Self-repair promotes microtubule rescue

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The dynamic instability of microtubules is characterized by slow growth phases stochastically interrupted by rapid depolymerizations called catastrophes. Rescue events can arrest the depolymerization and restore microtubule elongation. However, the origin of these rescue events remains unexplained. Here we show that microtubule lattice self-repair, in structurally damaged sites, is responsible for the rescue of microtubule growth. Tubulin photo-conversion in cells revealed that free tubulin dimers can incorporate along the shafts of microtubules, especially in regions where microtubules cross each other, form bundles or become bent due to mechanical constraints. These incorporation sites appeared to act as effective rescue sites ensuring microtubule rejuvenation. By securing damaged microtubule growth, the self-repair process supports a mechanosensitive growth by specifically promoting microtubule assembly in regions where they are subjected to physical constraints.

Oriented growth of the microtubule network is a key process in the establishment of cell polarity1. The asymmetry of microtubule-network organization exists in various forms and usually involves differences in the protection of microtubules against depolymerization. Examples include the selective stabilization and orientation of microtubules during neuronal axon determination2–4; the selective stabilization of microtubules toward the leading edge of migrating cells5–7 or the basal pole of epithelial cells8; the selective bundling of microtubules toward the immune synapse9; and the stabilization of a specific subset of microtubules for spindle assembly and orientation10,11. Most attention has been paid to the role of components such as Par312, mDia/APC13, GSK314, IQGAP15 or Lis116 that promote microtubule capture and stabilization in specific subcellular regions containing defined actin-network compositions. In particular, at the leading edge of migrating cells, microtubules appear to be protected from disassembly that could potentially be induced by the physical barrier of the plasma membrane17. The rescuing of microtubules from depolymerization is frequent in such regions where actin retrograde flow is active5,6,18,19, and thus the rescue events could actively contribute to local differences in microtubule-network growth. However, the mechanisms controlling the occurrence and modulation of those rescue events are still poorly understood20.

The frequency of rescue events increases with the concentration of free tubulin21 and in the presence of MAP2, CLIP170 or CLASP22–24. Their locations appear to be correlated with the presence of short GTP-tubulin stretches along the microtubule shaft25,26, but the origin of these stretches is still debated25. Recently, in response to bending arising from mechanical perturbations, microtubules have been shown to be capable of self-repair by the incorporation of free tubulin dimers into fractured points of the microtubule lattice27. This process of self-repair generates stretches of new tubulin dimers along the microtubule length reminiscent of those GTP-tubulin stretches described previously25. It is therefore tempting to hypothesize that microtubule repair sites act as rescue sites. However, microtubule self-repair has not yet been described in vivo and the mechanism by which it could promote microtubule rescue, alone28 or with the help of microtubule-associated proteins23,24,29,30 (MAPs), still needs to be investigated.

RESULTS

Microtubule repair in living cells

The investigation of microtubule repair requires free tubulin dimers to be distinguishable from polymerized dimers so that their incorporation into the microtubule lattice can be detected. Local photoconversion of mEOS2-tubulin was used to convert some of the green-fluorescent dimers into red-fluorescent dimers (Fig. 1a; where red fluorescence is depicted as magenta). These red-fluorescent dimers rapidly diffused through the cell cytoplasm and became available for incorporation throughout the entire cell. A few minutes after photoconversion (Supplementary Video 1), spots of red-fluorescent tubulin could be observed at the growing tips of microtubules, as well as along the shafts of pre-existing microtubules (Fig. 1a-I and Supplementary Video 2). Microtubules often grow along pre-existing ones. Therefore,
Figure 1 Microtubule self-repair in living cells. (a) A PtK2 cell expressing mEos2 pre- and post-conversion. Converted free tubulin dimers (magenta) diffused through the cytoplasm. The signal of converted dimers (magenta) was observed at the growing tips as well as in spot-like structures along the length of pre-existing microtubules (green). (I–V) Enlarged regions of a according to the boxes in the upper right panel. (I) Bundled microtubules with photo-converted tubulin spots within the bundle (arrowhead) and at the growing tip (arrow). Image taken 1 min 35 s after photo-conversion. (II,III) Incorporation of converted dimers (magenta) in pre-existing microtubules (green) at microtubule crossing sites. Images taken respectively 6 min 38 s or 6 min 30 s after photo-conversion, respectively. (IV) Incorporation of converted dimers (magenta) in pre-existing microtubules (green) at bent sites. Image taken 6 min 38 s after photo-conversion. (V) Incorporation along pre-existing microtubules (arrowheads, 3 min after photo-conversion) with the corresponding kymograph. Bottom white arrowhead in (V) is represented by the first left arrowhead of the kymograph in (V - kymograph). (b) After photo-conversion the magenta tubulin signal was observed at growing microtubule tips (arrows). True incorporation was not distinguishable from growing tips within bundled microtubules (arrowheads). Arrows indicate growing microtubule tips after photo-conversion; arrowheads indicate sites of incorporation of converted tubulin dimers (magenta) in pre-existing microtubules. Images are representative of five independent experiments. Scale bars, 5 μm.

A local patch of red tubulin along a green microtubule shaft may correspond to a growing secondary microtubule rather than a site of red-tubulin incorporation into the green primary microtubule. However, in the former case, fluorescence intensity should increase over time along the original microtubule, concomitantly with the local incorporation of red tubulin at the growing tip. By observing microtubule fluorescence over several minutes before and after the appearance of red patches, we considered that no change in the fluorescence at the site of the initial red-fluorescent patch implied that tubulin was incorporated into the pre-existing lattice (Fig. 1a-V and V-kymograph). From this analysis, most of the tubulin-incorporation sites were located at microtubule crossovers (Fig. 1a-II, a-III) or in highly curved regions (Fig. 1a-IV). Interestingly, microtubule crossovers are known to recruit katanin\(^31,32\), which has a strong affinity for sites of damaged lattice\(^33,34\), suggesting that the incorporation sites were genuine repair sites. Incorporation sites were also frequently observed along microtubules forming bundles (Fig. 1a-I,1b), although in those cases, it was hard to confirm that the sites did not correspond to growing plus ends.

Microtubule rescue at repaired sites in living cells

When following microtubule dynamics next to the repair sites, we found that with a microtubule undergoing catastrophe, depolymerization was arrested and regrowth was initiated precisely at the location of the repair site (Fig. 2a and Supplementary Video 3). Unfortunately, it was experimentally challenging to repeat these observations in regions where the network was sufficiently sparse.
to follow single microtubules, and in regions where rescues were likely to occur. Alternatively, we decided to record the locations of microtubule rescue events near the cell margin where microtubules show a high rescue frequency and compare the locations of rescue sites with those of incorporation sites (Fig. 2e). We found that most rescues occurred close to the microtubule tip (Fig. 2d) at microtubule crossovers (60%) (Supplementary Video 4) or at curved regions (30%) (Fig. 2e). Strikingly, the relative frequencies of microtubule rescues with respect to three different types of microtubule structure (crossover, curved and straight) precisely matched the relative frequencies of incorporation sites (Fig. 2e).

**Microtubule rescue at laser-induced photodamaged sites**

To further validate this correlation at the single-microtubule level, and more directly, to test whether the repair of structural damage in the microtubule lattice was responsible for the rescue events, we selected a method to modulate microtubule damage and repair in space and time. This method used focused laser light, which was above the power required for bleaching but below the severing threshold, and can induce local damage and promote further self-repair of microtubules (Supplementary Fig. 1a). The method both ensured the genuine occurrence of lattice damage on targeted sites and allowed the monitoring of potential consequential changes in microtubule dynamics at the same sites. We first confirmed that laser-induced photodamage could trigger lattice self-repair by photo-converting mEOS2-tubulin and then focusing the laser on a straight and central section of the microtubule, more than 2 µm away from the microtubule end, where rescues were unlikely (Supplementary Fig. 1b). Photo-converted free tubulin was rapidly incorporated at the photodamaged sites, confirming the effectiveness of the laser to...
Figure 3 Photodamaged sites are repair and rescue sites in living cells. Yellow stars indicate photodamage sites; white arrows indicate sites of incorporation of photo-converted tubulin dimers (magenta). Yellow arrows track the depolymerization, rescue and growth events. (a) Laser-induced photodamage sites get repaired (arrowheads) by converted tubulin dimers over time. Scale bar, 5 μm. (b) Time-lapse sequence with corresponding kymographs of microtubules after laser-induced photodamage. Incorporation of converted tubulin dimers occurs at the photodamage sites and these act as rescue sites. Note within the kymographs that rescue occurs at the exact position of the repair site, highlighted by yellow arrows. Scale bar, 2 μm. (c) Representative sites of laser-induced photodamage within the cell margin and near the nucleus of PtK2 GFP-tubulin cells. Time-lapse sequence and kymograph of the rescue event of the microtubule labelled with an encircled star is shown in d. Scale bar, 5 μm. (d) Representative time-lapse sequence and corresponding kymograph of an analysed microtubule after photodamage. Microtubule rescues at the photodamage site. Images are representative of 5 independent experiments. Scale bar, 2 μm. (e) Histogram of the localization of the 111 rescue events with respect to the 51 photodamage sites in 20 PtK2 GFP-tubulin cells. All rescue events occurring along the microtubule were taken into account. The centre of the photodamage site is x= zero; average size of photodamage was 1.2 μm. Fifty per cent of the rescues occurred within the damage site. Only one rescue event was observed a short distance outside the damage site. The other 50% of rescues were observed along the microtubule closer to the tip, where the frequency of rescue is reported to be high. (f) Multiple rescues at a photodamage site were observed occasionally. Microtubules depolymerized eventually over the photodamage site after rescue events (bottom image). 1 t, elapsed time after photodamage. (g) Histogram of the time between the induction of photodamage (t=0s) and observation of the rescue or depolymerization event in 10 cells showing 52 rescues at damaged sites and 47 absences of rescue at damaged sites. Rescue events are most frequent within 250 s after photodamage. No rescue was observed after 550 s.

induce microtubule damage and repair (Fig. 3a). Furthermore, we observed rescue events at the incorporation sites that were subsequent to microtubule depolymerization events (Fig. 3b). This behaviour was further quantified by recording the location of the rescue sites with respect to the damaged site (Fig. 3c–e and Supplementary Videos 5 and 6). Photodamaged microtubules rescued more frequently...
Figure 4 Microtubule self-repair induces rescue events in vitro. (a) Rescue at crossing microtubules. Time-lapse sequence of three microtubules crossing each other. The kymograph highlights the crossing sites (yellow arrowhead pointing at the bright white vertical lines) and the occurrence of multiple rescue events at this site (red arrowheads). (b) The graph shows the frequency of rescue events for crossing microtubules as a function of distance from the crossing site. Data represent mean ± s.d. from n=8 independent experiments. (c) Repair at crossing microtubules. Observation of the incorporation of green tubulin dimers along red microtubules. White arrowheads point to crossing sites where accumulation of green tubulin was detected. The image is representative of three independent experiments. Scale bar, 5 μm. (d) Illustration of the microfluidic device. Short biotinylated microtubule seeds were fixed on neutravidin-coated micropatterns and elongated using red or green free tubulin. To exchange or remove the solution of free tubulin, a flow was induced parallel to the microtubules. (e) Photodamage sites can induce rescue. The time-lapse sequences and kymographs show microtubule dynamics with (right) and without (left) laser-induced damage (yellow star). The green arrows indicate the seed. Red arrowheads indicate rescue events. (f) The graph shows the frequency of rescue events for photodamaged microtubules as a function of distance from the centre of the damage (green bars) and for microtubules without damage, as distance from the centre of the observed microtubule (magenta bars). Data represent mean values ± s.d. from n=4 independent experiments. (g) Tubulin incorporation at photodamaged sites is associated with rescue. Green microtubule seeds were elongated with red free tubulin (step I). A GMPcPP cap was grown at the microtubule tip to avoid spontaneous depolymerization (step II). Photodamage was induced in the presence of green tubulin (step III). Depolymerization was initiated by removing the GMPcPP cap with a laser pulse at high intensity (step IV). The kymograph shows rescue (red arrowhead) at the damaged site (yellow star) where green tubulin was incorporated. GMPCPP, Guanosine-5′-[(α,β)-methylene]triphosphate, sodium salt. The image is representative of four independent experiments.
(75%, 41/55, Fig. 3g) within a time frame of 4 min than non-
photodamaged microtubules (39%, 24/62). The increased frequency is
due to additional rescue events occurring next to the photodamaged
site, regardless of its position along the microtubule (Fig. 3d,e and
Supplementary Videos 5 and 6). It is noteworthy that repair by
incorporation of new dimers was tightly focused and did not occur
over the entire length of the targeted region, but rescues systematically
occurred at the exact position of the repair site either on the right or
on the left of the targeted region (Fig. 3b). These results clearly showed
that microtubule damage and repair provided the microtubule with
protection from depolymerization. Interestingly, some observations
suggested that the protective effect did not last indefinitely (Fig. 3f).
More quantitative experiments of longer duration further revealed
that protection was quite effective within a two-minute period after
photodamage. However, protection was progressively less effective
from 2 to 8 min after photodamage (Fig. 3g). This effect of time
showed that the microtubule repair and rescue events were not
due to permanent structural changes and suggested that additional
biochemical regulation was involved at longer timescales.

**Microtubule self-repair and rescue in vitro**

At the repair site, newly incorporated dimers were certainly associated
with GTP-tubulin, as is the case for dimer assembly at the growing
end of the microtubule. Hence, the subsequent rescue event could
have depended on intrinsic structural factors, such as the specific
conformation of GTP-tubulin dimers, and extrinsic factors, such as
the numerous proteins preferentially interacting with GTP-
tubulin dimers. To elucidate the mechanism of microtubule
rescue after damage and repair events, and to identify the minimal
conditions sufficient to support the mechanism, we performed in vitro
experiments based on purified tubulin dimers that self-assembled to
form microtubules on glass slides. This set-up provided more defined
conditions than in vivo and could be used to challenge the role of MAPs
during rescue at incorporation sites.

We first investigated the rescue events in networks of dynamic
microtubules. They appeared preferentially localized at crossing sites
(Fig. 4a,b and Supplementary Video 8, see Methods) confirming our
observations in vivo (Fig. 2c,e). To test whether these sites corre-
sponded to self-repair events based on free tubulin incorporation, we
screened long microtubules, assembled from red tubulin and stabilized
with a GMPCPP cap, in a medium containing free green tubulin
dimers. Self-repair indeed occurred at crossing sites (Fig. 4c). Half
of the 300 crossing sites (from 3 independent experiments) displayed
incorporation of free tubulin in the seven minutes following free
tubulin exchange.

To further quantify the repair and rescue events in single
dynamic microtubules in a controlled environment, microtubules
were assembled in a microfluidic device. Short microtubule fragments
were adsorbed onto micropatterned lines and used as seeds to induce
microtubule polymerization. Micropatterned lines were surrounded
with PEG to minimize microtubule interactions with the glass-slide
surface. The micropatterned glass coverslips were mounted on a
microfluidic circuit to modulate the addition and removal of soluble
components without moving the microtubules under observation (Fig. 4d).
Laser light was used to generate photodamage on dynamic
microtubules. As expected from our previous work, photodamaged
microtubules self-repaired by incorporating free tubulin in the
damaged site (Supplementary Fig. 2). In the absence of photodamage,
rescue events were occasional (in 18 out of 78 microtubules) and
randomly dispersed along the microtubule (Fig. 4f). By contrast,
most photodamaged microtubules underwent depolymerization that
was subsequently rescued (50 out of 76 microtubules, Fig. 4e and
Supplementary Fig. 3 and Supplementary Video 7). These rescue
events were precisely located at the sites of damage (Fig. 4e) where
self-repair occurred (Fig. 4g) showing that damage/repair sites could
protect microtubules from depolymerization independently of the
presence of MAPs or any other cellular compound.

**Incorporation and hydrolysis of free tubulin**

We then tested whether repair was actually required for rescue or
whether damage in the lattice was sufficient to block microtubule
depolymerization. Microtubules were photodamaged in the absence
of free tubulin to prevent lattice self-repair. We ensured that the laser
power and frequency were appropriate and did not induce micro-
tubule breakage or lattice cauterization (Supplementary Fig. 4). Elong-
gated microtubules were capped with non-hydrolysable GMPCPP-
tubulin to protect the ends from initiating depolymerization that
would otherwise occur in the absence of free tubulin (Fig. 5a). After
a microtubule was photodamaged, the laser power was increased
to sever the cap from the microtubule and trigger microtubule de-
polymerization (Methods). Under these conditions, no rescue events,
pauses or arrests in depolymerization were observed at the damaged
and non-repaired sites (Fig. 5a). This showed that lattice self-repair by
the incorporation of free tubulin dimers was essential to protect the
microtubule from depolymerization. Moreover, this dependency on
free tubulin could account for the observations that rescue frequency
also depends on the concentration of free tubulin. Interestingly, it
appeared that the protective capacity provided by the repair site, in
the presence of free tubulin, was limited by time, similar to what was
observed in vivo. Rescue events were frequent within 3 min after
the damage/repair event (73%, 38/52), but less frequent from 3 to 20 min
after the damage/repair event (30%, 14/47) (Fig. 5b). Nucleotide hy-
drolysis following tubulin polymerization at the microtubule tip effects
the conformation and stability of the lattice structure; we therefore
tested whether hydrolysis of incorporated GTP tubulin was involved
in rescue regulation. We compared the rescuing frequency at repair sites
depending on the insertion of free tubulin dimers in the presence of
control (GTP) and non-hydrolysable (GMPCPP) nucleotide 5 to
10 min after damaging microtubules with the laser (Fig. 5b). As tubulin
bound to GMPCPP formed numerous seeds, the rescue effect had to
be tested in the presence of a sub-critical concentration of free tubulin
dimers to prevent the growth of too many additional microtubules
next to our observation region. The pauses at the laser-damage site,
following cap removal and microtubule depolymerization, were twice
more frequent when microtubules were repaired with tubulin dimers
bound to GMPCPP (Fig. 5c), confirming that nucleotide hydrolysis
was limiting the rescue lifetime.

**Recruitment of MAPs with affinity for GTP-tubulin**

At the microtubule growing tip, newly incorporated tubulin
dimers together with specific proteins that have high affinity for
GTP-bound tubulin form a cap that protects microtubules from
Figure 5 Incorporation and hydrolysis of free tubulin. (a) Damage without repair. Red microtubule seeds were elongated with green free tubulin (step I). A GMPCPP cap was grown at the microtubule tip to avoid spontaneous depolymerization (step II). Photodamage was induced in the absence of free tubulin (step III). Depolymerization was initiated by removing the GMPCPP cap with a laser pulse at high intensity (step IV). The time-lapse sequence and kymograph show no rescue nor pause at the damaged (yellow star), and non-repaired, site. See quantification in Supplementary Fig. 4. (b) Rescue lifetime. The kymograph shows multiple rescues (red arrowheads) at a photodamage site (yellow star) eventually followed by microtubule depolymerization (green arrowhead). The graph represents the time between inducing the damage and observation of the rescue or depolymerization event. Rescue events become less frequent as the time after damage increases. No rescue could be observed 17 min after inducing the damage. (c) Hydrolysis of incorporated tubulins. Red microtubules were laser damaged and repaired with non-hydrolysable (GMPCPP, left panels) or hydrolysable (GTP, right panels) tubulin. After an additional delay of 3 to 6 min, the cap was removed in the presence of a sub-critical concentration of free tubulin. The graph shows the frequency of pauses (highlighted in the left panel with a red arrowhead) in both cases. Non-hydrolysable tubulins increased the lifetime of repair and rescue sites. A chi-square test was used to compare pause frequencies for GTP and GMPCPP. n represents the number of microtubules that were allowed to repair in the presence of GTP and GMPCPP, respectively. (d) EB3 recruitment at repair sites. Red microtubules were photodamaged in the presence of red-fluorescent free tubulin dimers and EB3-GFP. The images show microtubule fluorescence in the red (left) and green (right) channels, before (top) and after (bottom) the laser-induced damage. The yellow star represents the damaged site. More examples are shown in Supplementary Fig. 5 as well as absence of EB3 recruitment in the absence of free tubulin. Images are representative of five independent experiments.
Figure 6 Self-repair biases microtubule dynamic instability in vitro. (a) The kymograph on the left shows a typical non-damaged microtubule with infrequent rescue events. On the right, the microtubule was damaged several times close to the tip as soon as it grew out long enough. Although catastrophe events were frequent, this microtubule was protected from complete depolymerization by the photodamage. The yellow stars indicate the photodamage sites. The red arrowheads indicate rescue events. (b) Damage increases microtubule lifetime. The graph shows the distribution of 28 laser-damaged (green) and 133 non-damaged (magenta) microtubule lifetimes. Damaged microtubule lifetime was found to be considerably longer than the lifetime of non-damaged microtubules. (c) Damage increases microtubule length. The graph shows the length of laser-damaged (green) and non-damaged (magenta) microtubules after 20 min. Error bars show mean ± s.d. for n=22 microtubules per condition, pooled from 4 independent experiments.

depolymerization. These proteins could then be recruited to repair sites, which are also made of GTP-tubulin, and form some cap-like structures that could act as rescue factors. To test this hypothesis, we grew red-fluorescent microtubules and followed microtubule repair after laser-induced damage, in the presence of red-fluorescent tubulin and GFP-tagged end-binding protein 3 (EB3). We found that lattice damage, which we could visualize by the reduction of fluorescent tubulin along the lattice, was associated with a specific recruitment of EB3 at the repair site (Fig. 5d). Interestingly, this recruitment was firmly dependent on the incorporation of free tubulin at the damaged site since it could not be detected in the absence of free tubulin (Supplementary Fig. 5). Therefore, the self-repair process induced GTP islands resembling cap structures along the microtubule lattice that are able to recruit proteins with high affinity for GTP-tubulin. Although we could not detect such recruitment in cells following laser-induced damage because of the high background of cytoplasmic EBs, this cap could contribute to the rescue process in vitro. However, our previous data in vitro showed that the incorporation of free tubulin was necessary (Fig. 5a) and sufficient (Fig. 4b,c) to promote microtubule rescue. Microtubule repair by free tubulin incorporation conditioned the recruitment of EBs and associated proteins, which may contribute but did not appear to be required to promote microtubule rescues.

Repeated damage lengthens microtubule protection from depolymerization

In vitro, a single event of microtubule repair had a time-limited effect on microtubule dynamics. We reasoned that in vivo the duration of protection against depolymerization could be extended in situations when repair events occur frequently, for example, at the cell margin where microtubules often crossover their neighbours, form bundles and are repeatedly bent by the retrograde flow of actin. Indeed, repeated photodamage near the tip of microtubules growing in vitro in the presence of 12 μM of tubulin could prevent catastrophic depolymerization for more than 20 min, whereas, in the absence of damage, depolymerization frequently occurred within 5 min (Fig. 6a,b and Supplementary Fig. 6 and Supplementary Video 9). Therefore, damaged microtubules became much longer (Fig. 6c). Remarkably, the targeting of individual microtubules using the same approach in vivo had similar consequences. We focused on peripheral cell parts where microtubules were sparse. To compare the effects of laser-induced damage on microtubules with the nonspecific damage the laser may
cause to all surrounding components, pulses were simultaneously sent to two distinct subcellular regions. In one the laser impacts targeted the microtubules, in the other they were focused next to microtubules (Fig. 7a). The evolution of the microtubule network was followed over 10 to 15 min during which both regions were subjected to repeated laser pulses (Fig. 7a and Supplementary Video 10). Laser-induced photodamage of every microtubule that approached the cell margin robustly protected those microtubules from depolymerization and promoted their elongation whereas no net extension of the network was seen when impacts were o-target (Fig. 7b and Supplementary Video 10). The damaged microtubules underwent multiple rescue events, continued to grow and eventually aligned parallel to the cell edge (Supplementary Fig. 7 and Supplementary Video 10). These effects were quantified by measuring the ratio of fluorescent tubulin before and after the laser-induced damage on the two windows of laser pulses. No net growth was detected when laser impacts were o-target whereas an average of 50% increase was measured when impacts were focused on microtubules (Fig. 7b). Strikingly, cells then tend to move toward this newly defined leading edge (Fig. 7a and Supplementary Fig. 8 and Supplementary Videos 10 and 11). These observations were consistent with the known enhancement of cell motility in response to microtubule overgrowth following downregulation of microtubule severing enzymes. Therefore, damage and self-repair appeared capable of sustaining microtubule polymerization in cells and affect higher-order processes such as modulating the polarity of a motile cell.

**DISCUSSION**

Our results have revealed a mechanical and structural mechanism by which a damaged microtubule can acquire a capacity to prevent its catastrophic depolymerization through the incorporation of new dimers of tubulin. Incorporation could be directly visualized thanks to the monitoring of photo-converted tubulin dimers. It occurred not only at microtubule ends but also along the microtubule shaft. It was not randomly distributed along the microtubule length but preferentially located in regions where the lattice is likely to be submitted to geometrical and mechanical constraints such as microtubule crossover, bundle and bending sites (Fig. 8a). Solid friction between adjacent microtubules is a mechanical necessary condition for the incorporation of tubulin dimers. This notion is coherent with the mechanical properties of the microtubule lattice (Fig. 8b), which show that the network is more densely packed at the cell edge than in the middle of the cell (a). Capture of tubulin into the microtubule lattice is a plausible mechanism for the incorporation of tubulin. The effect is not surprising since it is widely accepted that microtubules are dynamic structures capable of undergoing growth, depolymerization, and disassembly.

**Figure 7** Self-repair biases microtubule dynamic instability in vivo. (a) Repeated microtubule shooting ‘on-’ and ‘off-target’. Laser-induced photodamage was targeted either on the microtubules, in the red region, or next to the microtubules, in the green region. The images show the cell before (top) and after (bottom) the shooting. A comparison of the left and right cell margin pre- and post-photodamage is shown. Scale bar, 5 μm. (b) Preferential microtubule-network growth in damaged regions. Images show the microtubule network in the ‘on-target’ (red) and ‘off-target’ (green) regions before (t = 0 s) and after (t = 680 s) the shooting. The graph shows the after/before ratio of the mean microtubule fluorescence intensity in each region. Lines represent mean values from n = 16 cells from 4 independent experiments. The P value was generated by a Wilcoxon paired test. The total microtubule length increased in the regions where the laser impacts were targeted on the microtubules.
Damage could also be recruited and further contribute to the rescue mechanism. Additional MAPs with high affinity for GTP-tubulin, such as CLASP, documented the ability of newly incorporated dimers to recruit EB3. This is sufficient to rescue microtubule depolymerization. However, we found that the incorporation of new tubulin dimers in the absence of MAPs can protect the microtubule from depolymerization and support subsequent elongation. Importantly, our in vitro experiments show that the incorporation of new tubulin dimers in the absence of MAPs is sufficient to rescue microtubule depolymerization. However, we documented the ability of newly incorporated dimers to recruit EB3. Additional MAPs with high affinity for GTP-tubulin, such as CLASP, could also be recruited and further contribute to the rescue mechanism.

Finally, the repair and rescue mechanism we described biases the dynamic instability of the microtubule in a direction where the lifespan and maximal length are greater than would have occurred in a stochastic process without damage. What is also notable is that the repair process provides a mechanosensitive feedback loop that specifically promotes microtubule extension in intracellular regions where network entanglement and physical constraints are higher, and hence supports the directed growth of the microtubule network within the cell.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of this paper.

Note: Supplementary Information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

C.A. performed experiments in cells. L.S. and J.G. performed the experiments in vitro. L.B. and M.T. directed the work. C.A., L.S., K.J., L.B. and M.T. analysed the data. M.T. wrote the paper.

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The authors declare no competing financial interests.

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Figure 8 Microtubule self-repair and rescue. (a) Microtubule self-repair. The schemes show the preferential conformation of microtubules in which free tubulin incorporation (orange–red) was observed along pre-existing microtubules (green–blue) in living cells. (b) Microtubule rescue at self-repair sites. The schemes show the interruption of microtubule depolymerization at the repaired site (shown with orange-red dimers) and the induction of microtubule regrowth.

Microtubule lattices can protect the microtubule from depolymerization and support subsequent elongation. Importantly, our in vitro experiments show that the incorporation of new tubulin dimers in the absence of MAPs is sufficient to rescue microtubule depolymerization. However, we documented the ability of newly incorporated dimers to recruit EB3. Additional MAPs with high affinity for GTP-tubulin, such as CLASP, could also be recruited and further contribute to the rescue mechanism.

Thus, microtubule rejuvenation by incorporation of new tubulin dimers could synergize with MAPs recruitment to enhance the autonomous rescuing capacities of repair sites.

In summary, the repair and rescue mechanisms we described biases the dynamic installation of the microtubule in a direction where the lifespan and maximal length are greater than would have occurred in a stochastic process without damage. What is also notable is that the repair process provides a mechanosensitive feedback loop that specifically promotes microtubule extension in intracellular regions where network entanglement and physical constraints are higher, and hence supports the directed growth of the microtubule network within the cell.
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METHODS

Imaging. Microtubules were visualized using an objective-based azimuthal ilas2 TIRF microscope (Nikon Eclipse Ti, modified by Roper Scientific) and an Evolve 512 camera (Photometrics). The cell culture conditions on the microscope stage were controlled with a 512 Camera Temperature Controller (at 37 °C and 3% CO2). For in vitro experiments, the microscope stage was kept at 37 °C by means of a warm stage controller (LINKAM MC60). Excitation was achieved using lasers with wavelengths of 491 and 561 nm (Optical Insights). Time-lapse recording was performed using Metamorph software (version 7.7.5, Universal Imaging). Videos were processed to improve the signal/noise ratio (subtract background, smooth and PureDenoise functions of Image), version 1.47n5). The kymographs corresponding to the time-lapse sequences were drawn using ImageJ. Images were taken every 300 ms to 5 s (in vitro) and 3 s to 7 s (microtubule dynamics in vivo) or 10 s to 2 min (cell migration). For in vitro experiments showing incorporation of labelled tubulin in photodamaged microtubules, 30 successive individual images were overlaid and background subtracted.

Live cell imaging. PtK2 cells stably expressing GFP-tubulin (a gift from F. Perez, Curie Institute, Paris, France) were cultured on glass coverslips for microscopy. The PtK2 cell line (Sigma Aldrich) was transfected with Lipofectamine 2000 (Thermo Fisher Scientific) according to a protocol with the photo-conversion protein fused to tubulin mEOS2-tubulin (a gift from M. Davidson, Florida State University, Florida, USA; Addgene plasmid no. 57431). All cell lines were tested monthly for mycoplasma contamination. No cell lines used in this study were found in the database of commonly misidentified cell lines that is maintained by ICLAC and NCBI Biosample. We did not attempt to authenticate them. For long-time imaging (10 min, time frame 4 s) after photo-conversion the stable PtK2 GFP-tubulin cell line was transfected with the mEOS2-tubulin construct to overcome bleaching of the unconverted green signal. Photo-conversion was achieved with a 100 mW/405 nm laser used at 9% power, performing 15 repetitions within a field size varying between 12 μm × 12 μm to 30 μm × 30 μm.

Fixation. Cells expressing mEOS2-tubulin were fixed 2 min after photo-conversion. Cells were detergent-extracted with BRB80 + 4 mM EGTA supplemented with 0.2% Triton X-100 at 37 °C for 15 s. Glutaraldehyde and Triton X-100 were added to a final concentration of 0.5% each and cells were fixed for 10 min. Cells were rinsed twice using PBS supplemented with 1% Triton X-100 and incubated twice with 1% NaBH4 in PBS for 7 min. Cells were rinsed in PBS and imaged. Ten cells from four independent experiments were analysed.

Laser-induced microtubule photodamage. Laser-induced microtubule photodamage was performed using a Laser illuminator Ilas2 (Roper Scientific) set up on an inverted microscope (Ti-E, Nikon) with a ×100 Nikon APO TIRF oil-immersion objective plus an optical lens ×1.45. Ilas2 is a dual-axis galvanometer-based optical scanner that focuses the laser beam on the sample (diffraction-limited spot size) over the whole field of view of the camera. Laser displacement, exposure time, frequency and repetition rate were controlled using Metamorph software (version 7.7.9.0, Universal Imaging). To damage the microtubules of the GFP-tubulin or mEOS2-tubulin cell line, a 200 mW/491 nm laser was used at 40% power, performing 300 repetitions within a field size of approximately 1 μm × 1 μm. To analyse the long-term effect of laser-induced photodamage with respect to rescue, microtubules were repeatedly photodamaged along the lifetime (twice or three times). This increased the lifetime of the microtubule and the damage farthest away from the plus tip was taken into account for the analysis.

We then analysed the effect of locally increased microtubule lifetime due to laser damaged in cells. PtK2 GFP-tubulin cells were imaged over 10 min. We photodamaged single microtubules within a region of the cell margin for 5–7 min. Within the same time frame we simultaneously applied the same laser energy next to microtubules at a distant cell margin region (Supplementary Fig. 7). The mean fluorescent intensity of GFP-tubulin was measured in both areas before (0 min) and after (10 min) laser impact. After subtraction of the intensity decrease due to photobleaching, the ratio of fluorescent intensity before and after laser damage was calculated for the site within the cells where microtubules were damaged and the site where the laser was applied next to the microtubules. Sixteen cells were analysed from four different experiments.

For in vitro experiments, ATTO-488-labelled microtubules were damaged using a 100 mW/491 nm laser with a ×60 Nikon APO TIRF oil-immersion objective. To test the effects of frequency (corresponding to the number of points per second) and laser power, GMPCPP-capped microtubules were damaged in the absence of free tubulin and the frequency was varied between 1,000 s⁻¹ and 10,000 s⁻¹ and the laser power between 3% and 100% of the maximum power (Supplementary Fig. 3). The GMPCPP cap was removed using a laser pulse to initiate depolymerization. At low laser power and frequency, microtubules depolymerized without pausing. At high laser power and low frequency, microtubules were cut at the laser-damage site and immediately depolymerized afterwards. At low laser power and high frequency, most microtubules either broke, pausing for a short time before depolymerization, or paused at the laser-damage site. At high laser power and frequency, microtubules mostly paused at the damage site. For all other experiments involving laser damage in vitro, low laser power (4–9%) and frequency (2,500 s⁻¹) were chosen.

Tubulin purification and labelling. Tubulin was purified from fresh bovine brain by three cycles of temperature-dependent assembly and disassembly in Brinkley buffer 80 (BRB80 buffer: 80 mM PIPES pH 6.8, 1 mM EGTA and 1 mM MgCl2, plus 1 mM GTP). MAP-free neurotubulin was purified by cation-exchange chromatography (EMD SO, 650 M, Merck) in 50 mM PIPES, pH 6.8, supplemented with 1 mM MgCl2, and 1 mM EGTA. Purified tubulin was obtained after a cycle of polymerization and depolymerization. Fluorescent tubulin (ATTO-488- and ATTO-565-labelled tubulin) and biotinylated tubulin were prepared as previously described. Microtubules from neurotubulin were polymerized at 37 °C for 30 min and layered onto cushions of 0.1 M NaHEPES, pH 8.6, 1 mM MgCl2, 1 mM EGTA, 40% glycerol and labelled by adding 1/10 volume 100 mM ATTO-488 NHS ester; we stopped the reaction by adding 2 volumes 160 mM PIPES, 2 mM MgCl2, 2 mM EGTA, 100 mM potassium glutamate and 40% v/v glycerol, and then microtubules were sedimented on cushions of BRB80 supplemented with 60% glycerol. Microtubules were resuspended in BRB80, and a second cycle of polymerization and depolymerization was performed before use.

Cover glass micropatterning. The micropatterning technique was adapted from ref. 37. Cover glasses were cleaned by successive chemical treatments: 30 min in acetone, 15 min in ethanol (96%), rinsing in ultrapure water, 2 h in Hellmanex III (2% in water, Hellmanex), and rinsing in ultrapure water. Cover glasses were dried using nitrogen gas flow and incubated for three days in a solution of tri-ethoxy-silane-PEG (30 kDa. Creative PEGWorks) or a 1:10 mix of tri-ethoxy-silane-PEG-biotin and tri-ethoxy-silane-PEG at 1 mg ml⁻¹ in ethanol 96% and 0.02% HCl, with gentle agitation at room temperature. Cover glasses were then successively washed in ethanol and ultrapure water, dried with nitrogen gas, and stored at 4 °C. Passivated cover glasses were placed into contact with a photomask (Toppan) with a custom-made vacuum-compatible holder and exposed to deep ultraviolet light (7 mW cm⁻² at 184 nm, Kelight) for 3 min. Deep ultraviolet exposure through the transparent micropatterns on the photomask created oxidized micropatterned areas on the PEG-coated cover glasses.

Microfluidic circuit fabrication and flow control. The microfluidic device was fabricated in PDMS (Sylgard 184, Dow Corning) using standard photolithography and soft lithography. The master mould was fabricated by patterning 50-μm-thick negative photoresist (SÜB 3050, Microchem) by photolithography using a custom-made photolithographic mask (La Composite). A positive replica was fabricated by replica moulding PDMS against the master. Before moulding, the master mould was vapour silanized (trichloro(1H,1H,2H,2H-perfluorooctyl)silane, Sigma) for easier lift-off. Two inlet/outlet ports were made in the PDMS device using 0.5 mm soft substrate punches (UniCore 0.5, Ted Pella). Connectors to support the tubing were made out of PDMS cubes (0.5 cm side length) with a 1.2-mm-diameter through-hole. The connectors were bonded to the chip ports using still liquid PDMS as glue, which was used to coat the interface between the chip and the connectors, and was then rapidly cured on a hotplate at 120 °C. Teflon tubing (Tefzel, inner diameter: 0.03", outer diameter: 1/16", Upchurch Scientific) was inserted into the port serving as an outlet. Tubing with 0.01" inner and 1/16" outer diameter was used to connect the inlet, via a three-way valve (Onmitf Labware) that could be opened and closed manually, to a computer-controlled microfluidic pump (MFCS-FC, Fluigent). Flow inside the chip was controlled using the MFCS-Flex control software (Fluigent).

Microtubule growth on micropatterns. Microtubule seeds were prepared at 10 μM tubulin concentration (20% ATTO-565- or ATTO-488-labelled tubulin and 80% biotinylated tubulin) in BRB80 supplemented with 0.5 mM GMPCPP at 37 °C for 1 h. The seeds were incubated with 1 μM Taxotere (Sigma) at room temperature for 30 min and were then sedimented by centrifugation at 30 °C and resuspended in BRB80 supplemented with 0.5 mM GMPCPP and 1 μM Taxotere. Seeds were stored in liquid nitrogen and quickly warmed to 37 °C before use.

For experiments involving exchange of the solution after microtubule growth, the PDMS chip was placed on a micropatterned cover glass and fixed on the microscope stage. The chip was perfused with neutrinavin (25 μg ml⁻¹ in BRB80; Pierce) and washed with BRB80. Microtubule seeds were flowed into the chamber at high flow rates perpendicularly to the micropatterned lines to ensure proper orientation of the seeds. Non-attached seeds were washed out immediately using BRB80 supplemented with 1% BSA. Seeds were elongated with a mix containing 12–20 μM of tubulin (20% labelled) in BRB80 supplemented with 50 mM NaCl, 25 mM NaP, 1 mM GTP, an oxygen scavenger cocktail (20 mM diethothreitol,
1.2 mg ml\(^{-1}\) glucose, 8 µg ml\(^{-1}\) catalase and 40 µg ml\(^{-1}\) glucose oxidase), 0.1% BSA and 0.025% methyl cellulose (1500 cp, Sigma). For experiments showing tubulin incorporation after photodamage, a solution of 100% labelled tubulin was perfused before applying the laser damage. GMPCPP caps were grown by supplementing the before mentioned buffer with 0.5 mM GMPCPP (Jena Bioscience) and using 10 µM tubulin (100% labelled). Only microtubules growing in the direction of flow were analysed. For depolymerization experiments, the same mix as for microtubule elongation was used, without adding free tubulin to it.

For rescue experiments after photodamage, a flow cell chamber with an approximate volume of 40 µl was constructed with double-sided tape (70 µm height) between a micropatterned cover glass and a passivated glass slide. The chamber was perfused with 25 µg ml\(^{-1}\) neutravidin (Pierce) and washed with 300 µl of BRB80. Microtubule seeds were perfused and non-attached seeds were removed by washing with 300 µl BRB80 supplemented with 1% BSA. Seeds were elongated with a mix containing 12 µM of tubulin (20% labelled and 80% unlabelled tubulin) as described above. Only microtubule plus ends were considered for analysis.

For repair and rescue experiments after photodamage, microtubules were grown with red-fluorescent tubulin (20% labelled with ATTO-565 and 80% unlabelled, 20 µM) from micropatterns. Microtubules were then protected from depolymerization by growing a cap at microtubule ends with 0.5 mM GMPCPP and 10 µM tubulin. The solution was then replaced by 20 µM ATTO-488-labelled tubulin (100% labelled) and microtubules were photodamaged. After waiting approximately 2 min, the solution was replaced by red-fluorescent tubulin (18 µM, 20% labelled) and microtubule dynamics were initiated by removing the GMPCPP cap with a laser pulse at high intensity.

For repair and pause experiments in the presence of GMPCPP- and GTP-tubulin, microtubules were grown with red-fluorescent tubulin at 20 µM (20% labelled). Microtubules were then capped as described above with GMPCPP-tubulin. Laser damage was initiated either in the presence of 10 µM green-fluorescent GMPCPP-tubulin or 15 µM green-fluorescent GTP-tubulin (100% labelled). After waiting approximately 2 min, the solution was replaced by 100 µM red-fluorescent GTP-tubulin (20% labelled) and depolymerization was initiated by cutting the GMPCPP cap with a laser pulse at high intensity after a delay of 200–400 s.

**Microtubule crossing experiment.** For microtubule crossing and rescue experiments, microtubules were grown from seeds randomly attached via neutravidin to cover glasses passivated with a mix of tri-ethoxy-silane-PEG-biotin and tri-ethoxy-silane-PEG. Seeds were elongated with red-fluorescent free tubulin (20% labelled, 20 µM) in a microfluidic chamber from red-fluorescent seeds attached to micropatterns. Microtubule dynamics were imaged for 30 min. A flow cell chamber with an approximate volume of 40 µl, and two entry and two exits sites was constructed with double-sided tape (70 µm height) between a glass coverslip functionalized with SIPEG-Biotin\(^4\) and a passivated glass slide (SiPEG 30 kDa). The chamber was perfused with 25 µg ml\(^{-1}\) neutravidin (Pierce) for 1 min and washed with 300 µl of BRB80. Microtubules were polymerized for 30 min from microtubule seeds (0.5 µM) in a tube with an elongation mix containing BRB80, 1 mM GTP and 20 µM of tubulin (10% ATTO-565-labelled and 90% biotinylated tubulin).

The long microtubules were perfused into the chamber from two sites with a 90° orientation towards each other. A stabilization mix containing BRB80, 0.5 mM GMPCPP and 10 µM ATTO-565-labelled tubulin was perfused into the chamber from both entry sites. After incubation for 5 min the stabilization mix was washed with 300 µl BRB80. The incorporation mix containing BRB80, 1 mM GTP and 20 µM ATTO-488-labelled tubulin was perfused from both sites and incubated for 7 min before washing with 300 µl BRB80 and imaging. The incorporation of green tubulin within a distance of 1 µm from the crossing site was taken into account as repair sites. Three hundred crossing sites from three independent experiments were analysed.

**EB3 binding to photodamage sites in vitro.** Red-fluorescent microtubule seeds were randomly attached via neutravidin to cover glasses passivated with a mix of tri-ethoxy-silane-PEG-biotin and tri-ethoxy-silane-PEG. Seeds were elongated with red-fluorescent free tubulin (20% labelled, 16 µM) in the presence of 100 nM EB3-GFP and standard buffer (see above) with 60 mM NaCl.

For experiments in the absence of free tubulin, microtubules were grown in a microfluidic chamber from red-fluorescent seeds attached to micropatterns. Microtubules were grown with red-fluorescent free tubulin (20% labelled, 20 µM) and protected from depolymerization with a cap of GMPCPP-tubulin (0.5 mM GMPCPP, 10 µM tubulin). Before photodamage, the solution was replaced by 100 nM EB3-GFP\(^5\) in standard buffer with 60 mM NaCl without free tubulin.

**Statistics and reproducibility.** All statistical analyses were performed using GraphPad Prism 6.0 or Microsoft Excel software. All results presented in graphs are the mean ± s.d. Each exact n value is indicated in the corresponding figure or figure legend. No statistical method was used to predetermined sample size. No samples were excluded from the analyses. The investigators were not blinded to allocation during experiments and outcome assessment. A paired t-test was used in Fig. 7b. A Chi-square test was used in Fig. 5c. All experiments without quantification were independently performed at least three times and the representative data are shown.

**Data availability.** All data that support the conclusions are available from the authors on request.

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