Microtubule catastrophe and rescue

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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.

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Introduction

Microtubules are long cylindrical polymers composed of tubulin subunits, whose dynamic ends contribute to the establishment of cellular architecture, and to cell motility. 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.
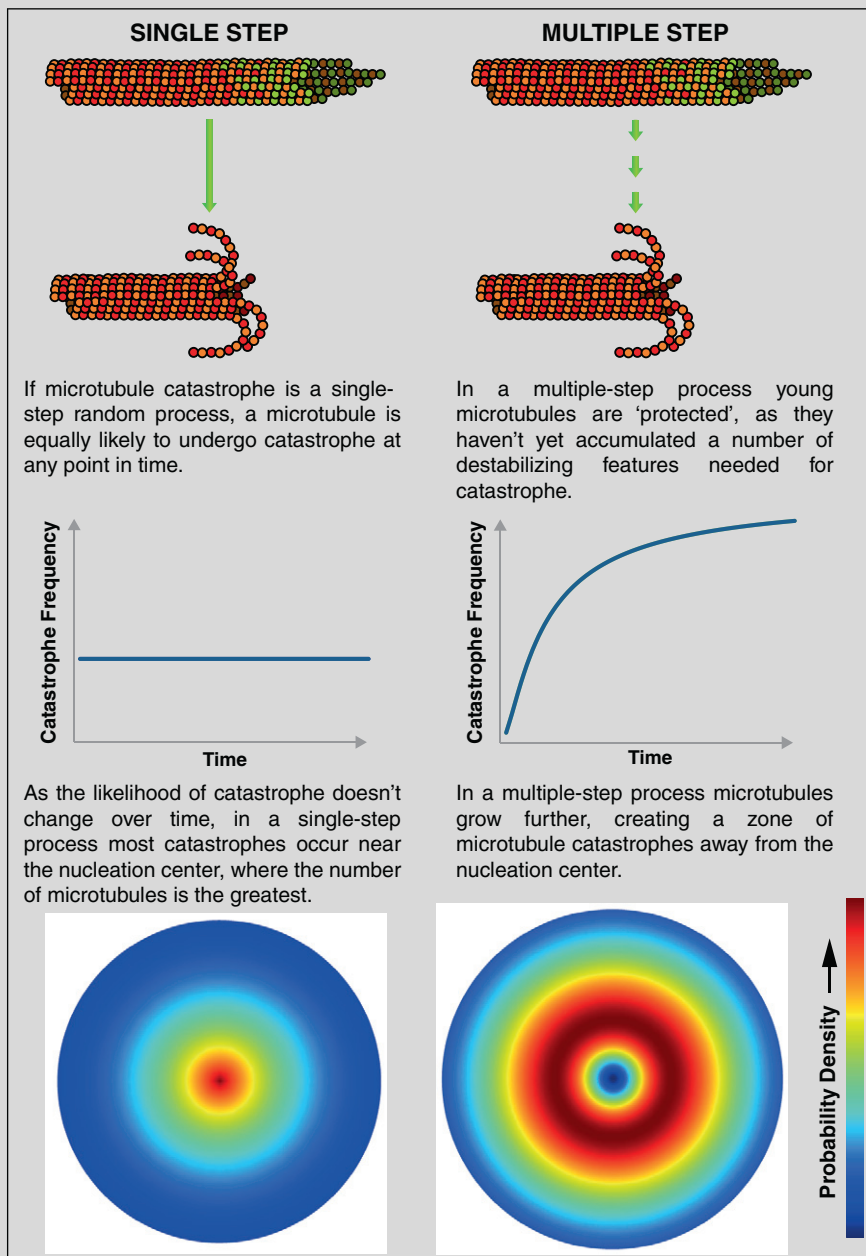
However, measurements of microtubule length and lifetime distributions both *in vitro* and *in vivo* do not display a simple exponential decay [4–13]. Although the distribution of microtubule lengths appears to decay exponentially at longer lengths, the predicted exponential distribution is not observed 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 catastrophe events at short time and length scales is real. These results confirm earlier predictions by Odde and co-workers [9,15].

The finding that young microtubules are less probable 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-dependence 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

Box 1 Microtubule catastrophe: single vs. multi-step process

SINGLE STEP

If microtubule catastrophe is a single-step random process, a microtubule is equally likely to undergo catastrophe at any point in time.

As the likelihood of catastrophe doesn't change over time, in a single-step process most catastrophes occur near the nucleation center, where the number of microtubules is the greatest.

MULTIPLE STEP

In a multiple-step process young microtubules are 'protected', as they haven't yet accumulated a number of destabilizing features needed for catastrophe.

In a multiple-step process microtubules grow further, creating a zone of microtubule catastrophes away from the nucleation center.

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., a small standard deviation) of longer mean microtubule lengths is required.

By contrast, in '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

Figure 1
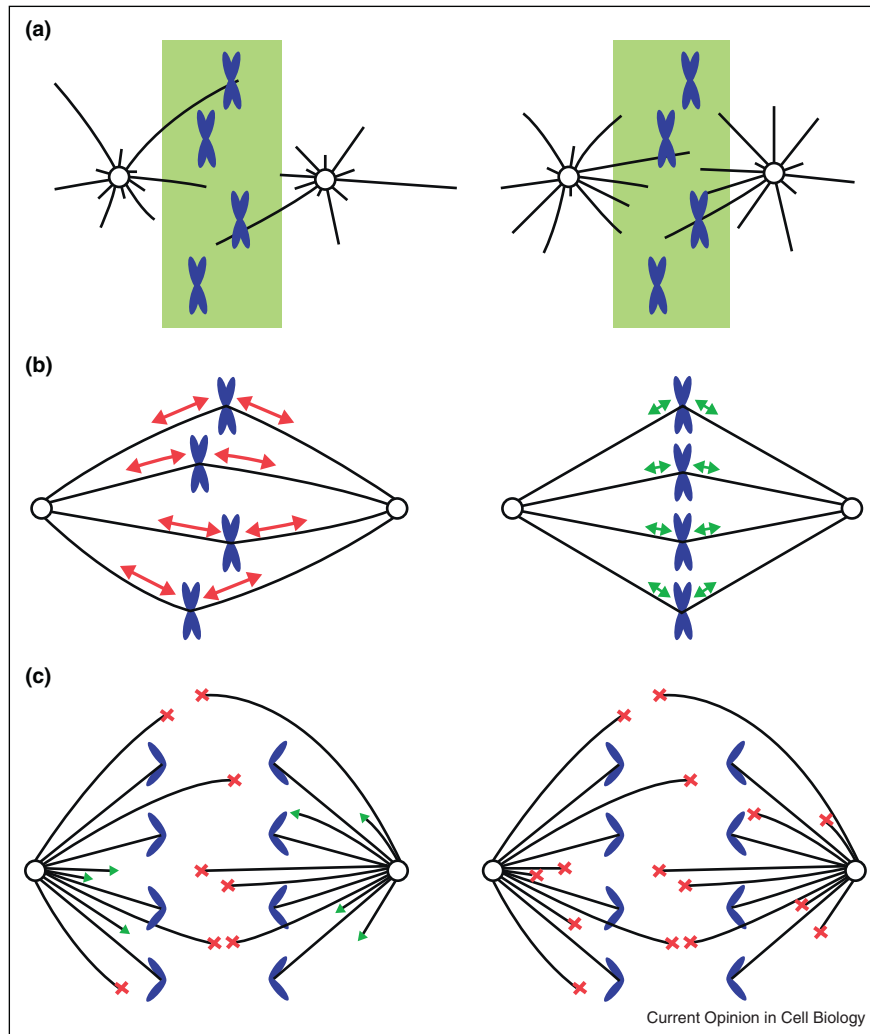
Cellular effects of microtubule aging regulation. **(a)** In a single step process, the microtubule length distribution is exponential (left), while microtubule aging leads to a non-exponential length distribution of microtubules (right), allowing for a more effective search-and-capture mechanism. **(b)** 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)** Owing 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).

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 *et al.* [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 could 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 [11,45,46,47•]. 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 *Schizosaccharomyces 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 *et al.* [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

Figure 2
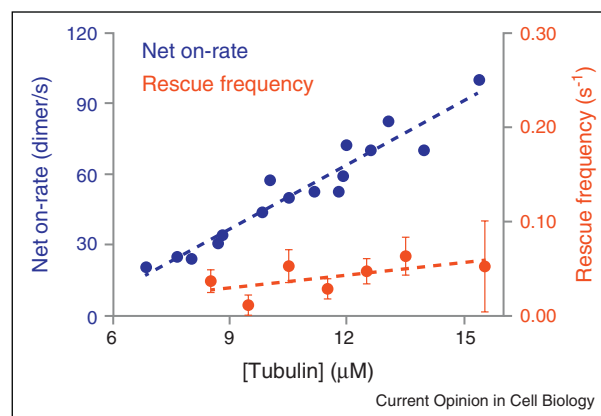
Rescue events are not strongly correlated to tubulin concentration. In data reproduced from Walker *et al.*, the net on-rate of tubulin subunits during microtubule growth increases monotonically as a function of tubulin concentration. However, rescue frequency remains relatively constant regardless of the tubulin concentration.

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/s (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 may 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.
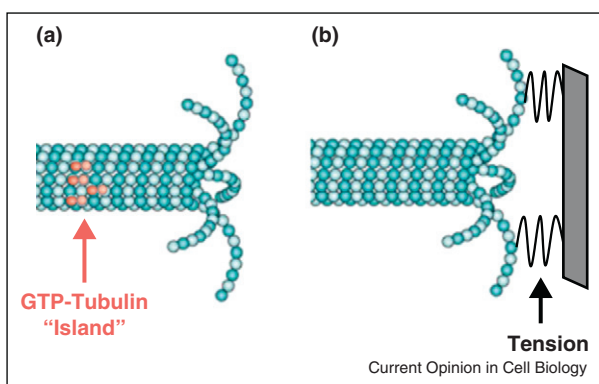
GTP-tubulin islands promote rescue events

Given that rescue events may occur as a result of features that are embedded within the microtubule lattice, work by Dimitrov *et al.* [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 partly 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 β5-tubulin isotype showed an increased frequency of rescue events, which corresponded with an increase in GTP-tubulin remnant staining by hMB11. Conversely, HAβ5-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 γTuRC 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 3b). For example, islands produced using 50% GMPCPP relative to GTP-tubulin were ~2-fold less likely to produce rescue events than islands that were

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.

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•; Figure 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 may 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••,62,63]. In addition, rescue events of kinetochore microtubules probably contribute to the chromosome oscillations that are important for proper chromosome segregation during mitosis [22•,64]. In each of these cases, recent data suggest 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 probable 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 *in vivo* observations will continue to be an important goal.

Acknowledgements

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- •• of outstanding interest

1. Mitchison TJ, Kirschner MW: **Dynamic instability of microtubule growth**. *Nature* 1984, **312**:237-242.

2. Desai A, Mitchison TJ: **Microtubule polymerization dynamics**. *Annu Rev Cell Dev Biol* 1997, **13**:83-117.

3. Howard J, Hyman AA: **Growth, fluctuation and switching at microtubule plus ends**. *Nat Rev Mol Cell Biol* 2009, **10**:569-574.

4. Cassimeris L, Wadsworth P, Salmon ED: **Dynamics of microtubule depolymerization in monocytes**. *J Cell Biol* 1986, **102**:2023-2032.

5. Du Y, English CA, Ohi R: **The kinesin-8 Kif18A dampens microtubule plus-end dynamics**. *Curr Biol* 2010, **20**:374-380.

6. Foethke D, Makushok T, Brunner D, Nédélec F: **Force- and length-dependent catastrophe activities explain interphase microtubule organization in fission yeast**. *Mol Syst Biol* 2009, **5**:241.

7. Fygenson D, Braun E, Libchaber A: **Phase diagram of microtubules**. *Phys Rev E Stat Nonlin Soft Matter Phys* 1994, **50**:1579-1588.

8. Janson ME, de Dood ME, Dogterom M: **Dynamic instability of microtubules is regulated by force**. *J Cell Biol* 2003, **161**:1029-1034.

9. Odde DJ, Cassimeris L, Buettner HM: **Kinetics of microtubule catastrophe assessed by probabilistic analysis**. *Biophys J* 1995, **69**:796-802.

10. Stepanova T, Smal I, van Haren J, Akinci U, Liu Z, Miedema M, Limpens R, van Ham M, van der Reijden M, Poot R *et al.*: **History-dependent catastrophes regulate axonal microtubule behavior**. *Curr Biol* 2010, **20**:1023-1028.

11. Tischer C, Brunner D, Dogterom M: **Force- and kinesin-8-dependent effects in the spatial regulation of fission yeast microtubule dynamics**. *Mol Syst Biol* 2009, **5**:250.

12. Voter WA, O'Brien ET, Erickson HP: **Dilution-induced disassembly of microtubules: relation to dynamic instability and the GTP cap**. *Cell Motil Cytoskeleton* 1991, **18**:55-62.

13. Walker RA, Pryer NK, Salmon ED: **Dilution of individual microtubules observed in real time in vitro: evidence that cap size is small and independent of elongation rate**. *J Cell Biol* 1991, **114**:73-81.

14. Gardner MK, Zanic M, Gell C, Bormuth V, Howard J:
•• **Depolymerizing kinesins Kip3 and MCAK shape cellular microtubule architecture by differential control of catastrophe**. *Cell* 2011, **147**:1092-1103.
The authors report that microtubule catastrophe *in vitro* relies on a multiple-step aging process. The aging process is differentially modulated by different catastrophe factors: the authors find that Kinesin-8 Kip3 accelerates the aging, while Kinesin-13 MCAK abolishes the aging and reverts catastrophe to a single-step random process.

15. Odde DJ, Buettner H, Cassimeris L: **Spectral analysis of microtubule assembly dynamics**. *AIChE J* 1996, **42**:1434-1442.

16. Howard J, Hyman AA: **Microtubule polymerases and depolymerases**. *Curr Opin Cell Biol* 2007, **19**:31-35.

17. Akhmanova A, Steinmetz MO: **Tracking the ends: a dynamic protein network controls the fate of microtubule tips**. *Nat Rev Mol Cell Biol* 2008, **9**:309-322.

18. Wollman R, Cytrynbaum EN, Jones JT, Meyer T, Scholey JM, Mogilner A: **Efficient chromosome capture requires a bias in the ''search-and-capture'' process during mitotic-spindle assembly**. *Curr Biol* 2005, **15**:828-832.

19. Gupta ML, Carvalho P, Roof DM, Pellman D: **Plus end-specific depolymerase activity of Kip3, a kinesin-8 protein, explains its role in positioning the yeast mitotic spindle**. *Nat Cell Biol* 2006, **8**:913-923.

20. Varga V, Helenius J, Tanaka K, Hyman AA, Tanaka TU, Howard J: **Yeast kinesin-8 depolymerizes microtubules in a length-dependent manner**. *Nat Cell Biol* 2006, **8**:957-962.

21. Varga V, Leduc C, Bormuth V, Diez S, Howard J: **Kinesin-8 motors act cooperatively to mediate length-dependent microtubule depolymerization**. *Cell* 2009, **138**:1174-1183.

22. Stumpff J, Wagenbach M, Franck A, Asbury CL, Wordeman L:
• **Kif18A and chromokinesins confine centromere movements via microtubule growth suppression and spatial control of kinetochore tension**. *Dev Cell* 2012, **22**:1017-1029.
Using *in vitro* reconstitution assays and live-cell imaging, the authors report that kinesins from Kinesin-4, Kinesin-8 and Kinesin-10 families regulate centromere positioning by modulating microtubule dynamics.

23. Mayr MI, Hümmer S, Bormann J, Grüner T, Adio S, Woehlke G, Mayr TU: **The human kinesin Kif18A is a motile microtubule depolymerase essential for chromosome congression**. *Curr Biol* 2007, **17**:488-498.

24. Stumpff J, Dassow von G, Wagenbach M, Asbury CL, Wordeman L: **The kinesin-8 motor Kif18A suppresses kinetochore movements to control mitotic chromosome alignment**. *Dev Cell* 2008, **14**:252-262.

25. Zhu C, Zhao J, Bibikova M, Leverson JD, Bossy-Wetzel E, Fan J-B, Abraham RT, Jiang W: **Functional analysis of human microtubule-based motor proteins, the kinesins and dyneins, in mitosis/cytokinesis using RNA interference**. *Mol Biol Cell* 2005, **16**:3187-3199.

26. Stumpff J, Du Y, English CA, Maliga Z, Wagenbach M, Asbury CL, Wordeman L, Ohi R: **A tethering mechanism controls the processivity and kinetochore-microtubule plus-end enrichment of the Kinesin-8 Kif18A**. *Mol Cell* 2011, **43**:764-775.

27. Wargacki MM, Tay JC, Muller EG, Asbury CL, Davis TN: **Kip3, the yeast kinesin-8, is required for clustering of kinetochores at metaphase**. *Cell Cycle* 2010, **9**:2581-2588.

28. Garcia MA, Koonrugsa N, Toda T: **Two kinesin-like Kin I family proteins in fission yeast regulate the establishment of metaphase and the onset of anaphase A**. *Curr Biol* 2002, **12**:610-621.

29. West RR, Malmstrom T, Mcintosh JR: **Kinesins klp5(+) and klp6(+) are required for normal chromosome movement in mitosis**. *J Cell Sci* 2002, **115**:931-940.

30. Desai A, Verma S, Mitchison TJ, Walczak CE: **Kin I kinesins are microtubule-destabilizing enzymes**. *Cell* 1999, **96**:69-78.

31. Goshima G, Vale RD: **Cell cycle-dependent dynamics and regulation of mitotic kinesins in Drosophila S2 cells**. *Mol Biol Cell* 2005, **16**:3896-3907.

32. Maney T, Hunter AW, Wagenbach M, Wordeman L: **Mitotic centromere-associated kinesin is important for anaphase chromosome segregation**. *J Cell Biol* 1998, **142**:787-801.

33. Rogers GC, Rogers SL, Schwimmer TA, Ems-McClung SC, Walczak CE, Vale RD, Scholey JM, Sharp DJ: **Two mitotic kinesins cooperate to drive sister chromatid separation during anaphase**. *Nature* 2004, **427**:364-370.

34. Tournebize R, Popov A, Kinoshita K, Ashford AJ, Rybina S, Pozniakovsky A, Mayer TU, Walczak CE, Karsenti E, Hyman AA: **Control of microtubule dynamics by the antagonistic activities of XMAP215 and XKCM1 in Xenopus egg extracts**. *Nat Cell Biol* 2000, **2**:13-19.

35. Walczak CE, Mitchison TJ, Desai A: **XKCM1: a Xenopus kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly**. *Cell* 1996, **84**:37-47.

36. Kline-Smith SL, Walczak CE: **The microtubule-destabilizing kinesin XKCM1 regulates microtubule dynamic instability in cells**. *Mol Biol Cell* 2002, **13**:2718-2731.

37. Montenegro Gouveia S, Leslie K, Kapitein LC, Buey RM, Grigoriev I, Wagenbach M, Smal I, Meijering E, Hoogenraad CC, Wordeman L *et al.*: **In vitro reconstitution of the functional interplay between MCAK and EB3 at microtubule plus ends**. *Curr Biol* 2010, **20**:1717-1722.

38. Domnitz SB, Wagenbach M, Decarreau J, Wordeman L: **MCAK activity at microtubule tips regulates spindle microtubule length to promote robust kinetochore attachment**. *J Cell Biol* 2012, **197**:231-237.

39. Helenius J, Brouhard GJ, Kalaidzidis Y, Diez S, Howard J: **The depolymerizing kinesin MCAK uses lattice diffusion to rapidly target microtubule ends**. *Nature* 2006, **441**:115-119.

40. Friel CT, Howard J: **The kinesin-13 MCAK has an unconventional ATPase cycle adapted for microtubule depolymerization**. *EMBO J* 2011, **30**:3928-3939.

41. Bakhoum SF, Genovese G, Compton DA: **Deviant kinetochore microtubule dynamics underlie chromosomal instability**. *Curr Biol* 2009, **19**:1937-1942.

42. Kline-Smith SL, Walczak CE: **Mitotic spindle assembly and chromosome segregation: refocusing on microtubule dynamics**. *Mol Cell* 2004, **15**:317-327.

43. Wordeman L, Wagenbach M, von Dassow G: **MCAK facilitates chromosome movement by promoting kinetochore microtubule turnover**. *J Cell Biol* 2007, **179**:869-879.

44. Rankin KE, Wordeman L: **Long astral microtubules uncouple**
• **mitotic spindles from the cytokinetic furrow**. *J Cell Biol* 2010, **190**:35-43.
Here, the authors deplete a catastrophe factor MCAK (Kinesin-13) to investigate the effects of astral microtubules in mitosis. As a result, they observe intense oscillations of entire spindle with chromosomes between the daughter cells in early anaphase.

45. Komarova YA, Vorobjev IA, Borisy GG: **Life cycle of MTs: persistent growth in the cell interior, asymmetric transition frequencies and effects of the cell boundary**. *J Cell Sci* 2002, **115**:3527-3539.

46. Wittmann T, Waterman CM: **Spatial regulation of CLASP affinity for microtubules by Rac1 and GSK3beta in migrating epithelial cells**. *J Cell Biol* 2005, **169**:929-939.

47. Myers KA, Applegate KT, Danuser G, Fischer RS, Waterman CM:
• **Distinct ECM mechanosensing pathways regulate microtubule dynamics to control endothelial cell branching morphogenesis**. *J Cell Biol* 2011, **192**:321-334.
Here, the authors investigate the role of dimensionality and extra-cellular-matrix (ECM) compliance on microtubule dynamics *in vivo*. The authors find local and global modifications of microtubule growth rates and growth persistence in response to the changes in ECM properties.

48. Erent M, Drummond DR, Cross RA: ***S. pombe* Kinesins-8 promote both nucleation and catastrophe of microtubules**. *PLoS ONE* 2012, **7**:e30738.

49. Mimori-Kiyosue Y, Grigoriev I, Lansbergen G, Sasaki H, Matsui C, Severin F, Galjart N, Grosveld F, Vorobjev I, Tsukita S *et al.*: **CLASP1 and CLASP2 bind to EB1 and regulate microtubule plus-end dynamics at the cell cortex**. *J Cell Biol* 2005, **168**:141-153.

50. Efimov A, Schiefermeier N, Grigoriev I, Ohi R, Brown MC, Turner CE, Small JV, Kaverina I: **Paxillin-dependent stimulation of microtubule catastrophes at focal adhesion sites**. *J Cell Sci* 2008, **121**:196-204.

51. Laan L, Pavin N, Husson J, Romet-Lemonne G, van Duijn M,
•• López MP, Vale RD, Jülicher F, Reck-Peterson SL, Dogterom M: **Cortical dynein controls microtubule dynamics to generate pulling forces that position microtubule asters**. *Cell* 2012, **148**:502-514.
The authors use microfabricated barriers for *in vitro* reconstitution of cortical dynein interactions with microtubules. They report that dynein-generated pulling forces alter the microtubule dynamics and lead to centering of microtubule asters.

52. Walker RA, O'Brien ET, Pryer NK, Soboeiro MF, Voter WA, Erickson HP, Salmon ED: **Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies**. *J Cell Biol* 1988, **107**:1437-1448.

53. Dimitrov A, Quesnoit M, Moutel S, Cantaloube I, Poüs C, Perez F: **Detection of GTP-tubulin conformation in vivo reveals a role for GTP remnants in microtubule rescues**. *Science* 2008, **322**:1353-1356.

54. Thoma CR, Matov A, Gutbrodt KL, Hoerner CR, Smole Z, Krek W,
• Danuser G: **Quantitative image analysis identifies pVHL as a key regulator of microtubule dynamic instability**. *J Cell Biol* 2010, **190**:991-1003.
In this paper, the authors use *in vivo* microtubule growth tracking and find pVHL to be a microtubule stabilizing factor which promotes microtubule rescue. Additionally, the authors report that cells expressing pVHL have more GTP-tubulin remnants in the microtubule lattice.

55. Bhattacharya R, Yang H, Cabral F: **Class V β-tubulin alters dynamic instability and stimulates microtubule detachment from centrosomes**. *Mol Biol Cell* 2011, **22**:1025-1034.

56. Nakata T, Niwa S, Okada Y, Perez F, Hirokawa N: **Preferential binding of a kinesin-1 motor to GTP-tubulin-rich microtubules underlies polarized vesicle transport**. *J Cell Biol* 2011, **194**:245-255.

57. Bouissou A, Vérollet C, Sousa A, Sampaio P, Wright M, Sunkel CE, Merdes A, Raynaud-Messina B: **{gamma}-Tubulin ring complexes regulate microtubule plus end dynamics**. *J Cell Biol* 2009, **187**:327-334.

58. Tropini C, Roth EA, Zanic M, Gardner MK, Howard J: **Islands**
• **containing slowly hydrolyzable GTP analogs promote microtubule rescues**. *PLoS ONE* 2012, **7**:e30103.
In this paper, the authors use *in vitro* reconstitution assay to investigate the effects of GMPCPP-tubulin islands on microtubule dynamics. The authors report that such islands directly induce rescue events, depending on the size and the composition of the island.

59. Seetapun D, Castle BT, McIntyre AJ, Tran PT, Odde DJ:
• **Estimating the microtubule GTP cap size in vivo**. *Curr Biol* 2012, **22**:1681-1687.
Here, the authors use quantitative imaging in living cells to estimate the *in vivo* microtubule GTP-tubulin cap size, based on the assumption that Eb1

recognizes the GTP hydrolysis state of tubulin subunits within the microtubule lattice.

60. Al-Bassam J, Kim H, Brouhard GJ, van Oijen A, Harrison SC, Chang F: **CLASP promotes microtubule rescue by recruiting tubulin dimers to the microtubule**. *Dev Cell* 2010, **19**:245-258.

61. Hendricks AG, Lazarus JE, Perlson E, Gardner MK, Odde DJ,
•• Goldman YE, Holzbaur ELF: **Dynein tethers and stabilizes dynamic microtubule plus ends**. *Curr Biol* 2012, **22**:632-637.
In this paper the authors investigate *in vitro* interaction of dynein-bound beads and growing microtubule plus ends. The authors report that dynein stabilizes captured microtubule ends against catastrophe.

62. Moore JK, Li J, Cooper JA: **Dynactin function in mitotic spindle positioning**. *Traffic* 2008, **9**:510-527.

63. Levy JR, Holzbaur ELF: **Dynein drives nuclear rotation during forward progression of motile fibroblasts**. *J Cell Sci* 2008, **121**:3187-3195.

64. Tirnauer JS, Canman JC, Salmon ED, Mitchison TJ: **EB1 targets to kinetochores with attached, polymerizing microtubules**. *Mol Biol Cell* 2002, **13**:4308-4316.

65. Gardner MK, Pearson C, Sprague B, Zarzar T, Bloom K, Salmon ED, Odde DJ: **Tension-dependent regulation of microtubule dynamics at kinetochores can explain metaphase congression in yeast**. *Mol Biol Cell* 2005, **16**:3764-3775.

66. Jaqaman K, King EM, Amaro AC, Winter JR, Dorn JF, Elliott HL, Mchedlishvili N, McClelland SE, Porter IM, Posch M *et al.*: **Kinetochore alignment within the metaphase plate is regulated by centromere stiffness and microtubule depolymerases**. *J Cell Biol* 2010, **188**:665-679.

67. Ribeiro SA, Gatlin JC, Dong Y, Joglekar A, Cameron L, Hudson DF, Farr CJ, McEwen BF, Salmon ED, Earnshaw WC *et al.*: **Condensin regulates the stiffness of vertebrate centromeres**. *Mol Biol Cell* 2009, **20**:2371-2380.

68. Dumont S, Salmon ED, Mitchison TJ: **Deformations within moving kinetochores reveal different sites of active and passive force generation**. *Science* 2012, **337**:355-358.

69. Wan X, Cimini D, Cameron LA, Salmon ED: **The coupling between
•• sister kinetochore directional instability and oscillations in centromere stretch in metaphase PtK1 cells**. *Mol Biol Cell* 2012, **23**:1035-1046.
By tracking the position of kinetochores and their poles in metaphase cells, the authors report that chromosome stretching correlates with switching of leading kinetochore from a shrinking to a growing state.

70. Franck AD, Powers AF, Gestaut DR, Gonen T, Davis TN, Asbury CL: **Tension applied through the Dam1 complex promotes microtubule elongation providing a direct mechanism for length control in mitosis**. *Nat Cell Biol* 2007, **9**:832-837.

71. Akiyoshi B, Sarangapani KK, Powers AF, Nelson CR, Reichow SL, Arellano-Santoyo H, Gonen T, Ranish JA, Asbury CL, Biggins S: **Tension directly stabilizes reconstituted kinetochore-microtubule attachments**. *Nature* 2010, **468**:576-579.