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Microtubule Catastrophe and Rescue

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Abstract

Microtubules are long cylindrical polymers composed of tubulin subunits. In cells, microtubules play an essential role in architecture and motility. For example, microtubules give shape to cells, serve as intracellular transport tracks, and act as key elements in important cellular structures such as axonemes and mitotic spindles. To accomplish these varied functions, networks of microtubules in cells are very dynamic, continuously remodeling through stochastic length fluctuations at the ends of individual microtubules. The dynamic behavior at the end of an individual microtubule is termed “dynamic instability”. This behavior manifests itself by periods of persistent microtubule growth interrupted by occasional switching to rapid shrinkage (called microtubule ‘catastrophe’), and then by switching back from shrinkage to growth (called microtubule ‘rescue’). In this review, we summarize recent findings which provide new insights into the mechanisms of microtubule catastrophe and rescue, and discuss the impact of these findings in regards to the role of microtubule dynamics inside of cells.

Microtubule catastrophe: an aging process

A microtubule “catastrophe” event manifests itself by the sudden switch of a growing microtubule into a rapidly shortening state. The widely accepted view of microtubule catastrophe is that it involves a single random event, such as the sudden loss of a protective end structure [1–3]. This single-step mechanism implies that a microtubule has the same probability of undergoing catastrophe at any given point in time, irrespective of how long it has been growing already. In this model, the ‘catastrophe frequency’, which is the number of observed catastrophes divided by the total period of microtubule growth, remains constant over time, and the distribution of microtubule lifetimes and lengths is predicted to follow a decaying exponential distribution.

However, measurements of microtubule length and lifetime distributions both in vitro and in vivo do not display a simple exponential decay [4–13]. Although the distribution of microtubule lengths appears to decay exponentially at longer lengths, the predicted exponential distribution is not observed at shorter lengths. This was often attributed to limitations in spatial and temporal imaging resolution, which would render short-lived microtubule growth events undetectable. Recent work by Gardner et al. [14] used large data sets of high-resolution microtubule lifetime measurements to confirm that the lack of

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catastrophe events at short time and length scales is real. These results confirm earlier predictions by Odde and coworkers [9,15].

The finding that young microtubules are less likely to undergo catastrophe means that microtubules age: catastrophe frequency is not a constant, but rather increases with time. This behavior can be explained by a model in which a microtubule catastrophe event is viewed as a multi-step process that requires several independent random events to occur before the microtubule can switch from a growing to a shrinking state (Box 1). In light of the age- and length-dependence of catastrophe, special care must be taken when characterizing microtubule lifetimes in vivo and in vitro, because sampling different populations of microtubules may lead to a difference in apparent mean lifetime and length.

Implications of microtubule aging inside of cells

In cells, catastrophe frequency is modulated by a number of microtubule-associated-proteins [16,17], and microtubule lengths can be increased or decreased by suppressing or promoting catastrophe, respectively. However, if microtubule catastrophe is a single-step process, the cellular length distribution of microtubules would always have a broad exponential shape, which means that the standard deviation of microtubule lengths would be equal to the mean length. In addition, most microtubules would catastrophe close to the nucleation center, and only a small fraction of microtubules would reach very long lengths (much larger than the mean length). Therefore, a single-step catastrophe process (where the mean microtubule length is equal to the standard deviation of microtubule lengths) poses a problem for those times in the cell cycle when tight regulation (i.e., small standard deviation) of longer mean microtubule lengths is required.

In contrast, by 'protecting' the young microtubules, the microtubule aging process results in most catastrophe events happening away from the microtubule nucleation center, allowing a greater proportion of microtubules to grow longer and better explore the cellular space. This could greatly improve the efficiency of proposed 'search and capture' mechanisms for capturing of kinetochores by dynamic microtubules during mitotic spindle assembly [18] (Figure 1A).

In a multi-step catastrophe process, not only the mean microtubule length, but also the shape of microtubule length distributions can be regulated by microtubule-associated-proteins. This could be accomplished by regulating one or both parameters of the microtubule aging process: the rate of aging, or the number of events needed for catastrophe. In fact, two prominent microtubule catastrophe factors — Kip3, a member of Kinesin-8 family, and MCAK, a member of Kinesin-13 family — have very different effects on microtubule length distributions in vitro [14].

Kip3 is a highly processive microtubule plus-end-directed motor which is a catastrophe factor in vivo [11,19] and a microtubule depolymerase in vitro [20,21]. Because Kip3 accumulates at the ends of longer stabilized microtubules, Kip3 depolymerizes longer microtubules faster than shorter ones. Consistent with its effect on stabilized microtubules, Kip3 slows down the in vitro microtubule growth rate in a length-dependent manner, and additionally promotes microtubule catastrophe by increasing the rate at which destabilizing effects occur [14]. In this way, Kip3 tightens the distribution of microtubule lengths, which is of particular importance during mitosis where fine-tuning of microtubule lengths is required.

Indeed, recent work by Stumpff and collaborators [22] identified Kif18A, another member of Kinesin-8 family, to be essential for spatial confinement of kinetochore movements. Consistent with previous reports [23–25], Stumpff et al. [22] found that Kif18A depletion

led to large sister kinetochore oscillations and increased the oscillation velocities. Interestingly, the authors reported that directional switches of kinetochores in HeLa cells coincide with K-fiber catastrophes, which are known to be modulated by kinesin-8 motor proteins [5,19,23,24,26–29]. The tight spatial regulation of sister kinetochore switching events [22] confirms an important role for Kinesin-8 in shaping microtubule length distributions in vivo (Figure 1B).

MCAK (mitotic centromere-associated kinesin) is a molecular motor from the Kinesin-13 family. This protein is known to affect spindle and astral microtubule lengths [30–35] and to increase catastrophe rates in cells [36]. By binding to microtubule tip-tracking EB proteins [37,38], as well as by diffusion on the microtubule lattice [39], MCAK targets microtubule ends and utilizes its ATPase cycle for potent microtubule depolymerization [39,40]. Unlike Kip3, which speeds up the rate of aging, MCAK exerts its destabilizing effect by reducing the number of events needed for microtubule catastrophe, such that only a single random event is sufficient to induce microtubule disassembly [14]. This results in rapid depolymerization of microtubules, regardless of their age. Such a process is likely to be of particular importance for correction of improper microtubule-kinetochore attachments [41–43], as well as for spindle breakdown at the end of mitosis [44] (Figure 1C).

Microtubule catastrophe at the cell boundary

The multi-step model of microtubule catastrophe predicts a reduction in the number of catastrophe events close to nucleation centers, and could therefore, on its own, help explain the higher number of catastrophes observed at the cell periphery as compared to the cell body [11,45–47]. Additionally, there are a number of possible mechanisms for the enhancement of catastrophe by interaction with a barrier, such as the cell cortex.

Actively growing microtubules that contact the cell cortex in an “end-on” configuration may exhibit a reduced growth rate, as free tubulin subunits may be unable to gain access to the growing microtubule end, effectively reducing the local tubulin concentration. This reduced growth rate could result in catastrophe events if the rate of tubulin subunit addition is insufficient to maintain a robust GTP-tubulin “cap” [6,8]. Another interesting possibility is described in recent work by Erent et al [48]. In this work, the authors find that the *S. Pombe* Kinesin-8 molecular motor Klp5/6 walks too slowly on the microtubule lattice to be able to catch up with in vivo growing microtubule plus-ends. Therefore, Erent and coworkers [48] predict that interaction of microtubule ends with the cell cortex could result in slowing of the net microtubule growth rate, which would allow Klp5/6 to catch up to the microtubule plus-end and thus promote depolymerization and catastrophe. Finally, a third possibility is that proteins such as CLASP [49], paxilin [50], and dynein [51] could be localized to the cell cortex and induce catastrophes of incoming microtubules.

In summary, microtubule catastrophe is a major mechanism that is employed for cellular microtubule length regulation. Microtubule aging provides a direct mechanism for shaping microtubule length distributions, both by allowing for the uninterrupted growth of young and short microtubules and by fine-tuning the lengths of longer microtubules. The intrinsic microtubule aging process can be globally and locally modified by extrinsic factors, such as microtubule-associated proteins and/or mechanical force in the context of cellular environment.

Rescue Dependence on Tubulin Concentration In Vitro

Rescue events during microtubule dynamic instability are those events in which a shortening microtubule suddenly and stochastically ceases shortening, and the microtubule switches to a polymerization state. In vitro rescue events were quantitatively described as a function of

tubulin concentration by Walker et al [52]. These early in vitro studies provided hints as to the mechanism of rescue. Specifically, Walker et al found that, although the plus-end growth rate of a microtubule increases substantially as a function of tubulin concentration, the rescue frequency is relatively insensitive to tubulin concentration. For example, in going from 7 to 14 μM tubulin, the net on-rate of tubulin dimers during growth increased nearly 5-fold, from 20 to 100 dimers/sec (Figure 2). However, the frequency of rescue was insensitive to this large increase in tubulin subunit on-rate (slope is not significantly different than 0, $p=0.14$) (Figure 2). Because a 5-fold increase in GTP-tubulin subunit addition rates does not promote rescue, this suggests that rescue does not occur as a result of stochastic GTP-tubulin addition during rapid microtubule depolymerization. Rather, in vitro microtubule rescue events likely occur when the depolymerization process is disrupted as a result of a feature that is previously embedded within the microtubule lattice. Recent work has now shed light on possibilities for this embedded lattice feature, and also examined how external forces and molecules could act to disrupt the rapid microtubule depolymerization process.

GTP-Tubulin Islands Promote Rescue Events

Given that rescue events may occur as a result of features that are embedded within the microtubule lattice, work by Dimitrov and colleagues [53] provided an interesting possible mechanism for rescue. In this work, a recombinant antibody was developed that may specifically recognize GTP-tubulin in microtubules. The antibody cosedimented specifically with GMPCPP microtubules at low concentrations of taxol, suggesting that it recognizes a conformation associated with GTP-tubulin and not GDP-tubulin. Using this antibody (hMB11), the authors found that, as expected, GTP-tubulin was present at the tips of growing in vitro microtubules. Surprisingly, hMB11 also labeled discrete dots along the lengths of both in vitro and in vivo polymerized microtubules. This finding suggests that “remnants” of GTP-tubulin may remain buried within the microtubule lattice. By observing in vivo rescue events and then staining cells with hMB11, it was found that rescue events frequently occurred at the GTP-tubulin remnant locations. Thus, this work proposed a model for rescue in which GTP-tubulin subunits buried within the lattice act to initiate rescue events during microtubule depolymerization (Figure 3A).

Interesting recent work by Thoma et al [54] used RPE-1 cells to elucidate the effect of pVHL on microtubule dynamics. In this work, the authors found that pVHL is a strong rescue promoter, which may act in part by slowing the rate of GTP hydrolysis. Strikingly, the authors stained cells using the hMB11 antibody, and found that the distance between GTP-tubulin remnants was shorter in the presence of the rescue-promoting pVHL protein, and longer in cells expressing no pVHL. This work provided an independent verification that GTP-tubulin remnants may correlate with the frequency of rescue events inside of cells. In separate work, Bhattacharya et al [55] found that microtubules in cells depleted of a $\beta 5$ -tubulin isotype showed an increased frequency of rescue events, which corresponded with an increase in GTP-tubulin remnant staining by hMB11. Conversely, HA $\beta 5$ -overexpressing cells grown without paclitaxel showed very little evidence of GTP-tubulin remnant staining by hMB11, consistent with their observation that rescue events were rare for microtubules in these cells. In other work using cultured hippocampal neuronal cells, hMB11 staining was found to be present along the length of axonal microtubules, providing evidence for the presence of GTP-tubulin remnants within the lattice of axonal microtubules [56]. An argument for a separate type of rescue-promoting structure was made by Bouissou et al, who found that γ TuRC localizes along interphase microtubules and that these spots tended to be the sites of rescue events [57].

One question that remained was whether or not the presence of a GTP-tubulin remnant in the lattice could indeed directly promote a rescue event. This question was addressed in recent work by Tropini et al [58], in which the authors introduced GMPCPP islands into dynamic microtubules during growth, and asked whether or not these islands would consistently result in rescue of depolymerizing microtubules after catastrophe. Indeed, it was found that the GMPCPP “islands” could directly produce rescue events, and that the frequency of rescue directly correlated with the size and composition of the island (Figure 3A). For example, islands produced using 50% GMPCPP relative to GTP-tubulin were ~2-fold less likely to produce rescue events than islands that were produced using 74% GMPCPP relative to GTP-tubulin. Consistent with the in vitro result that islands which contained relatively low concentrations of GMPCPP were able to promote rescue, recent in vivo results suggest that rescue sites in cells could contain as little as 6.5% GTP-tubulin relative to lattice-incorporated GDP-tubulin [59]. These in vivo and in vitro results raise the question of size: how large would a minimal remnant need to be in order to consistently produce a rescue event?

The mechanism for how lattice-incorporated tubulin subunits could avoid hydrolysis remains unclear. Recent in vivo evidence suggests that the tail end of a long GTP-cap could provide a GTP-tubulin rich region 500–2000 nm from the microtubule tip that would tend to promote rescue events [59]. However, it is interesting to consider whether the aging process that leads to catastrophe could be related to features in the microtubule lattice that ultimately lead to rescue events. For example, if lagging protofilaments could act to destabilize the microtubule tip and lead to catastrophe events, it could also be possible that slow addition of GTP-tubulin at the tips of these lagging protofilaments could result in “islands” of GTP-tubulin that are at a distance from the more rapidly growing microtubule tip. It is important to note that the GTP-tubulin “island” theory of rescues is based on one antibody and is so far not confirmed by other reagents that may preferentially recognize GTP-tubulin (such as EB proteins). However, recent in vivo evidence does suggest that a basal level of EB1 [59; Fig. 4D] can be observed on the microtubule lattice even at positions that are >2000 nm from the microtubule tip.

Another possibility for promotion of rescue events in vivo that would not rely on lattice-incorporated GTP-tubulin remnants is through rescue-promoting microtubule-associated proteins (MAPs). Candidates for in vivo rescue factors are members of the CLIP-170 and CLASP families. The mechanism for rescue-promotion via CLASP was recently examined in vitro for the *S. pombe* CLASP, Cls1p [60]. In this work, the authors find that CLASP binds to the microtubule lattice, where it recruits free tubulin subunits. By recruiting tubulin subunits to the lattice, CLASP is then able to locally promote rescue events. Consistent with the hypothesis that microtubule rescue events likely occur when protofilament depolymerization is disrupted as a result of a feature that is pre-embedded within the microtubule lattice, this new work proposes that CLASP promotes rescue events by pre-loading GTP-tubulin subunits onto the lattice, which subsequently acts to “cap” depolymerizing protofilaments and thus disrupt disassembly.

Rescue Events and Tension

One important consideration regarding rescue events is whether or not these relatively rare events are important in the context of properly regulating important cellular processes. One possibility is that rescue events play an important role in stabilizing and anchoring microtubules at the cell cortex to mediate mitotic spindle positioning and cell polarity [51,61–63]. In addition, rescue events of kinetochore microtubules likely contribute to the chromosome oscillations that are important for proper chromosome segregation during

mitosis [22,64]. In each of these cases, recent data suggests that rescue events may be mediated by mechanical tension at the microtubule plus-end.

Two recent papers investigated the effect of cortical dynein pulling forces in regulating microtubule dynamics. Laan et al [51] performed an impressive technical feat by coating micro-fabricated gold barriers with cortical dynein, and then by growing microtubules from centrosomes near the barriers. Thus, the centrosome-attached microtubule plus-ends subsequently contacted the dynein-coated barrier. Strikingly, microtubules which contacted the dynein-coated barrier in an “end-on” configuration were captured by the dynein molecules and were stabilized against disassembly for many minutes. This result suggests that motor-generated mechanical pulling forces could act to slow disassembly and promote rescue of depolymerizing microtubules (Figure 3B). A similar result was obtained in the recent work by Hendricks et al [61]. In this work, the authors found that dynein-bound polystyrene beads could tether microtubule plus-ends which grew into the beads, transiently stabilizing these ends against catastrophe. Thus, the Laan et al and the Hendricks et al papers together strongly suggest that mechanical tension as supplied by minus-end directed motor proteins could act to promote rescue events inside of cells. This type of tension-based rescue event may be important for processes which involve microtubule plus-end contact with the cell cortex, such as in mitotic spindle positioning.

Similarly, a range of in vivo studies implicate mechanical tension-dependent rescue events in kinetochore oscillations during mitosis, from yeast [65] to mammalian cells [66,67]. Here, it is likely that that chromosome stretching is the major source of mechanical tension which could promote rescue events of chromosome-attached kinetochore microtubules during mitosis (Figure 3B). However, an interesting recent study also revealed active force generation within the kinetochore, although it is unclear whether this force could act to promote microtubule rescue events [68]. Perhaps the most convincing evidence to date which correlates microtubule rescue events with chromosome stretch is the recent study by Wan et al [69]. In this study, the authors performed careful quantitative analysis of PtK1 sister kinetochore oscillations, and found that the maximum chromosome stretch occurred when the leading kinetochore switched from depolymerization to polymerization, strongly suggesting that chromosome stretching tension resulted in rescue events of depolymerizing kinetochore microtubules that were associated with the leading kinetochore. These in vivo results are consistent with previous in vitro studies which demonstrated that tension applied through the Dam1 complex and through purified kinetochore particles could suppress catastrophe and promote net microtubule assembly [70,71].

In summary, recent studies suggest that both mechanical and chemical cues may contribute to the regulation and origination of rescue events inside of cells. Thus, important future studies may focus on how these signals could be integrated to regulate rescue events inside of cells.

Summary and Outlook

Although dynamic instability of microtubules was discovered nearly 30 years ago, the mechanism for how catastrophe and rescue events occur both in vivo and in vitro remains an area of active study. Because in vitro and computational studies provide information on basic mechanisms of catastrophe and rescue events, the integration of these studies with vivo observations will continue to be an important goal.

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Figure 1. Cellular effects of microtubule aging regulation

(A) In a single step process, the microtubule length distribution is exponential (left), while microtubule aging leads to a non-exponential length distribution of microtubules (right), allowing for a more effective search-and-capture mechanism. (B) Lack of fine-tuning of microtubule lengths leads to improper metaphase chromosome alignment during mitosis (left, red arrows denoting the magnitude of kinetochore oscillations), while tight spatial regulation of microtubule lengths by the effects of a Kinesin-8 molecular motor on microtubule growth rate and microtubule aging allows for proper alignment (right, green arrows denoting the magnitude of kinetochore oscillations). (C) Due to the aging process, short (young) microtubules continue growth at the completion of mitosis (left, green arrows denote growing, while red crosses denote shrinking microtubules). By turning catastrophe into a single-step process, MCAK leads to indiscriminatory microtubule disassembly (right).

Figure 2. Rescue events are not strongly correlated to tubulin concentration

In data reproduced from Walker et al, the net on-rate of tubulin subunits during microtubule growth increases monotonically as a function of tubulin concentration. However, rescue frequency remains relatively constant regardless of the tubulin concentration.

Figure 3. Possible rescue mechanisms

(A) In one mechanism, GTP-tubulin "islands" remain embedded within the microtubule lattice (blue=GDP-tubulin, red = GTP-tubulin), and provide a nucleation point for rescue events. (B) In another mechanism, tension at the depolymerizing microtubule end could promote rescue by straightening the curled GDP-tubulin subunits during microtubule depolymerization.

Box 1.
Microtubule Catastrophe: Single vs. Multi-Step Process