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

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1 Introduction

Each cell contains a mechanical framework vital for cellular processes such as vesicular transport and cell division. This framework, called the cytoskeleton, is very dynamic and constantly reorganises to adapt to changing cellular needs. Proper regulation is therefore crucial and is ensured partly by forces that originate from the assembly and disassembly of cytoskeletal filaments. Forces often arise from the interaction of filaments with structural components within the cell, such as the cell boundary. The rearrangement of the cell interior is further aided by the biochemical machinery directly modulating the dynamics of cytoskeletal filaments. In this thesis, we examined the dynamic regulation of one of these filaments, the microtubules. Specifically we were interested in the combined effect of force and proteins binding specifically to the microtubule's ends. For our *in vitro* experiments we used total-internal-reflection-fluorescence microscopy and a high resolution optical tweezers approach including force-feedback.

1.1 Cellular organisation

Life is very diverse. There are innumerable different organisms and until today new ones are being discovered. Yet, when looking at one organism, let's say a spider, we see a very complex creature in itself. It has glassy eyes, a fang, often soft hair, glands extruding silk and rigid legs which, due to the absence of muscles in most cases are moved by internal pressure changes [Parry and Brown, 1959, Barnes et al., 2001]. But if you think about it: the amazing part is that the spider is basically composed of one type of building block: the cell. Consequently, there must be many different types of cells, ranging from lung cells to extract oxygen, brain cells to transmit signals and photoreceptor cells to measure light intensities. Though cells have a lot of different shapes, sizes and functions, their internal organisation and structure has much in common. For example, cells comprise, among others, a nucleus containing the hereditary information in the form of DNA (the chromosomes), a cell membrane to separate and protect it from its surrounding and a scaffolding of filaments, named the cytoskeleton, to give it structure and aid internal organisation [Alberts et al., 2002].

The cytoskeleton is composed of three different types of filaments: actin filaments, intermediate filaments and microtubules (MTs). MTs are relatively stiff and are therefore well suitable to provide support to the cell. On the other hand, MTs are very dynamic filaments that can extend and reduce their length modulating their many functions. During cell

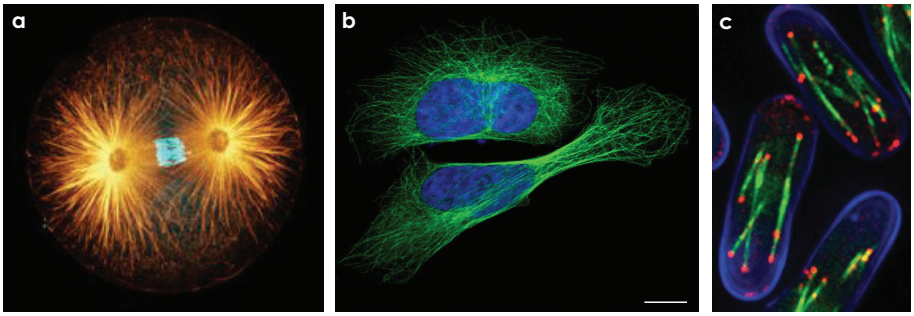

Figure 1.1: MTs in the cell. a. Microtubules are fixed and stained in sand dollar zygotes (urchin egg) during mitosis. The MT plus ends are connected to the chromosomes in order to pull them apart before cell division. Image obtained from [web, a]. **b.** Microtubules are stained in human cells. Scale bar represents 10 μm. Image obtained from [web, c]. **c.** The Clip-170 homologue tip1 (red) localises to the plus ends of MTs (green) in fission yeast cells (blue). Image taken from [web, b].

division, MTs originating from the centrosome (the main MT organising centre) connect to filaments at the cell cortex (actin) and to the chromosomes, see figure 1.1a. Molecular motors, attached to the cell cortex and to MTs, can then pull towards the cell periphery while other MTs assist the chromosome separation by decreasing their length [Alberts et al., 2002]. But MTs can also act as intracellular "highways". Motor proteins can walk on them or bind and rebind to move vesicles and organelles through the cell. There are motor proteins which walk from the cell centre to the periphery and others which follow the opposite direction.In non-dividing, migrating cells MTs furthermore have a key role in cellular organisation leading to directed movement. Finally, the combination of even other proteins and MTs can together form complex structures called flagella and cilia used by cells to swim [Mcintosh and Mcdonald, 1989, Burakov et al., 2002, Vallee and Stehman, 2005, Yeaman et al., 1999, Lye and McIntosh, 1987, Vale, 1985].

All structures such as organelles, filaments, proteins and membrane compartments in the cell need space. However, cells are small and – very crowded. How is it possible that a cell can perform its task with so much precision though there is considerably little free space? The answer is: tight spatial regulation. To fulfil its various tasks, which even alter during the cell cycle, the dynamic elongation and shrinking phases by MTs and the affinity of proteins need to be carefully organised. How this functions in detail is an intriguing question and an on-going topic in research.
Also this thesis focuses on MT regulation. More specifically it looks at MT regulation by a set of proteins in combination with mechanical cues. But let us first take a closer look at MTs.

1.2 Microtubules (MTs)

1.2.1 Microtubule structure

Microtubules are composed of the protein subunit tubulin which shows little variation at the amino-acid level between different organisms. This hints at evolutionary conservation arising from important biological functions. Tubulin forms an 8 nm long dimer composed

of an α- and a β-monomer. A microtubule grows in two phases, nucleation and elongation. It usually nucleates from a template which is in cells primarily a centrosome [Kellogg et al., 1994], but can also be an axoneme and *in vitro* a short stabilised MT. Afterwards, the MT elongates by the head-to-tail association of tubulin dimers [Amos and Klug, 1974] or possibly short oligmers [Kerssemakers et al., 2006]. Tubulin assembles into the MT in linear protofilaments which bind laterally to form a hollow cylindrical tube of 25 nm diameter, as shown in figure 1.2a. Microtubules nucleated by centrosomes and axonemes have usually 13 protofilaments but in general, the protofilament number has been seen to vary between 9 to 17, even within the same microtubule [Chretien and Wade, 1991, Chretien et al., 1992]. Adjacent protofilaments are bound with a pitch of 1.5 times the length of a dimer. Therefore, neighbouring monomers follow a helical path where β-β monomers meet each other, constituting the so-called B-lattice. After one turn around the MT, due to the pitch between protofilaments, an α- is adjacent to a β-monomer, which is named the seam and forms an A-lattice structure [Mandelkow et al., 1986].

Though α- and β-monomers are similar at the amino acid level, they have structural and functional differences. This polarity of the tubulin dimer also gives rise to polarity of the MT. The microtubule grows faster at the end exposing its β-monomer (referred to as plus end). The other slower growing end, exposing its α-monomer, is called the minus end [Allen and Borisy, 1974]. In the cell, however, the minus end is usually connected to the centrosome or other nucleating structure and is therefore static. The plus end, on the other hand, can explore intracellular space. Microtubule polarity serves different functions, from giving directionality to motor proteins following the MT, to formation of protein gradients or local accumulation at MT ends [Browning et al., 2003].

Due to its architecture and relative thickness, the MT is relatively rigid. The persistance length l_p, a measure of stiffness, is in the order of millimeters and can hence span a significant fraction of the cell diameter (10-50 μm). As a result, MTs serve as a framework for the cell and can transport signals over its whole length. Note however, that the stiffness of a MT is not a universal number but can vary depending on MT lifetime and polymerisation rate (shorter and fast growing MTs are less stiff [Janson and Dogterom, 2004, Pampaloni et al., 2006]), drugs (taxol-stabilised MTs are less stiff [Felgner et al., 1996]), or microtubule-associated proteins [Mickey and Howard, 1995, Felgner et al., 1996, Felgner et al., 1997, Sandblad et al., 2006].

1.2.2 Microtubules are dynamic

MTs are dynamic structures becoming apparent by the constantly varying length over the life of a MT. By the assembly and disassembly of their subunit tubulin MTs can remain in alternating, prolonged states of slow growth and rapid shrinkage (see fig 1.2b.). The abrupt switch from the assembling to the disassembling state is called catastrophe while the reverse, more infrequent event is termed rescue [Desai and Mitchison, 1997]. This intrinsic mechanism of switching between different states, termed "dynamic instability" by Mitchison and Kirschner in 1984 [Mitchison and Kirschner, 1984a], can be described by four parameters: the growth speed (*in vitro* typically in the order of 1-5 μm/min), the shrinkage speed (approximately 10-50 μm/min), the time the MT spends in a growing state until catastrophe, the catastrophe time, and, the rescue time describing the duration of a shrinking phase until rescue. An example of these processes is presented in figure 1.2e.
As the MT never reaches a steady-state length, dynamic instability is in fact an non-equilibrium process. As such it needs to be driven which results from nucleotide

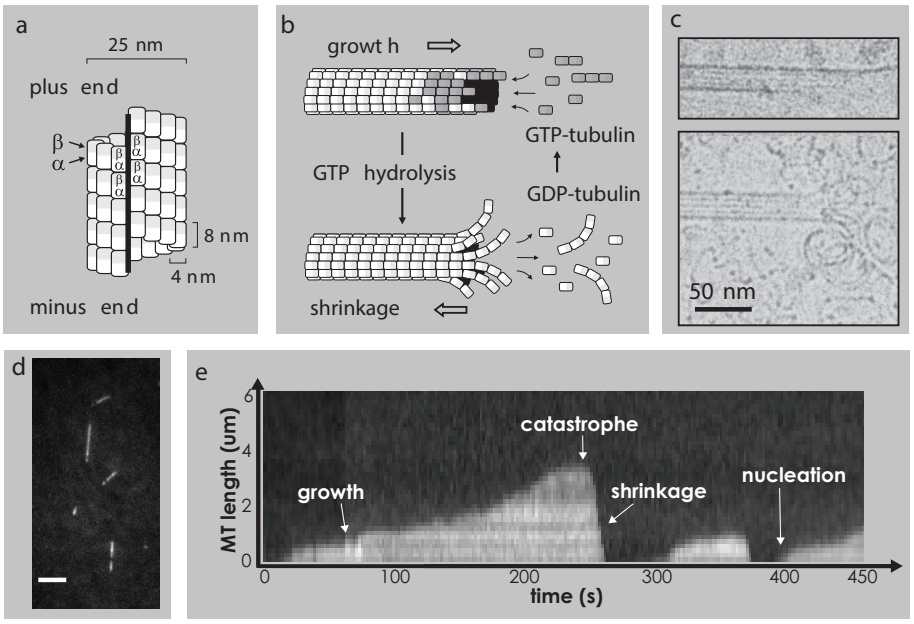

Figure 1.2: MT structure and dynamics. a. A MT is formed by an array of laterally bound protofilaments each composed of α-β tubulin dimers. The polarity on the dimer evokes polarity of the MT. Its ends are called plus and minus end. The major part of the MT forms a B-lattice where an α-monomer is adjacent to a β-monomer. An exception is the "seam" (indicated by the thick black line), where monomers of the same type are next to each other. **b.** MTs elongate by the addition of GTP-tubulin. In the lattice the GTP hydrolysis into GDP giving rise to mechanical strain within the lattice. Eventually, this leads to rapid de-polymerisation of the MT. In solution, the GDP is replaced by a GTP nucleotide. **c.** *top.* Electron microscopy image of a growing MT which suggests the existence of sheet-like structures which later close into a tubular shape. *below.* A shrinking MT, characterised by its protofilaments peeling off. Images adapted from [Mandelkow et al., 1991] and [Munteanu, 2008]. **d.** TIRF-M image of fluorescently labelled rhodamine-MTs. The seed, a short stabilised MT serving as nucleation template, labelled with tubulin-HiLite635 is not visible. Clearly visible are both growing plus ends and (on average shorter) minus ends. Scale bar 5 μm. **e.** A space-time plot (kymograph) of the lower MT presents "dynamic instability". MTs alternate between phases of slow growth and rapid shrinkage where the transition is called catastrophe. Before undergoing catastrophe MTs often display a stalling or slow-growth phase. The reverse transition is termed rescue (not shown) as rescues are rarely seen *in vitro*. MTs were nucleated from a seed (not shown).

hydrolysis. MTs grown in the presence of the non-hydrolisable nucleotide GMPCPP only elongate [Hyman et al., 1992] whereas MTs grown in the presence of the hydrolisable guanosine-5-triphosphate (GTP) display the typical dynamic instability pattern. Roughly this works as follows: every tubulin dimer associates to the MT with a guanosine-5-triphosphate (GTP) bound to its β-tubulin monomer [Carlier et al., 1984] (see fig 1.2b.). Once bound in the MT lattice the nucleotide hydrolises, after a short delay, into the GDP-state. During the shrinkage phase the tubulin falls off from the MT where, in solution, the GDP is replaced by a fresh GTP nucleotide [Desai and Mitchison, 1997].

Does GTP hydrolysis have a role other than providing energy for dynamic instability? In fact, the nucleotide state of tubulin goes along with a specific tubulin conformation. GTP hydrolysis provokes a conformational change of the tubulin dimer from an almost straight to a kinked state [Wang and Nogales, 2005]. But since the GDP-dimer is constrained in the MT lattice it cannot recuperate its preferential shape. This bending energy remains therefore in the MT lattice as mechanical strain [Caplow et al., 1994]. It is only liberated when the MT shrinks, where its protofilaments peel outward in a curved fashion [Chretien et al., 1995, Mandelkow et al., 1991] which is a driving force for the rapid depolymerisation (see fig 1.2c.). The fact that the MT protofilaments hold together in a growing MT without immediate release of this energy is thought to be ensured by a MT "cap". Theoretical efforts have shown that only one to two layers of GTP-bound tubulin or even a few incomplete GTP-tubulin rings can stabilise the cap [Janosi et al., 2002, Molodtsov et al., 2005a]. However, whether such a cap is enough to prevent the MT from de-polymerisation still needs to be clarified. Another scenario for the MT cap comes from other experimental results suggesting a difference between a cap in the GTP nucleotide state than the (much larger) size of the tubulin cap mimicking a "GTP-conformational", straight state [Maurer et al., 2012] (we will come back to this in more detail in chapter 3.3, page 33).
Similarly, it is still unclear how the MT gets its tubular shape during growth. There are several models. One, derived from electron microscope images, envisions tubulin dimers to bind first longitudinally into protofilaments which then hold together to form a sheet like structure (see fig 1.2c.). This sheet would then close at the seam [Wang and Nogales, 2005, Wang et al., 2005]. Other models imagine MTs to grow as a tube where the terminal GTP-dimers' association fluctuates resulting in very dynamic and fast "growth" and "shrinkage" pattern [VanBuren et al., 2005, Schek et al., 2007, Margolin et al., 2012]. An agreement on these details is still missing.

How does the MT transition between its different dynamic states? One possibility are GTP-tubulin remnants in an otherwise GDP-lattice bering thought responsible for MT rescues [Dimitrov et al., 2008]. Yet, how the MT transitions from a growing state to a period of disassembly is not clear. More specific, for long it has been questioned whether catastrophe is a stochastic process with first order kinetics. This implies that the catastrophe time is independent of the microtubule age. It also means that catastrophe is a one-step process and that events are independent. While some literature regarded catastrophes as randomly distributed and thus first-order events [Hill, 1984, Mitchison and Kirschner, 1987, Dogterom and Leibler, 1993, Flyvbjerg et al., 1994, Howard, 2001, Phillips R., 2008], others showed evidence of catastrophe being a multi-step process [Odde et al., 1995, Odde et al., 1996, Stepanova et al., 2010, Gardner et al., 2011b]. Nonetheless, all efforts so far could only suggest on the nature of the catastrophe process.

1.2.3 Microtubule catastrophes

During the last ten-odd years the view on MT catastrophes has widened without coming to a clear consensus. Originally, the "standard" GTP cap model implicates that tubulin dimers not yet hydrolyzed into a GDP state are responsible for the stability of the MT tip. Thus, it has been commonly accepted that the size of the GTP cap is an indicator for MT stability. However, recent experimental, theoretical and computational models, especially those with microtubule-associated proteins, challenge the simplicity of this model [Duellberg et al., 2016a, Duellberg et al., 2016b, Bowne-Anderson et al., 2015]. As mentioned before, a differentation has been made between the region of EB-binding and the smaller cap necessary for stability. Researchers suggest a minimal end density threshold: for a catastrophe to occur the GTP cap has to decrease in size until the critical density is reached [Duellberg et al., 2016a]. Several catastrophe concepts have been proposed.
Many experiments have confirmed during the last years that short MTs are more stable against catastrophe than longer ones, in other words, the time a MT is growing, the MT lifetime, has an influence on its stability. This created the aforementioned concept of "MT age" and with it the idea of catastophe being a multi-step "defect" accumulation process [Gardner et al., 2011b, Gardner et al., 2013, Bowne-Anderson et al., 2013, Coombes et al., 2013, Zakharov et al., 2015, Bowne-Anderson et al., 2015]. These defects could be the formation of cracks [Flyvbjerg et al., 1996, Li and Kolomeisky, 2014, Margolin et al., 2012] or protofilaments unable to bind new dimers. GTP tubulin from solution is thought improbable to associate to protofilaments with a terminal GDP-tubulin, thus rendering the MT instable after several such protofilaments are formed [Gardner et al., 2011b, Gardner et al., 2013].
Another concept to influence catastrophe stems from binding of tubulin dimers from solution. Not all dimers permanently attach to the MT tip, with a certain probability a portion of them dissociates after a short binding period resulting in a nanoscale irregular growth pattern [Kerssemakers et al., 2006, Schek et al., 2007, Rickman et al., 2017, Gardner et al., 2011b]. These rapid growth fluctuations create a fast turnover at the tip and can create periods of critically short MT caps [Bowne-Anderson et al., 2015, Kok et al., 2021], switching the MT into the dissociation phase. We describe this effect in detail in chapter 7 on page 79.
Finally, during growth many conformational changes take place at the MT tip. It is still not completely clear how tubulin dimers associate to the MT and thus also under debate how the MT forms a tubular shape. Further experiments have revealed the MT end to become more and more tapered while it grows. Young MTs have thus more blunt tips while older MTs tend to have longer tapered ends up to a single protofilament. With that the sites of GTP-tubulin are effectively smeared out reducing the density of binding sites in the course of the MT lifetime and thus rendering older MTs more unstable [Duellberg et al., 2016b].

1.3 Microtubule end-binding proteins (EBs)

By employing dynamic instability MTs can fulfil a variety of functions in the cell [Desai and Mitchison, 1997]. The ability to adapt appropriately and rapidly to changing cellular needs stems from a precise regulation of the dynamic instability of MTs. *In vivo* MT regulation is ensured by a variety of proteins, sometimes in combination with other cues, for example forces. In this and the following section we discuss these MT regulators in more detail.

In the cell, MT dynamics and the MT network can be regulated by the biochemical machinery. Their mechanism of action has different manifestations: it can be globally in the whole cell but it can also be a local effect. As such, MT dynamics can be modulated

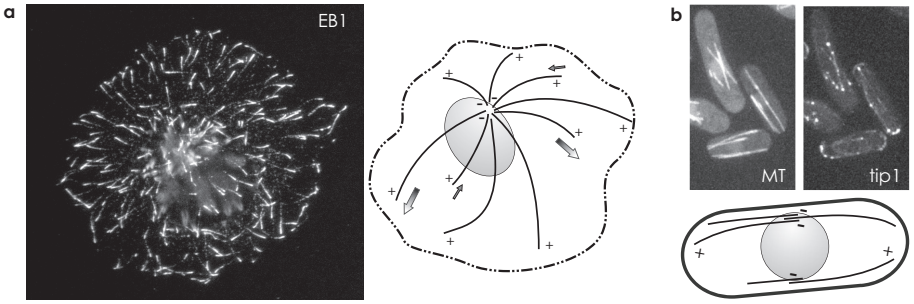

Figure 1.3: End-binding proteins in the cell. a. Mammalian end-binding 1 proteins (EB1) specifically bind to the growing ends of MTs in an interphase COS-7 cell. Clearly visible are the typical comets at the MT tips formed by EBs. Image courtesy of Anna Akhmanova. *right.* Schematic drawing in a comparable fibroblast cell. While the MT minus ends are connected to the nucleus the plus ends can explore intracellular space. EBs have high affinity for the growing plus ends directed towards the cell periphery. **b.** MT bundles composed of several MTs made up of tubulin-mCherry in fission yeast cells. The growing MT ends are bound by EBs, here tip1-GFP. Image courtesy of Christian Tischer. *below.* Schematic view of a fission yeast cell where the MT plus ends are growing towards the cell poles. Image taken from [Munteanu, 2008].

by MT length or age-dependent protein accumulations [Dogterom et al., 1996, Holy et al., 1997, Tischer et al., 2009], so that dynamics is different in the cytoplasm compared to at the boundaries where MTs are longer. Second, proteins can also be bound locally to intracellular structures such as the membrane and from there interact with MTs that transiently move into their vicinity [Mimori-Kiyosue et al., 2005]. A third possibility of locally regulating MT's state is the joint effort of proteins and forces which are generated when MTs grow against intracellular obstacles. We will look at MT force generation in more detail in section 1.4.

Numerous proteins binding specifically to MT ends (end-binding proteins, EBs) have been identified. Each class of proteins has specific functions, e.g. modulating the interaction of MTs with intracellular organelles (CLIP-170/CLIP150) [Perez et al., 1999, Arnal et al., 2004], stabilising MTs and attaching them to the cortex (CLASPs) [Akhmanova et al., 2001, Maiato et al., 2003, Lansbergen et al., 2006] assisting in tumor suppression in association with the MT (APC) [Mimori-Kiyosue et al., 2000]. Specifically, end-binding protein 1 (EB1), a highly conserved protein between different organisms, has been found to localise to centrosomes and to the MT ends [Mimori-Kiyosue et al., 2000,Akhmanova and Steinmetz, 2008], as shown in figure 1.3a. Importantly, it is targeting other MT-associated proteins to the MT plus end by recognising a structural SxIP-motif [Honnappa et al., 2009] and thereby modulating MT's dynamic state. As a consequence it regulates interactions with organelles, the kinetochore, the cortex and the nucleus. Due to the multitude of protein binding partners it is considered the core component of MT plus-end protein networks [Akhmanova and Steinmetz, 2008].
In fission yeast dynamic instability is mediated, among others, by the EB1-homologue mal3 [Busch and Brunner, 2004] (see fig 1.3b.). Its importance for the cell has been shown in mal3 depletion by RNA-mediated inhibition (RNAi) experiments leading to branched, bent and curved cells [Beinhauer et al., 1997]. The discovery that this was caused by very short MTs never reaching the cell poles suggested mal3 to be a prominent catastrophe regulator [Busch

and Brunner, 2004]. Another protein, closely associated with mal3 is the Clip170-homologue tip1. (An image of a fission yeast cell with fluorescent tip1 is presented in figure 1.1c.) *In vivo* the deletion of tip1 also caused bent and branched cells and shortened MTs. Together with mal3, tip1 functions as a MT regulator suggested to prevent catastrophes at locations other than the cell poles [Brunner and Nurse, 2000a].

1.4 MTs and forces

Various experiments have shown that MTs can generate pulling and pushing forces [Dogterom and Yurke, 1997, Grishchuk et al., 2005]. Pulling forces can be created when a protein binds to the shrinking MT end and an intracellular structure. While the MT end is retracting, the protein, stationary located, pulls on its binding partner, thereby performing mechanical work [Inoue and Salmon, 1995, Dogterom et al., 2005]. This is for example the case in mitosis when proteins connect the MT end to the kinetochore of chromosomes [Maiato et al., 2004]. Pushing forces, on the other hand, are used for positioning processes. There are many examples: placement of microtubule organising centres and organelles [Tran et al., 2001] or the central alignment of the nucleus [Tran et al., 2001, Foethke et al., 2009] or the spindle [Tolic-Norrelykke et al., 2004, Vogel et al., 2007] by microtubules pushing against the cell boundary. The interest in MT force generation and its diversity is shown by the variety of published experimental and theoretical work [Inoue and Salmon, 1995, Dogterom and Yurke, 1997, Mogilner and Oster, 1999, van Doorn et al., 2000, Stukalin and Kolomeisky, 2004, Janson et al., 2003, Janson and Dogterom, 2004]. Since this thesis deals specifically with pushing forces we want to give more insight into this topic in the following section.

How does a statically located filament generate a pushing force? First, thermodynamic calculations show that tubulin association to the MT gives rise to enough energy to create a significant force [Hill and Kirschner, 1982, Hill, 1987, van Doorn et al., 2000]. (This is a different energy than the one derived from GTP hydrolysis, which was explained above. GTP hydrolysis creates internal mechanical strain and is the driving force for rapid depolymerisation and pulling forces.) Further, a mechanistic way of force generation is given by the Brownian ratchet model [Peskin et al., 1993, Mogilner and Oster, 1999]. As presented in figure 1.4 the simplest model imagines the MT as a linear arrangement of tubulin dimers in contact with a mobile barrier. Though most of the time the MT is in close contact with the barrier, thermal fluctuations of the MT and the barrier occasionally open a small gap. If the space is transiently large enough a new tubulin dimer can be incorporated. As now the barrier cannot move back to its original position it is translated by the length of the dimer, resulting in work being performed. Since the space between barrier and MT end to insert a new tubulin dimer appears less often with increasing force, the MT reduces its growth speed. Therefore the Brownian ratchet model can explain the decaying force - growth speed relationship as shown in chapter 6 and [Dogterom and Yurke, 1997, Janson et al., 2003]. The compressive force experienced by the MT results in slight compression of the filament. When the barrier is static as is the case in our *in vitro* experiments, the applied force comes from MT compression only.

If MTs are long enough they can release the compressive force by buckling (the mechanism of buckling is presented in fig. 3.2c). A condition for buckling is the compressive force to overcome the critical buckling force $F_c \cong 20.19\kappa L^{-2}$, where κ is the flexural rigidity and L the MT length [Gittes et al., 1996] assuming the nucleation site is clamped (i.e. not pivoting). Using the typical value for κ=25 pN$\,\mu$m^2, a MT of length 1 μm will buckle at a force of ~500 pN while a MT longer than 20 μm needs only ~1 pN.

If the MTs are too short to buckle they will eventually stop growth. The force necessary to

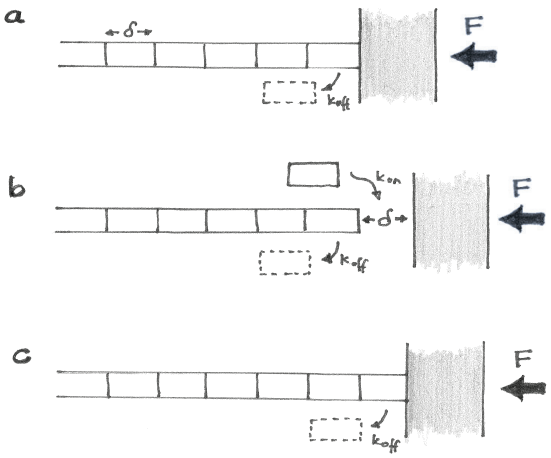

Figure 1.4: The Brownian ratchet model explains MT force generation. a. Schematic view of a "MT" just reaching a barrier to which a compressive force F is applied. **b.** Thermal motion creates transient gaps between the barrier and the MT end. If such a gap is large enough a tubulin dimer of length δ can bind to the MT end with rate k_{on}. **c.** The insertion of the tubulin dimer moved the barrier a distance δ and thus performed work. At all times a tubulin dimer can disassociate from the MT with rate k_{off}. Image adapted from [Dogterom et al., 2002].

prevent the new incorporation of tubulin subunits where only occasionally a dimer detaches from the MT is termed "stall force".

1.5 Thesis layout

Despite the wealth of experimental and theoretical information, it is not yet clear what is the combined effect of end-binding proteins (EBs) and forces on MT dynamics. The scope of this thesis is to obtain a more detailed understanding of the catastrophe process and the accompanying role of EBs and forces. We set out to tackle this problem with *in vitro* experiments. What the specific research questions are, how we approach them, and what insights we gain is shown below:

Chapter 2: Methods

- How do we create flow chambers with embedded micro-fabricated barriers to study the interaction of dynamic MTs with physical structures?
- How are the assays prepared?
- Describing the TIRF and optical tweezers setup

Chapter 3: Fluorescence intensity measurements of the end-binding protein mal3

why?
- How is the binding of mal3 to the MT end related to catastrophe?
- What influence do head-on forces have on this?

how?
- TIRF-M experiments of fluorescently labelled rhodamine-tubulin MTs and mal3-Alexa488.
- MTs interacting with micro-fabricated barriers.

what?
- Mal3 starts unbinding from the MTs on the order of 10 s before catastrophe.
- Barrier contact induces the loss of mal3 within an average of 10 s.

Chapter 4: The catastrophe process of free MTs

why?
- What is the nature of the catastrophe process?
- How is this process influenced by mal3 and the protein complex mal3-tea2-tip1?

how?
- TIRF-M experiments of MTs and EBs.
- Fit catastrophe times to several models and compare to simulations.

what?
- In the absence of EBs catastrophe is a two-step process.
- Before catastrophe mal3 starts unbinding from the MT.

Chapter 5: The catastrophe process of MTs under force

why?
- How is the catastrophe process in the absence and presence of EBs affected by head-on forces?

how?
- TIRF-M experiments of MTs and EBs with microfabricated barriers.
- Fit barrier-contact times of MTs having a catastrophe at a barrier with several distributions.

what?
- The catastrophe process is a multi-step process in the absence of EBs.
- The presence of EBs renders MTs under force a one-step process.

Chapter 6: Force velocity relationship of MTs with EBs

why?
- What is the force-velocity relationship of growing MTs under force, in the presence of EBs?

how?
- Measure polymerization dynamics of MTs with optical tweezers with force-feedback.

what?
- The effect of EBs on MT growth is limited by forces. For forces above 0.5 pN we cannot determine substantial influence on growth by EBs.

Chapter 7: EB3-informed dynamics of the MT cap during stalled growth

why?

- How do EBs inform MT dynamics during stalled growth?

how?

- TIRF-M experiments of MTs and EB3 growing against microfabricated barriers with an overhang.
- Monte Carlo Simulations of 1D MTs during growth and stalling.

what?

- Noisy growth, a single EB3-dependent hydrolysis rate and a density of GTP dimers at the MT tip are enough to reproduce a range of experimental outcomes.

Chapter 8: Discussion and outlook

- Remarks on catastrophe-describing distributions, other than the ones presented in chapter 4.
- Different concepts of MT catastrophe.
- Preliminary results of correlated dynamics at MT ends of mal3 and tip1.
- Possible additional research directions.