kirschner et al.; Mandelkow et al., 1985). It may be difficult for the bead to negotiate the corner formed by such a depolymerizing protofilament, as to do so would require breaking many bonds at once. We make no attempt to simulate such details here. Instead, we just make it an assumption of the model, as stated above, that the bead cannot dissociate from the tubule.

Our goal is to compute the speed at which the bead moves toward the minus end of the tubule, ratcheted along by the depolymerization process. We shall calculate this speed as a function of a load force (directed to the right) that may be acting on the bead opposing the depolymerization-driven motion. Such a load can be applied by putting the whole system in a fluid flow or by using a laser trap. In addition to calculating the mean velocity, we shall also determine certain statistical properties of the motion, specifically the statistics of runs and pauses. These results can be used to test the model and to determine the model parameters from experimental data.

RESULTS

The load-velocity relationship is not monotonic

In Appendix A we derive and solve a system of differential difference equations that govern the evolution of an ensemble of systems of the type depicted in Fig. 1. The steady-state solution of these equations yields the following load-velocity relationship:

$$v(\omega) = \delta\gamma e^{-\omega/2} \frac{p\gamma(e^{\omega/2} - e^{-\omega/2}) + \beta}{\gamma(e^{\omega/2} - pe^{-\omega/2}) + \beta}, \tag{1}$$

where $\omega = f\delta/k_B T$ is the dimensionless work performed by the load force f (directed toward the plus end of the microtubule) in moving the bead one step to the right. Here, k_B is Boltzmann's constant and T is the absolute temperature. The constant γ is the common value of γ_+ and γ_- when $f = 0$. As defined above, β is the depolymerization rate in the absence of the bead, δ is the step length of the bead in its random walk along the microtubule, and p is the probability of detachment of a subunit when the bead takes a step away from the depolymerizing end of the microtubule. In deriving this form of the force velocity curve, we have made use of the thermodynamic relationship $\gamma_+/\gamma_- = e^{\omega}$, which is valid only in the case of a passive (nonmotor) linkage between the bead and the microtubule, such as NK350, or kinesin without ATP. The active case is considered below.

Aside from $k_B T$ (which is set by the temperature of the experimental conditions), there are four parameters in the model: γ, β, δ, and p. Table 1 gives the values we have used in our calculations. β is measured by observing the depolymerization rate of microtubules without beads. γ can be determined directly by measuring the diffusion coefficient on stable microtubules. Lombillo et al. (1995) did not determine γ directly; however, we can place bounds on it as described in Table 1. The value of δ depends on the density of proteins on the bead; we have taken δ to be the length of a tubulin dimer (~8 nm). The probability of the bead detaching a terminal subunit, p, is not directly measurable; however, we can estimate it from the unloaded velocity:

$$v(0) = \delta\gamma \frac{\beta}{\gamma(1 - p) + \beta}. \tag{2}$$

If β, γ, and δ are known, this expression can be used to compute p, from $v(\omega = 0)$.

The load-velocity relationship given in Eq. 1 is plotted in Fig. 2 *a* for beads coated with kinesin and the chimeric protein NK350. The reasons that the curves are different are discussed below. The load-velocity relationship described

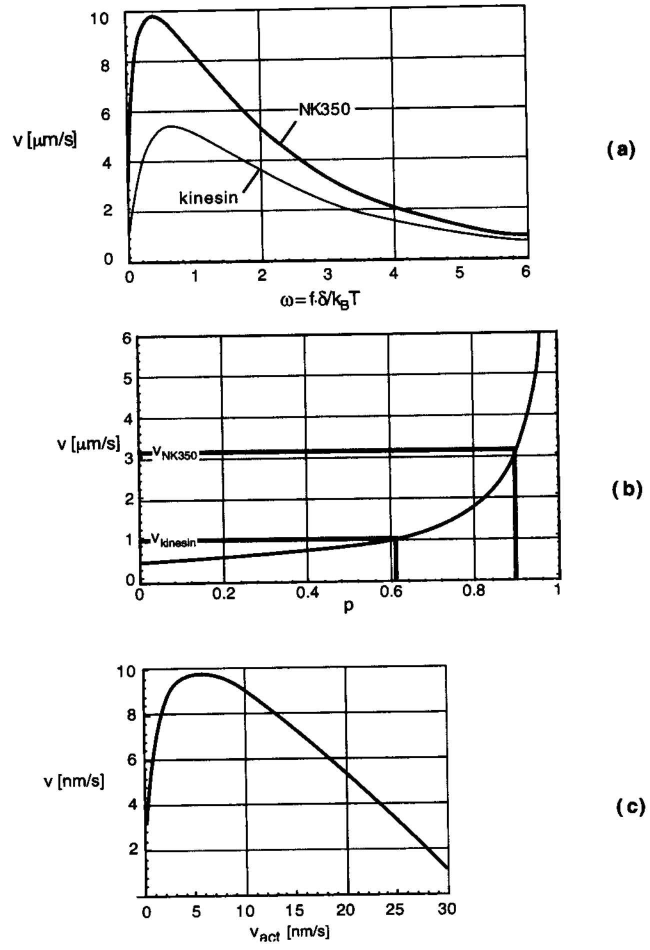

FIGURE 2 (*a*) The load velocity curves for NK350 (*heavy line*) and kinesin (*light line*). They are different because of their differing depolymerization efficiencies, which are deduced in *b*. The velocity enhancement is evident for loads given by $\omega < 1$ (which correspond to forces of less than 0.5 pN. (*b*) The bead velocity as a function of the depolymerization probability, p, under no-load conditions, $\omega = 0$, using the parameter values given in Table 1. Assuming that $\gamma = 2000/\mathrm{s}$, the observed no-load velocity for NK350 of $v = 3.4\ \mu\mathrm{m/s}$ corresponds to $p \approx 0.9$, and for kinesin $v = 0.97\ \mu\mathrm{m/s}$ corresponds to $p \approx 0.6$. Using these values in Eq. 1 gives the load-velocity curves shown in *a*. (*c*) The velocity of unloaded kinesin-coated beads as a function of their active velocity, $v_{\mathrm{act}} \equiv \delta(\gamma_+ - \gamma_-)$, also shows a maximum as their plus-end-directed motility keeps them near the tip where they facilitate depolymerization. Here we have assumed that $\Gamma \equiv \gamma_+ + \gamma_-$ is independent of ATP for illustrative purposes only.

by Eq. 1 has the unusual feature that the application of a moderate load (directed to the right) can actually speed the movement of the bead to the left. Physically, this comes about because the loading force causes the bead to spend a greater fraction of its time on the terminal subunit, where it is effective in facilitating the depolymerization of the tubule. We can give a condition for this speed-up effect to occur by examining the derivative of v with respect to ω at $\omega = 0$. This derivative is positive if

$$\frac{\gamma}{\beta} > \frac{1}{2p}\left(1 + \sqrt{\frac{1+p}{1-p}}\right). \tag{3}$$

When this inequality is satisfied, the depolymerization velocity increases with increasing applied force up to some optimal load, and then, as $v \rightarrow 0$ as $\omega \rightarrow \infty$, the velocity decreases with additional increases in the applied force. Note that the model's force-velocity curve does not show a stall load; for all finite ω we have $v > 0$. This is because we have treated depolymerization as an irreversible process. Similarly, we have not allowed the load force to pull the bead off the end of the microtubule, no matter how large the load force becomes.

Different attachment proteins behave differently in the bead assay. The inactive chimeric protein NK350 moved toward the minus end more than six times faster than the free depolymerization rate, whereas kinesin's minus-directed motion was one sixth of the free depolymerization rate in the absence of ATP. Cytoplasmic dynein had no influence on depolymerization, and beads fell off the end of the tubule when the depolymerizing tip caught up with the bead. We attribute this to differences in binding affinity, which has two effects. The diffusion of the bead on the microtubule clearly depends on the binding affinity of the proteins to the tubulin dimers. If the affinity is too large, the bead cannot diffuse; if it is too small, the bead simply detaches from the microtubule. The binding affinity also affects the depolymerization efficiency of the bead as the binding of the bead to the terminal dimer presumably destabilizes it (e.g., by allosterically competing with the dimer's bonds to its three neighbors). Therefore, there must be an optimal affinity that would produce the highest depolymerization velocity. Because the γ values of kinesin and NK350 have not yet been independently measured, we have simply assumed them to be equal. As shown in Fig. 2 *b*, if γ were the same for both kinesin and NK350, their estimated p values would be quite different, reflecting the greater depolymerization efficiency of NK350. This is reflected in their differing load-velocity curves, as shown in Fig. 2 *a*. Cytoplasmic dynein, on the other hand, does not work at all as a depolymerization enhancer. We attribute this to its binding affinity being too high, so that it cannot diffuse away from the tip. Thus, rather than destabilizing the terminal subunit, it simply detaches along with the protofilament to which it is attached.

The peculiar shape of the load-velocity curve accounts qualitatively for the paradoxical observation that the depolymerization velocity of kinesin-coated beads increased when the kinesin was supplied with ATP (Lombillo et al., 1995). That is, by attempting to walk in the plus direction, the bead's minus end velocity is enhanced from 23 μm/min (380 nm/s) to 58 μm/min (970 nm/s). Because we have assumed that the bead diffusively rolls along the microtubule, individual motor molecules must dissociate from the tubule to permit rolling in either direction. An individual kinesin molecule executing an active power stroke introduces a small forward translational bias to the rolling diffusion before it dissociates. Thus the active power strokes provide a drift velocity superimposed upon the rolling, which is an isotropic random walk. Note that, for these beads, the velocity without ATP is about one sixth of the free depolymerization rate of the microtubule (148 μm/min = 2460 nm/s). As presently formulated, the model cannot account for this observation; a more detailed description of bead binding and of the rolling diffusion process is required.

From Eq. 2 the condition $v(0) = \delta\beta$ corresponds to $\gamma p = \beta$. This is the condition for no depolymerization facilitation at zero load. That is, when the bead is at the tip, the terminal subunit cannot detach until the bead rolls away (the tip is actually stabilized when the bead is exactly at the end). When $p > \beta/\gamma$, the probability of pulling off the terminal subunit while rolling away from the end of the tubule is large enough to overcome the stabilization and to give enhanced depolymerization. When $p = \beta/\gamma$, these two effects exactly cancel each other, leaving the system with the same depolymerization velocity as if the bead were not there at all.

We can recast the load velocity curve in terms of the bead's active motile velocity (cf. Appendix A):

$$v = \frac{(\delta\Gamma - v_{\mathrm{act}})(\beta\delta + p v_{\mathrm{act}})}{\delta(2\beta + \Gamma(1-p)) + v_{\mathrm{act}}(1+p)}, \tag{4}$$

where $\Gamma \equiv (\gamma_+ + \gamma_-)$, v is the observed bead velocity, and $v_{\mathrm{act}} \equiv \delta(\gamma_+ - \gamma_-)$ is the active bead velocity. Fig. 2 *c* shows that active motility has the same qualitative effect as imposing a load.

Note that the model as formulated has an infinite stall load. This is because we assumed that, during depolymerization, the concentration of tubulin subunits in solution was essentially zero; that is, the depolymerization is irreversible ($k_{\mathrm{on}} = 0$). As always, when using an irreversible approximation, one has an infinite free energy source available. This can easily be remedied by including the polymerization reaction, at the expense of more cumbersome mathematics, and with little effect on the answer as, under conditions of the experiment, $k_{\mathrm{on}} \ll k_{\mathrm{off}}$. Ultimately, the free energy that drives the load comes from the binding free energy of dimers into the tubule lattice, and this is embodied in the ΔG of the polymerization reaction. Inclusion of polymerization will produce a finite stall load, $f_0([\mathrm{M}], K)$, where [M] is the monomer concentration, and K is the dissociation constant for the terminal subunit. Because the experiments were done with very low monomer concentration, we felt that this was an unnecessary complication in the model. For experiments done under conditions where the polymerization reaction proceeds at a significant rate, the theory can be generalized to take such polymerization into account.

Next, we turn our attention to showing how to use the statistics of the bead's motion to establish two additional, independent equations that can be used to estimate the parameters.

The statistics of the bead motion can be used to estimate parameters

The speed-up mechanism, either by active motility, as in kinesin, or by imposition of an external load, can be understood by examining the statistics of the bead's motion. The more time a bead spends in the depolymerizing zone the more effective it is in enhancing depolymerization. This effect is seen clearly in the stochastic simulation shown in Fig. 3, which shows the location of the microtubule tip in relation to the bead position. The unloaded trajectory exhibits the same characteristic runs and pauses as observed in the experiments. We see that the runs occur when the bead is at the tip, whereas the pauses correspond to those periods when the bead has diffused away from the tip. In this latter situation the bead's random diffusive motions look nearly stationary. Eventually, the bead diffuses to the tip, or the slowly depolymerizing tip catches up to the bead and another run ensues. The average velocity is just the mean slope of the trajectory. When a small plus-end-directed load is applied to the bead, the bead spends most of its time near the tip, facilitating depolymerization, and the pauses nearly disappear, so that the trajectory appears as a single long run. Note that these runs and pauses are distinct from those caused by the intermittent character of the depolymerization process itself (dynamic instability) (Mitchison et al., 1984). The intrinsic depolymerization pauses can be eliminated experimentally by reducing the monomer concentration sufficiently; moreover, the time scale for depolymerization pauses should be much longer than the bead pauses discussed here.

The statistics of the runs and pauses provide additional information about the bead's motion. In Appendix B we

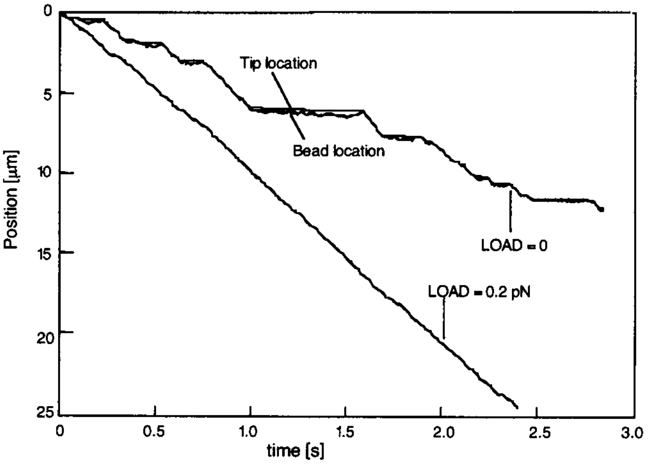

FIGURE 3 A stochastic simulation of the depolymerization ratchet. $N(t)$ = number of subunits in the microtubule at time t; $K(t)$ = location of the (right-most) subunit to which the bead is attached at time t. N is monotone decreasing (the tubule only depolymerizes) whereas K is not. The unloaded (*upper*) trajectory consists of fast runs of depolymerization during which the bead is at the tip, $N = K$, separated by slower depolymerization when the bead diffuses away from the tip, $N > K$. The loaded (*lower*) trajectory lacks the pauses as the bead is held near the tip, and so the mean velocity is greater.

derive the probability distribution for pause times, τ_p, from which we obtain the following expressions for the mean and variance of the pauses:

$$\langle \tau_p \rangle = \frac{1}{\gamma_+ - \gamma_- + \beta} \tag{5}$$

$$\mathrm{var}(\tau_p) = \left(\frac{\gamma_+ + \gamma_- + \beta}{\gamma_+ - \gamma_- + \beta} \right) \langle \tau_p \rangle^2. \tag{6}$$

Note that the variance can be much greater than the square of the mean. For example, if $\gamma_+ = \gamma_- = 2000\ \mathrm{s}^{-1}$ and $\beta = 48\ \mathrm{s}^{-1}$, the variance of the pause duration is ~80 times the square of the mean pause duration. As the full distribution has quite a long tail, occasional long pauses can arise. These equations together with those derived previously can be used to determine the model parameters and also to check the model. As an example, consider the unloaded, passive case. Then, $\gamma_+ = \gamma_- = \gamma$. The parameter β can therefore be found from Eq. 5 and γ can be found from Eq. 6. Let us fix the value of δ at 8 nm, the length of a tubulin dimer. These choices of β, γ, and δ can be subjected to two independent checks. The parameter β can be found by observing the unfacilitated depolymerization of microtubules. The parameters γ and δ should be related by $\gamma\,\delta^2 = D$, where D is the experimentally observed diffusion coefficient of the bead on a stable (nondepolymerizing) microtubule. The parameter p can be found from the statistics of the runs of rapid depolymerization that occur when the bead is at the end of the microtubule. The probability that such a run involves exactly k steps (of length δ) is $(1 - p)\,p^k$. With γ, β, δ, and p known, Eq. 2 can be used to predict the mean depolymerization velocity at zero load. Moreover, Eq. 1 can be used to predict the entire load-velocity curve, with no further adjustment of parameters. Note that this last prediction rests on the assumption that the parameter γ defined as $\sqrt{\gamma_+ \gamma_-}$ is independent of load. This assumption can be independently checked by measuring the diffusion coefficient and drift velocity of the bead on a stable microtubule at a variety of loads, see Appendix A.

As an additional test of the model, the program that we have just outlined can be applied in a variety of experimental situations. Note that it is not necessary to know in advance how much each parameter has changed, as the parameter identification procedure that we have described can be applied in each case. For example, changing the bead size will change the rolling diffusion coefficient of the bead on the microtubule. The precise change may be difficult to predict, a priori, because of the complication introduced by bonds to the tubule, but one can measure the rolling diffusion coefficient directly by observing the random walk of the bead on a stable microtubule. Similarly, in experiments on active motors, the ATP concentration may be varied, thus affecting the biased random walk of the bead in a manner that is difficult to predict a priori but can be measured directly. In both instances, and in others like them, these variations provide a means of testing the model under a variety of experimental conditions.

DISCUSSION

We have presented a model that explains how a microtubule, depolymerizing at its plus end, can generate a directed force that can drive a protein-coated microsphere toward the minus end. The mechanism that we have proposed is based on the assumption that the microsphere facilitates the depolymerization process by straining the bonds on the terminal dimer of a protofilament, thus lowering the activation energy for depolymerization. In the experimental situations modeled here, separation of the microsphere from the microtubule is rarely seen, and this is imposed as a constraint in our model.

The mechanism that we have described has an autocatalytic character, as the microsphere catalyzes the depolymerization process that propels it along, and because this very process keeps the depolymerizing tip of the microtubule close to the microsphere where the catalysis is most effective. As we have shown, this can have paradoxical consequences. When a force is applied in a direction that would seem to oppose the depolymerization-driven motion of the microsphere, such force may actually speed the microsphere on its way by holding it more effectively against the depolymerizing tip of the microtubule and hence speeding up the depolymerization process. Thus the model predicts an unusual load-velocity curve, which initially rises to a maximum and then falls.

The model explains several unusual observations on this type of motility. (1) The motion is saltatory, with intermittant runs and pauses. (2) Different proteins exhibit different depolymerization velocities. (3) Beads coated with kinesin and supplied with ATP move actively toward the plus end; however, under depolymerizing conditions, active beads are driven faster toward the minus end than inactive beads. This is a consequence of the non-monotone load-velocity curve mentioned above.

The model contains several parameters (four in the case of a passive linkage between the bead and the microtubule; five if the linkage functions as an active molecular motor). We have shown how these parameters may be identified, how some of their values may be independently verified, and how additional predictions may be made to test the model once the parameters have been found.

The mechanism of depolymerization-driven transport discussed in this paper may be the same one that drives chromosomes toward the spindle poles during anaphase mitosis, and one may speculate that the paradoxical velocity enhancement generated by a moderate opposing load, or more likely by the corresponding action of an opposing motor protein, may be used by the cell to speed the chromosomes on their way.

The authors are indebted to S. R. S. Varadhan for suggesting the method of analysis used to study the pause statistics (Appendix B) and to Vivian Lombillo for sharing her unpublished data. The reviewers' comments also greatly improved the manuscript. A preliminary version of this work has already been published (Peskin et al., 1995).

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APPENDIX A

The load-velocity relationship

In this Appendix we derive the load-velocity relationship given in Eq. 1. Let $N(t)$ be the number of subunits in the tubule at time t. Let $K(t)$ be the index of the subunit to which the bead is attached at time t. (If the bead is attached to more than one subunit at a time, $K(t)$ is the index of the right-most subunit to which it is attached; see Fig. 1). We place the origin of our coordinates at the end of the tubule by setting $J(t) = N(t) - K(t)$. Thus $J(t)$ is a non-negative integer that measures the distance (in subunits) of the bead from the depolymerizing end of the tubule. Then $J(t)$ describes the random walk of the bead along the tubule; it obeys the transition rules shown in Fig. 1 *b*. Note in particular that J is reduced by 1 not only when the bead moves right but also every time that a subunit dissociates from the end of the tubule; such dissociation brings the end of the tubule closer to the current position of the bead. This is the reason that the rate constants for the rightward transitions include the term β in addition to γ_+. The transition from the state $J = 0$ to $J = 0$ corresponds to the case in which the bead rolls leftward, away from the terminal subunit, but pulls the terminal subunit off the tubule in the process. In that case the bead finds itself again at the end of the tubule despite its motion to the left; thus J remains 0. If, on the other hand, the terminal subunit does not come off, the bead ends up one subunit away from the end, so $J = 1$. The rate constants for the transitions $0 \rightarrow 0$ and $0 \rightarrow 1$ are $p\gamma_-$, and $(1-p)\,\gamma_-$, respectively. In these rate constants, p is the probability that the terminal subunit is pulled off by the leftward roll of the bead away from the terminal subunit.

We compute the mean velocity of depolymerization by considering an ensemble of such systems (each system is a microtubule with a bead attached). Let $C_j(t)$ be the number of systems in the ensemble for which $J = j$ (i.e., the number of systems in which the bead is j subunits from the plus end of the tubule) at time t. Then the following equations for $C_j(t)$ may be derived directly from the transition diagram in Fig. 1 *b*:

$$\frac{dC_0}{dt} = (\gamma_+ + \beta)C_1 - (1-p)\gamma_- C_0 \tag{A1}$$

$$\frac{dC_1}{dt} = (\gamma_+ + \beta)C_2 + (1-p)\gamma_- C_0 - (\gamma_- + \gamma_+ + \beta)C_1 \tag{A2}$$

$$\frac{dC_j}{dt} = (\gamma_+ + \beta)C_{j+1} + \gamma_- C_{j-1} - (\gamma_- + \gamma_+ + \beta)C_j, \qquad j = 2, 3, \cdots. \tag{A3}$$

Here we have taken the limit $N \rightarrow \infty$, so this is an infinite system of differential equations. One can check by direct substitution that a steady-state solution of Eqs. A.1–A3 is given by

$$C_0 = 1, \quad C_j = (1-p)\left(\frac{\gamma_-}{\gamma_+ + \beta}\right)^j, \quad j = 1, 2, \cdots. \tag{A4}$$

Another steady-state solution is given by C_j = constant, but this is rejected because it does not decay as $j \rightarrow \infty$. The solution given by Eq. A4 does exhibit such decay provided that $\gamma_-/(\gamma_+ + \beta) < 1$. This is assured in our case, as all rate constants are positive and because the applied force, if any, is to the right, so that γ_+ is at least as large as γ_-. As the equations are linear, we could multiply the solution given by Eq. A4 by any constant (independent of j). The choice $C_0 = 1$ is an arbitrary normalization that has no effect on the results derived below. Note further that $p = 1$ is a degenerate case in which the bead remains at the end of the tubule. This is because $J = 0$ is an absorbing state when $p = 1$. If $p = 1$, a bead reaching the end of the tubule stays there because every time it rolls leftward it dissociates a subunit and so finds itself at the (new) end of the tubule. In this situation, depolymerization proceeds at the rate γ_-, catalyzed by the constant presence of the bead at the terminal subunit of the tubule.

When $p < 1$, the situation is more complicated. If $\gamma_- \gg \beta$, and if p is reasonably large, then depolymerization will proceed in fast runs of rate γ_- interrupted by intervals in which the depolymerization rate is much slower (equal to β). The durations of the fast runs will be exponentially distributed with mean $[\gamma_-\,(1-p)]^{-1}$ (see Fig. 1 *b*). The intervals between the fast runs will have more complicated statistics that can be computed from the transition diagram of Fig. 1 *b*; this will be treated in Appendix B. We consider now the mean depolymerization rate and the resulting mean velocity at which the bead is transported.

The model does not specify the mechanism by which the bead dissociates the terminal subunit. Previously, we suggested that the free energy of binding to the terminal dimer competes with the dimer's binding to its neighbors in the lattice. Whatever the mechanical mechanism, the effect of the bead on the terminal subunit is felt reciprocally by the bead. Strictly speaking, therefore, the motion of the bead away from the tip should reflect this effect; that is, γ_- for jumps from $N \rightarrow N-1$ should be somewhat smaller than when the bead is away from the tip. However, because the bead is much larger than the microtubule, this effect is likely to be undetectable compared with the other statistical properties of the motion, and so we have ignored it here.

Let r be the mean depolymerization rate (in subunits per second). Because there are two disjoint ways that depolymerization can occur,

$$r = p\gamma_- \Pr(J = 0) + \beta \Pr(J > 0). \tag{A5}$$

Here $\Pr(\cdot)$ denotes the probability of the indicated event. In general,

$$\Pr(J = j) = C_j / \sum C_i. \tag{A6}$$

Once r is known, the mean velocity of the tip of the depolymerizing tubule, and hence the mean velocity of the bead, is given by

$$v = \delta r, \tag{7}$$

where δ is the size of a subunit. Substituting the solution given by Eq. A4 into these expressions, we find (after some algebra) that

$$v = \delta\gamma_- \frac{p(\gamma_+ - \gamma_-) + \beta}{(\gamma_+ - p\gamma_-) + \beta}. \tag{A8}$$

The parameters γ_+ and γ_- may be related to observable quantities in two different ways, depending on whether the protein that joins the bead to the microtubule is functioning as an active molecular motor, or not. In the passive case (NK350, or kinesin without ATP), we know from thermodynamics that

$$\gamma_+/\gamma_- = \exp(\omega), \tag{A9}$$

where

$$\omega = f\delta/(k_B T). \tag{A10}$$

Here f is the applied force (pushing to the right, against the direction of depolymerization), k_B is Boltzmann's constant, δ is the step size, and T is the absolute temperature. We can ensure that Eq. A9 will always be satisfied if we introduce a new parameter, γ, and write

$$\gamma_+ = \gamma e^{\omega/2} \qquad \gamma_- = \gamma e^{-\omega/2}. \tag{A11}$$

Thermodynamics cannot specify how γ depends on ω; for this, one requires a knowledge of the spatial arrangement of the energy barriers that must be overcome as the bead makes its transition from one subunit to the next. In the absence of such knowledge we make the simplest possible assumption, that γ is a constant. This is equivalent to the assumption that the product $\gamma_+\gamma_-$ is independent of load. The value of γ can be determined by measuring the diffusion coefficient, D, of the bead on a stable microtubule (i.e., not depolymerizing) with no applied force. To see the relation between γ and D, set $\gamma_+ = \gamma_-$ and $\beta = 0$ in Eq. A3. The result is a discretization of the diffusion equation with $D = \gamma\,\delta^2$. Substituting Eq. (A11) into Eq. A8, we obtain the force velocity curve of a depolymerization ratchet with a passive (non-motile) attachment protein. This is given by Eq. 1 of the text and is plotted in Fig. 2 *a*.

In the active case (kinesin with ATP) one can determine γ_+ and γ_- by observing the random walk of the bead on a stable microtubule. The applied force (if any) during this observation should be the same as the applied force for which γ_+ and γ_- are sought. Such a walk will be characterized (at least approximately) by a drift velocity that we shall call v_{act} (as it is generated by the activity of the motor protein) and by a diffusion coefficient, D. By manipulation of Eq. A3 with $\beta = 0$, one can see that

$$\gamma_+ + \gamma_- \equiv \Gamma = \frac{2D}{\delta^2} \qquad \gamma_+ - \gamma_- = \frac{v_{\mathrm{act}}}{\delta}. \tag{A12}$$

Solving for γ_+ and γ_- in terms of Γ and v_{act}/δ, and substituting the result into Eq. A8, we obtain a relationship between the depolymerization velocity v and the motor velocity v_{act}. This is stated in Eq. 4 of the text and is plotted in Fig. 2 *c*.

APPENDIX B

Statistics of the motion

In this Appendix we derive the mean and variance of the pauses, when the bead is apparently stationary. These occur when the bead diffuses away from the end of the depolymerizing microtubule so that its observed velocity is its rolling diffusive motion, which appears almost stationary.

Let us denote by $\hat{\gamma}_+ \equiv \gamma_+ + \beta$, where β is the intrinsic depolymerization rate. Note that $\hat{\gamma}_+ > \gamma_-$, as β is positive and because the applied force (if any) is directed to the right so that $\gamma_+ \geq \gamma_-$. Also, $0 < p < 1$, with the interesting case being p close to 1. The transition diagram for the system is shown in Fig. 4. To an excellent approximation, a pause begins when the bead rolls to the left away from the terminal subunit without detaching the terminal subunit, and the pause ends when the bead returns to the terminal subunit of the tubule. Thus we may analyze the statistics of a pause by assuming that the system is in state 1 of Fig. 4 at $t = 0$ and by calculating the distribution of $\tau_{\mathrm{p}} \equiv T_{10}$, the time of the first passage from state 1 to state 0. Because we are interested only in the first time that the transition $1 \rightarrow 0$ occurs, we regard state 0 as an absorbing state in this analysis. The description of a a pause given here ignores two short time intervals at the beginning and end of the pause: the time waiting in state 0 at the end of the preceding run before making the transition $0 \rightarrow 1$, and the time waiting in state 0 at the end of the pause before starting the next run. These two times have the same exponential distribution with mean given by $1/\gamma_-$; they are brief in comparison with the rest of the pause, and we ignore them here.

Let $q_{\mathrm{j}}(t)$ = probability that the system is in state j at time t, $j = 1,2,\cdots$

$$\rho_{10} = \text{probability density function for } T_{10}.$$

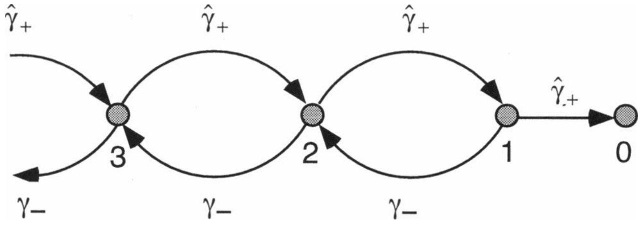

FIGURE 4 Reduced transition diagram for computing the pause time statistics.

Note that $\rho_{10}(t) = \hat{\gamma}_+\, q_1(t)$.

The $q_{\mathrm{j}}(t)$ are governed by the differential equations:

$$\begin{aligned} \frac{dq_1}{dt} &= -(\hat{\gamma}_+ + \gamma_-)q_1 + \hat{\gamma}_+ q_2 \\ \frac{dq_{\mathrm{j}}}{dt} &= \gamma_- q_{\mathrm{j}-1} - (\hat{\gamma}_+ + \gamma_-)q_{\mathrm{j}} + \hat{\gamma}_+ q_{\mathrm{j}+1}, \quad j \geq 2 \end{aligned} \tag{B1}$$

with initial conditions $q_1(0) = 1$ and $q_{\mathrm{j}}(0) = 0$ for $j > 1$. Note that

$$\frac{\mathrm{d}}{\mathrm{d}t}\sum_{\mathrm{j}=1}^{\infty} q_{\mathrm{j}}(t) = -\hat{\gamma}_+ q_1 = -\rho_{10}(t)$$

since

$$\sum_{\mathrm{j}=1}^{\infty} q_{\mathrm{j}}(t) = \Pr(t < T_{10}),$$

and ρ_{10} is the probability density function for T_{10}. We solve this system of differential equations using the Laplace transform:

$$\tilde{q}_{\mathrm{j}}(\lambda) = \int_0^{\infty} q_{\mathrm{j}}(t)\mathrm{e}^{-\lambda t}\,\mathrm{d}t$$

and make use of the property that

$$\left(\frac{\mathrm{d}\tilde{q}_{\mathrm{j}}}{\mathrm{d}t}\right)(\lambda) = \int_0^{\infty}\left(\frac{\mathrm{d}q_{\mathrm{j}}}{\mathrm{d}t}\right)(t)\mathrm{e}^{-\lambda t} = -q_{\mathrm{j}}(0) + \lambda\tilde{q}_{\mathrm{j}}(\lambda).$$

The equations for $\tilde{q}_{\mathrm{j}}$ are:

$$\begin{aligned} (\lambda + \hat{\gamma}_+ + \gamma_-)\tilde{q}_1 - \hat{\gamma}_+\tilde{q}_2 &= 1 \\ -\gamma_-\tilde{q}_{\mathrm{j}-1} + (\lambda + \hat{\gamma}_+ + \gamma_-)\tilde{q}_{\mathrm{j}} - \hat{\gamma}_+\tilde{q}_{\mathrm{j}+1} &= 0, \quad j \geq 2. \end{aligned} \tag{B2}$$

We look for solutions of the form constant $\times z^{\mathrm{j}}$, with $|z| < 1$ for real non-negative λ. This leads to the following expression for $\tilde{q}_1$:

$$\tilde{q}_1(\lambda) = \frac{2}{\lambda + \hat{\gamma}_+ + \gamma_- + \sqrt{(\lambda + \hat{\gamma}_+ + \gamma_-)^2 - 4\hat{\gamma}_+\gamma_-}}. \tag{B3}$$

This yields the transformed probability density function

$$\begin{aligned} \tilde{\rho}_{10}(\lambda) &= \hat{\gamma}_+\tilde{q}_1(\lambda) \\ &= \frac{2\hat{\gamma}_+}{\lambda + \hat{\gamma}_+ + \gamma_- + \sqrt{(\lambda + \hat{\gamma}_+ + \gamma_-)^2 - 4\hat{\gamma}_+\gamma_-}}. \end{aligned} \tag{B4}$$

From this the moments can be computed by differentiation:

$$\langle T_{10}^{\mathrm{n}}\rangle = \int_0^{\infty} t^{\mathrm{n}}\rho_{10}(t)\,\mathrm{d}t = \left(-\frac{\mathrm{d}}{\mathrm{d}\lambda}\right)^{\mathrm{n}}\tilde{\rho}_{10}(\lambda)|_{\lambda=0}. \tag{B5}$$

Carrying out the differentiation yields Eqs. 5 and 6 for the mean and variance of the pause times. Inverting the Laplace transform gives an expression for the complete probability density function:

$$\rho_{10}(t) = \mathrm{e}^{-(\hat{\gamma}_+ + \gamma_-)t} Q(2\sqrt{\hat{\gamma}_+ \gamma_-}\, t)$$

where (B6)

$$Q(x) = \frac{2}{\pi} \int_{-1}^{1} \mathrm{e}^{sx} \sqrt{1 - s^2}\, ds.$$

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