Insights into microtubule catastrophes: the effect of end-binding proteins and force

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Summary

(in interview form)

This thesis deals with “microtubules” and “catastrophes”? Can you explain in simple words for a layman what is meant with these terms?

Let’s first look at a spider. It is a complex creature with many different body parts but basically all of them are composed of one building block: the cell. That means that cells exist in many different shapes and sizes with many different functions. Still, these cells have much in common. Almost all of them contain a nucleus containing the hereditary information and a scaffold to give it shape and assist internal organisation. One of the filaments which comprise this scaffold are “microtubules” (MTs). They look like long rods and can span the whole cell. One special feature is that they are dynamic: like building a tower from Lego, the building blocks for children, MTs assemble from a smaller subunit, termed "tubulin". Also similar to children’s play with Lego, the MT alternates between periods of build-up and breakdown. The sudden switch to a disassembly period is called catastrophe.

Why do you want to study MT catastrophes?

As I said, the cell has many different functions. Failure in proper execution can ultimately lead to sickness or even death of the organism. To avoid this, it is important that the cell’s tasks are fulfilled properly which can only be achieved by tight regulation. MTs play a crucial role in this. As they infer a framework to an ever-changing cell and serve as guiding structures for delivering cargo and signals, they need to be built up (which we call “grow”) at the right time and space. Similarly, their other dynamic instability parameters: their breakdown (which we call “shrinkage”) and the switch from growth to shrinkage, catastrophe, or vice versa from shrinkage to growth, need to be regulated.

Can you be more specific in what you want to know about catastrophes?

Yes. It was thought for long time that catastrophes occur suddenly, at a random time [Hill, 1984, Mitchison and Kirschner, 1987, Dogterom and Leibler, 1993, Flyvbjerg et al., 1994, Howard, 2001, Phillips R., 2008]. Later, another idea in research came up thinking that a catastrophe happens progressively in several steps [Odde et al., 1995, Odde et al., 1996, Stepanova et al., 2010, Gardner et al., 2011b]. We want to examine whether this is indeed the case and what is the cause of catastrophe. How many steps is the catastrophe process composed of, what is the nature of the step(s). As I mentioned MTs need tight regulation for proper functioning. This thesis deals with two MT regulators: proteins and compressive forces generated

when MTs encounter an intracellular obstacle. We want to understand how both these factors influence MT catastrophes.

What are these proteins?

Cells contain a lot of different proteins associated with microtubules. In order to uncouple their regulatory effect we do *in vitro* experiments with three highly conserved proteins known to influence MT dynamics [Beinhauer et al., 1997, Brunner and Nurse, 2000a]. This makes them biologically very relevant. Our proteins are known to interact with each other and are all from fission yeast: mal3, tea2 and tip1 [Busch et al., 2004, Busch and Brunner, 2004]. They belong to a class of microtubule-associated proteins which bind specifically to the MT ends. That's why they are called "end-binding proteins" (EBs).

What are your methods to examine MT catastrophes, EBs and force?

The methods are described in chapter 2 of this thesis. All the experiments are performed in-vitro in flow chambers containing on the bottom surface rigid, custom-made grooves. MTs assembled in these grooves from stabilised MT seeds grow against the rigid barriers where they experience compressive forces. This imitates the interaction of MTs and the cell wall. In the flow chamber is tubulin and also the EBs. Using a TIRF microscope we can specifically illuminate the fluorescently-tagged EBs and tubulin at the chamber surface between the grooves. We also use optical tweezers where we nucleated MTs from an axoneme (serving as a MT nucleation template) which is attached to a trapped bead. We direct the MTs against a rigid barrier in the presence and absence of EBs. The growth of the MTs against the barrier results in displacement of the bead from the trap centre which we can measure with high spatial and temporal resolution. We can thus deduce MT growth and force generation from these measurements. Furthermore, we also do experiments at constant force conditions where a growth of the MT results in translation of the barrier and constant location of the bead.

So what do you observe with the microscope? What do you learn from it?

We first looked at the binding dynamics of the end-binding protein mal3 in chapter 3. We observed the fluorescent accumulation at the growing MT end and examined how it altered before, during and after catastrophe. This we did for free MTs and for MTs in contact with a barrier. We saw, as already mentioned in [Maurer et al., 2011], that mal3 starts unbinding from the free MT, on average 15 s before catastrophe. This decrease of mal3 intensity stops about 5 s after catastrophe when the lattice intensity is reached. When MTs are under force it is similar, just that the decrease of intensity is now timed with the establishment of barrier-contact instead of with catastrophe.

Why do you see mal3 unbinding with a free catastrophe or establishment of barrier-contact?

Maurer et al [Maurer et al., 2011, Maurer et al., 2012] proposed a model where the tubulin at the very tip of the MT is in another nucleotide conformation than tubulin farther from the tip (termed the "EB1-competent state") or the tubulin in the lattice. The authors suggest that mal3 only binds to the nucleotide state of tubulin in the EB1-competent state. The decrease of mal3's affinity to the MT must therefore mean the loss of its binding sites [Maurer et al., 2012]. As a consequence we reason catastrophe or a compressive force (upon barrier contact) to provoke the omission of the EB1-competent state.

What do you know about the catastrophe process?

In chapter 4 we examined the catastrophe process of free MTs. We measured the catastrophe time, the time spanned from nucleation off the seed until catastrophe, in three conditions: free MTs in the absence of EBs, in the presence of mal3 and in the presence of the three EBs: mal3, tea2, and tip1. However, since it is experimentally difficult to determine the lifetime of very short MTs we have an uncertainty in the short catastrophe events. To account for this we fitted our unbinned data with several truncated distributions. The distributions describe multistep reactions with irreversible sequential and parallel steps, where the steps are or are not restricted to have the same timescale (gamma, (parallel) two-step exponential and multistep exponential distributions). We observed that in all our three conditions catastrophe is a multistep process as suggested before [Gardner et al., 2011b]. Moreover, we propose catastrophe to consist of (almost) two steps of unequal timescales. Comparing the observed timescales to the results from chapter 3, the timescales of mal3 unbinding, we conclude that the shorter step of the two must be characterised by the loss of mal3. Therefore, it cannot be the first step. At the moment we cannot say more about the nature of the other, longer step nor can we be sure about the steps being consecutive.

Special about your data is that you let MTs grow against a barrier. Do compressive forces influence the catastrophe process?

That we examined in chapter 5. We measured the time spanned from establishment of barrier-contact until catastrophe of stalling MTs. As expected, we measured that the force speeds up the catastrophe process. More interestingly, under force there is a clear effect of the EBs: in their absence, the catastrophe process consists of more than two steps, while in their presence the catastrophe process seems to become a random reaction. Taking the results from chapter 3, the loss of mal3 with establishment of force, we suggest that the random step in the presence of EBs is connected to the loss of the EB1-competent state. As for the MTs in pure tubulin: we do not have an explanation for the increase of number of steps at the moment.

Previous data suggested that an increase in the catastrophe rate stems from a decrease of the tubulin on-rate [Janson et al., 2003]. Could that be the case in your experiments?

Indeed, that is what we examined in chapter 6. By using optical tweezers, optionally with force-feedback, we could measure both the growth speed and the generated force with high resolution. Surprisingly, the absence or presence of EBs did not cause an obvious difference in the force – growth speed relationship in the force range we measured (F>0.5pN). We assume that this is caused by the loss of EBs upon establishment of force (see chapter 3) and the subsequent growth of the MT in the absence of EB binding.

There are still a few open questions...

Indeed, we examined the effect of mal3, tea2 and tip1 on the catastrophe process. However, we do not know much about the correlated binding dynamics of the three EBs. Further, there might be a connection between growth speed and the accomplishment of catastrophe-promoting events. For these problems we provide preliminary data. We also do not know the nature of all the steps leading to catastrophe. Connected to this we cannot resolve the puzzle why a force increases the number of steps of force-loaded MTs. We discuss possible, putative scenarios like a third step, non-detected in the free MT data but recovered at the barrier, and further distributions involving fixed-duration or reversible steps. We do not think that the former is the case while both latter scenarios seem realistic.