ARTICLE

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On the stall force for growing microtubules

Abstract The assembly of microtubules generates forces that play a role in cellular motility processes such as the motion of chromosomes during mitosis. Recently, Mogilner and Oster proposed a model for the growth of microtubules that agrees quantitatively with the force-velocity relation measured for individual microtubules. In addition, the authors predicted that the stall force for any polymer consisting of N independently growing protofilaments should increase as the square root of N. We simulated this model and found that the stall force increases linearly with N, and is in fact consistent with the maximum force predicted by thermodynamic arguments. We show that this discrepancy can be explained by a more careful treatment of the "off-term" in the Mogilner-Oster model.

Key words Microtubules · Stall force · Force-velocity relation · Brownian ratchet

Introduction

Forces generated by the assembly of microtubules are thought to play a role in cellular motility processes such as the motion of chromosomes during cell division (Inoué and Salmon 1995). Both in vivo and in vitro, microtubules switch between growing and shrinking phases in a process termed dynamic instability (Desai and Mitchison 1997). It has been shown that, during this process, microtubules are able to generate both pushing and pulling forces (Inoué and Salmon 1995). The so-called force-velocity relation for a single growing microtubule has recently been measured in vitro (Dogterom and Yurke 1997). To understand this rela-

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tion and to be able to predict the magnitude of the forces that can be generated by protein assembly in general, several models can be proposed.

Thermodynamic arguments

Let us consider a polymer consisting of N protofilaments, where each protofilament is a linear aggregate of protein subunits. A microtubule, for instance, consists of 13 parallel protofilaments forming a hollow tube, whereas actin filaments have two protofilaments forming a helix. The growth or shrinkage of such a polymer results from the imbalance between the addition and removal of protein subunits at the ends of the protofilaments. Thermodynamic arguments state that the ratio between the rate of subunit addition, k_{on}, and the rate of subunit removal, k_{off}, is related to ΔG, the gain in (Gibbs) free energy upon addition of one subunit, in the following way (Hill 1987):

$$\frac{k_{on}}{k_{off}} = \exp(\Delta G/k_B T) \tag{1}$$

where k_B is Boltzmann's constant and T is the absolute temperature. Note that, for microtubules, ΔG is expected to be different depending on whether the polymer is in a growing or shrinking phase. Growth of the polymer involves the net assembly of tubulin-GTP, whereas shrinkage involves the net disassembly of tubulin-GDP. Here we do not consider sudden changes in polymerization dynamics due to switching between these two phases (dynamic instability), but only consider the polymerization process in the growing phase itself. When ΔG is positive in this phase, the on-rate exceeds the off-rate, resulting in a net growth of the polymer with a velocity given by: $v = \delta(k_{on} - k_{off})$, where δ is the size of the protein subunit.

These same thermodynamic arguments predict that the maximum force that can be generated by the assembly of a polymer consisting of N protofilaments (the

stall force) is given by ΔG divided by δ/N, the added polymer length per subunit (Hill 1987):

$$F_{\text{stall}} = \frac{\Delta G}{\delta/N} = \frac{Nk_BT}{\delta}\ln\left(\frac{k_{\text{on}}}{k_{\text{off}}}\right) \qquad (2)$$

When a load force equal to F_{stall} is applied to the end of a growing polymer the amount of work, $F_{\text{stall}}\delta/N$, that has to be performed on average for the addition of a subunit is exactly equal to the free energy of polymerization. The system is in equilibrium and no net growth occurs. In the presence of a finite force, $F < F_{\text{stall}}$, one expects that the ratio between the on- and the off-rate changes in the following way:

$$\frac{k_{\text{on}}^*}{k_{\text{off}}^*} = \exp(\Delta G/k_BT)\exp(-F\delta/Nk_BT)$$

$$= \frac{k_{\text{on}}}{k_{\text{off}}}\exp(-F\delta/Nk_BT) \qquad (3)$$

If the (arbitrary) assumption is made that the force has no effect on the off-rate, this leads to the following prediction for the force-velocity relation:

$$v = \delta(k_{\text{on}}\exp(-F\delta/Nk_BT) - k_{\text{off}}) \qquad (4)$$

Brownian ratchet models

Thermodynamic arguments ignore the possible effects of geometrical details of the growth process on the force-velocity relation. More mechanistic arguments that take these details into account are based on "Brownian ratchet" models (Peskin et al. 1993). In these models a barrier is pushed against the end of the growing polymer with a force F. Subunits can add to the polymer only when thermal fluctuations of either the polymer tip or the barrier position allow for a gap between the end of the growing polymer and the barrier large enough to insert a new subunit. In the limit that thermal fluctuations are fast compared to the frequency at which new subunits attach, the on-rate is simply multiplied by the probability for a gap of the correct size l to occur: $k_{\text{on}}^* = k_{\text{on}}\exp(-Fl/k_BT)$.

For a single protofilament ($N = 1$), these models predict a force-velocity relation identical to the one given in Eq. (4), and no clear distinction exists between the two approaches. For $N = 2$ or larger, this result may still be expected to hold if the geometry of the growing polymer is such that the gap size needed for the addition of every subunit is equal to δ/N (Peskin et al. 1993). Or, in other words, if the applied force affects the addition of every subunit and the growth of every protofilament contributes to the work. However, experimental measurements of the force-velocity relation suggest that this is not the case for growing microtubules (Dogterom and Yurke 1997). In these experiments it was found that the velocity decreases faster with the applied force than predicted by Eq. (4). One way to interpret this result is that in fact only a few of the protofilaments are able to contribute to

the work, and that therefore the exponential decay of the velocity is faster than predicted by Eq. (4).

Recently, Mogilner and Oster proposed a model that explicitly takes into account the growth geometry of a multi-protofilament polymer in the simplest way. They introduced a generalized ratchet model in which they considered a polymer consisting of N independently growing protofilaments with a barrier pushed against the longest filament (Mogilner and Oster 1999) (Fig. 1). In this model the protofilament length distribution is free to evolve, depending on the local rates of addition and removal of subunits, while possible interactions between the neighboring filaments are ignored. It is assumed that the force affects the rate of subunit addition only for filaments whose tips are within a distance smaller than δ from the barrier, and that the local on-rate for such filaments only depends on the gap that needs to be created between the leading tip and the barrier.

When the authors treated the filament tip distribution as a continuum, they were able to solve this model numerically in the limit that k_{off} is zero. They found that for any finite force a steady state filament tip distribution is reached. The number of filament tips within a distance δ from the leading filament, considered to be
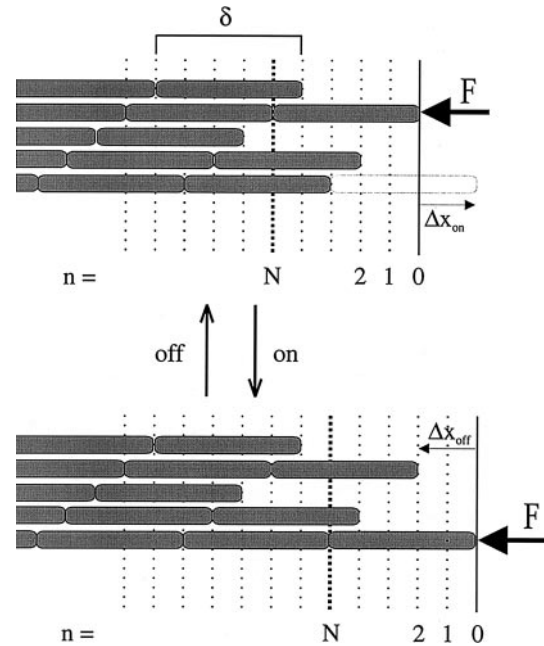
Fig. 1 Discrete version of the Mogilner-Oster model for a polymer with $N = 5$ independently growing filaments. A force F is applied at a barrier supported by the leading filament. Initial conditions fix the distance between neighboring filaments at $\sigma = \delta/N$, so that at every position denoted by n ($x = n\sigma$) at most one filament tip can be found. The probability to find a filament tip at position n is given by p_n, where $p_0 \equiv 1$. The on-rate is given by k_{on} for every filament whose tip is at a position $n \geq N$. When $n < N$, the on-rate is given by $k_{\text{on}}\exp(-F\Delta x_{\text{on}}/k_BT)$, where Δx_{on} is the length added to the polymer by the growth of that filament. The off-rate is given by k_{off} for every filament. Δx_{off} is the length subtracted from the polymer when the leading filament loses a subunit. At the stall force the two configurations shown are in equilibrium: $p_3\Delta x_{\text{on}}k_{\text{on}}\exp(-F_{\text{stall}}2\sigma/k_BT) = k_{\text{off}}\Delta x_{\text{off}}p_2(1 - p_1)$ [see Eq. (11b)]

the number of "working" filaments, was indeed found to be smaller than N and to increase with increasing force. For $N = 13$ (the case of microtubules) the resulting force-velocity relation was found to be remarkably consistent with the experimental data, despite the fact that k_{off} was taken to be zero.

The authors then used this model to predict the stall force for a multi-protofilament polymer. They introduced a finite k_{off} in the force-velocity relation and found that the stall force depends on the square root of the number of filaments:

$$F^*_{stall} = \frac{k_B T}{\delta} \sqrt{\frac{k_{on}}{k_{off}}} \sqrt{N} \qquad (5)$$

Here we argue that within this model the stall force is instead linear in N as predicted by thermodynamic arguments [Eq. (2)], as can be readily seen from a simulation of the Mogilner-Oster model for finite k_{off}. We show how this result can be obtained analytically from a discrete version of the model by a more careful treatment of the term describing the removal of subunits in the force-velocity relation (the "off-term").

Methods

Simulations of the Mogilner-Oster model

We simulated the growth of a polymer consisting of N parallel filaments (see Fig. 1 for $N = 5$), each consisting of a linear array of subunits with size δ. As an initial condition the first (upper) filament was chosen to be the longest filament and a barrier was placed at its tip. The tip of the second filament was placed a distance δ/N from the barrier, the third at $2\delta/N$, and so forth. This choice of tip positions corresponds to an initial condition in which all the tips are evenly distributed over an interval of size δ. A load force F was applied to the barrier.

For each time step we choose N times randomly one of the filaments. If the filament tip was at a distance larger than δ from the barrier, a subunit was added with probability $k_{on}d\tau$, and a subunit was removed with probability $k_{off}d\tau$, where $d\tau$ is the duration of the time step. For filament tips within a distance δ from the barrier these probabilities were $k_{on}d\tau \exp(-F(\delta - x)/k_B T)$ and $k_{off}d\tau$, respectively, where x is the distance between the filament tip and the barrier. Note that the effect of force on the on-rate is related to the gap size needed between the longest filament and the barrier, given by $\delta - x$. After addition of a subunit to any filament within a distance δ from the barrier, this filament became the new leading filament and the barrier was moved to the position of its tip.

After the distribution of filament tips was allowed to reach steady state, the growth velocity was computed by dividing the displacement of the barrier over m time
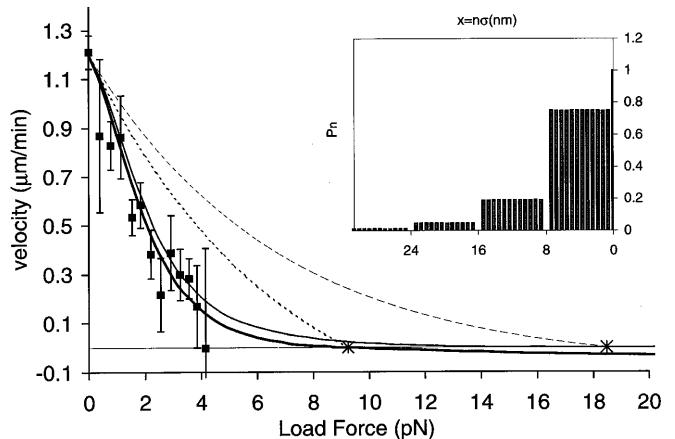
Fig. 2 The force-velocity relation for growing microtubules. *Squares* are the experimental data from Dogterom and Yurke (1997). *Solid lines* are simulation results of the discrete Mogilner-Oster model for two different values of k_{off}. *Thick line*: $k_{off} = 50 \, \text{min}^{-1}$ ($k_{on} = 200 \, \text{min}^{-1}$); *thin line*: $k_{off} = 10 \, \text{min}^{-1}$ ($k_{on} = 160 \, \text{min}^{-1}$). Other parameters: $N = 13, \delta = 8 \, \text{nm}, k_B T = 4.1 \, \text{pN nm}, d\tau = 10^{-3} \, \text{min}, m = 5 \times 10^5$. *Dashed lines* are the corresponding curves predicted by Eq. (4). *Stars* indicate the stall forces predicted by Eq. (2) for the two cases. Note that the simulation results are not very sensitive to the value of k_{off} in the range where the experimental data were taken. *Inset*: simulation result for the probability distribution of filament tip positions at the stall force predicted by Eq. (2) for $k_{off} = 50 \, \text{min}^{-1}$. The x-axis gives the distance of the tip to the barrier

steps by $md\tau$. This was repeated for different values of F to obtain the force-velocity relation (Fig. 2). The stall force was estimated by locating the force where this relation crosses the zero-velocity axis. At the stall force the distribution of filament tips was averaged over m time steps to obtain the probability distribution of tip positions (inset Fig. 2).

The force-velocity relation

Analytically, the velocity of the growing polymer is obtained by considering the average change in length of the polymer (or position of the barrier) per unit of time, assuming the system is in a steady state. This change is just the difference between the length added due to subunit assembly and the length subtracted due to subunit removal:

$$v = v_{on} - v_{off} = \langle k^*_{on}(F)\Delta x_{on}\rangle - k_{off}\langle \Delta x_{off}\rangle \qquad (6)$$

To compute the velocity we need to introduce ρ_x, the average number of filament tips present at distance x from the barrier. It is important to note that our choice of initial conditions imposes a number of restrictions on ρ_x. First of all, filament tips can only be found at distances given by multiples of δ/N from the barrier. It is therefore convenient to choose the shift $\sigma = \delta/N$ as our unit of length, and measure distances as multiples of σ. Furthermore, at most one filament can be at a given distance from the barrier at any given time. ρ_x can therefore be replaced by the probability $0 \leq p_n \leq 1$ to

find a filament at position n, at a distance $x = n\sigma$ from the barrier. Note that p_0 has to be equal to 1, since there is always one filament tip at the barrier. At any given time, the tip of any filament can only be found at distances from the barrier that are separated by multiples of $\delta = N\sigma$, and finally, the total number of tips has to be equal to N. We can therefore impose:

$$\sum_{n=0}^{\infty} p_n = N \tag{7a}$$

$$\sum_{i=0}^{\infty} p_{n+iN} = 1 \quad \text{for } n < N \tag{7b}$$

With this in mind, we compute the on-term in Eq. (6). Any filament tip located at a position $n < N$ from the barrier will add a length $\Delta x_{\text{on}} = (\delta - x) = \sigma(N - n)$ to the polymer at a rate given by:

$$k_{\text{on}}^*(F) = k_{\text{on}} \exp(-F(N-n)\sigma/k_{\text{B}}T) \tag{8}$$

The total average contribution due to addition of subunits is thus given by:

$$v_{\text{on}} = \sum_{n=0}^{N-1} p_n(N-n)\sigma k_{\text{on}} \exp(-F(N-n)\sigma/k_{\text{B}}T) \tag{9}$$

This expression is the discrete analogue of the term for the average growth velocity derived by Mogilner and Oster (1999).

To compute the off-term in Eq. (6) we note that only the removal of a subunit from the longest filament leads to a decrease in polymer length. The decrease in length Δx_{off} upon removal of a subunit from the longest filament is equal to the distance of the next longest filament from the barrier. This distance equals σ with probability p_1, 2σ with probability $p_2(1 - p_1)$, etc. The average decrease in polymer length per unit time is thus given by:

$$v_{\text{off}} = k_{\text{off}} \left[\sigma p_1 + \sum_{n=2}^{N-1} \left(n\sigma p_n \prod_{j=1}^{n-1}(1 - p_j) \right) \right. $$
$$\left. + N\sigma \prod_{j=1}^{N-1}(1 - p_j) \right] \tag{10}$$

where the final term accounts for the possibility that the distance between the two longest filaments is larger than $\delta = N\sigma$. In the article by Mogilner and Oster the off-term was simply taken to be $k_{\text{off}}\delta$, leading to the prediction of the stall force given by Eq. (5).

Results

We found in our simulations of the Mogilner-Oster model that the stall force depends linearly on the number of filaments, and is in fact exactly given by the thermodynamic stall force given by Eq. (2) (Fig. 3). This same result can also be obtained analytically. At the stall force F_{stall} the net velocity is zero and $v_{\text{on}} = v_{\text{off}}$. The
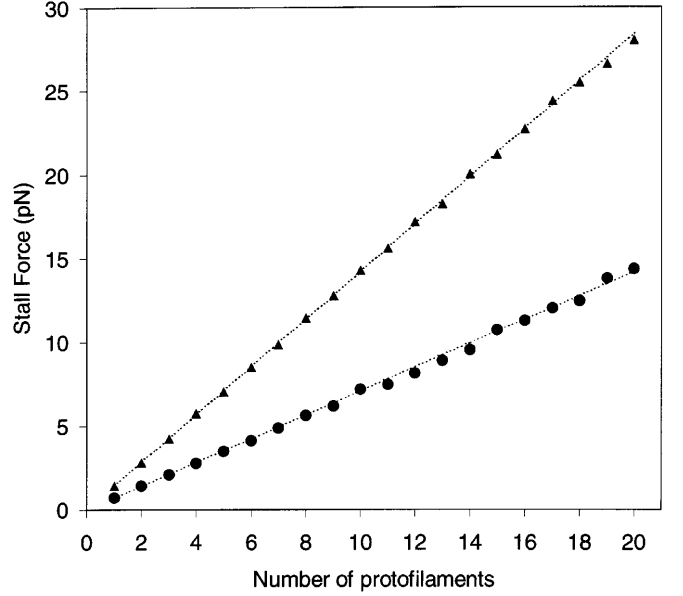
Fig. 3 Stall force as a function of the number of filaments for the same values of k_{off} as in Fig. 2. The *symbols* are simulation results and the *dashed lines* are the values predicted by Eq. (2)

system is in equilibrium, which means we can impose a detailed balance condition between all pairs of tip configurations that can be transformed into each other by a single subunit addition or removal event. An example is given in Fig. 1: the two configurations shown are in equilibrium so that we can write $p_3\Delta x_{\text{on}}k_{\text{on}} \exp(-F_{\text{stall}}2\sigma/k_{\text{B}}T) = k_{\text{off}}\Delta x_{\text{off}}p_2(1 - p_1)$ for $\Delta x_{\text{on}} = \Delta x_{\text{off}} = 2\sigma$. All terms in the on-term [Eq. (9)] and the off-term [Eq. (10)] can thus be matched in the following manner:

$$p_0 N\sigma k_{\text{on}} \exp(-F_{\text{stall}}N\sigma/k_{\text{B}}T) = k_{\text{off}}N\sigma \prod_{j=1}^{N-1}(1 - p_j) \tag{11a}$$

$$p_{N-n}n\sigma k_{\text{on}} \exp(-F_{\text{stall}}n\sigma/k_{\text{B}}T)$$
$$= k_{\text{off}}n\sigma p_n \prod_{j=1}^{n-1}(1 - p_j) \quad \text{for } 0 < n < N \tag{11b}$$

$$k_{\text{on}}p_{n+N} = k_{\text{off}}p_n \quad \text{for } n > 0 \tag{11c}$$

where the last equation gives the detailed balance condition for subunit addition and removal events that do not affect the length of the polymer. With the conditions imposed by Eqs. (7) we only need Eq. (11c) to find the full solution for p_n: given that p_0 is equal to 1, Eq. (7b) says that $p_{iN} = 0$ for every $i > 0$; for every $n > 0$ we have

$$p_{n+N} = \frac{k_{\text{off}}}{k_{\text{on}}}p_n$$

[Eq. (11c)], which, for $0 < n < N$, gives

$$\sum_{i=0}^{\infty} p_{n+iN} = \sum_{i=0}^{\infty} \left(\frac{k_{\text{off}}}{k_{\text{on}}} \right)^i p_n = 1$$

[Eq. (7b)]. With

$$\sum_{i=0}^{\infty} \left(\frac{k_{\text{off}}}{k_{\text{on}}}\right)^i = \frac{k_{\text{on}}}{k_{\text{on}} - k_{\text{off}}}$$

this leads to the following solution:

$$p_0 = 1 \tag{12a}$$

$$p_{iN} = 0 \quad \text{for } i > 0 \tag{12b}$$

$$p_{n+iN} = \frac{k_{\text{on}} - k_{\text{off}}}{k_{\text{on}}} \left(\frac{k_{\text{off}}}{k_{\text{on}}}\right)^i \quad \text{for } 0 < n < N \text{ and } i \geq 0 \tag{12c}$$

This solution was also found in our simulations (see inset Fig. 2). For any n, Eqs. (11a) and (11b) now give the same stall force as predicted by thermodynamic arguments [Eq. (2)]:

$$F_{\text{stall}} = \frac{N k_{\text{B}} T}{\delta} \ln\left(\frac{k_{\text{on}}}{k_{\text{off}}}\right)$$

Discussion

We have shown in this article that a discrete version of the Mogilner-Oster generalized ratchet model leads to the same prediction for the stall force of a multi-protofilament polymer as predicted by simple thermodynamic arguments. We believe that the different prediction given by Mogilner and Oster (1999) is the result of an oversimplification of the off-term in the force-velocity relation.

This result leads to the suggestion that geometrical details of the growth process may influence the force-velocity relation for forces smaller than the stall force, but that they do not influence the stall force itself. In Fig. 2 we compare the full force-velocity relation predicted by simple thermodynamic arguments [Eq. (4)] with our simulations of the Mogilner-Oster model. The two curves converge for $F = 0$ and $F = F_{\text{stall}}$, but give different results in between. In our simulations of the Mogilner-Oster model we found the same stall force

independent of the exact choice of the initial shift between neighboring protofilament tips. We also found the same result when we assumed that the force affected both the on- and the off-rate in a way consistent with Eq. (3) or when we introduced an energy term connected to lateral interactions between neighboring subunits (a full description of the discrete model will be published elsewhere).

Experimentally, it may thus be expected that information about the details of the growth process and the geometry of the polymer tip can be obtained from the shape of the force-velocity curve, but not from the end points. For the different situations mentioned above we always found the same stall force, whereas clear differences were found elsewhere in the force-velocity curve (to be published). Figure 2 furthermore clearly shows the danger of trying to infer information about the stall force from experimental data in the range available so far. In this range the theoretical curve predicted by the present model shows very little sensitivity to the exact value of k_{off}.

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