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Microtubule self-repair

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Abstract

The stochastic switching between microtubule growth and shrinkage is a fascinating and unique process in the regulation of the cytoskeleton. To understand it, almost all attention has been focused on the microtubule ends. However, recent research has revived the idea that tubulin dimers can also be exchanged in protofilaments along the microtubule shaft, thus repairing the microtubule and protecting it from disassembly. Here, we review the research describing this phenomenon, the mechanisms regulating the removal and insertion of tubulin dimers, as well as the potential implications for key functions of the microtubule network, such as intracellular transport and cell polarization.

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Microtubule, Dynamics, Self-repair, Self-healing, Rescue, Molecular motors, Mechanosensation, Polarisation.

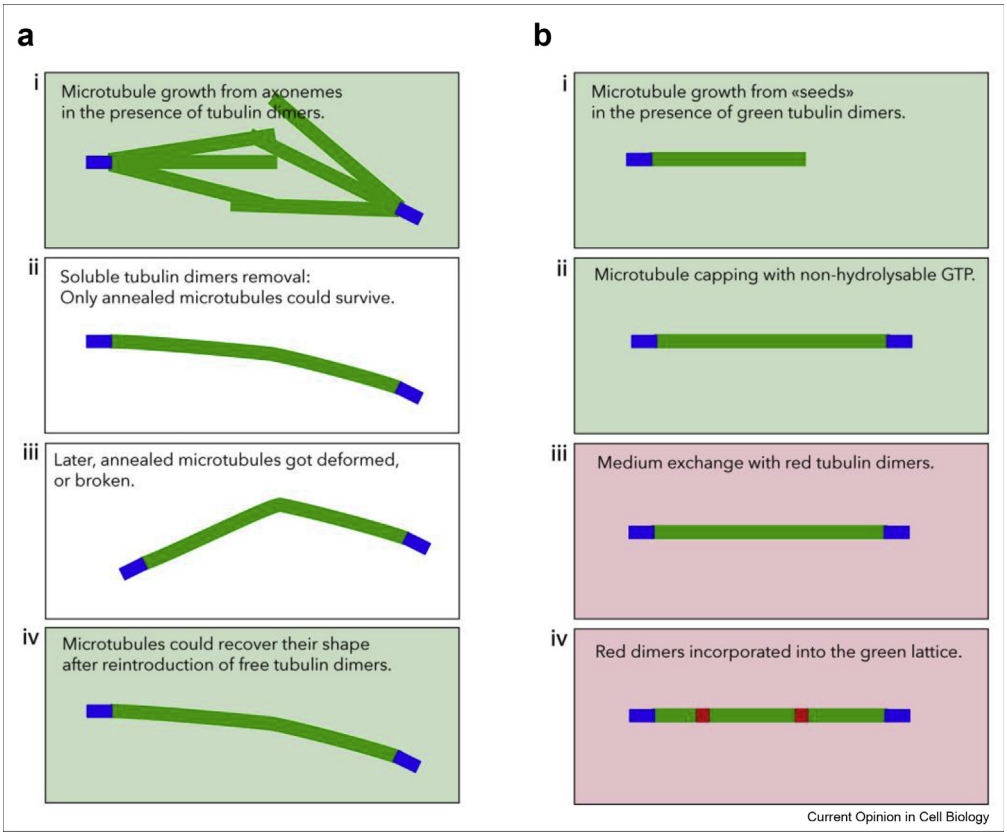
Introduction

A microtubule consists of longitudinally aligned protofilaments twisted in a helix to form a wall surrounding a hollow tube. Each protofilament is a linear polymer of alpha and beta tubulin dimers. Thus a protofilament has a polarity dictated by the orientation of these dimers, and all protofilaments in a microtubule align in accordance with this polarity, resulting in the microtubule having a plus end (beta-tubulin exposed) and a minus end (alpha-tubulin exposed). The regular spacing of tubulin dimers, the lateral interactions of tubulins between protofilaments and the strict angular orientation between adjacent protofilaments confer a crystal-like structure to the walls of microtubules [1]. Because of the similarity with the regular and periodic structure of metals, the structural arrangement of the microtubule wall is described as a lattice. This lattice-like structure of the microtubule wall supported the idea that the addition or loss of tubulin dimers only occurs at the microtubule ends [2–4].

In the early 90s, certain observations raised the possibility that the lattice could be weakened by the loss of dimers, and subsequently repaired by dimer reincorporation at a distance from the microtubule ends [5]. The initial observations came from the in vitro analysis of microtubules, where dense arrays of microtubules were formed by the addition of tubulin dimers to microtubule nucleation templates (i.e. axonemal fragments isolated from the tails of sea-urchin sperm). Microtubule disassembly in these dense arrays was triggered by washing out soluble dimers. However, some microtubules remained because they were annealed end-to-end to each other, and their other ends were secured by the nucleation templates (Figure 1a, i-ii). A few minutes later, these microtubules displayed kinks or hyper-flexible sections in their lattice, which later broke and led to complete microtubules disassembly (Figure 1a, iii). This process was inhibited by the reintroduction of soluble dimers, with deformed microtubules recovering their initial straight shape (Figure 1a, iv). In recently repeating these experiments with a slightly different protocol, we observed the same microtubule weakening and disassembly upon soluble dimer washout, and the same process of repair [6]. microtubules were stabilized by the addition of tubulin caps (tubulin bound to nonhydrolysable GTP) before and after microtubule-growth (Figure 2a, i-ii), and the tubulin dimers were labelled green in the the microtubule-growth phase and red in the microtubule repair phase. After the repair phase, red-labelled dimers were identified in the pre-existing green lattice (Figure 2a, iii-iv) [6], demonstrating the exchange of tubulin dimers in the lattice at distance from microtubule ends.

Given this microtubule repair process is hidden in typical experimental setups, it has been generally overlooked for decades. Nevertheless, some several recent studies have shined some light on this microtubule repair process, and in this opinion piece we will address how this process works; what are the conditions

Figure 1



Microtubule self-repair. These schematics illustrate the damage and self-repair of microtubules described in a study by Dye et al. [5] (**a**) and in a study by Schaedel [6] (**b**). Microtubules are green, caps are blue and repair sites are red.

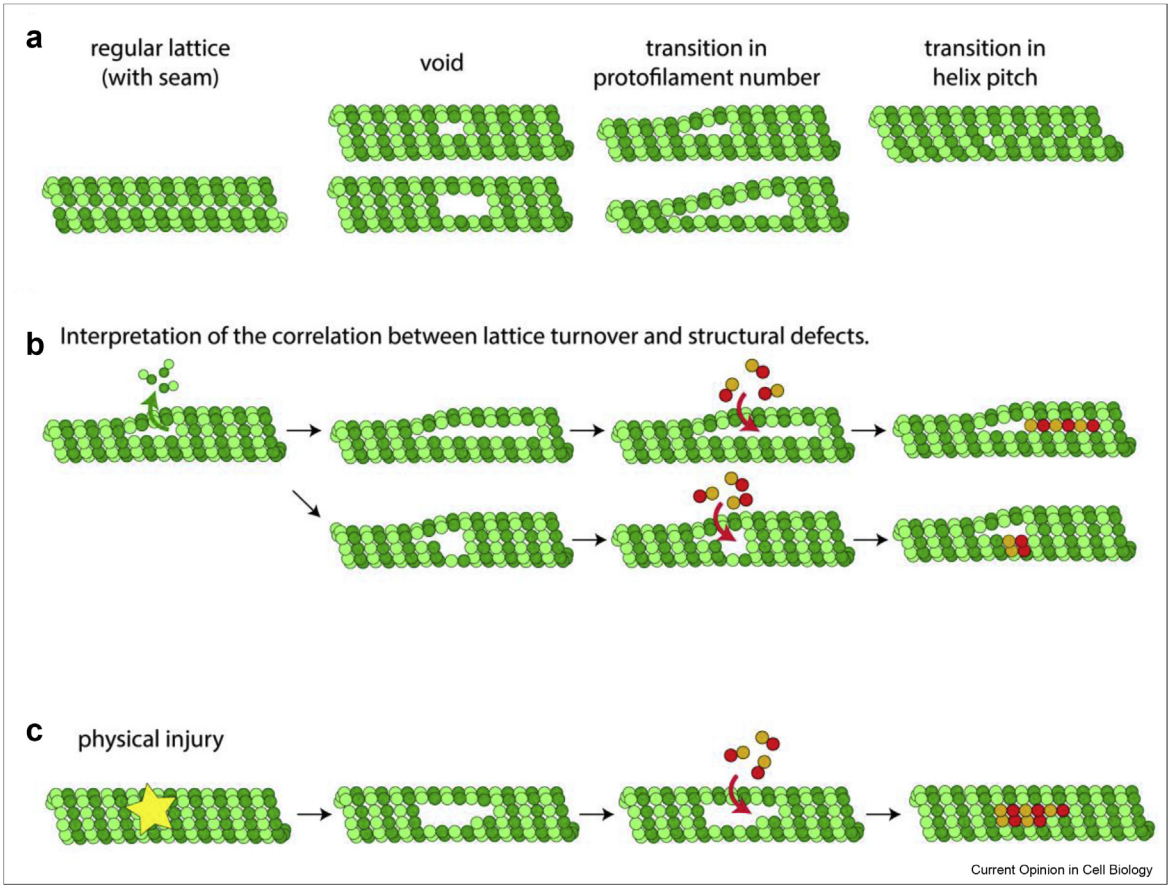
fostering or blocking it, how does it impact microtubule stability and dynamics; and how it can influence key microtubule network functions.

Spontaneous self-renewal at lattice defects

Although the energies of the longitudinal and lateral bonds between tubulin dimers have not been measured experimentally, these values can be estimated from the mathematical modelling of observed parameters associated with microtubule dynamics, such as growth rate, depolymerization rate, frequencies of switching between polymerization and depolymerization; and the sensitivity of those rates to tubulin concentration [7]. Interestingly, numerical simulations based on these parameters show that spontaneous dimer renewal in the lattice is possible, but so energetically unfavourable that it would take days to observe the renewal of a few dimers [6]. Indeed, it is unlikely that a dimer would come out of the lattice, and instead would require a physical intervention, e.g. by using an atomic force microscope tip [8]. However, under experimental conditions, such spontaneous dimer renewal events are observed within minutes and are clearly dependent on the classic assembly parameters, such as tubulin concentration [6]. Yet such high renewal rates could be obtained in numerical simulations if lattice defects are included in the modelling [6].

Lattice defects can include missing dimers (vacancies), dislocations (points at which the number of protofilaments changes in the microtubule), transitions in periodicity of the lattice (a change in the pitch of the helix) or a combination of those (Figure 2a). These defects appear frequently along the length of a microtubule and have been well documented with electronic microscopy [9,10] and atomic force microscope [11]. They are likely to stem from lateral interactions of growing protofilaments at the microtubule end [12]. Defects can be observed in microtubules assembled in vitro with pure tubulin, as well as in egg extract where tubulin, in addition to numerous proteins regulating microtubule-growth, are present [9]. Interestingly, in these experimental set ups, the frequency of lattice defects decreases with time, suggesting that, either (i) repeated cycles of polymerization/depolymerization eliminate them or (ii) the displacement and encountering of opposite defects annihilate them. Dislocations in the microtubule-lattice

Figure 2



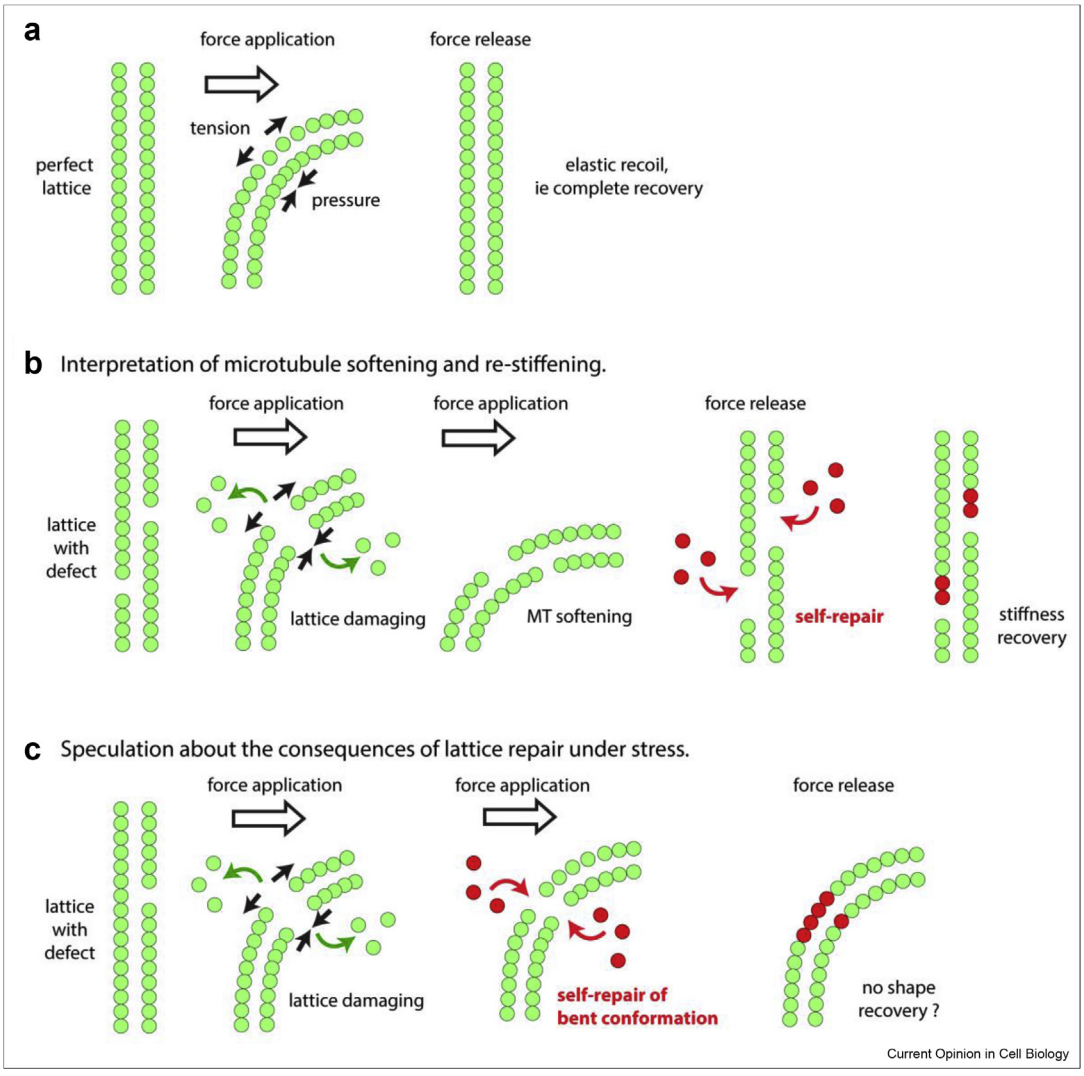
Spontaneous self-renewal at microtubule lattice defects. These schematics illustrate various types of lattice defects (**a**). A speculative interpretation of the mechanism supporting self-renewal at the sites of lattice defects, inspired by a study by Schaedel [6] (**b**). The repair in response to laser-induced damage described in a study by Schaedel et al. [21] (**c**). Polymerized tubulins are green and free dimers involved in the repair are red.

can also be stabilized by the microtubule-associated protein (MAP) Tau [13], whereas the addition of taxol during polymerization induces major structural defects and numerous sites of lattice renewal [14].

Lattice defects represent energetically favourable sites for dimer loss and readdition because these sites are associated with gaps in the structure where the number of bonds between dimers has been reduced. In addition, the lattice is under mechanical stress because of protofilament skewing and because of the conformation changes associated with GTP hydrolysis [12]. Therefore a lattice defect is likely to represent a weak point in the structure, in which the loss of a dimer could release that stress, thus propagating the structural damage, further dimer loss, and hence leaving more space for external dimers to be incorporated (Figure 2b) [6]. Although, the frequency of lattice defects is correlated with the frequency of lattice renewal sites (e.g. high concentrations of tubulin favour the occurrence of defects and increase the number of sites of lattice renewal [6]), there is currently no direct evidence that tubulin dimers incorporate precisely at sites of lattice defects. Electronic microscopy and gold labelling of dimers are required to further investigate this and to visualize the size and shape of the sites of lattice renewal.

Whether structural defects exist in the nascent microtubule lattice in vivo, remains difficult to establish. The elongation of microtubule plus-ends [4] is supported by numerous proteins, suggesting that mechanisms may be in place to secure the assembly of a regular lattice. However, using egg extract in which all these proteins should be present, the frequencies of microtubule defects and sites of renewal were similar to that observed with microtubules generated from tubulin alone [6]. Furthermore, microtubules in neurons display major structural defects [15], even though it remains unclear whether those defects arose during the formation of the lattice or due to subsequent physical damage. Here also, further cryo-EM (electron-microscopy) studies are required to characterize in cells, subtle defects, such as

Figure 3



Microtubule self-repair in response to external bending forces. These schematics illustrate the elastic bending of a regular microtubule lattice (**a**). The inelastic bending cycle of a lattice containing a defect, and the interpretation of its relationship to the self-repair process described in a study by Schaedel et al. [21] (**b**). A speculation about the consequence of microtubule self-repair in bent rather than straight conformation. Polymerized tubulins are green and free dimers involved in the repair are red.

the transition of protofilament numbers in a given microtubule. Three-dimensional cryo-ET (electron-tomography) can now be applied to relatively thick tissue samples and a wide variety of culture cells also opening the possibility of more thoroughly investigating these defects in cells [16,17].

Overall, these studies revealed that the interactions between tubulin dimers in the lattice are so weak that small imperfections in the lattice, subjected to nothing else but thermal energy, are sufficient to trigger the loss of dimers. Because all noncovalent bonds can rupture under force [18], any external forces on the dimer will add to the forces generated by thermal energy and accelerate bond rupture.

Microtubule self-repair in response to external bending forces

Hydrodynamic flow can be used to apply controlled forces on microtubules [19]. Surface micropatterning offers the possibility to anchor one end of the microtubule, while the rest of the microtubule can fluctuate freely above a nonadhesive surface [20]. The combination of the two methods allows cycles of bending forces to be applied on multiple microtubules in parallel [21]. In a regime of low forces and small strains, an microtubule can be considered elastic if it recovers its initial shape after the bending cycle (Figure 3a). This complete recovery assumes that the structure of the microtubule has not been altered in any way by the

deformation. However, repeated bending cycles induces ever increasing deformation on the microtubule, showing that the microtubule becomes softer after each bending cycle [21] (Figure 3b). By contrast, microtubules with low number of defects [6], appear to remain elastic and not display any softening in response to bending forces [21]. In metals and other crystals, the development of inelasticity and material fatigue are well known to result from point defects [22].

In microtubules, structural defects are likely to contribute to the local loss of dimers and to the softening of the microtubule. However, a 10 min incubation period with soluble tubulin dimers is sufficient for lattice repair and for the microtubule to recover its stiffness in the regions of the microtubules that have experienced the maximal deformation [21] (Figure 3b). It is notable that in those experiments, the lattice repair process was performed when the microtubules were straight and in their rest conformation. It would be interesting to test whether the repair process in an microtubule subjected to mechanical force would stabilize the bent conformation (Figure 3c). This is because in cells, microtubules are generally bent or curved. Hence, in cells, to what extent is the curvature of microtubules due to stress or due to repair processes stabilizing those deformations? It should also be noted that the microtubule curvature in those in vitro experiments was much lower than that observed in cells, where the activation of depolymerization and lattice repair mechanisms would be expected to be more frequent. The exact relationship between curvature and dimer incorporation needs further investigation. It remains unclear whether dimer incorporation occurs at the interior or exterior surface of the microtubule, and whether the incorporation of a new GTP dimer is dependent on the recruitment of specific proteins in curved regions [23,24]. If other proteins are recruited then do they have some affinities for specific parts of the tubulin that become exposed because of damage or contortion in the regular lattice (as with the exposure of tubulin surfaces at the microtubule tip [25])?

Motors and severing enzymes damage and thereby catalyse the self-repair process

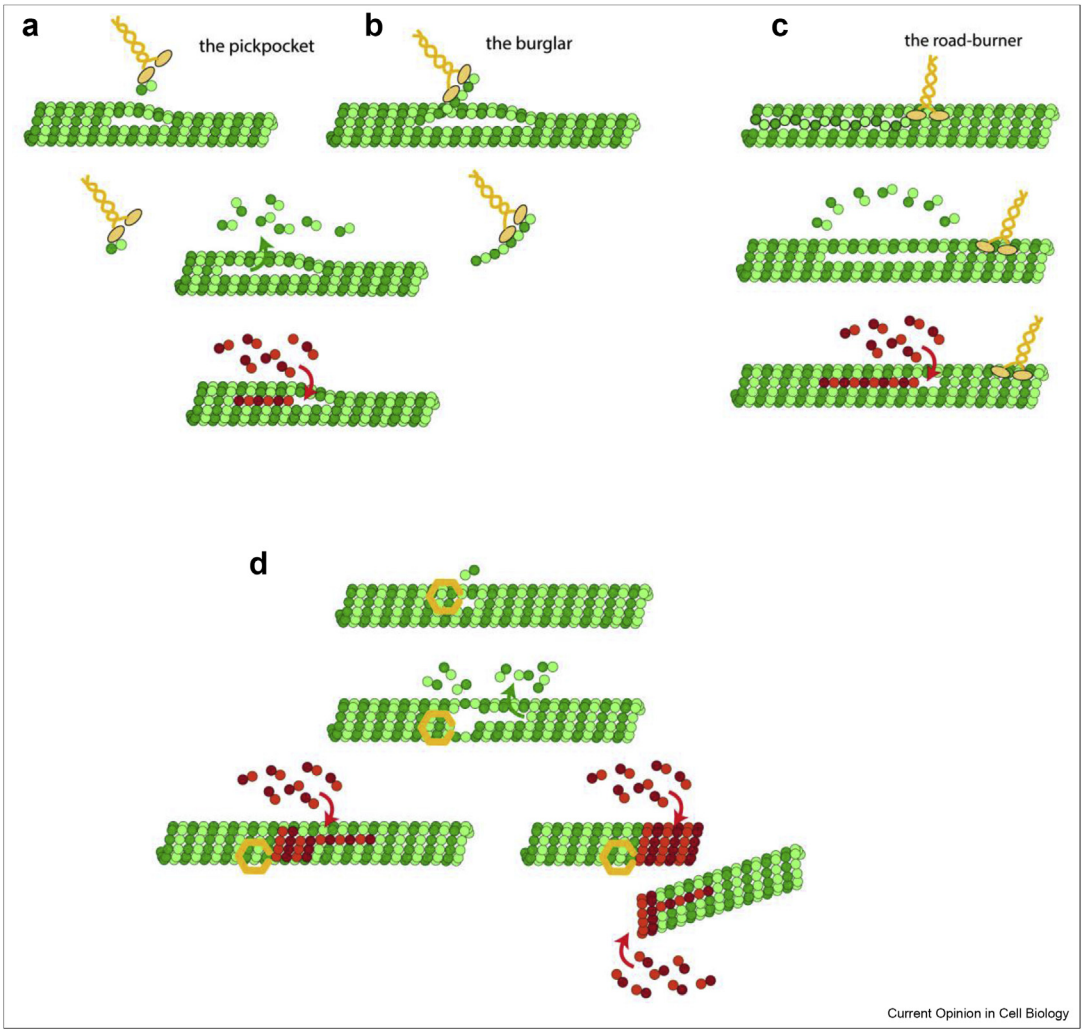
MAPs and molecular motors continually bind and unbind from microtubules. Hence, in forming or breaking bonds with tubulin dimers, they can promote or hinder the processes of disassembly and lattice repair.

In the absence of ATP, nonmotile motors can stretch the lattice upon binding tubulin [26]. In the presence of ATP and coated on the surface of a glass slide (ie 'gliding assays'), the mechanical work generated by these motors is sufficient to split apart the protofilaments of taxol-stabilized microtubules [27], and to disassemble nonstabilized microtubules, as they are translocated by the motors [28]. Conversely, with motors interacting with surface-bound microtubules (ie 'motility assays'), the microtubule can be disassembled within minutes after the addition of ATP [28]. Motors are capable of pulling out dimers from the lattice, leading to the propagation of dimer release and complete microtubule disassembly. Interestingly, free dimers can repair the lattice concomitantly and protect the microtubule from catastrophic disassembly [28]. Consequently, the frequency of repair sites along an microtubule increases with time to reach one every 10–20 microns after 30 min of incubation with motors and ATP [28].

The mechanism by which motors breakdown the microtubule lattice is not yet known. Can a motor remove a dimer anywhere on the lattice or only at sites with a structural defect? Defects are likely to be preferential sites. Motor-bound cargos travelling along microtubules regularly pause or come off the microtubule at the same location, suggesting that motors are actually sensitive to some signals from the lattice [29]. Furthermore, the higher the number of engineered defects on the microtubule lattice, the shorter the travel distance travelled by the motor-bound cargo [29,30]. However, there is no direct evidence that motors come off precisely at the site of the lattice defect. It is likely that the disassembly mechanism originating from within the lattice shares some similarities with motor-induced disassembly at the microtubule ends [31]. It has been shown that motors can only depolymerize stabilized microtubule from their ends. Therefore, it is plausible that motors could disassemble nonstabilized microtubules anywhere along the microtubule, and in particular, at sites containing structural defects, such as where some protofilaments end. Other questions remain: do motors come off the microtubule with the displaced tubulin attached, as has been shown for kinesin-8 [32,33] (Figure 4a)? Does the motor open the lattice by curling out a protofilament, as has been shown for kinesin-13 [34,35] (Figure 4b)? Is part of the energy from ATP hydrolysis that is not used to move the motor, transferred to the lattice thereby weakening the lattice and promoting its disassembly (Figure 4c)?

Severing enzymes also catalyse microtubule lattice breakdown and lattice repair. Katanin and spastin hydrolyse ATP in changing the conformation of the lattice and in removing dimers [36,37], and therefore catalyses lattice repair [38] (Figure 4d). In the presence of free dimers, the newly generated protofilament plus-ends do not depolymerize but are stabilized by the newly added GTP-bound dimers, which promote shaft elongation. Consequently, the activity of these severing enzymes increase rather than reduce the total number and length of microtubules [38].

Figure 4



Motors and severing enzymes damage the microtubule and thereby catalyse the self-repair process. These schematics illustrate some speculations about possible mechanisms involved in microtubule damage and self-repair induced by a molecular motor moving along at, as described in a study by Triclin et al. [28] (**a, b,c**); and by severing enzymes, as described in a study by Vemu et al. [38]. (**d**) Polymerized tubulins are green and free dimers involved in the repair are red.

Why taxol-treated microtubules are not broken down by motors remains unclear [28]. It may be due to taxol reinforcing the links between dimers in the lattice, and thus preventing the occurrence of damages, or to the stabilisation of links around sites of lattice damage. In any case, the effect of lattice stabilization by taxol suggests that a similar function could be performed by MAPs. Because MAPs and motors recruit and influence each other [39,40], it is plausible that these factors compete or synergize to promote or temper lattice destruction and self-repair [12]. As discussed in the following context, such mechanisms could further impact microtubule stability and the polarization of intracellular transport.

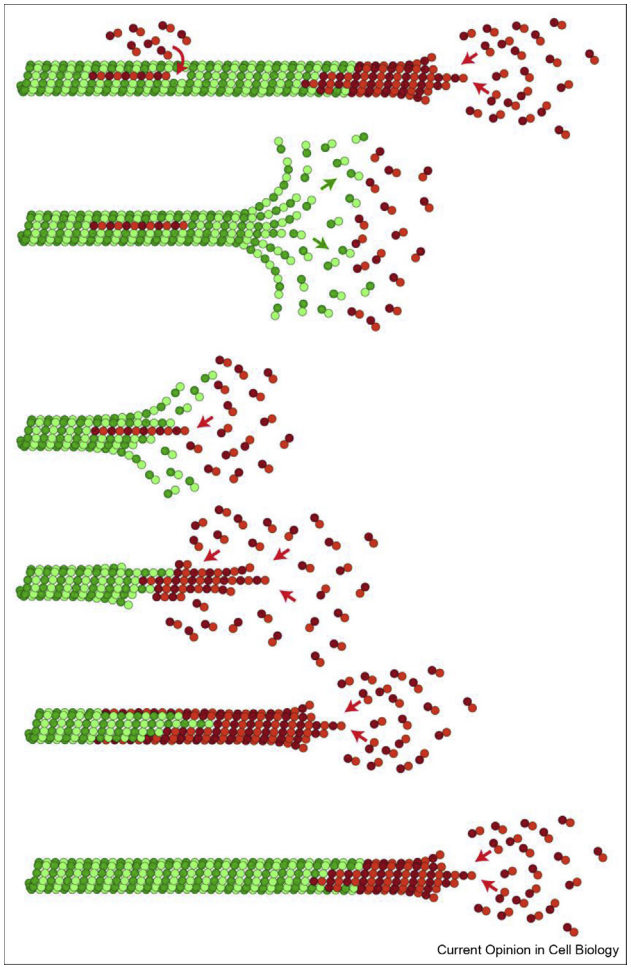
Plus-end-tracking proteins are recruited to damaged sites where they boost the self-repair process

In many respects, the openings in the microtubule lattice are similar to microtubule plus-ends because of the presence of free protofilament ends and freshly incorporated GTP dimers. As is the case for microtubule plus-end elongation, the lattice repair process does not require the contribution of any other protein apart from tubulin dimers, but lattice repair can be accelerated and regulated by plus-end-tracking proteins. Clip170 is recruited at the site of damage when two microtubules cross each other or when a microtubule is pressed against a nanoengineered barrier [41]. EB1 is recruited

at sites of damage generated by spastin or katanin [38]. Lattice repair can also be studied by using laser irradiation to create sites of damage (Figure 2c). Using this method, EB3 [21] and CLASP2 [42] are rapidly recruited to the irradiated site. Even in the case of a major damage, CLASP2 can stabilize the damaged lattice and prevent lattice disassembly [42]. As described previously, sites of damage can also occur at existing sites containing structural defects in microtubules bent by an external force, due to the concentration of mechanical stress. In such bent microtubules, CLASP can protect the structural defects from damage and prevent force-induced softening [42].

An alternative method to study lattice damage is to grow microtubules in the presence of taxol and then wash out free tubulin [43]: taxol increases the frequency of structural defects, and those defects are expanded by removal of free tubulin [14]. At the site of damage, CLASP improves the spontaneous recruitment of free tubulin and promotes the complete repair of the lattice [42]. From the data obtained in controlled biochemical conditions with purified and selected proteins [42], it is plausible that in the cytoplasm, the entire plus-end machinery [4] would be recruited to the sites of damaged lattice. Because a site of repair would thus become a plus-end–like complex in the middle of the microtubule, it is also plausible that the site of repair could initiate microtubule branching. In line with this view, it would be interesting to test whether lattice damaged sites can also recruit microtubule branching proteins such as augmin [44,45] or SSNA1 [46].

Figure 5



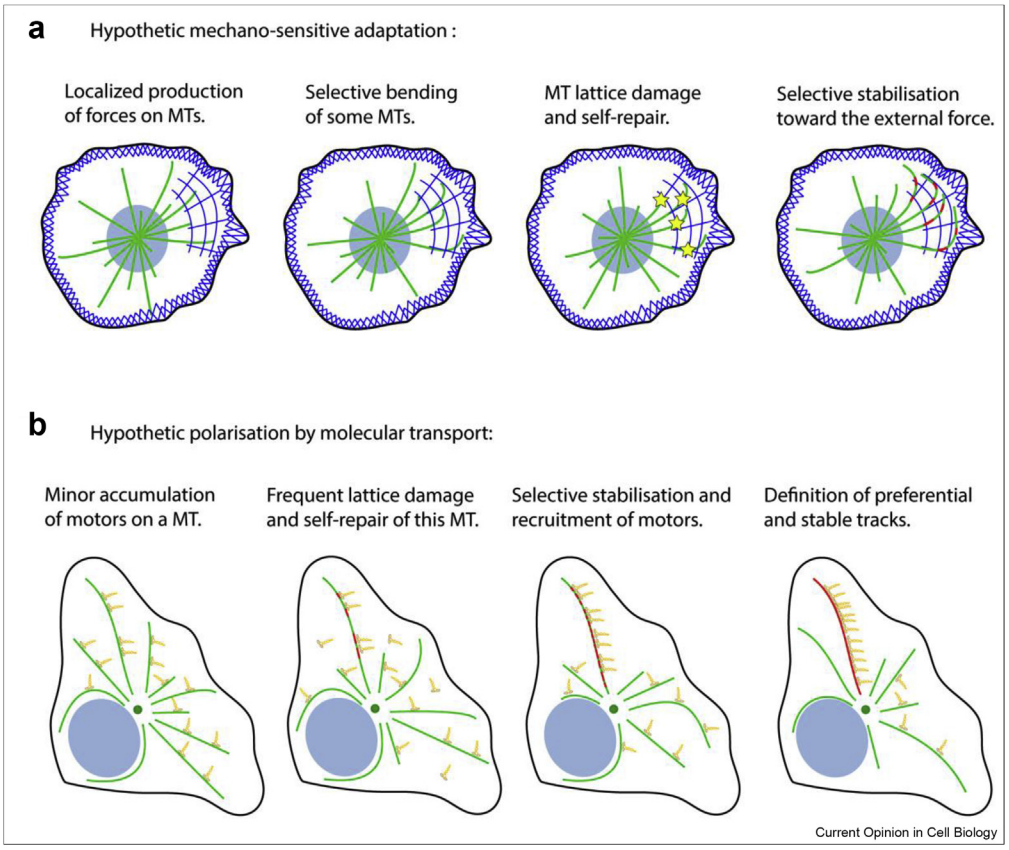
Repair sites promote rescue events and increase microtubule lifespan. These schematics illustrate our interpretation of the mechanism by which lattice self-repair sites block catastrophic depolymerisation of the microtubule and rescue its regrowth, as described in a study by Aumeier et al. [47]. Polymerized tubulins are green and free dimers involved in the repair are red.

Repair sites promote microtubule rescue events and increase microtubule lifespan

The identification of repair sites is challenging and their location is difficult to predict. Laser irradiation is an artefactual way to damage the lattice, but it can induces a genuine self-repair process [42]. It is thus a convenient method to control the location of self-repair sites and study the effect of damage and repair on microtubule network dynamics and architecture (Figure 2c). Laser irradiation of isolated microtubules in vitro and of microtubules in living cells induces local rescue events; i.e. at the irradiated sites, microtubule disassembly is arrested and microtubule-growth resumed [47]. This effect is likely due to the incorporation of new GTP-bound dimers in the lattice, which form straighter and strongly interacting protofilaments that are capable of resisting lattice disassembly and promoting further nucleation and elongation of new protofilaments (Figure 5). Indeed, GTP islands in the GDP lattice are potent rescue sites [48] and are likely to be sites of self-repair. The rescue potential of the GTP islands is limited in time suggesting that the hydrolysis of GTP removes the potential. Accordingly, the rescue potential can be extended in time by repairing laser-induced damage with nonhydrolysable GTP tubulin, or by creating GTP islands in engineered microtubules the sequential addition of hydrolysable GTP tubulin to microtubules comprising nonhydrolysable GTP tubulin dimers [47,49]. The location of damage and repair sites can also be generated by centrifugation of multiple microtubules, whereby the points at which microtubules are pressed together, also mark points of potential damage. At the sites of damage, new GTP tubulin is recruited where it promotes MT rescue [41].

As with the process of microtubule repair, the process of microtubule rescue can also be regulated and augmented by the recruitment of MAPs, and in particular, by plus-end-tracking proteins. Clip-170 is a strong promoter of microtubule rescue in living cells [4,50].

Figure 6



Possible implications in cell physiology. These schematics illustrate speculations about the involvement of microtubule self-repair in microtubule mechanosensation (**a**) and the organization of intracellular transport (**b**). Prepolymerized microtubules are green and repair sites are red. In (a) repair sites allow microtubules to be protected from catastrophic microtubule depolymerization and promote further growth of the microtubule. In (b), repair sites allow microtubules to be protected from catastrophic microtubule depolymerization and promote the recruitment of more motors.

Clip-170 is recruited at microtubule intersections in living cells, where microtubule rescue events are frequent, suggesting that repair-induced rescue events can be promoted by Clip-170 [41]. Clip-associated proteins, CLASPs, also promote microtubule rescue by recruiting new tubulin dimers for regrowth [51]. In particular, CLASPs boost the recruitment of new dimers to the repair site [42].

The consequence of the self-repair–induced rescue is remarkable and appears to follow Friedrich Nietzsche's maxim 'was mich nicht umbringt macht mich stärker': the microtubule that gets damaged also gets rescued, and thus survives longer than an microtubule that is not damaged about which we will speculate later. Hence, repeated laser ablations on microtubules in living cells can lead to local overstabilization of microtubules and growth of the microtubule network and can effect cell functions such as migration [47]. Thus, self-repair–induced rescue can play a key role in the polarization of the microtubule network.

Self-repair in living cells

As acknowledged earlier, laser-induced damage to a microtubule should be considered as an experimental artefact, so it is important to know whether microtubule self-repair naturally happens in living cells. The difficulty is that there is currently no fully reliable way of identifying repair sites in living cells.

EMB11 is an antibody that has been raised against GTP tubulin, so it can potentially detect all plus-ends and all repair sites [48]. However EMB11 localization revealed numerous spots in cells that were not microtubule plus-ends, raising some doubts about the specificity of the antibody [48]. In vitro, many EMB11-positive dots on isolated microtubules were not associated with the repair process. This showed that the antibody does not recognize the presence of GTP itself, because GTP is present on all freshly incorporated dimers at the repair sites [41]. Many EMB11-positive dots were located at microtubule–microtubule intersections, suggesting that the antibody might detect a particular non-regular

lattice conformation, including the bent conformation of tubulin dimers at the microtubule growing tip [52,53].

The incorporation of soluble tubulin into a pre-existing microtubule in a living cell can be visualized by photoconversion of a fluorescent probe attached to the tubulin molecules [47]. This method can reveal the incorporation of new dimers at sites of laser damage and the occurrence of rescue events at these sites. To observe the incorporation of dimers in pre-existing microtubules in the absence of externally induced damage is more challenging because microtubules often overlap, and sites of potential damage are difficult to distinguish from growing ends. Although the compelling evidence that microtubules can undergo spontaneous self-repair in living cells is still missing, dimer incorporation could be detected in isolated microtubules using this methodology.

Possible implications for cell physiology

The self-repair process, by inducing the incorporation of GTP dimers in a GDP lattice, provides a different path for regulating key functions of the microtubule network. In this section, we speculate about two functions that appear particularly exciting: mechanosensation and cell polarization.

The association of the two processes of force-induced damage and subsequent repair can be considered as conferring a mechanoresponsive property to the microtubule network, enabling the network to grow towards regions where it is mechanically stimulated. Hydrodynamic bending forces in a microfluidic device can damage imperfect microtubule lattices; and the repair of the sites of damage can rescue microtubule disassembly and can extend the lifespan of the microtubule. In cells, bending forces can be produced by the contraction and retrograde flow of the actin network [54]. A regionalized flow of actin in polarized or migrating cells can stimulate a subset of microtubules, which get selectively bent, damaged and repaired. These microtubules then become stabilized and protected by rescue events, so they can extend further than microtubules that have not been subjected to damage and repair cycles (Figure 6a). Indeed, microtubules switch often between states of growth, rescue and quiescence in regions of intense actin-based constraints [54,55]; a behaviour that has been attributed to the presence of proteins associated with the actin network [56–58] but may actually be intrinsic to microtubule dynamics thanks to their self-repair properties.

As a molecular motor moves along a microtubule, the lattice structure is damaged. This damage can lead to microtubule self-repair and increase of lifespan of the microtubule hence favours further transport on that microtubule. In addition, some kinesins are preferentially recruited on GTP-rich microtubules [59]. Therefore, the incorporation of GTP tubulin during the repair process may also increase the local recruitment of additional motors. These two positive feedback loops can break the symmetry of the microtubule network by stabilizing preferential routes for intracellular trafficking. This concept of positive feedback of intracellular trafficking on motor recruitment has already been proposed to occur in response to the conformational change that motors induce in the lattice [60]. It is also clear that the microtubule network is heterogeneous and kinesins use preferential tracks [61–63]. Here, we propose that it is the processes of damage and repair that stabilize and select microtubules for intracellular trafficking (Figure 6b).

Conclusion

Recent research is beginning to shine some light on the origin and mechanisms supporting microtubule self-repair. The incorporation of new dimers in the lattice of the microtubule at sites distant from the microtubule ends appears to play important roles in the regulation of microtubule mechanics and dynamics. Investigations are needed at the molecular level, using cryo-electronic microscopy and atomic force microscopy, to further understand the exact relationship between the lattice structure and the insertion of new dimers. In living cells, the existence of self-repair remains to be fully established, and this will be aided by the development of appropriate markers of this process. How the dynamics of dimer incorporation in the lattice compare with those at microtubule ends will help assess their relative contribution to the dynamics in the microtubule network. We anticipate that these studies will explain established microtubule phenomena, such as biased dynamic instability at the cell front, and reveal some novel phenomena-like microtubule mechanosensation. The investigation of tubulin dynamics at the ends of the microtubule has been exciting, but has been preoccupied with 1% of its structure. The exploration of 99% of the rest of the microtubule opens up a new vista of excitement and may potentially reveal how the shaft of the microtubule is a genuine sensor that integrates information from throughout the cytoplasm.

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Conflict of interest statement

Nothing declared.

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