Biophysical Journal, Volume 98

Supporting Material

Force transduction by the microtubule-bound Dam1 ring

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Supporting material for "Force transduction by the microtubule-bound Dam1 Ring"

Here we give several results that are supplementary to the main body of our analysis. In particular, we examine (1) the role of a drag force, such as might arise from a moving chromosome; (2) the detachment time for a Dam1 ring, and (3) present the data we use to fit our model.

1 Chromosome drag

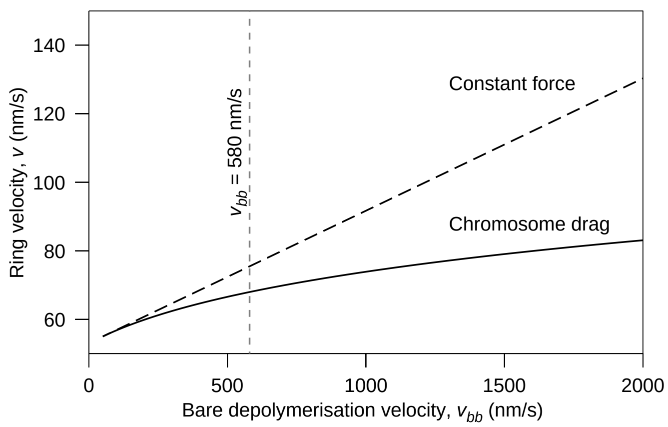

Figure 1: Ring velocity v dependence on bare depolymerization velocity $v_{\rm bb}$ of both models under $f_0 = 1$ pN compared to velocity dependent chromosome drag $f(v) = v \cdot (k_B T/D_c) + f_0$. Under constant load, velocity increases linearly. With an additional chromosome drag ($D_c = 0.0004\ \mu\text{m}^2/\text{s}$ (1)) the total load increases proportionally to v and consequently v follows the form of the Lambert-W function.

The effect of occlusion of the MT end by the ring on v is given by

$$v = (v_{\rm bb} - v_{\rm ps}) \int_{\delta}^{\infty} \phi(x)\, dx + v_{\rm ps}, \tag{1}$$

To account for the viscous drag caused by moving a chromosome at constant velocity through the cytosol of a cell, the load force f must have a component that is proportional to velocity, where the constant coefficient is given by the Einstein relation $\xi = k_B T/D$ (2). Thus

$$f(v) = \xi v + f_0, \tag{2}$$

where f_0 is the constant (external) load, and $f(v)$ is directed towards the chromosome. To solve Eqs. 1 and 2 self-consistently for v we consider the Dam1 ring diffusing on the MT, as Eq. 1 in the main article, with a reflecting (i.e. zero flux) boundary condition at the tip

$$\frac{\partial \phi}{\partial t} = D\frac{\partial}{\partial x}\left(\frac{\partial \phi}{\partial x} + \frac{f(v)}{k_B T}\phi\right). \tag{3}$$

At steady state, assuming the quasi-equilibrium condition $\lambda/v(f) \gg \lambda^2/D$ holds, the flux must vanish, according to

$$\frac{d\phi}{dx} + \frac{f(v)}{k_B T}\phi = 0. \tag{4}$$

giving the quasi-equilibrium distribution

$$\phi(x) = \frac{f(v)}{k_B T}\exp\left(-\frac{f(v)}{k_B T}x\right). \tag{5}$$

Substituting Eq. 5 into Eq. 1 and solving for v

$$v = \frac{D}{\delta}W\left[\frac{(v_{\mathrm{bb}} - v_{\mathrm{ps}})\delta}{D}\exp\left\{-\left(\frac{v_{\mathrm{ps}}}{D} + \frac{f_0}{k_B T}\right)\delta\right\}\right] + v_{\mathrm{ps}}, \tag{6}$$

where $W(\cdot)$ is the Lambert-W function (3). A comparison of Eq. 6 and Eq. 4 from the main article is shown in Fig. 1.

2 First passage time

The average distance of the ring from the tip is

$$\lambda = \frac{k_B T}{f}$$

and the characteristic time to diffuse this length is $t_D = \lambda^2/D$. For the assumption that distribution of the ring position is quasi-static to hold we require $t_D \ll t_{\mathrm{unzip}}$, or equivalently

$$f \gg k_B T\left(\frac{k_{\mathrm{unzip}}}{D}\right)^{1/2} = f_{\mathrm{min}} \tag{7}$$

If this condition is met then the probability that the ring does *not* reach the tip before further unzipping takes place is negligible (for $v_{\mathrm{bb}} = 580$ nm/s we find $f_{\mathrm{min}} = 0.15$ pN). Otherwise, we must include the probability that the first passage time t_{fp}, the time it takes the ring to first reach the tip, satisfies $t_{\mathrm{fp}} < t_{\mathrm{unzip}}$, for which detachment occurs. Following Redner (4), we can calculate this probability. The distribution of t_{fp} for a particle at λ at time $t = 0$ is

$$t_{\mathrm{fp}}(t) = \frac{\lambda}{(4\pi D t^3)^{1/2} e^{-\lambda^2/4Dt}}. \tag{8}$$

The probability the particle reaches the end before the protofilaments grow is then

$$\begin{aligned} P_{\mathrm{fp}}(t_{\mathrm{unzip}}) &= \int_0^{t_{\mathrm{unzip}}} t_{\mathrm{fp}}(t)\,dt \\ &= 1 - \mathrm{erf}\left(\frac{\lambda}{(4Dt_{\mathrm{unzip}})^{1/2}}\right). \end{aligned} \tag{9}$$

We can thereby calculate the mean residence time of a ring on a shrinking MT

$$\tau = \frac{\langle t_{\mathrm{unzip}} \rangle}{P_{\mathrm{detach}} P_{\mathrm{fp}}(\langle t_{\mathrm{unzip}} \rangle)}, \tag{10}$$

where P_{detach} is the probabilty of detachment during a step as calculated in the main text.

3 Velocity of ring under load

Force f (pN)	0.5 ± 0.2	2.0 ± 0.2
# measurements	44	28
Total depolymerization time (h)	0.212 ± 0.036	0.068 ± 0.013
# detachments	32	20
Detachment frequency (1/h)	150 ± 30	290 ± 70
Velocity v (nm/s)	158 ± 26	56 ± 10
Runtime τ_{res} (sec) [a]	23.9 ± 6.2	12.2 ± 4.1
Runlength y (μm) [b]	3.8 ± 1.0	0.7 ± 0.2

[a] Calculated by dividing total depolymerization time by # detachments.
[b] Calculated by multiplying runtime by velocity.

Table 1: Velocity of ring during MT disassembly under various loads. Data obtained from Table 1 of Franck et al. (5). Runtime and runlength defined as the time and distance, respectively, between switching to depolymerization and detachment.

We constrain our model by comparison with recent experiments where load has been applied to a Dam1 ring attached to a depolymerizing MT by way of an optical trap (5–7). The nature of MT depolymerization and ring motion is highly stochastic. The available data from (5) is displayed in Table 1.

References

1. Nicklas, B. R. 1983. Measurements of the force produced by the mitotic spindle in anaphase. J. Cell Biol. 97:542–548.

2. Landau, L. D., and E. M. Lifshitz. 1980. Statistical Physics. Butterworth-Heineman, Oxford, third edition.

3. Corless, R. M., G. H. Gonnet, D. E. G. Hare, D. J. Jeffrey, and D. E. Knuth. 1996. On the Lambert *W* function. Adv. Comp. Math. 5:329–359.

4. Redner, S. 2001. A Guide to First Passage Processes. Cambridge University Press, Cambridge.

5. Franck, A. D., A. F. Powers, D. R. Gestaut, T. Gonen, T. N. Davis, and C. L. Asbury. 2007. Tension applied through the Dam1 complex promotes microtubule elongation providing a direct mechanism for length control in mitosis. Nat. Cell Biol. 9:832–837.

6. Asbury, C. L., D. R. Gestaut, A. F. Powers, A. D. Franck, and T. N. Davis. 2006. The Dam1 kinetochore complex harnesses microtubule dynamics to produce force and movement. Proc. Natl. Acad. Sci. USA 103:9873–9878.

7. Grishchuk, E. L., A. K. Efremov, V. A. Volkov, I. S. Spiridonov, N. Gudimchuk, S. Westermann, D. Drubin, G. Barnes, J. R. McIntosh, and F. I. Ataullakhanov. 2008. The Dam1 ring binds microtubules strongly enough to be a processive as well as energy-efficient coupler for chromosome motion. Proc. Natl. Acad. Sci. USA 105:15423–15428.