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Tension applied through the Dam1 complex promotes microtubule elongation: a direct mechanism for length control in mitosis

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Abstract

In dividing cells, kinetochores couple chromosomes to the tips of growing and shortening microtubule (MT) fibers^{1, 2} and tension at the kinetochore-MT interface promotes fiber elongation³⁻⁶. Tension-dependent MT fiber elongation is thought to be essential for coordinating chromosome alignment and separation^{1, 3, 7-10}, but the mechanism underlying this effect is unknown. Using optical tweezers, we applied tension to a model of the kinetochore-microtubule interface composed of the yeast Dam1 complex¹¹⁻¹³ bound to individual dynamic microtubule tips¹⁴. Higher tension decreased the likelihood that growing tips would begin to shorten, slowed shortening, and increased the likelihood that shortening tips would resume growth. These effects are similar to the effects of tension on kinetochore-attached microtubule fibers in many cell types, suggesting that we have reconstituted a direct mechanism for microtubule length control in mitosis.

For decades, a central problem for biologists has been to understand how MT lengths are controlled during mitosis^{15, 16}. MTs are protein polymers that switch stochastically between phases of assembly and disassembly, during which tubulin subunits are added or lost from the filament tips¹. This behavior, called ‘dynamic instability’, can be described by four parameters: the speeds of growth and shortening, and the rates of switching from growth to shortening and from shortening to growth, transitions known as ‘catastrophes’ and ‘rescues’^{1, 17}. In dividing cells, chromosomes are linked to the tips of MT fibers through specialized structures called kinetochores and their movements are coupled to fiber growth and shortening^{1, 2}. Remarkably, kinetochores maintain persistent, load-bearing attachments to microtubule tips even as the filaments assemble and disassemble under their grip^{1, 2, 18}. Classic micromanipulation experiments show that tension at the kinetochore-MT interface promotes MT fiber elongation³⁻⁶, and this effect is widely believed to be essential for controlling fiber lengths and thereby coordinating chromosome alignment and separation^{1, 3, 7-10, 16}. Very little is known about the mechanism underlying this tension-dependent length control. However, the dynamic behavior of kinetochore-attached MT tips¹⁸ implies that the mechanism underlying tension-dependent length control *in vivo* may act by altering one or more of the parameters of dynamic instability in response to load.

A key unanswered question is whether tension promotes elongation via an *indirect* mechanism, where force transmitted through load-bearing kinetochore components regulates the activity of separate MT-modifying components, or by a *direct* mechanism, where the load-bearing

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components themselves modulate MT behavior. Indirect regulation through MT-modifying enzymes has been implicated in corrective detachment of aberrant kinetochore-MT attachments¹⁹⁻²¹, but it is unknown whether an analogous indirect mechanism also controls the lengths of MTs that remain persistently attached. Evidence for a simpler direct mechanism comes from cell-free assays showing that compressive force caused by pushing MT tips against glass barriers slows filament growth^{22, 23}, and that brief pulses of tension < 1 pN transmitted through avidin-biotin linkages can delay detachment of terminal subunits²⁴. However, these approaches do not allow measurement of MT dynamic parameters under continuous tension, so their relevance to length control of kinetochore MTs—which are usually under tension^{5, 25}—is uncertain.

To overcome these limitations, we relied on a motility assay using the Dam1 complex¹¹ (also known as DASH, or DDD), a putative load-bearing component of kinetochores in yeast^{11-14, 26}. Like kinetochores, pure recombinant Dam1 complex forms robust connections to growing and shortening MT tips that remain attached even when tension is applied¹⁴. This persistent tip tracking has enabled us to measure the effects of continuous tension on MT dynamic parameters in a reconstituted system for the first time. In our assay, beads coated with the Dam1 complex are attached to the tips of individual dynamic MTs grown from stabilized seeds anchored to a glass coverslip^{14, 22, 23} (Fig. 1a). Tension is applied to the filament tip using an optical trapping-based force clamp that moves the specimen stage under feedback control¹⁴. Changes in MT length are accommodated while keeping a fixed offset between the tip-attached bead and the center of the optical trap, thus maintaining a constant tensile force. MT growth and shortening is recorded by monitoring the position of the stage.

When a Dam1-coated bead is attached to a growing MT tip and placed under tension, it moves slowly away from the coverslip-anchored seed as the intervening portion of the filament lengthens (840 ± 80 nm on average, $N = 298$ records). Sometimes the bead detaches from the growing MT (Fig. 1b, red trace). However, as reported previously¹⁴, it will sometimes remain attached long enough for the filament to undergo catastrophe. After catastrophe, the shortening MT carries the bead back toward the seed (1400 ± 330 nm, $N = 72$), performing work against the tension applied by the optical trap (Figs. 1b and 2, Movie 1). To allow frequent catastrophes to be observed, we operated at relatively low tubulin concentration ($9 - 12$ μ M). Under these conditions, the filament usually disassembles completely back to the seed after catastrophe and the bead usually detaches during shortening. However, occasionally the filament will rescue and the bead will resume assembly-coupled movement (Fig. 1b, green trace, Fig. 2b, Movie 2). These observations show that growth, shortening, catastrophe and rescue can all be recorded with continuous tension using our Dam1-based motility assay.

To determine whether MT dynamic parameters are sensitive to tension, we recorded movement of tip-attached beads at two levels of force, either 0.5 ± 0.2 pN or 2.0 ± 0.2 pN (Fig. 3, Table 1). Growth and shortening speeds were obtained for each record by plotting traces of position over time and fitting lines to the appropriate segments. The distributions of growth speed were very similar at the two levels of force (Fig. 3a), with mean values consistent with previous *in vitro* measurements¹⁷. The mean growth speed at 0.5 pN was 6.0 ± 0.8 nm s⁻¹, not significantly different ($p = 0.42$) from the mean speed at 2.0 pN, 5.8 ± 0.3 nm s⁻¹ (Fig. 3c). The insensitivity of growth speed to tension indicates that the effective binding rate of free tubulin to the MT tip is load-independent under the conditions of our assay. In contrast, the distribution of MT shortening speeds at 2.0 pN was shifted towards lower values compared to 0.5 pN (Fig. 3b). The mean shortening speed at 2 pN was 56 ± 10 nm s⁻¹, significantly slower ($p = 0.00022$) than the mean speed at 0.5 pN, 158 ± 26 nm s⁻¹ (Fig. 3d). The observation that, on average, filaments under higher tension shorten more slowly suggests that MT disassembly is sensitive to tension.

If the effect of tension on disassembly is direct, then abrupt changes in the level of tension should cause immediate changes in shortening speed. To test this hypothesis, we programmed our force clamp to automatically switch the level of tension during episodes of MT disassembly. After a specified amount of shortening occurred at the initial tension, the force was either increased (Fig. 4a,c) or decreased (Fig. 4b). Switching the force required ~ 0.2 s, during which elastic elements (e.g. seed-coverslip linkage, Dam1-bead linkage, etc.) stretched or relaxed depending on whether the force was increased or decreased. Thereafter, disassembly continued at the new, constant level of force. Fig. 4c shows an example where the force was increased from 0.5 to 2.0 pN, causing an immediate reduction in shortening speed, from 100 to 63 nm s⁻¹, which persisted > 25 s before bead detachment. Fig. 4b shows an example where the force was decreased from 1.9 to 0.3 pN, and the speed increased from 104 to 322 nm s⁻¹. To estimate the sensitivity of shortening speed to force we collected many records ($N = 27$) where the force was switched between 0.5 ± 0.2 and 2.0 ± 0.2 pN. For each record we quantified the sensitivity by computing $\Delta \text{speed} \Delta \text{force}^{-1}$, the change in shortening speed divided by the change in tension. The distribution of sensitivity values was broad, with a mean sensitivity of -40 ± 7 nm s⁻¹ pN⁻¹ (Fig. 4c, inset). This average agrees well with the mean sensitivity estimated from our prior measurements at constant force, -68 ± 28 nm s⁻¹ pN⁻¹ (Fig. 3d, Table 1), and indicates that shortening speeds are reduced when tension is increased. The observation that force can cause an immediate and persistent change in shortening speed confirms that tension affects MT disassembly in a direct manner.

MT length distributions are exquisitely sensitive to catastrophe and rescue frequencies²⁷⁻²⁹, so we sought to determine whether these switching rates are also altered by changes in tension. To estimate catastrophe and rescue rates, we counted the number of switching events observed under constant force and divided by the total observation time. At 0.5 pN we recorded 44 catastrophes during 87 episodes of MT growth with a combined duration of 4.594 hrs. On this basis, we estimate a catastrophe frequency of 9.6 ± 1.4 hr⁻¹ (Table 1). At 2.0 pN we recorded 28 catastrophes during 211 growth episodes lasting 7.888 hrs, giving a catastrophe frequency of 3.5 ± 0.7 hr⁻¹, significantly lower ($p = 0.00008$) than at 0.5 pN (Fig. 3e). Rescues were comparatively rare in our experiments, partly due to frequent bead detachment during disassembly (Fig. 3h). Nevertheless, we recorded 7 rescue events in 0.068 hrs of shortening at 2.0 pN, whereas at 0.5 pN we recorded only 3 events in 0.212 hrs (Table 1). From these observations we estimate a rescue frequency of 14 ± 8 hr⁻¹ at 0.5 pN and a 7-fold higher frequency, 103 ± 39 hr⁻¹, at 2.0 pN ($p = 0.013$, Fig. 3f).

The lower catastrophe and higher rescue frequencies at 2 pN imply that for a population of MTs, the proportion of time spent in the growth phase increases as tension increases. The lower shortening speed will also reduce the extent of disassembly during episodes of shortening. Numerous other studies have shown that similar changes, particularly reductions in the catastrophe frequency, dramatically promote microtubule elongation²⁷⁻²⁹. Depending on switching frequencies and growth and shortening speeds, a population of MTs polymerized from stable seeds can exhibit two very different classes of behavior: steady state, where the average MT length reaches a constant value, and unbounded growth, where the population grows indefinitely (until the free tubulin is depleted)²⁹. The rate parameters we measured at 0.5 pN are consistent with the steady state scenario, with an average length of 2400 ± 500 nm. In contrast, the rates at 2.0 pN are consistent with unbounded growth, with an average length for the population that increases by 3.7 ± 1.0 nm s⁻¹. (These population-average values are calculated from the measured growth, shortening, catastrophe, and rescue rates as described in Methods.) In light of these considerations, our observations indicate that the microtubules in our assay are strongly biased toward elongation at higher levels of tension.

The tension dependent behavior we observed here with Dam1 and individual MTs is strikingly similar to the behavior of kinetochores and their attached MT fibers in a wide variety of

cells^{3-6, 9, 30}. For example, tugging on chromosomes in grasshopper spermatocytes using microneedles causes the kinetochore-attached fibers opposing the load to lengthen³, consistent with our central conclusion that tension promotes net MT elongation. Likewise, our observation that tension inhibits catastrophe and promotes rescue is consistent with observations of kinetochore ‘directional instability’ in PtK₁ and newt lung cells^{4-6, 9, 30}, where increased tension causes kinetochore-attached fibers to switch from shortening to growth⁴, and loss of tension causes switching from growth to shortening⁴⁻⁶. These similarities suggest that tension at kinetochore-MT interfaces *in vivo* modulates the dynamics of attached MTs in a direct manner analogous to our *in vitro* experiments. We note, however, that an alternative explanation for the above-mentioned experiments with animal cells, in which each kinetochore binds multiple MTs, is theoretically possible³¹. Kinetochore switching could occur without tension-dependent changes in MT dynamic rates if shortening filaments under high tension detach and are subsequently replaced by new, growing filaments. But this detachment-driven mechanism is untenable in budding yeast, where each kinetochore binds a single MT tip³² and tension-dependent control of MT dynamics is strongly implicated^{8, 32}. Our experiments show that such control can be exerted directly, through the Dam1 complex or other load-bearing kinetochore components, perhaps acting in concert with indirect mechanisms mediated by additional MT-modifying agents.

It is instructive to compare our results with previous measurements of MT dynamics under compressive force. MT tips growing against rigid barriers can generate a few pN of compressive force, which is enough to reduce their growth speed ~3-fold^{22, 33} and to promote catastrophe²³. The catastrophe frequency in such experiments scales with the reciprocal of growth speed over a range of tubulin concentrations independently of whether compressive force is applied, suggesting that compression promotes catastrophe only by limiting the rate of tubulin addition²³. This relatively simple interpretation, however, cannot explain our results. Whereas forces of similar magnitude are sufficient to alter MT dynamics in either experiment (2 pN of tension versus 2 – 4 pN of compression^{22, 33}), the net effect of tension in our assay is to promote MT growth by altering filament behavior, including the catastrophe frequency, without significantly changing the speed of growth. This difference suggests that the two methods of applying force differ not only in the direction of force, but also in the underlying mechanism by which force alters MT dynamics.

Understanding the structural basis for coupling between the Dam1 complex and MT tips is of great interest, and it is critical for understanding mechanistically how tension transmitted through the complex affects filament dynamics. The data presented here provide some clues about the nature of this interaction, but considerable uncertainty remains. The tips of shortening MTs are frayed because individual rows of tubulin subunits, called protofilaments, curl outward and peel away from the main filament before breaking off³⁴. The Dam1 complex may transmit tension to these outwardly curling protofilaments, tending to straighten them. Disassembly would be slowed in this interpretation because the protofilaments must overcome the tension as they peel away from the main filament. The same scenario could explain how tension affects catastrophe and rescue frequencies, since these transitions may also involve protofilament curling and straightening, respectively. The ring model proposed recently for Dam1-based coupling to MT tips^{12-14, 26} is consistent with this picture. When pure recombinant Dam1 complex is mixed with stabilized MTs it oligomerizes into rings encircling the filaments^{12, 13}. To drive movement, peeling protofilaments may act as a ‘conformational wave’² that propagates in the direction of shortening and pushes continuously against a ring of Dam1 and also against any external tension applied to the ring. We have confirmed that under the buffer conditions of our experiments free Dam1 complex (i.e. not bead-bound) is capable of forming rings (Fig. S1). Additional experiments will be required to determine if the bead-bound Dam1 complex in our assays can also form rings and if so, whether they are necessary for the tension-dependent effects reported here.

Tension-induced MT elongation is widely believed to be essential for controlling mitotic chromosome movements in organisms as diverse as yeast^{8, 32}, insects^{3, 7, 10}, and vertebrates^{6, 9}. Our work demonstrates that this effect can be reconstituted in a simple system. The effect is direct, in the sense that pure recombinant Dam1 complex without additional factors is sufficient to form load bearing attachments that also modify MT dynamics in response to the load. Important questions remain about the underlying mechanism, particularly regarding the structure of Dam1-based MT attachments. Regardless of the structural basis, however, our finding that a load-bearing kinetochore component alters filament dynamics in a tension-dependent manner suggests a general mechanism for MT length control *in vivo*. In principle, such a mechanism could operate wherever dynamic MTs form tension-bearing tip attachments (e.g., at kinetochores, spindle poles and the cell cortex).

METHODS

Protein purification

All 10 subunits of the Dam1 complex were expressed in *E. coli* (BL21 Rosetta, Novagen) from a single plasmid (gift of J.J. Miranda, Harvard) and purified essentially as described¹²⁻¹⁴. The gene for one subunit (Spc34p) included a His₆-tag to facilitate purification and bead binding. Cells harboring the plasmid were induced to express the complex in mid-log phase by addition of 0.2 mM IPTG, grown for 5 hrs at 37 °C, pelleted, and snap frozen. Pellets were re-suspended in PB (20 mM phosphate, pH 7.0, 500 mM NaCl, 1 mM PMSF and Complete protease inhibitors without EDTA, (Roche)), lysed in a French press, and clarified by centrifugation. The supernatant was mixed with metal-affinity resin (Talon, BD Biosciences), washed in PB, and eluted with 200 mM imidazole. The eluate was purified on a size-exclusion column (Superdex 200 10/300GL, Amersham Biosciences), equilibrated with PB, and snap frozen in PB plus 10% glycerol.

Motility assays

To bind the His₆-tagged complex, 0.44- μ m-diameter streptavidin-coated polystyrene beads (SpheroTech) were further functionalized by incubation with biotinylated penta-His antibody (Qiagen) and washed in BRB80 (80 mM Pipes, 1 mM MgCl₂, 1 mM EGTA, pH 6.9) plus 8 mg ml⁻¹ bovine serum albumin (BSA), included as a blocking protein. Dilute suspensions of anti-His beads (0.01% solids) were coated with Dam1 by mixing with 24 nM of the heterodecameric complex in BRB80 plus BSA and incubating 90 min. Free Dam1 was then removed by centrifugation before introducing the beads into the flow chamber. Stable MT seeds were grown by incubating 68 μ M bovine brain tubulin, 1 μ M biotinylated tubulin (Cytoskeleton), and 1 mM GMPCPP (Jena Bioscience) in BRB80 plus 10% glycerol at 37 °C for > 30 min. KOH-cleaned coverslips were fixed with double-stick tape to glass slides to create flow chambers, which were functionalized by incubation with 5 mg ml⁻¹ biotinylated BSA (Vector Labs) followed by 0.3 mg ml⁻¹ avidin DN (Vector Labs). Seeds were bound and then washed with GB (BRB80 plus BSA with 1 mM GTP) prior to introduction of Dam1 beads and 9 – 12 μ M tubulin in GB supplemented with 1 mM DTT and an oxygen scavenging system consisting of 250 μ g ml⁻¹ glucose oxidase, 30 μ g ml⁻¹ catalase, and 4.5 mg ml⁻¹ glucose. After MT growth, thermal fluctuations tended to bend the extensions slightly away from the coverslip, making them distinguishable from the seeds and their tips accessible for bead binding. Beads were attached exclusively to MT plus ends, identified by their greater extension length as compared to minus ends. The accuracy of this assignment was > 98%, judging from control experiments in which kinesin-coated beads were used to determine unambiguously which end was the plus end (data not shown). We chose to work at low tubulin concentration in order to maximize the catastrophe frequency while still allowing MT extensions of sufficient length to grow from the coverslip-anchored seeds. The data in Table 1 and Fig. 3 were generated using two different preparations of tubulin. Because the fraction of active tubulin typically

varies slightly between preparations^{17, 33}, we adjusted the relative concentrations to match MT growth rates. With this adjustment, all MT dynamic parameters measured with applied tension for the two preparations were statistically indistinguishable, so the data were combined. Assays were performed at 22 °C.

Instrumentation and data collection

The optical trap was essentially as described previously¹⁴. Position sensor response was mapped using the piezo stage to raster-scan a stuck bead through the beam, and trap stiffness was calibrated along the two principle axes using the drag force, equipartition, and power spectrum methods. Feedback was implemented with custom LabView software. During clamping, bead-trap separation was sampled at 40 kHz while stage position was updated at 50 Hz to maintain the desired load. Bead and stage position data were decimated to 200 Hz before storing to disk. For experiments where force was changed during microtubule-driven movement, the instrument was programmed to automatically switch to the new level of tension after the bead traveled a user-specified distance in the direction of disassembly (typically 250 – 500 nm).

Data analysis

Periods of slow MT growth and rapid shortening were easily identified in the records of relative bead position versus time. Growth and shortening speeds were obtained from best-fit lines to portions of these records. For constant force experiments (Figs. 1 – 3), shortening speeds were obtained from fits to the last half of the disassembly-driven movement to avoid brief periods of acceleration, usually lasting ~1 s, which sometimes occurred just after the onset of shortening. Catastrophes were scored and shortening speeds were recorded only when a bead moved in the direction of disassembly by an amount that clearly exceeded the level of random noise in the trace (i.e., by > 40 nm). Similarly, rescues were scored only when a clear episode of disassembly-driven movement (> 40 nm) was followed again by slow growth. For the force-switch experiments (Fig. 4), shortening speeds immediately before and after the change in tension were obtained by fitting 100 nm of movement on either side of the switch. Uncertainties reported for mean speeds represent s.e.m. and corresponding significance levels were estimated using both the one-tailed student's t-distribution and the Kolmogorov-Smirnov test. Both methods yielded essentially the same results, so only p-values from the t-tests are reported here. Uncertainties reported for catastrophe and rescue rates represent counting errors, computed as $\delta r = t^{-1}\sqrt{N}$ where t is the total observation time and N is the number of transition events. These uncertainties were used to calculate the corresponding significance levels, assuming normally distributed rates with standard deviations given by δr .

Two formulas from the literature were used to estimate how the tension-dependent changes in MT dynamic parameters we measured would affect net length for a population of MTs. Depending on parameter values, a MT population can exist in either a steady state regime, where the average length reaches a constant value, or an unbounded growth regime, where the population grows indefinitely (until the free tubulin is depleted)²⁹. The first formula, adapted from Verde et al. (ref 29), represents the mean length, $\langle L \rangle$, for a population of MTs in the steady state regime:

$$\langle L \rangle = v_s v_g (v_s f_c - v_g f_r)^{-1}$$

The second formula, adapted from Walker et al. (ref 17), represents the mean rate of growth, $\langle J \rangle$, for a population in the unbounded growth regime:

$$\langle J \rangle = (v_g f_c^{-1} - v_s f_r^{-1}) (f_c^{-1} + f_r^{-1})^{-1}$$

Here, v_g and v_s are the growth and shortening speeds, and f_c and f_r are the catastrophe and rescue frequencies, respectively.

Electron microscopy

Dam1 complex was mixed with taxol-stabilized MTs to final concentrations of 20 nM Dam1 and 80 nM tubulin in motility buffer (BRB80 supplemented with 8 mg ml⁻¹ BSA, 1 mM GTP, 1 mM DTT, and an oxygen scavenging system consisting of 250 µg ml⁻¹ glucose oxidase, 30 µg ml⁻¹ catalase, and 4.5 mg ml⁻¹ glucose) plus 10 µM taxol, incubated 10 min, and fixed by addition of 3 volumes of 2% glutaraldehyde. Samples were then negatively stained and viewed on a transmission electron microscope, as follows. Carbon coated electron microscopy copper grids (EMS) were positively charged on a plasma cleaner (EMS-100, EMS). A 4 µl droplet of each sample was pipetted onto a clean grid, washed with water and stained with 2% uranyl acetate in water. Grids were air-dried and viewed on a 120kV JEM 1230. Images were recorded on a 2k × 2k Gatan slow-scan charge coupled device camera.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Recording microtubule dynamics with tension applied by an optical trapping-based force clamp. **(a)** Schematic showing experimental geometry and force clamp operation. A polystyrene bead (blue) is held by an optical trap (orange). Dam1 complex (green) on the bead surface mediates attachment to the tip of a dynamic microtubule (red). A portion of the microtubule (dark red) is anchored to the coverslip. As the microtubule grows and shortens, the coverslip is moved via computer to keep a fixed separation (Δx) between the bead and trap, thereby maintaining a constant level of tension. **(b)** Three (of $N = 298$) representative records showing position against time for tip-attached beads under tension. Increasing position represents bead movement away from the anchored portion of the filament during microtubule

growth. Decreasing position represents movement toward the anchored seed during filament shortening. Arrows mark catastrophe and rescue events. More example records are shown in Fig. 2.

Figure 2. Additional records showing microtubule dynamics with applied tension. **(a)** Three records showing growth, catastrophe (arrows), and shortening at the indicated levels of tension. **(b)** Two records showing growth, catastrophe (downward arrows), shortening, and rescue (upward arrows).

Figure 3.

Tension slows shortening, inhibits catastrophe, and promotes rescue. **(a, b)** Distributions of growth and shortening speeds at two levels of tension. Growth speed distributions at 2.0 pN (**a**, red histogram) and 0.5 pN (**a**, blue histogram) are similar in shape. The distribution of shortening speeds, however, is shifted toward lower values at 2.0 pN (**b**, red histogram) as compared to 0.5 pN (**b**, blue histogram). **(c, d)** Mean growth (**c**) and shortening speeds (**d**) plotted against tensile force. Uncertainties in speed and force represent s.e.m. and s.d., respectively. **(e, f)** Catastrophe (**e**) and rescue (**f**) rates plotted against tensile force. Uncertainties in transition rates represent counting errors. **(g)** Distributions of tensile force for the dataset. **(h)** Rates of bead detachment from shortening and growing tips, estimated by

counting the number of detachment events and dividing by the total observation time in each phase. Uncertainties in detachment rate represent counting errors.

Figure 4. Changing the level of tension during movement immediately alters shortening speed. (a – c) Three (of $N = 27$) representative records showing tensile force (upper plots) and bead position (lower plots) against time during disassembly-driven movement. In these force-switch experiments, the instrument was programmed to automatically change the level of tension after a pre-specified amount of movement occurred. Dashed vertical lines denote intervals (~ 0.2 s duration) when the force was changing. Shortening speeds before and after the change in force are indicated (gray triangles). In c, the arrow marks a catastrophe and the dashed red line shows a least-squares fit to the portion of the trace preceding the change in force. The inset shows the

distribution of sensitivities, $\Delta speed \Delta force^{-1}$, computed from each record by dividing the change in speed by the change in force.

Table 1

Microtubule dynamic parameters measured at different levels of tension

Tensile force (pN)	0.5 ± 0.2	2.0 ± 0.2
Number of growth events	87	211
Mean growth speed (nm s ⁻¹)	6.0 ± 0.8	5.8 ± 0.3
Total growth time (hr)	4.594	7.888
Number of catastrophes	44	28
Catastrophe frequency (hr ⁻¹)	9.6 ± 1.4	3.5 ± 0.7
Number of shortening events	44	28
Mean shortening speed (nm s ⁻¹)	158 ± 26	56 ± 10
Total shortening time (hr)	0.212	0.068
Number of rescues	3	7
Rescue frequency (hr ⁻¹)	14 ± 8	103 ± 39