3 Function

A variety of cell functions are carried out by proteins. A number of biochemical functions are necessary to the survival of the cell, such as enzymatic activity in breaking down sugars, or transporting oxygen via Hemoglobin. Other functions pertain to sensing the environment, signaling its state, and mounting necessary responses, as in the case of transcription factors interacting with proteins. Still other functions are biophysical, related to force and motion, e.g. transporting organelles, maintaining cell shape, and producing motion. We shall explore examples of the latter in the context of microtubules and motors.

3.1 Filaments of the cytoskeleton

The interior of most cells is far from a fluid bag, but assumes specific shapes and functionalities through the active dynamics of protein fibers forming its *cytoskeleton*. Long fibers are formed by assembling protein units; much like polymers but considerably stiffer due to larger subunits. The thinnest *microfilaments* are made of the monomer *actin* and are roughly 8nm in diameter. *Actin filaments* have a specific directionality (like an arrow), and are typically in a state of dynamic equilibrium in which monomers dissociate from one end (minus side) and assemble at the other end (plus side), via a process called *tread-milling*. In the following, we shall focus on a related dynamic equilibrium in *microtubules*. These are much larger filaments, with diameter of 25nm, assembled from dimers of α and β *tubulin*. Microtubules are cylinders formed by stacking 13 proto-filaments around a hollow core. They are directional, and of variable length, growing up to 25,000 nm in length. *Intermediate filaments* in the size range of 8 to 25nm occur in a variety of cells, for example strengthening the long axons of neurons.

3.1.1 Dynamic Instability of Microtubules

Discovered in the 1980s, *dynamic instability* is a phenomenon whereby a microtubule grows at a slow, steady rate, then a "catastrophe" is initiated whereby it shortens at a much faster rate, until a "rescue" event restores the original slow growth. This cycle repeats itself (stochastically) on a timescale of several minutes. Key to appreciating the behavior of microtubules, indeed as with the cytoskeleton and the entire cell, is that there is a constant influx of energy. In the case of miccrotubules, this energy is provided by the hydrolysis of GTP (guanosine tri-phosphate) to GDP (guanosine di-phosphate). The tubulin monomers need to be attached to GTP before they can be added to the growing end of a microtubule. The growing (+) end of the microtubule is thus composed of GTP bound tubulin. Further behind this cap, some GTP molecules can hydrolyze to GDP, releasing around $10k_BT$ of energy in the process. The resulting GDP rich region is less stable, and GDP bound tubulin can detach from the other (-) end. We shall first address how the addition and detachment of tubulins can lead to a dynamic steady state, and then inquire if the rapid dynamic changes are due to instabilities in the cap.

3.1.2 Microtubule Growth and Regulation

To study the dynamic behavior of a microtubule (MT), we examine a simple two state model:[1] The MT is either slowly growing with velocity v_+, or rapidly shrinking with velocity v_-. Assuming that one end of the MT is attached to a stationary support at $z = 0$, the growing (+) end can be at a distance z at time t. With probability $p_+(z,t)$ the MT is growing, while with probability $p_-(z,t)$ it has lost its GTP cap and is shrinking. There is a probability that a catastrophe occurs, switching from a growing to a shrinking MT, at a rate f_{+-}, while the converse rescue events occur at rate f_{-+}.
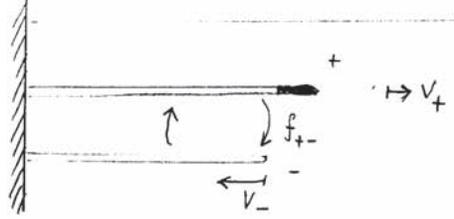
The two probability functions evolve according to the coupled equations

$$
\begin{aligned}
\frac{\partial p_+(z,t)}{\partial t} &= -f_{+-}p_+(z,t) + f_{-+}p_- - \frac{\partial}{\partial z}(v_+ p_+) \,, \\
\frac{\partial p_-(z,t)}{\partial t} &= +f_{+-}p_+(z,t) - f_{-+}p_- + \frac{\partial}{\partial z}(v_- p_-) \,.
\end{aligned}
\tag{3.1}
$$

For simplicity, we have assumed a continuously variable length z; a more microscopic representation would describe the evolution of $p_\pm(n,t)$ for n tubulin units, with rates for addition/subtraction of units. The above coupled partial differential equations are linear, and hence easily solved by Fourier transforming in space and time. Effectively this amounts to the replacements

$$
\frac{\partial}{\partial t} \rightarrow -i\omega, \ \ \frac{\partial}{\partial z} \rightarrow ik,
\tag{3.2}
$$

which after simple manipulations lead to the matrix equation

$$
\begin{pmatrix} i\omega - f_{+-} - ikv_+ & f_{-+} \\ f_{+-} & i\omega - f_{-+} + ikv_- \end{pmatrix} \begin{pmatrix} \tilde{p}_+(k,\omega) \\ \tilde{p}_-(k,\omega) \end{pmatrix} = 0.
\tag{3.3}
$$

For the matrix equation to have non-zero solutions, the determinant of the 2×2 matrix must be zero. This condition leads to a dispersion equation of the form

$$
\omega(k) = vk - Dk^2 + \cdots .
\tag{3.4}
$$

The term linear in k represents the net drift velocity, while the quadratic term describes an effective diffusion due to the switchings between the two states. The details of this calculation

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[1]M. Dogterom and S. Leibler, *Phys. Rev. Lett.* **70,** 1347 (1993).

are left to the problem sets. We shall instead derive expressions for v and D by appealing to physical arguments.

Consider the limit where exchanges between $+$ and $-$ states take place rapidly. This quickly leads to an equilibrium between the two states such that

$$\frac{p_+}{p_-} \approx \frac{f_{-+}}{f_{+-}}, \qquad \Longrightarrow \qquad p_+ = \frac{f_{-+}}{f_{+-} + f_{-+}}, \quad \text{and} \quad p_- = \frac{f_{+-}}{f_{+-} + f_{-+}}. \tag{3.5}$$

The evolution of the net probability $p(z,t) = p_+(z,t) + p_-(z,t)$ for MTs of length z is then obtained by adding the two evolution Eqs. (3.1), as

$$\frac{\partial p(z,t)}{\partial t} = -\frac{\partial}{\partial z}\left(v_+ p_+ - v_- p_-\right), \tag{3.6}$$

which using Eq. (3.5) turns into

$$\frac{\partial p(z,t)}{\partial t} = -\frac{\partial}{\partial z}\left[\left(v_+ \frac{f_{-+}}{f_{-+} + f_{+-}} - v_- \frac{f_{+-}}{f_{-+} + f_{+-}}\right) p\right]. \tag{3.7}$$

The solution to the above equation is a traveling wave $p(z,t) = p(z - vt)$ describing a net probability that drifts to higher z with a velocity

$$v = \frac{f_{-+} v_+ - f_{+-} v_-}{f_{-+} + f_{+-}}. \tag{3.8}$$

Of course, given the barrier at $z = 0$, the above solution only makes sense as long as v is positive. When the value of v from Eq. (3.8) is negative, the MTs tend to shrink towards zero. In this case fluctuations lead to a time independent steady state in which

$$p_\pm(z,t) = p_\pm^*(z) = c_\pm e^{-\alpha z}. \tag{3.9}$$

This can be verified by substituting Eq. (3.9) into Eqs. (3.1) to obtain the matrix form

$$\begin{pmatrix} -f_{+-} + \alpha v_+ & +f_{-+} \\ +f_{+-} & -f_{-+} - \alpha v_- \end{pmatrix} \begin{pmatrix} c_+ \\ c_- \end{pmatrix} = 0. \tag{3.10}$$

Once again, the determinant of the matrix must be zero to allow non-zero solutions, leading to

$$\alpha = \frac{f_{+-} v_- - f_{-+} v_+}{v_- v_+}. \tag{3.11}$$

The probability to find a MT of length z in this state is a simple exponential with

$$\langle z \rangle = \frac{1}{\alpha} = \frac{v_- v_+}{f_{+-} v_- - f_{-+} v_+}, \tag{3.12}$$

while the fraction of time the MT is in the growing/shrinking state is easily observed to be

$$\frac{c_+}{c_-} = \frac{v_-}{v_+}. \tag{3.13}$$

3.1.3 Caps & Catastrophes

In the last section we treated the transitions between the growing and shrinking states as instantaneous, effectively examining the MTs at time and length scales much longer than those of individual molecules. We would like to now take a closer look at the events that initiate the catastrophes. As noted earlier, the α and β tubulin molecules that attach to the + end are bound to GTP. The presence of the GTP at, and near, the growing tip stabilizes the MT. Further away from the tip, the GTP attached to β-tubulin (but not that attached to α-tubulin) can hydrolyze to GDP, releasing some energy. This renders the MT susceptible to breakdown and shrinkage. The cap region prevents this breakdown, maintaining the GDP bound portion in a metastable state. However, if through stochastic fluctuations the cap region disappears, the protofilaments start to fray and the MT rapidly shrinks until another cap is formed (a rescue event).
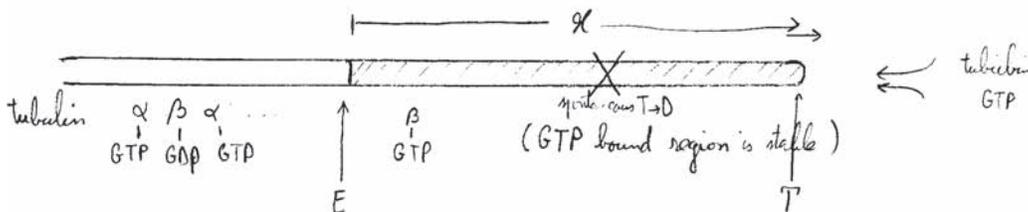
Let us focus on the cap region of the MT, assumed to span a length x from the tip T at the + end to a location E which marks the start of the GDP rich portion of the MT. The length x can change due to the following processes:

- The tip T grows at a rate $g(c)$ that depends on the concentration of tubulin.

- The end E grows at a rate h related to how fast the hydrolyzed front advances along the MT. This rate is presumably independent of the tubulin concentration.

If this is all, the cap region should grow/shrink with an average velocity of $v = g(c) - h$. However, there will certainly be stochastic fluctuations, which by analogy to the example considered earlier should be described by the equation

$$\partial_t p(x,t) = -\partial_x (vp) + D\partial_x^2 p \,, \tag{3.14}$$

for the probability $p(x,t)$ of a cap of length x, where D is some effective diffusion coefficient. However, for $v > 0$ the above equation predicts a cap that grows indefinitely and the probability of catastrophe becomes negligible, while for $v < 0$ a catastrophe should occur at a short time set by the initial cap length. These predictions of the model are at variance with experimental observations. For example, the model would predict longer cap lengths in solutions rich in tubulin (along with faster MT growth). If such rapidly growing MTs are then transferred to a solution poor in tubulin (such that v becomes negative), the prediction is that the onset of catastrophes should be later for solutions with faster growth (longer

initial caps). This contradicts the observation that the onset of catastrophes is independent of the quality of the initial solution.

One potential solution is to note that hydrolysis events may occur not just at the boundary point E, but throughout the cap.[2] Introducing hydrolyzed GDP at some point y along the cap, effectively fragments it, creating a shorter cap of length y (with the portion from y to x falling away). Since such events can occur at any point along the cap, they should be described by a *rate per unit length*, which we denote by r. The introduction of r modifies Eq. (3.14) to

$$\partial_t p(x,t) = -\partial_x(vp) + D\partial_x^2 p - rxp + r\int_x^\infty dy\, p(y,t). \qquad (3.15)$$

The negative term takes into account the reduction in probability of MTs of length x due to hydrolysis at a rate proportional to x; the positive term adds the probabilities for appearance of a segment of length x due to fragmentation of longer segments.

Equation (3.15) is an integral–differential equation, but in terms of the cumulative probability

$$\int_x^\infty dy\, p(y,t) \equiv \overline{P}(x,t) = 1 - P(x,t)\,, \qquad (3.16)$$

is equivalent to

$$\partial_t \overline{P}(x,t) = -\partial_x(v\overline{P}) + D\partial_x^2\overline{P} - rx\overline{P}\,, \qquad (3.17)$$

as can be verified by applying $-\partial_x$ to both sides. Equation (3.17) no longer has the integral character, and is thus easier to solve. Rather than a formal solution, we shall determine the key features of the behavior pertaining to catastrophes:

• In good solutions (rich in tubulin) $v > 0$, and the natural tendency of the cap is to grow longer. This is opposed mostly by the fragmentation events (and to a smaller extent by stochastic diffusion which we shall ignore initially). After a transient period, the competition between growth and fragmentation leads to a steady state distribution $p^*(x)$ for cap sizes. Setting $D = 0$, this stationary solution is easily obtained from Eq. (3.17) as

$$\log \overline{P}^*(x) = -\frac{rx^2}{2v}\,, \quad \Rightarrow \quad \overline{P}^*(x) = e^{-\frac{rx^2}{2v}}\,, \quad \Rightarrow \quad p^*(x) = -\frac{dP^*(x)}{dx} = \frac{rx}{v}e^{-\frac{rx^2}{2v}}\,. \qquad (3.18)$$

(Note the boundary conditions $\overline{P}^*(0) = 1$ and $\overline{P}^*(\infty) = 0$.) Because of fragmentation the cap does not grow indefinitely, but stabilizes to a typical size

$$\langle x \rangle = \sqrt{\frac{\pi v}{2r}}\,, \qquad (3.19)$$

which becomes longer as the tubulin concentration and v increases. For $v > 0$, there can be no catastrophes if diffusion is ignored. This is because while fragmentation can create short caps, the new cap starts growing away from $x = 0$. We thus reintroduce D as a small perturbation acting on this steady state, and note that Eq. (3.17) now gives

$$\partial_t \overline{P}(0,t) = -\frac{Dr}{v}\overline{P}(0,t)\,, \quad \Rightarrow \quad \overline{P}(0,t) = e^{-\frac{Dr}{v}t}\,, \qquad (3.20)$$

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[2]H. Flyvbjerg, T.E. Holy, and S. Leibler, Phys. Rev. Lett. **73**, 2372 (1994).

indicating a simple exponential decay. Catastrophes are thus Poisson distributed occuring at a rate Dr/v.

• The situation is very different in tubulin poor solutions with $v < 0$. In this case fragmented caps shrink to zero length, initiating catastrophes. Starting with a long cap, a catastrophe occurs at time t if a hydrolysis event takes place at some time $0 < \tau < t$ at a position $x(\tau) = (t - \tau)|v|$ so that the fragment can shrink to zero in $(t - \tau)$. The probability $p_{\text{cat}}(t)dt$ of a catastrophe in the interval $[t, t + dt]$ is thus the product of the probabilities that there are no hydrolysis events in the domain $0 < x < x(\tau)$ for $0 < \tau < t$, and that there is a hydrolysis event at the border of this region. Since the area of the domain is $|v|t^2/2$, and the hydrolysis events are Poisson distributed, we find

$$p_{\text{cat}}(t) = r|v|te^{-r|v|t^2/2} \,. \tag{3.21}$$

The mean time between catastrophes is thus

$$\langle t \rangle_{\text{cat}} = \sqrt{\frac{\pi}{2r|v|}} \,. \tag{3.22}$$

This result is quite insensitive the initial length of the cap, explaining experiments in which MTs were transported from good to poor solutions.

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