Functions generated by protein polymerization are important for various forms of cellular motility. Assembling microtubules, for instance, are believed to exert pushing forces on the assembly dynamics of microtubules and their motors. Forces generated by protein polymerization are important for various forms of cellular motility. Assembling microtubules, for instance, are believed to exert pushing forces on the assembly dynamics of microtubules and their motors. Forces generated by protein polymerization are important for various forms of cellular motility. Assembling microtubules, for instance, are believed to exert pushing forces on the assembly dynamics of microtubules and their motors.

It has long been speculated that the assembly and disassembly of cytoskeletal filaments, such as microtubules (MTs) and actin, can generate forces that are important for various forms of cellular motility. Examples include the motions of chromosomes during mitosis that depend on both the assembly and disassembly of MTs (I, 2), actin-dependent motility such as cell crawling and the propulsion of Listeria through a host cell (3), and possibly the MT-dependent transport of intracellular membranes (4). To understand the role of force production by protein polymerization in vivo, it is important to determine the maximum forces that can be generated and the effect of an opposing force on the assembly dynamics of microtubules.

Measurement of the Force-Velocity Relation for Growing Microtubules

Marileen Dogterom* and Bernard Yurke

Forces generated by protein polymerization are important for various forms of cellular motility. Assembling microtubules, for instance, are believed to exert pushing forces on the assembly dynamics of microtubules and their motors. Forces generated by protein polymerization are important for various forms of cellular motility. For instance, microtubules are involved in the movement of organelles and vesicles during cell division. The force generated by microtubules is essential for the proper movement of chromosomes during mitosis. The study of microtubule dynamics and the forces generated by their polymerization can provide insights into the mechanisms of cell division and the regulation of cellular processes.

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a protein polymer. In the case of MTs, there is clear experimental evidence that both their assembly (4–6) and disassembly (7) can generate force, but limited quantitative data are available on the actual magnitude of these forces. In this respect, the study of force production by the assembly of cytoskeletal filaments, or by protein aggregation in general, clearly lags behind the study of force production by motor proteins, for which a number of quantitative in vitro assays have been developed (8).

We created an experimental system in which growing MTs were made to push against an immobile barrier, and analyzed the subsequent buckling of the MTs to study the forces that were produced; the force calibration was provided by a measurement of the flexural rigidity of the MTs (9). We etched arrays of long channels (30 μm wide, 1 μm deep) in glass cover slips (10); the walls of these channels were used as barriers. Using materials with different etch rates, we produced walls with an “overhang” that prevented the MTs from sliding upward along the wall (Fig. 1, A and B). Short stabilized MT seeds, labeled with biotin, were attached to the bottom of the streptavidin-coated channels, and MTs were allowed to grow from these seeds (Fig. 1A) (11). Because the seeds were randomly positioned in the channels, the MTs approached the walls from different angles and distances. We scanned our samples for MTs that were growing roughly perpendicular to the walls and observed them as their growing ends approached the walls (Fig. 1, C and D) (12).

In many cases, the MT end was caught underneath the overhang on the wall, forcing the MT to encounter the wall. After encountering the wall, most MTs continued to increase in length, indicating a continuing addition of tubulin dimers at the growing MT ends. The virtually incompressible (9) MTs were observed to bend in two different ways to accommodate this continuing increase in length. In some cases, the MT end moved along the side of the wall while the MT bent roughly perpendicular to its original direction [these MTs were not followed any further (13)]. In other cases, the MT end, probably hindered by small irregularities in the shape of the wall, did not move along the side of the wall; this caused the MT to buckle with its end pivoting around a fixed contact point with the wall (Fig. 1, C and D). The force exerted by these MTs on the wall was large enough to overcome the critical buckling force (14). After the initiation of buckling, both the magnitude and the direction of the force f exerted by each MT on the wall (and therefore by the wall on the MT) were solely determined by the elastic restoring force of the buckled MT [initially this force should be roughly equal to the critical buckling force (14)]. A considerable component f_0 of this force was directed parallel to the direction of elongation of the MT, thereby opposing its growth (Fig. 2). Assuming that a MT behaves as a homogeneous elastic rod, the magnitude of the critical buckling force f_c normalized by the flexural rigidity k of the MT is given by f_c/k = A/L^2, where L is the length of the MT. The prefactor A depends on the quality of the clamp provided by the seed: A ≈ 20.19 (the maximum value) for a perfect clamp that fixes the initial direction of the MT exactly in the direction of the contact point with the wall, A = π^2 (the minimum value) for a seed that acts as a hinge around which the MT is completely free to pivot. Because there was no reason to assume that either of these conditions would be perfectly met, we expected buckling forces somewhere between these minimum and maximum values.

To determine the actual force acting on each buckling MT, we obtained a sequence of fits to the shape of an elastic rod from video frames spaced 2 s apart (Fig. 2) (15). When no assumptions were made about the quality of the clamp or the magnitude of f, these fits produced values for f/k, f_D/k, and L as a function of time. Fig. 3A shows the parallel component of the normalized force and the MT length as a function of time for five different examples, both before and after reaching the wall. The MT length before reaching the wall was determined by tracking the end of the growing MT (15)

![Figure 1](https://example.com/fig1.png)

**Fig. 1.** In vitro assay to study the force exerted by a single growing MT. (A) Schematic representation of the experiment (shown in perspective from a side view; not to scale). A biotinylated MT seed (black), attached to the streptavidin-coated bottom of a channel (indicated by black dots), templets the growth of a freely suspended MT (gray). An overhang was created on the walls of the channel to prevent the MT ends from sliding upward after encountering the wall. (B) DIC image of two buckling MTs (top view) (12). The upper panels show each a MT [arrowhead in top left of (C)] growing from a randomly positioned seed. The lower panels are snapshots (separated by 1 min) of each MT after the growing end has encountered the wall. Because of the contrast produced by the overhang on the walls (which vary in size between samples), the last few micrometers of the MTs cannot be seen. The sharp changes in contrast indicate the actual locations of the walls. Scale bar, 10 μm.

![Figure 2](https://example.com/fig2.png)

**Fig. 2.** Analysis of MT buckling shapes (15). Open squares show the hand-recorded shapes of the MT shown in Fig. 1D at 12-s intervals (shapes were analyzed at 2-s intervals). The dashed line on the left indicates the position of the seed (x_0). The dashed line on the right indicates the position of the wall (x_y) as judged by eye from the images (Fig. 1D). The solid lines show fits to the shape of an elastic rod. One (at the top) is shown as an example. We assumed that the MT was held at its seed and that a force f was applied at the contact point of the MT with the wall (x_y). This contact point remained fixed in time and was chosen to produce the best combined fit over the entire time sequence (this produces a value of x_y very close to x_0, x_y). We further assumed that the MT was free to pivot around the contact point, but we made no assumptions about the quality of the clamp provided by the seed. The fits produced the magnitude and the direction of the force f (normalized by the flexural rigidity k) of the MT at each time point, as well as the length of the MT given by the arc length between x_0 and x_y. MT growth is opposed by f_c, the component of the force that is directed parallel to the axis of the MT. Scale bar, 5 μm.
Fig. 4 is labeled with values for the normalizing force because this is the parameter obtained from our fits. An independent measure of $\kappa$ is needed to obtain values for the absolute force. The flexural rigidity of pure MTs has been measured using various methods; the values reported range over an order of magnitude, 4 to 40 pN·µm² (6, 19, 20). We used an analysis of the thermal fluctuations to measure the rigidity of our MTs (21) and found values at the upper end of this range: 34 ± 7 pN·µm². This means that the largest forces in Fig. 4 are on the order of 4 pN (the upper x axis is labeled with absolute values of force derived from our measurement of the flexural rigidity).

The force-velocity relation in Fig. 4 can be compared with theoretical predictions. In the absence of force, the growth velocity is given by the difference in the rate of addition and removal of subunits, $v = \delta(ac - \beta)$, where $\delta$ is the added MT length per dimer ($\delta = 8/13$ nm for an MT with 13 protofilaments), $ac$ is the rate of subunit addition (the on-rate), $c$ is the tubulin concentration, and $\beta$ is the rate of subunit removal (the off-rate). In principle, both $\alpha$ and $\beta$ may be affected by a force that opposes elongation of the MT ($f_e$ in our case). Thermodynamic arguments (22) show that their ratio (which gives the critical tubulin concentration $c_r$) must increase with force according to

$$c_r(f_e) = \frac{\beta(f_e)}{\alpha(f_e)}$$

where $\kappa_0$ is the Boltzmann constant and $T$ is temperature. This leads to

$$v(f_e) = \frac{\kappa_0 T}{\delta} \ln\frac{\alpha c}{\beta}$$

(3)

A similar result is obtained if the growth process is pictured as a “Brownian ratchet” (23). In this more mechanistic view, the on-rate depends on the force-dependent probability that thermal fluctuations (in the position of the MT end in this case) allow for a gap between the MT end and the barrier that is large enough for a dimer to attach to the growing MT end (under optimal conditions, the size of this gap along the direction of MT growth is equal to $\delta$, the added length per dimer). If the force is independent of the size of the gap and the time required to add a dimer is long relative to the time required for the MT end to diffuse over a distance $\delta$, then

$$v(f_e) = \delta(\alpha \exp(-f_e/\kappa_0 T) - \beta)$$

(4)

This relation assumes that the effect of force on the off-rate can be neglected. We performed a weighted least-squares fit of the data in Fig. 4 to both the function $v(f_e) = A - B \exp(Cf_e/\kappa)$ (assuming that only the off-rate is affected or $q = 0$) and the function $v(f_e) = A \exp(-Cf_e/\kappa) - B$ (assuming that only the on-rate is affected or $q = 1$), where $A$, $B$, and $C$ are fitting parameters. In the first case, the best fit ($\chi^2 = 1.5$)
produced extremely large values for the parameters $A$ and $B$, and a value for $C$ nearly equal to zero (corresponding to almost a straight line). Experimental results show, however, that $B$ is very small in the absence of force (25). Fixing the maximum value of $B$ at 0.5 $\mu$m m$^{-1}$ produced a fit that was much worse ($\chi^2 = 2.5$), and smaller values of $B$ produced fits that were even worse. Consequently, it is unlikely that the only effect of force is an increase in the off-rate (26). Although $B$ is expected to be small, its true value should be contained from the fit for $k_5$ of $B$ obtained:

$$indicated\ by\ the\ solid\ line\ in\ Fig.\ 4)\ was$$

$$obtained:$$

$$In\ the\ second\ case,\ a\ more\ reasonable\ result$$

$$was\ much\ worse\ (equal\ to\ zero\ (corresponding\ to\ almost\ a)$$

$$equal\ to\ zero\ (corresponding\ to\ almost\ a)$$

$$force\ ([7])\ with\ isolated\ chromosomes\ ([7])\ and\ repeat:\$$

$$This\ system\ can\ also\ be$$

$$studied\ by\ the\ presence\ of\ force\ even\ larger\ than$$

$$of\ a\ full\ dimer\ may\ be\ required\ to\ squeeze\ in$$

$$is\ not\ blunt\ but\ pointed,\ only\ a\ few proto-$$

$$result\ is\ not\ the\ case\ under\ our\ conditions.$$

$$we\ assume\ that\ we\ are\ not$$

$$length\ of\ the\ wall;\ some\ of\ the\ MTs\ that\ were$$

$$of\ a\ bad\ fit\ and\ two\ because\ they\ were\ curved\ before$$

$$on\ the\ wall;\ some\ of\ the\ MTs\ that\ were$$

$$of\ a\ bad\ fit$$(5.14))

$$where\ \rho =\ the\ arc\ length\ of\ the\ MT\ ([\rho = 0\ at$$

$$order\ of\ magnitude\ of\ the\ force,\ \phi_0\ is\ its\ direction,\ and\ \kappa$$

$$and\ growth\ from\ the\ minus-end\ of\ the\ seeds\ was\ rare.$$

$$L$$. Catas-$$$$

$$were\ often\ observed\ under\ these\ conditions,$$

$$Verde, M. Dogterom, E. Karsenti, S.$$
seriously overestimating the velocities.


21. For 12 MTs, each over a 2-min interval, we measured 860

22. B. Mickey and J. Howard,


24. The elastic restoring force of a MT that is slightly buckled against an immobile barrier is roughly equal to $f_b (14)$. This is still true when the end of the MT moves a small distance (relative to the length of the MT) away from the barrier because of thermal fluctuations. The force driving the gap between the MT end and the barrier to zero is therefore independent of the size of the gap. To leave a gap of size $a$ in the direction of MT growth, the MT end must be displaced by a distance $b$ cos $\phi$ against the buckling force $f_b$, where $\phi$ is the angle between the force and the growth direction of the MT. This is equivalent to saying that the MT end must be displaced by a distance $b$ cos $\phi$ equal to the component of the force $f_b$ that is directed parallel to the axis of the MT. The contact angle with the wall does not play any role because the direction of the force is determined by the shape of the nucleated MT not by the normal to the wall. The Brownian ratchet model for a MT that grows by bending perpendicular to its axis is described in A. Mogilner and G. Oster, Biophys. J. 71, 3030 (1996).


26. This does not exclude, of course, the possibility that force affects both rates. For instance, a fit to the function

$$v(I) = A \exp(-0.5C^2/k) - B \exp(0.5C^2/k)$$

with $A = 0.43$ with $A = 1.20 \pm 0.05$ mm min$^{-1}$, $B = -0.0003 \pm 0.010$ mm min$^{-1}$, and $C = 34 \pm 4$ mm. Note, however, that $B$ is much smaller than $A$, implying that the effect of force is in any case dominated by a decrease in the rate, a result that is obtained for every positive value of $q$.


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IIKK-1 And IIKK-2: Cytokine-Activated IkB Kinases Essential for NF-kB Activation

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Activation of the transcription factor nuclear factor kappa B (NF-kB) is controlled by sequential phosphorylation, ubiquitination, and degradation of its inhibitory subunit IkB. A large multiprotein complex, the IkB kinase (IKK) signalsome, was purified from HeLa cells and found to contain a cytokine-inducible IkB kinase activity that phosphorylates IkB-α and IkB-β. Two components of the IKK signalsome, IKK-1 and IKK-2, were identified as closely related protein serine kinases containing leucine zipper and helix-loop-helix protein interaction motifs. Mutant versions of IKK-2 had pronounced effects on RelA nuclear translocation and NF-kB-dependent reporter activity, consistent with a critical role for the IKK kinases in the NF-kB signaling pathway.

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ranscription factors of the NF-κB Rel family are critical regulators of genes that function in inflammation, cell proliferation, and apoptosis (1). The prototype member of the family, NF-κB, is composed of a dimer of p50 (NF-κB1) and p65 (RelA) (2). NF-κB exists in the cytoplasm of resting cells but enters the nucleus in response to various stimuli, including viral infection, ultraviolet irradiation, and proinflammatory cytokines such as tumor necrosis factor (TNF-α) and interleukin-1 (IL-1) (1, 3).

Activation of NF-κB is controlled by an inhibitory subunit, IkB, which retains NF-κB in the cytoplasm (4). NF-κB activation requires sequential phosphorylation, ubiquitination, and degradation of IkB as well as subsequent exposure of a nuclear localization signal (5, 6) for the activation of NF-κB. The IkB kinase (IKK) signalsome, was purified from HeLa cells by standard chromatographic methods (7). We assayed IKK kinase activity in each fraction by phosphorylating glutathione-S-transferase (GST)-IkB-α (1–54) or GST-IkB-β (1–44) (8). Kinase specificity was established by using (S32T, S36T) mutant GST-IkB-α (1–54) [GST–IkB-α (1–54; S32T, S36T)], and GST–IkB-β (1–44), in which Ser$^{19}$ and Ser$^{32}$ were mutated to Ala [GST–IkB-β (1–44; S19A, S23A)] (9). IkB kinase activity was not observed in unstimulated cell extracts but was strong in cells stimulated for 5 to 7 min with TNF-α (9). Gel-filtration chromatography resolved this IkB kinase activity in a broad peak of 500 to 700 kD (Fig. 1A). In contrast to the 600-kD IkB kinase complex that was observed after treatment of cell extracts with either okadaic acid or ubiquitin-conjugating enzymes (10), the IkB kinase activity described here displayed no requirement for ubiquitination (9). We refer to the protein complex that contains the inducible IkB kinase activity as the IKK signalsome.

NF-κB activation occurs under conditions that also stimulate mitogen-activated protein kinase (MAP kinase) pathways (11). We tested preparations containing the IKK signalsome for the presence of proteins associated with MAP kinase and phosphatase cascades (Fig. 1B). The MAP kinase signalsome (MEK-1) and two Tyr-phosphorylated proteins of 55 and 40 kD copurified with IkB kinase activity (Fig. 1B). A protein of 50 kD that reacted with an antibody to MAP kinase phosphatase–1 (anti–MKP-1) also copurified with the IkB kinase through several purification steps.

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