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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, and the potential implications for key functions of the microtubule network, such as intra-cellular transport and cell polarization.

Introduction

A microtubule (MT) 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 MT align in accordance with this polarity, resulting in the MT 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 MTs (1). Because of the similarity with the regular and periodic structure of metals, the structural arrangement of the MT wall is described as a lattice. This lattice-like structure of the MT wall supported the idea that the addition or loss of tubulin dimers only occurs at the MT 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 re-incorporation at a distance from the MT ends (5). The initial observations came from the in vitro analysis of MTs, where dense arrays of MTs were formed by the addition of tubulin dimers to MT nucleation templates (i.e. axonemal fragments isolated from the tails of sea-urchin sperm). MT disassembly in these dense arrays was triggered by washing out soluble dimers. However, some MTs 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 MTs displayed kinks or hyperflexible sections in their lattice, which later broke and led to complete MTs disassembly (Figure 1A, iii). This process was inhibited by the reintroduction of soluble dimers, with deformed MTs recovering their initial straight shape (Figure 1A, iv). In recently repeating these experiments with a slightly different protocol, we observed the same MT weakening and disassembly upon soluble dimer washout, and the same process of repair (6). MTs were stabilized by the addition of tubulin caps (tubulin bound to non-hydrolysable GTP) before and after MT growth (Figure 2A, i-ii), and the tubulin dimers were labelled green the MT-growth phase and red in the MT-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 MT ends.

Given this MT-repair process is hidden in typical experimental set ups, it has been generally overlooked for decades. Nevertheless, some several recent studies have shined some light on this MT-repair process, and in this opinion piece we will address how this process works; what are the conditions fostering or blocking it, how does it impact MT stability and dynamics; and how it can influence key MT 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 modeling of observed parameters associated with MT 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 unfavorable 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 (AFM) 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 MT), 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 MT and have been well documented with electronic microscopy (9)(10) and AFM (11). They are likely to stem from lateral interactions of growing protofilaments at the MT end (12). Defects can be observed in MTs assembled in vitro with pure tubulin, as well as in egg extract where tubulin, in addition to numerous proteins regulating MT 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 MT lattice can also be stabilized by the MT-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 favorable sites for dimer loss and re-addition, 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 favor 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 MT lattice in vivo, remains difficult to establish. The elongation of MT 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 MT defects and sites of renewal were similar to that observed with MTs generated from tubulin alone (6). Furthermore, MTs 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 studies are required to characterize in cells, subtle defects, such as the transition of protofilament numbers in a given MT. 3D Cryo-ET

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 non-covalent 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 MT, while the rest of the MT can fluctuate freely above a non-adhesive surface (20). The combination of the two methods allows cycles of bending forces to be applied on multiple MTs in parallel (21). In a regime of low forces and small strains, an MT can be considered elastic if it recovers its initial shape after the bending cycle (Figure 2A). This complete recovery assumes that the structure of the MT has not been altered in any way by the deformation. However, repeated bending cycles induces ever increasing deformation on the MT, showing that the MT becomes softer after each bending cycle (21) (Figure 2B). By contrast, MTs 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 MTs, structural defects are likely to contribute to the local loss of dimers and to the softening of the MT. However, a 10 minute incubation period with soluble tubulin dimers is sufficient for lattice repair and for the MT to recover its stiffness in the regions of the MTs that have experienced the maximal deformation (21) (Figure 2B). It is notable that in those experiments, the lattice repair process was performed when the MTs were straight and in their rest conformation. It would be interesting to test whether the repair process in an MT subjected to mechanical force would stabilize the bent conformation (Figure 2C). This is because in cells, MTs are generally bent or curved. Hence, in cells, to what extent is the curvature of MTs due to stress or due to repair processes stabilizing those deformations? It should also be noted that the MT 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 MT, 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 catalyze the self-repair process.

MAPs and molecular motors continually bind and unbind from MTs. 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, non-motile 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 MTs (27), and to disassemble non-stabilized MTs, as they are translocated by the motors (28). Conversely, with motors interacting with surface-bound MTs (ie “motility assays”), the MT 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 MT disassembly. Interestingly, free dimers can repair the lattice concomitantly and protect the MT from catastrophic disassembly (28). Consequently, the frequency of repair sites along an MT increases with time to reach one every 10-20 microns after 30 minutes of incubation with motors and ATP (28).

The mechanism by which motors break down the MT 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 MTs regularly pause or come off the MT 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 MT 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 MT ends (31). It has been shown that motors can only depolymerize stabilized MT from their ends. Therefore, it is plausible that motors could disassemble non-stabilized MTs anywhere along the MT, and in particular, at sites containing structural defects, such as where some protofilaments end. Other questions remain: do motors come off the MT 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 catalyze MT-lattice breakdown and lattice repair. Katanin and spastin hydrolyze ATP in changing the conformation of the lattice and in removing dimers (36)(37), and therefore catalyzes 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 MTs (38).

Why taxol-treated MTs 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 below, such mechanisms could further impact MT stability and the polarization of intra-cellular transport.

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

In many respects, the openings in the MT lattice are similar to MT plus ends because of the presence of free protofilament ends and freshly incorporated GTP-dimers. As is the case for MT 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 MTs cross each other or when a MT is pressed against a nano-engineered 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 above, sites of damage can also occur at existing sites containing structural defects in MTs 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 MTs 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 MT, it is also plausible that the site of repair could initiate MT branching. In line with this view, it would be interesting to test whether lattice damaged sites can also recruit microtubule branching proteins like augmin (44, 45) or SSNA1 (46).

Repair sites promote MT-rescue events and increase MT 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 induce 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 MT-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, MT disassembly is arrested and MT 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 non-hydrolysable-GTP tubulin, or by creating GTP-islands in engineered MTs the sequential addition of hydrolysable-GTP tubulin to MTs comprising non-hydrolysable-GTP tubulin dimers (47, 49). The location of damage and repair sites can also be generated by centrifugation of multiple MTs, whereby the points at which MTs 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 MT repair, the process of MT 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 MT rescue in living cells (4, 50). Clip-170 is recruited at MT intersections in living cells, where MT rescue events are frequent, suggesting that repair-induced rescue events can be promoted by Clip-170 (41). Clip-associated proteins, CLASPs, also promote MT 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 MT that gets damaged also gets rescued, and thus survives longer than an MT that is not damaged, about which we will speculate later. Hence, repeated laser ablations on MTs in living cells can lead to local overstabilization of MTs and growth of the MT 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 MT should be considered as an experimental artefact, so it is important to know whether MT 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 MT plus-ends, raising some doubts about the specificity of the antibody (48). In vitro, many EMB11-positive dots on isolated MTs 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 MT-MT intersections, suggesting that the antibody might detect a particular non-regular lattice conformation, including the bent conformation of tubulin dimers at the MT growing tip (52, 53).

The incorporation of soluble tubulin into a pre-existing MT in a living cell can be visualized by photo-conversion 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 MTs in the absence of externally induced damage is more challenging, because MTs often overlap, and sites of potential damage are difficult to distinguish from growing ends. Although the compelling evidence that MTs 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 mechano-responsive 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 MT lattices; and the repair of the sites of damage can rescue MT disassembly and can extend the lifespan of the MT. 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 sub-set of MTs, which get selectively bent, damaged and repaired. These MTs then become stabilized and protected by rescue events, so they can extend further than MTs that have not been subjected to damage and repair cycles (Figure 6A). Indeed, MTs switch often between states of growth, rescue and quiescence in regions of intense actin-based constraints (54, 55); a behavior that has been attributed to the presence of proteins associated with the actin network (56–58) but may actually be intrinsic to MT dynamics thanks to their self-repair properties.

As a molecular motor moves along a MT, the lattice structure is damaged. This damage can lead to MT self-repair and increase of lifespan of the MT hence favors further transport on that MT. In addition, some kinesins are preferentially recruited on GTP-rich MTs (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 MT 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 MT 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 MTs for intracellular trafficking (Figure 6B).

Conclusion

Recent research is beginning to shine some light on the origin and mechanisms supporting MT self-repair. The incorporation of new dimers in the lattice of the MT at sites distant from the MT ends appears to play important roles in the regulation of MT 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 MT ends will help assess their relative contribution to the dynamics in the MT network. We anticipate that these studies will explain established MT phenomena, such as biased dynamic instability at the cell front, and reveal some novel phenomena like MT mechano-sensation. The investigation of tubulin dynamics at the ends of the MT has been exciting, but has been pre-occupied with 1% of its structure. The exploration of 99% of the rest of the MT opens up a new vista of excitement, and may potentially reveal how the shaft of the MT is a genuine sensor that integrates information from throughout the cytoplasm.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure 1

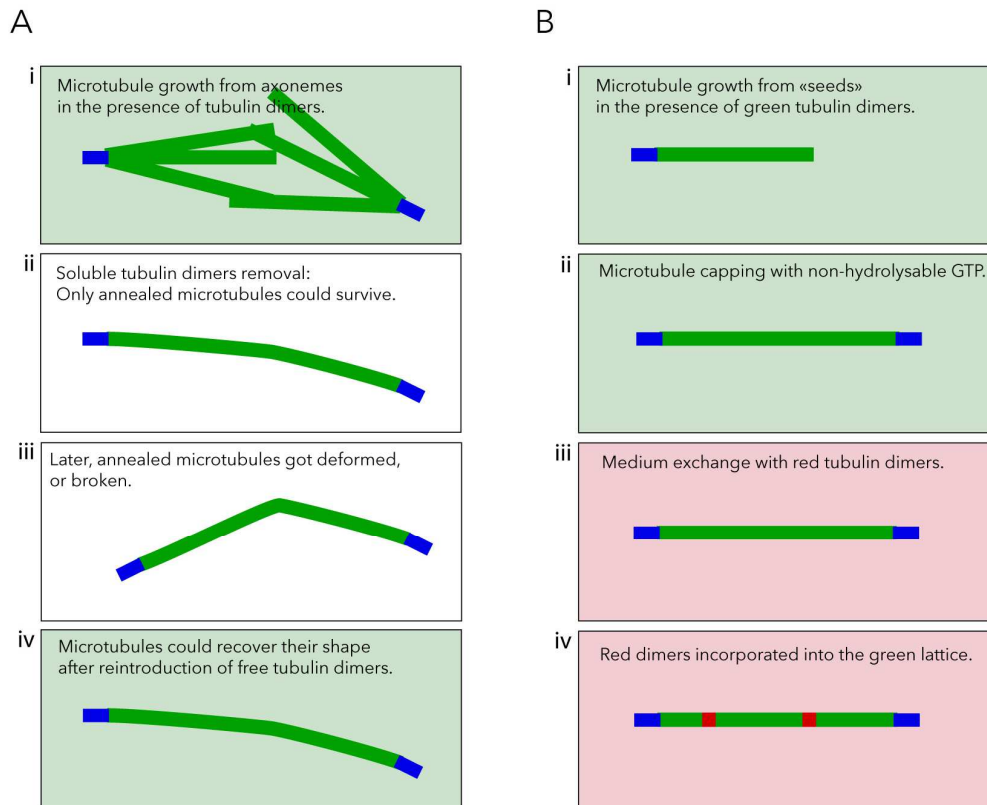


Figure 1 – MT self-repair.

These schematics illustrate the damage and self-repair of MTs described in ref (5) (A) and ref (6) (B). MTs are green, caps are blue and repair sites are red.

Figure 2

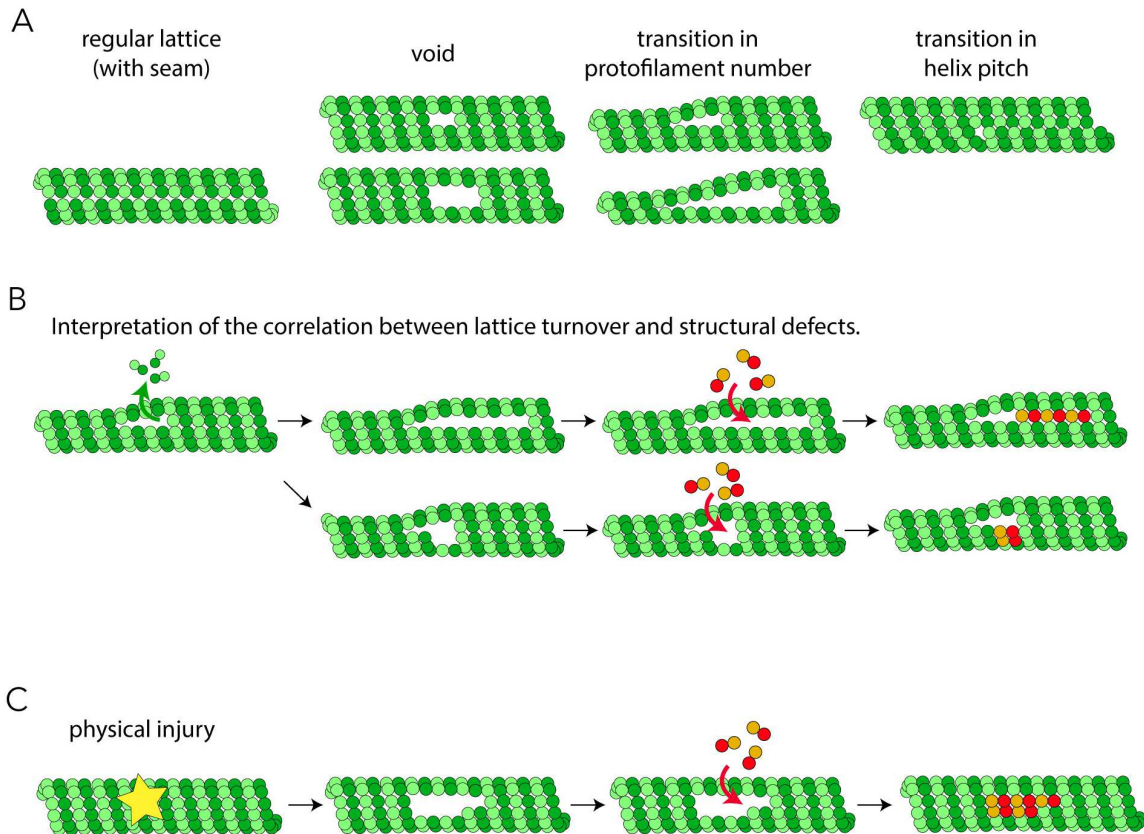


Figure 2 – Spontaneous self-renewal at MT lattice defects.

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 ref (6) (B). The repair in response to laser-induced damage described in ref (21) (C). Polymerized tubulins are green and free dimers involved in the repair are red.

Figure 3

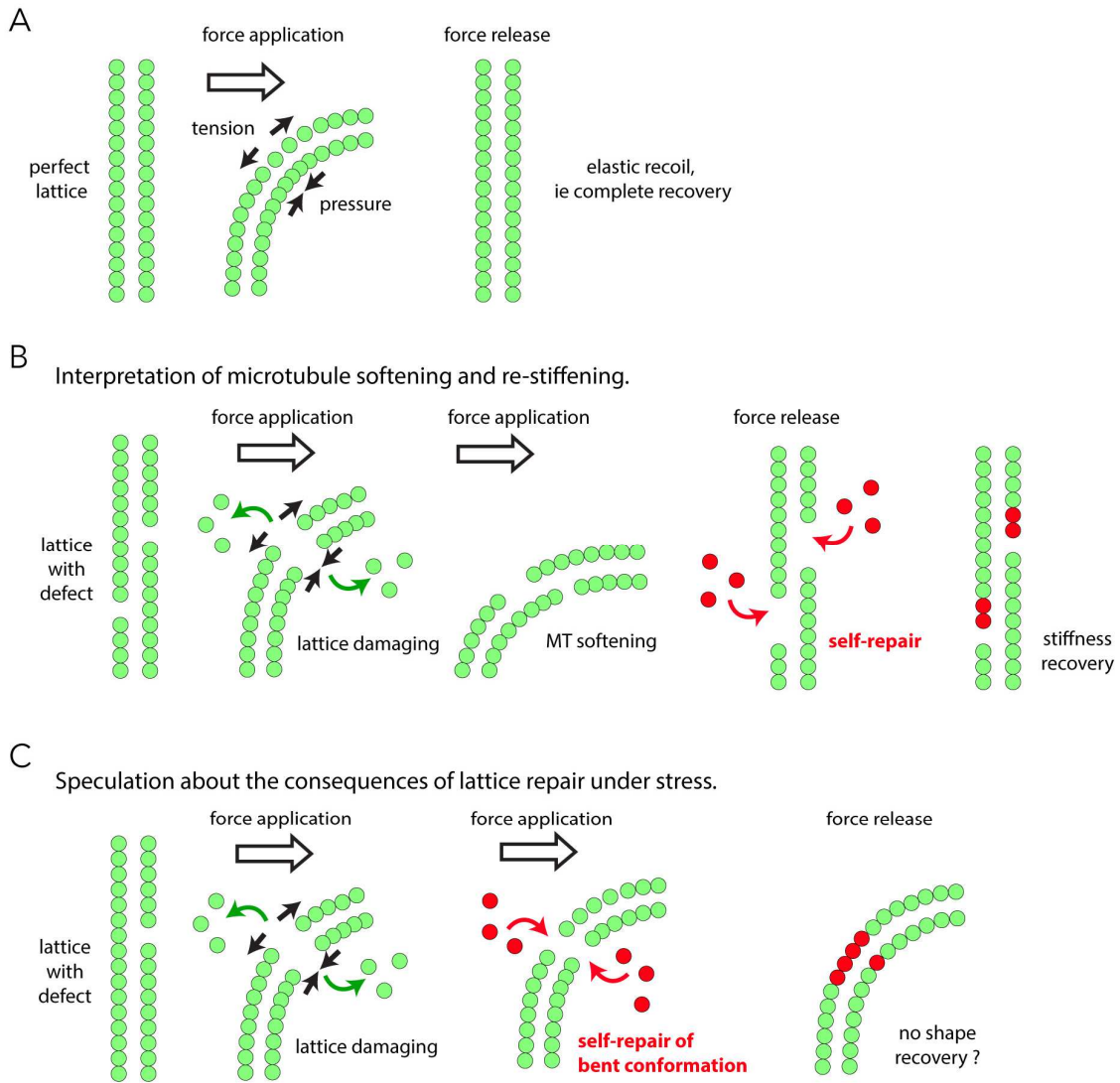


Figure 3 – Microtubule self-repair in response to external bending forces. These schematics illustrate the elastic bending of a regular MT lattice (A). The inelastic bending cycle of a lattice containing a defect, and the interpretation of its relationship to the self-repair process described in ref (21) (B). A speculation about the consequence of MT self-repair in bent rather than straight conformation. Polymerized tubulins are green and free dimers involved in the repair are red.

Figure 4

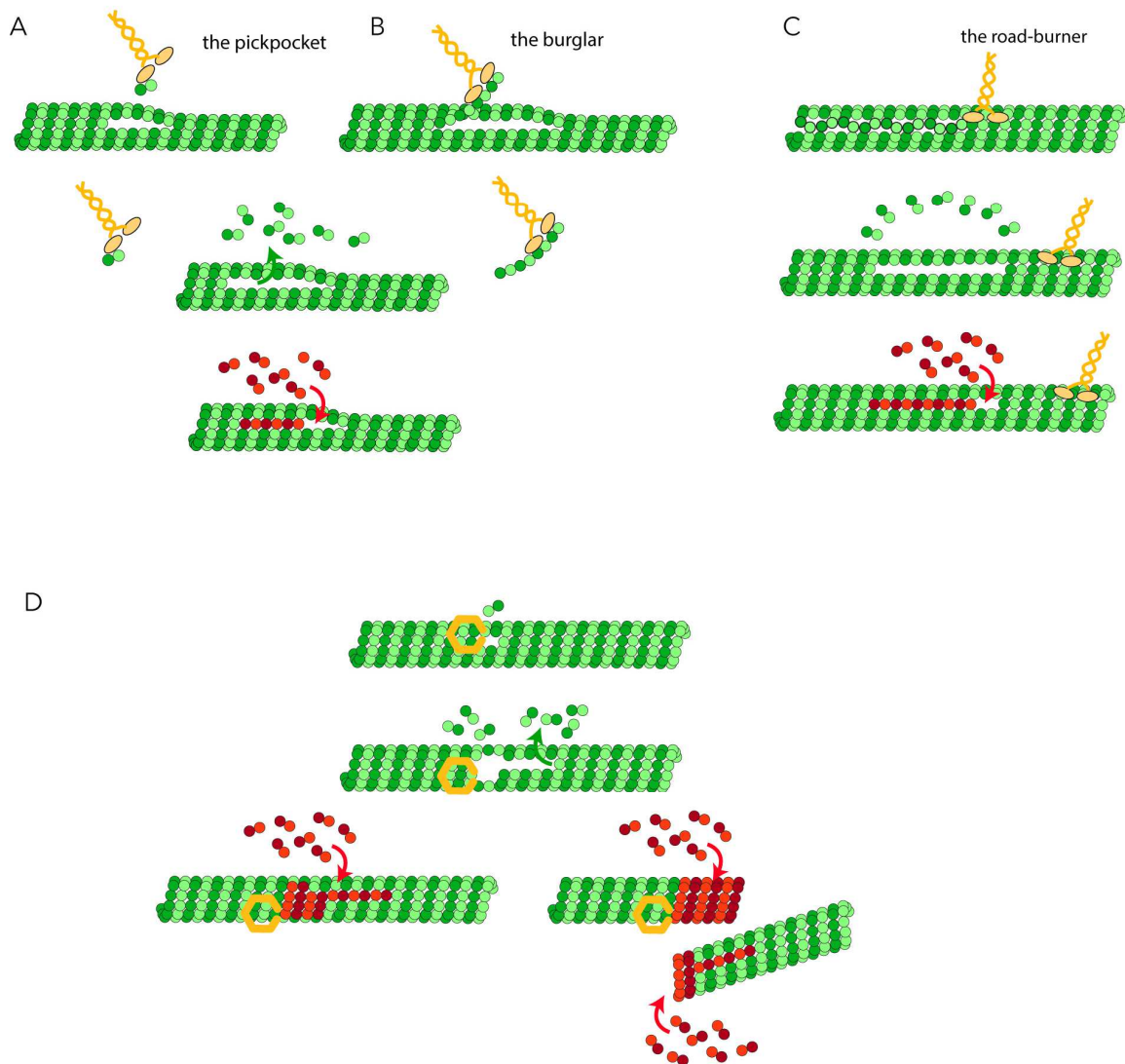


Figure 4 – Motors and severing enzymes damage the MT and thereby catalyse the self-repair process.

These schematics illustrate some speculations about possible mechanisms involved in MT damage and self-repair induced by a molecular motor moving along at, as described in (28) (A, B, C); and by severing enzymes, as described in (38) (D). Polymerized tubulins are green and free dimers involved in the repair are red.

Figure 5

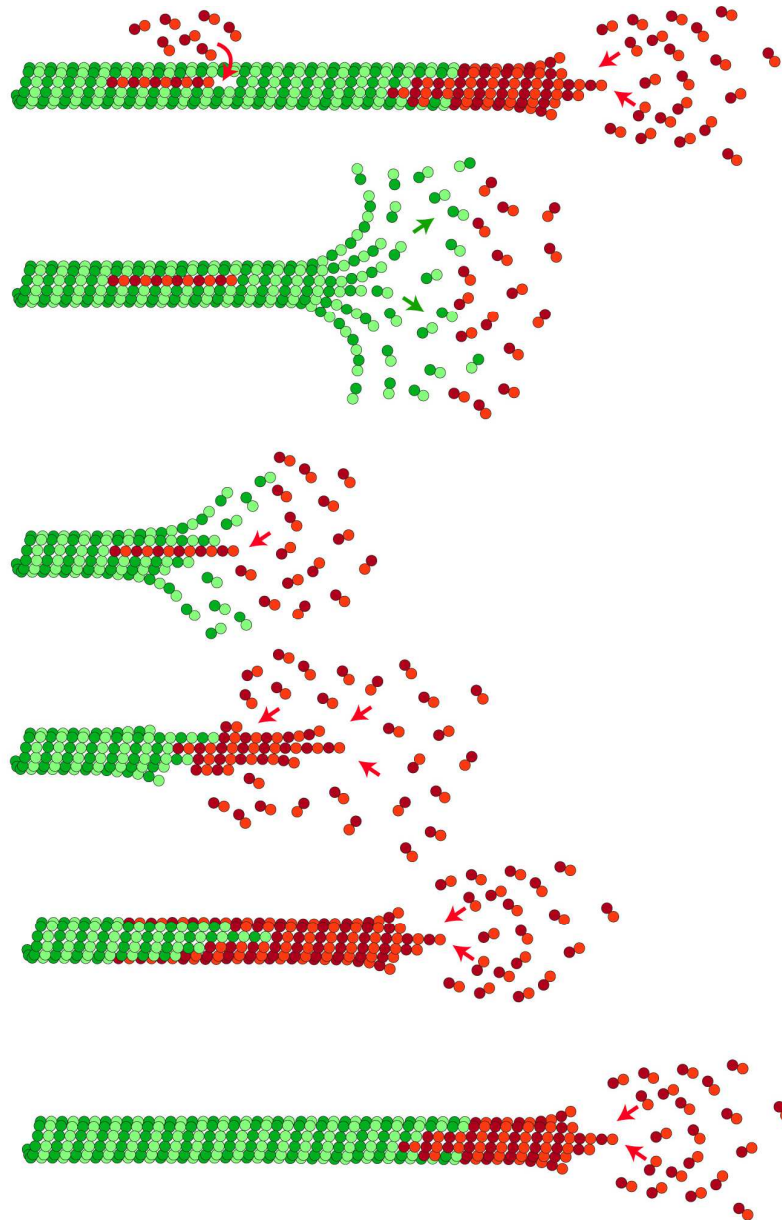


Figure 5 – Repair sites promote rescue events and increase MT lifespan. These schematics illustrate our interpretation of the mechanism by which lattice self-repair sites block catastrophic depolymerisation of the MT and rescue its regrowth, as described in (47). Polymerized tubulins are green and free dimers involved in the repair are red.

Figure 6

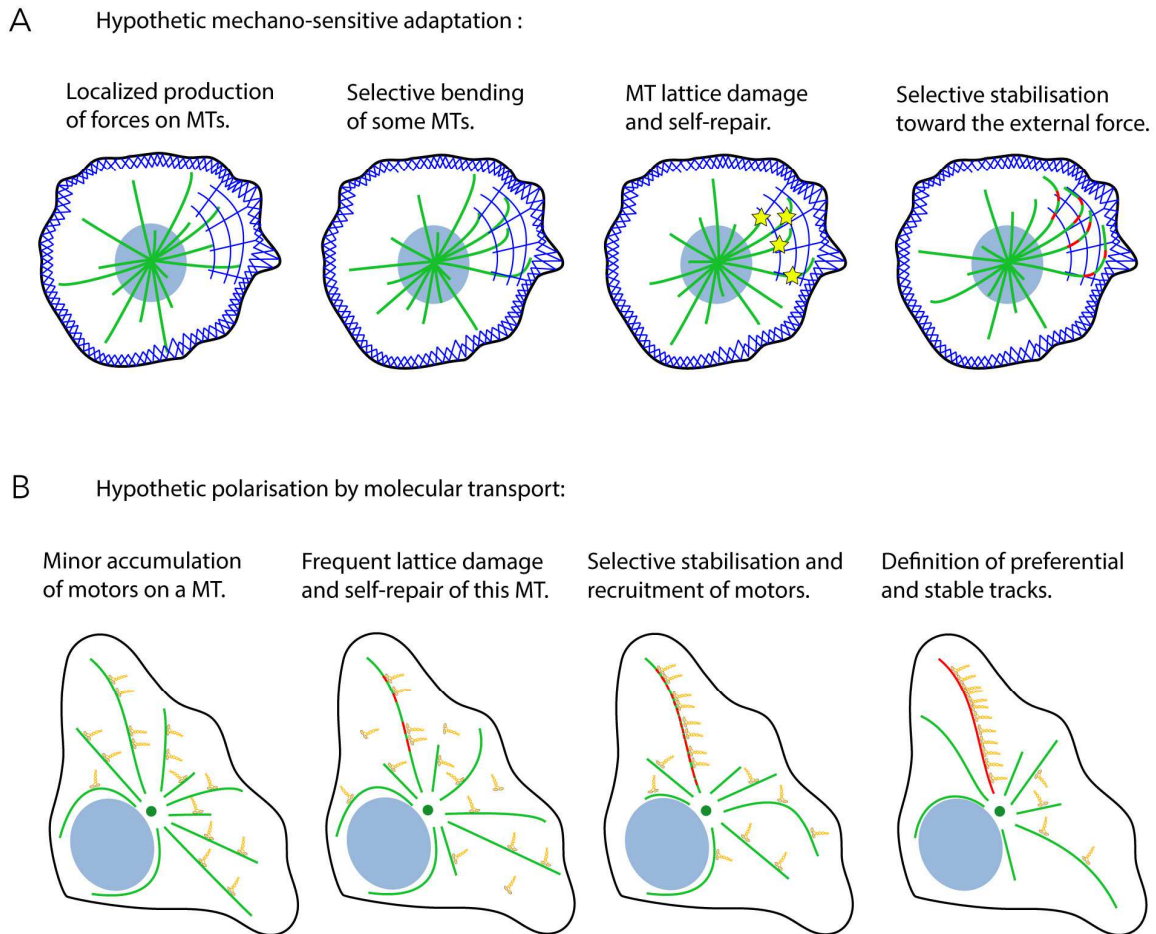


Figure 6 – Possible implications in cell physiology.

These schematics illustrate speculations about the involvement of MT self-repair in MT mechano-sensation (A) and the organization of intra-cellular transport (B). Pre-polymerized MTs are green and repair sites are red. In (A) repair sites allow MTs to be protected from catastrophic MT depolymerization, and promote further growth of the MT. In (B), repair sites allow MTs to be protected from catastrophic MT depolymerization, and promote the recruitment of more motors.