Dynamics and regulation at the tip : a high resolution view on microtubele assembly

Munteanu, L.

Summary

Cell division, internal cell organization, and intracellular transport rely on dynamic properties of microtubules. In cells, microtubule dynamics is highly regulated both spatially and temporally by a wide family of microtubule associated proteins (MAPs). In this thesis, an *in vitro* approach was used in order to shed light on the molecular details of the microtubule self-assembly process itself, as well as the molecular mechanism by which representative MAPs interact with and influence microtubule dynamics. The advantage of the *in vitro* minimal system is the possibility it offers to uncouple the regulation of different MAPs.

To zoom in with molecular resolution on the dynamic processes at the microtubule tip, we developed a high resolution technique that integrates optical tweezers, microfabricated rigid barriers and high-resolution video tracking of microbeads (described in chapter 2). In our experiments, microtubules are grown from a naturally occurring microtubule bundle, an axoneme, to which a polystyrene bead is attached near one end. The bead-axoneme construct is suspended in a 'keyhole' optical trap and positioned in front of a rigid barrier. The keyhole trap is used to control both the position of the bead and the direction of the axoneme. The rigid barriers are used to obstruct microtubule growth. Therefore microtubule elongation results in bead displacement. By detecting the position of the bead we can follow microtubule polymerization with near molecular resolution (~10 nm as compared with ~200 nm, the resolution of light microscopy). The experimental method, the special features of the optical trap, and considerations regarding microtubules in the context of our set-up are presented in chapter 2.

In chapter 3, molecular details of microtubule growth are discussed and how this process is altered, on a molecular scale, by the microtubule associated protein XMAP215 that is known to dramatically enhance the microtubule growth speed. We found that microtubule assembly is sometimes accompanied by fast length increases that correspond, in length, to 2-3 tubulin dimers. In the presence of XMAP215, we measured fast length increases equivalent to 7-8 tubulin dimers, corresponding to the size of the XMAP215 protein itself. These observations indicate that microtubule assembly might not always occur simply by the addition of individual tubulin dimers. Rather, small oligomers (2-3 dimers) seem to be able to attach to growing microtubules as well, an effect enhanced by the XMAP215 protein. XMAP215 might promote elongation of a tubulin protofilament along its length or could facilitate formation of long tubulin oligomers in

solution.

We also investigated another particular class of MAPs, the +TIPs, proteins that are specialized in tracking the microtubule dynamic ends. Their localization at the microtubule end enables direct regulation of microtubule dynamics. We focused our study on the complex of three fission yeast +TIPs: the EB1 homologue Mal3, the kinesin Tea2 and the cargo Tip1 (chapters 4 and 5). Preserving the protein functionality *in vitro* is a crucial requirement. Therefore, we first reconstituted *in vitro* the end-tracking behavior of the three proteins. From this experiment, presented in chapter 4, a hierarchy among the three +TIPs became clear. Mal3 is an autonomous end-tracker by recognizing a specific structure at the ends of growing microtubules. Tea2 and Tip1 need each other and Mal3 to efficiently track growing plus-ends: Mal3 acts as a loading factor on the microtubule for the Tea2-Tip1 complex and Tea2 motor activity ensures processive transport of the Tea2-Tip1 complex to the microtubule ends, where both proteins accumulate.

EB1 protein family members were shown to influence microtubule dynamics and organization *in vivo*, but it is still unclear whether the EB proteins solely have an effect on microtubule dynamics. As Mal3 is able to localize at microtubule growing ends independently of other proteins, we asked whether the EB1 homologue alone has an influence on microtubule dynamics. The results are presented in chapter 5. The experiments include three independent techniques: DIC microscopy to quantify the microtubule dynamic instability parameters in the presence of Mal3, fluorescence microscopy to quantify the Mal3 localization on microtubules, and the optical tweezers based technique to investigate the modifications induced by Mal3 at the microtubule dynamic tip. Mal3 had a complex effect on microtubules both at the lattice and at the tip. At the tip Mal3 bound efficiently and altered the end-structure in such a way that promoted tubulin dimer net addition and the chance for a microtubule to switch from a growing to a shrinking phase. Using the optical tweezers based technique we could identify microtubule end-structures that frequently disassembled before having the chance to close into a regular microtubule lattice. At the microtubule lattice Mal3 bound less potently than at the tip. The presence of Mal3 at the lattice hampered microtubule disassembly and promoted rescues. Our observations suggest a mechanism for Mal3-regulation based on local modification of the microtubule properties, which might be a reflection of the molecular mechanism underlying Mal3-microtubule interaction. Most probably, Mal3-tubulin binding sites are hidden within the lattice due to the protofilament lateral contacts and therefore, only occasional binding of Mal3 occurs at the lattice. At the seam, these binding sites are better accessible and at the tip, the exposed protofilaments offer most optimal binding of Mal3. This mechanism of regulation takes advantage of the complex architecture of the microtubule and enables proteins like Mal3 to act differentially at different locations on a dynamic microtubule.

Chapter 6 focuses on microtubule catastrophes, the transitions from a growing to a shrinkage phase. Knowledge about a mechanism for catastrophes has been limited to

models based on structural details of the microtubule ends imaged with cryo electron-microscopy. Information about how MAPs act on a molecular level to regulate catastrophes is even less extensive. Using our optical tweezers based technique, we observed a microtubule length decrease of several tens of nanometers prior to microtubule disassembly. This suggests the loss of a stabilizing structure. Two possible scenarios can be envisioned: either i) the sheet-like structure present at the growing end depolymerizes, leaving an exposed blunt end that is unstable and quickly collapses or ii) the stalled microtubule has a closed, cylinder configuration that becomes unstable only when the lateral contacts between two or more protofilaments are lost. This 'opening' of the cylinder would appear as a length decrease in our measurement. It is possible that the events leading to a catastrophe comprise a sequence of both scenarios. MAPs could regulate both aspects involved in catastrophes. Mal3, for example, could be a MAP that affects first events in catastrophes by altering the microtubule end-structure.

Chapter 7 describes future directions of investigation naturally emerging from the experiments presented in this thesis. (i) It is known that in living cells usually +TIPs interact with each other to affect microtubule dynamics. We performed preliminary experiments on microtubule dynamics regulation by the Mal3-Tea2-Tip1 complex. We found that Tea2-Tip1 need each other and Mal3, not only to localize at the microtubule ends, but also to regulate microtubule dynamics. We observed that Tip1 has a stabilizing effect by reducing the Mal3-enhanced catastrophe rate. (ii) We show that mammalian EB3, homologue of the fission yeast Mal3, is also an autonomous microtubule end-tracker *in vitro*. However, the details of EB3-microtubule interaction might be different and need further investigation. EB3, lacking the C-terminus tail, bound with high affinity to the microtubule lattice and promoted growth of intrinsically curved microtubules. (iii) The presence of MAPs might effect the forces generated by dynamic microtubules. In cells, microtubule pushing and pulling forces are constantly generated and constitute the driving forces for intracellular movement and transport of cellular components. Using the optical tweezers we measured microtubule force generation in the presence of the MAPs investigated here, XMAP215 and Mal3. Both proteins seemed to have an effect. The maximum forces generated by microtubules in the presence of Mal3 were lower than in the absence of the protein. With XMAP215 high forces were generated independently of the microtubule growth speed. More experiments are needed to confirm our first observations.

In conclusion we have investigated aspects of microtubule assembly and dynamics in the absence and in the presence of two representative microtubule associated protein systems. Our high-resolution technique combined with an *in vitro* approach allowed us to dissect the regulation of microtubule dynamics by individual MAPs and identify possible mechanisms of regulation.