Frequently Asked Questions

Q: `You investigate the behavior of microtubules only in wild type shaped cells. I would like to know what type of behavior the model predicts in the small wee1Δ mutant. What is the MT behavior for such cells with half the normal length?`

To know the answer, download our simulation package (www.cytosim.org), and change the parameters specifying the cell size. This can be done by editing "config.cym", setting:

```
boxsize  1 1.5
```

The first number specifies the half-length of the cylindrical part, and the second one is the radius of the cell. This will simulate a cell of length 5µm = 2 (1 + 1.5) and diameter 3µm = 2 * 1.5.

Q: `The model contains a large number of parameters. Is this really necessary to conclude that microtubules undergo catastrophe at cell ends... What else could happen?`

The model has 15 parameters, and this is what is needed in order to study the problem in a realistic way. Indeed, when a microtubule reaches the cell end, it has a few options:

1) it may grow slower or stall altogether
2) it may undergo a catastrophe
3) it could push the nucleus backward
4) it may curl around the cell end

The parameters of MT dynamics are needed for 1+2. The geometry and viscosity are needed for 3. The geometrical parameters and flexural rigidity of MT allow us to calculate 4. Altogether, the simulation predicts the fate of MTs in terms of 1+2+3+4 and this is why it needs the parameters. Actually, there are even other possibilities that are not included in the model, because their are thought to be rare:

5) the microtubule may break
6) the overlap near the minus-end may slip, or detach from the nucleus.

Having many unconstrained ('free') parameters is a serious problem, especially if they are used to fit the data (John von Neuman famous quote is "*with four parameters I can fit an elephant and with five I can make him wiggle his trunk*"). Yet, this was not our approach, because 14 parameters have been measured experimentally. The only parameter which is estimated theoretically is the mobility of the iMTOCs in the nuclear membrane, and we have determined by a systematic variation that it could be increased (or decreased) by a factor 1000 without affecting the system (data not shown).

Q: `How do the parameters describing microtubule dynamics compare with what has been measured, in particular the length-dependence observed by the accompanying paper by Tischer et al?`

Parameters in the simulation		Measurement
Growth speed	From 2 to 4 µm/min (range used for figure 2)	~2.5 µm/min (Tischer et al. Fig. 4E)
Shrinkage speed	0.15 µm/s = 9 um/min	~8.5 µm/min (Tischer et al. Fig. 3C)
Sensitivity to force	1.67 pN	1.67 pN (Dogterom and Yurke, 1997)

<table>
<tr><th colspan="2">Parameters in the simulation</th><th>Measurement</th></tr>
<tr><td>Flexural rigidity</td><td>30 pN µm^2</td><td>34 pN µm^2 for MT between 10 and 20 µm (Dogterom and Yurke, 1997 note 21). It is mentioned that shorter MT produced lower values, which is why we adopted 30.</td></tr>
<tr><td>Catastrophe time for stalled MTs</td><td>25s
(this is parameter a)</td><td>24±9s (Janson and Dogterom, JCB 2003, Fig. 3A)</td></tr>
<tr><td>Catastrophe rate</td><td>From 0.1 to 0.4/min
(range used for figure 2)</td><td>Length-dependent, between 0.2 and 0.3/min, for MT which do not make contact with the cell ends.</td></tr>
<tr><td colspan="3">Linear length-dependent catastrophe rate “c”
Because of the organization of the bundle, microtubules minus-ends are near the nucleus, and we can thus identify the length L of a MT with the distance x between its plus-tip and the center.</td></tr>
<tr><td colspan="2">Model FL assumed a catastrophe rate:
$\boldsymbol{c} = h\ \boldsymbol{L} / (a + bv_g)$
h is the inverse of the half-cell length, which is the average microtubule length at contact
a is the measured catastrophe time for stalled MTs (25s).
b is defined as in model F by the overall catastrophe rate c_o such that $c_o = 1 / (a + bv_g)$

In other words: $b = (1 / c_o - a) / v_g$

Hence, as long as the force on the microtubule tip is zero, then $\boldsymbol{c} = h\ \boldsymbol{L}\ c_o$ with h=0.2 / µm. The force by changing the value of v_g, further affects the catastrophe rate.

In the successful region of Figure 2A, the overall catastrophe rate is c_o = 0.3 / min, giving a slope:
c/L = $h\ c_o$ = 0.06 µm^{-1} min^{-1}
or:
$\boldsymbol{c}$ = 0.3 /min at L ~ 5 µm
$\boldsymbol{c}$ = 0.12 /min at L ~ 2 µm</td><td>Tischer et al, Fig. 2c shows the catastrophe rate as a function of x, the position of the plus tip from the cell center. The relationship is well fitted by a line. Their measured slope is:

c/x= 0.064±0.007 µm^{-1} min^{-1}.

Tischer et al, Fig. 1E is for cells which are not treated with Hydroxyurea. The range of length over which the relationship is measured is reduced but the results are similar:

c ~ 0.15 /min for x ~ 2 µm
c ~ 0.3 /min for x ~ 4 µm.
Hence a slope c/x ~ 0.07 µm^{-1} min^{-1}.</td></tr>
</table>

Q: Some of your parameters are derived from experiments performed with purified tubulin *in vitro* (Dogterom lab). How do you know that these properties are not significantly different *in vivo*?

We are indeed not certain that the molecules behave *in vivo* as they do *in vitro*. We see no alternative to using the available measurements, and they have been obtained *in vitro*.

Q: But, Brangwynne et al. (PNAS 2007 vol. 104 (41) pp. 16128-33) found that the critical buckling force of the microtubules in living cells is two orders of magnitude higher than that of the microtubules *in vitro*. Why did you not use this value?

We think we did. The work of Brangwynne (PNAS 2007 vol. 104 (41) pp. 16128-33) show that microtubules in the animal cells are constrained laterally, either by other filaments or simply by the visco-elastic cellular media. For this reason, they buckle at higher forces not because their flexural modulus is higher, but because they buckle in a different way. The main idea can be explained using Euler’s formula for buckling: Force = $K (\pi n / L)^2$, where L is the length, K the elastic bending modulus and ‘n’ is the mode of buckling. In Brangwynne et al., the first mode

(n=1) is not favorable because of lateral confinement, and indeed microtubules have a higher mode of deformation (n>1). In conclusion, the experiment does not show that the microtubule bending elasticity K is very different than measured *in vitro*. Our model of fission yeast includes the impediments from the nucleus and cell wall (it calculates the appropriate 'n').

Q: Ok, now you have convinced me that the model based on your assumptions can describe the chosen traits, but how do you know that this is how it happens in the cells?

We do not know. No matter how much verifications are made, it is never possible to be sure of a theory, as long as it relates to an experimental science. A model should be seen as a "working hypothesis" describing a number of observations at the time of its development. It is useful because it helps us to know which observations fit or do not fit our current understanding. It can indicate a direction of future research. What is really important, is that the theory makes clear predictions that are testable, such that it can be falsified in the future. Philosophically, it can be argued that we will never know exactly how it happens in the physical world. Anyway, what matters for the scientist is wether or not he is able to predict what he sees happening.

Q: The distinction between models F and FL hinges on two experimental observations which both involved centrifugation to attain unusual conditions. In principle any component in the cell could have its distribution spatially altered during the centrifugation. Can we really believe these results?

This cells indeed are made asymmetric by centrifugation, and this is what makes the results interesting. We have assumed in our model that only the nucleus was displaced. This is the simplest assumption, but we cannot indeed be sure that this is strictly true. However, the fact that our model can reproduce these observations, indicates that probably nothing unusual has occurred. These observations are explained by the length-dependence that was measured by *Tischer et al*. without centrifugation.

Q: The 10 traits are not independent and seem to be an arbitrary choice. How did you pick them?

Yes, they overlap partly. We do not think that it is possible to find a set of independent traits with similar information content. The traits are signatures of a 'system' that are not easy to take apart. This is exactly why we attempted to match them all. We picked the traits that were characteristics of microtubules in interphase cells. In particular, we included observations that lead some of us (and others) to previously propose that the cell end affect MT dynamics via specific protein activities. Our model includes force but nothing else at the cell ends. Hence because it faithfully reproduces all the traits, we conclude that this interpretation is not the only one. In the future, we will continue to test the physical mechanisms on which yeast cells rely to maintain their shape.

Q: The accepted wisdom is that microtubules are regulated by factors whose activities are restricted at the cell pole. Why did you not simulate this scenario? Can you rule it out?

No, we do not rule it out! Using simulations, we might exclude/confirm a model by comparing its predictions with the established measurements. However, this can be done only when the assumptions of the model are stated explicitly. This is not the case for the idea that microtubules are regulated by activities restricted to the cell poles. We can imagine an infinite number of models based on this idea, and we cannot test them all.