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Beyond uniformity: Exploring the heterogeneous and dynamic nature of the microtubule lattice

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ABSTRACT

A fair amount of research on microtubules since their discovery in 1963 has focused on their dynamic tips. In contrast, the microtubule lattice was long believed to be highly regular and static, and consequently received far less attention. Yet, as it turned out, the microtubule lattice is neither as regular, nor as static as previously believed: structural studies uncovered the remarkable wealth of different conformations the lattice can accommodate. In the last decade, the microtubule lattice was shown to be labile and to spontaneously undergo renovation, a phenomenon that is intimately linked to structural defects and was called "microtubule self-repair". Following this breakthrough discovery, further recent research provided a deeper understanding of the lattice self-repair mechanism, which we review here. Instrumental to these discoveries were *in vitro* microtubule reconstitution assays, in which microtubules are grown from the minimal components required for their dynamics. In this review, we propose a shift from the term "lattice self-repair" to "lattice dynamics", since this phenomenon is an inherent property of microtubules and can happen without microtubule damage. We focus on how *in vitro* microtubule reconstitution assays helped us learn (1) which types of structural variations microtubules display, (2) how these structural variations influence lattice dynamics and microtubule damage caused by mechanical stress, (3) how lattice dynamics impact tip dynamics, and (4) how microtubule-associated proteins (MAPs) can play a role in structuring the lattice. Finally, we discuss the unanswered questions about lattice dynamics and how technical advances will help us tackle these questions.

1. Introduction

Traditionally, a large part of research on microtubule dynamics has focused on a small part of the microtubule: its tip. Understandably, this focus stemmed from the fact that many known microtubule functions are intimately linked to this dynamic behavior. For a long time, the microtubule shaft (or microtubule lattice), far from the tips, was essentially considered to be static, serving as a passive transport track for molecular motors and providing microtubules with a high degree of rigidity.

However, a number of recent findings challenge the previously held view of the homogeneous and static nature of the microtubule lattice, unveiling a deeper complexity, and suggesting that regulation can occur all along a microtubule, not only at the tip. Considering that: first, the lattice constitutes most of the microtubule as opposed to the tip; second, the microtubule lattice is essential for many functions carried out by microtubules; and third, the lattice is under stress from different sources in a cell, it is likely that many important and physiologically relevant observations will keep emerging from studying the microtubule lattice.

Here, we explore the current understanding of the microtubule lattice. We dive into the lesser-explored realm of the dynamic and heterogeneous nature of the lattice, since post-translational modifications, the tubulin code and tip dynamics have been covered elsewhere (Brouhard and Rice, 2018; Janke and Magiera, 2020; Magiera et al., 2018). We focus on structural heterogeneity and defect formation as well as microtubule lattice dynamics, often termed "self-repair". Finally, we give an overview of microtubule-associated proteins (MAPs) that target and modulate the lattice. Notably, several key discoveries regarding the lattice and its interacting partners have emerged from *in vitro* reconstituted systems employing a minimal set of purified components. These systems have allowed researchers to control and manipulate parameters, providing mechanistic insight into the

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heterogeneous and dynamic nature of the microtubule lattice and its interplay with other proteins.

2. Microtubule structure (the lattice)

Microtubules are cylindrical biopolymers that are made of globular, structurally similar α and β tubulin monomers that align top-to-bottom to form protofilaments. Typically, in most cell types, a microtubule is comprised of 13 protofilaments, which connect *via* staggered lateral contacts to form a hollow tube (Amos and Schlieper, 2005). The slight stagger results in a helical arrangement of tubulin subunits with a left-handed twist (Fig. 1A). *In vivo*, protofilament numbers are tightly controlled by templated nucleation and accessory proteins (Roostalu and Surrey, 2017). However, even though 13 protofilaments prevail in intracellular microtubules (Ledbetter and Porter, 1964) and are also common in *in vitro* systems, counts ranging from 9 to 17 have been described (Chrétien and Wade, 1991; Evans et al., 1985; Wade et al., 1990). While 13 protofilaments run perfectly parallel with the microtubule wall, other protofilament numbers require a slight protofilament skewing, changing the lateral curvature of the microtubule wall (Hunyadi et al., 2005).

Since α-β tubulin dimers within a single microtubule filament share the same orientation, microtubules are polar structures with a plus end (β-tubulin exposed) and a minus end (α-tubulin exposed) (Akhmanova and Steinmetz, 2008a, 2008b; Mandelkow et al., 1986). The walls of microtubules have a crystal-like structure because of the regular spacing of tubulin dimers, the lateral contacts between tubulin dimers along protofilaments, and the precise angular orientation between neighboring protofilaments. Hence, due to the similarities between the microtubule wall and the regular and periodic structure of crystal lattices, the term "microtubule lattice" was adopted (Grimstone and Klug, 1966). Because the microtubule surface is a highly repetitive, generally acidic structure, it promotes processive movements but may also serve as a reaction platform.

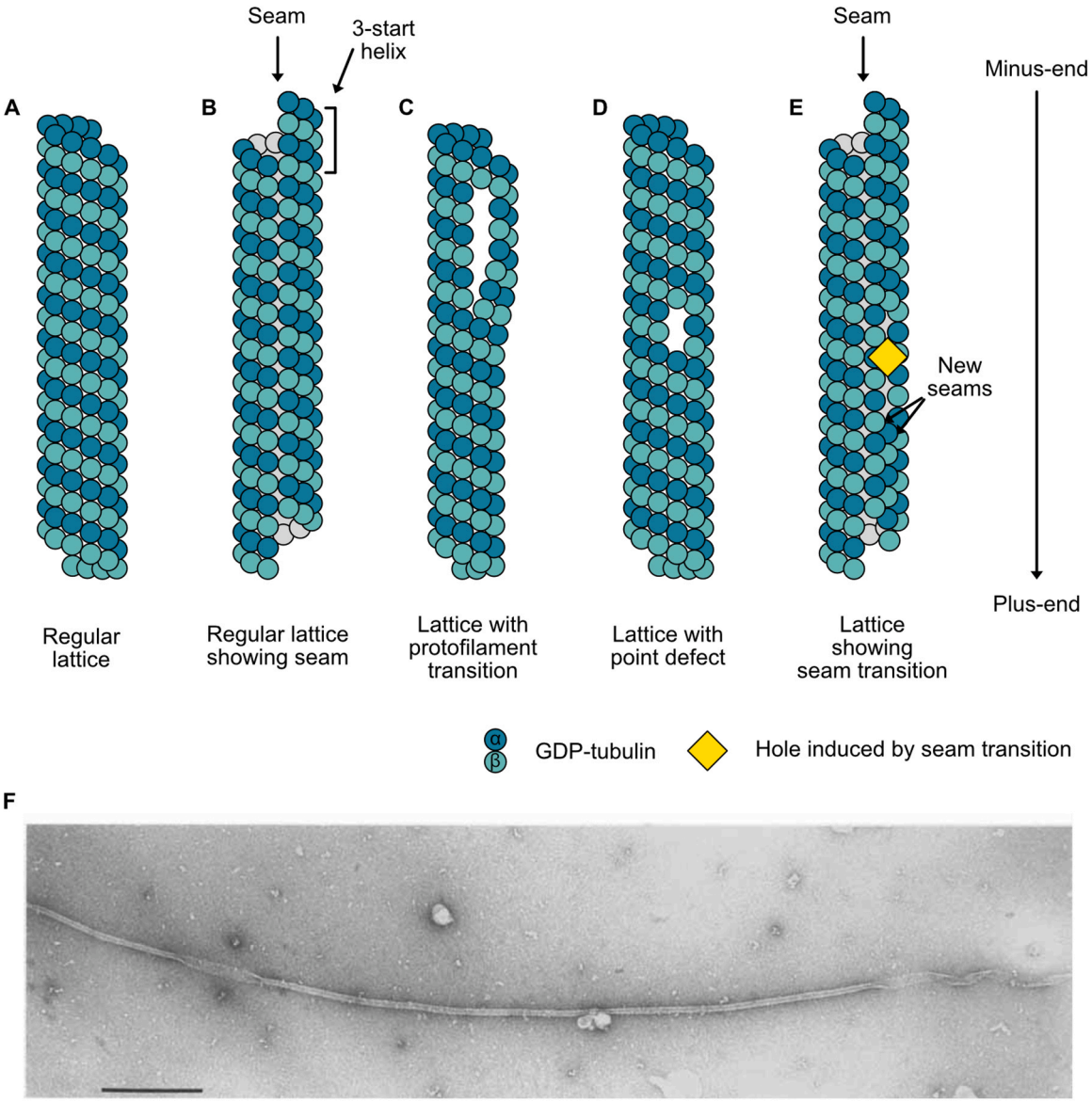

Fig. 1. Schematic representations of the microtubule lattice in its regular form and in the presence of defects. **(A)** A microtubule with a regular B-lattice configuration. **(B)** Rotated microtubule shown in (A) with a representation of the microtubule seam, where α-β-tubulin dimers make lateral contacts with dimers from other protofilaments in an A-lattice configuration. **(C)** A microtubule with a protofilament transition from 13 to 12 protofilaments. **(D)** A microtubule with a point defect, or a missing α-β-tubulin dimer, in the lattice. **(E)** A microtubule with missing α-β-tubulin dimers in the lattice due to the formation of new seams. **(F)** An EM image of end-stabilized microtubules grown from axonemal structures showing microtubule damage along the lattice (taken from Dye et al., 1992). Scale bar: 0.5 μm.

Apart from the protofilament number, another important criterion for defining the microtubule lattice is the helix start number – the number of monomers that each turn of the helix spans in the longitudinal direction. Helix start numbers of three or four are typical for microtubules (Amos and Schlieper, 2005; Sui and Downing, 2010).

Bonds between protofilaments can form between equal subunits (α-α and β-β), resulting in a so-called B-lattice configuration, or between unequal subunits (α-β), resulting in an A-lattice (Huber et al., 2015; Nogales et al., 1999; Sui and Downing, 2010). Both *in vitro* and in cells, B-lattice configurations are significantly more common: typically, protofilaments form B-lattice contacts, except for one A-lattice contact at the so-called seam (Fig. 1B). The seam is therefore structurally distinct from the rest of the microtubule, but its physiological relevance remains unclear.

Depending on the lattice configuration, helix start and protofilament number, microtubules can be helically symmetric or asymmetric. For instance, for microtubules with 13 or 14 protofilament numbers, microtubules of the A-lattice configuration are symmetrical when protofilament and helix start numbers are both even or odd (Mandelkow et al., 1986). On the other hand, microtubules of the B-lattice configuration are symmetrical with an even helix start number, independent of the protofilament number (Mandelkow et al., 1986).

Taken together, most microtubules *in vivo* are of the B-lattice configuration, with 13 protofilaments and a 3-start helix, as also confirmed in microtubules assembled with *Xenopus*-egg cytoplasmic extract (Guyomar et al., 2022). *In vitro*, microtubules have mostly the same characteristics except for the protofilament number which is often 14 instead of 13 (Chrétien et al., 1992).

3. Microtubule lattice defects

A range of structural variations (hereafter referred to as lattice defects) have been observed in microtubules. Although most of the observations referred to in this section were made *in vitro*, many of these seem to be relevant in cells too (see below).

Interestingly, the number of protofilaments can vary not only from microtubule to microtubule, but also along the length of the same microtubule (Chrétien et al., 1992). A protofilament mismatch necessarily leads to a discontinuity – a defect site where tubulin subunits have a reduced number of neighbors compared to a perfect lattice structure (Fig. 1C). Abrupt protofilament transitions were observed in *in vitro*-grown microtubules, typically as single increments, but double increments exist as well. The typical distance between lattice defects is a few up to hundreds of micrometers, depending on growth conditions: faster-growing microtubules exhibit significantly more lattice defects (Schaedel et al., 2019), which may explain why they are softer than slower growing microtubules that have fewer defects (Janson and Dogterom, 2004; Schaedel et al., 2015). Protofilament transitions were found in microtubules grown in cell-free extract from *Xenopus* eggs as well, albeit at a lower frequency (Chrétien et al., 1992). How defects emerge during microtubule growth is unclear; faster growth may promote lattice configurations that do not correspond to the energetically most favourable state, allowing randomly occurring imperfections that arise at the growing tip to remain stable (Chrétien and Fuller, 2000). This view is supported by the observation that faster growth rates coincide with larger proportions of lattices that differ from the typical 13- or 14-protofilament, 3-start configurations, exhibiting increasingly unusual lattices (see Schaedel et al., 2019, supplementary information). In addition, co-polymerizing microtubules with the stabilizing drug Taxol leads to frequent protofilament transitions (Arnal and Wade, 1995).

Following the discovery of protofilament transitions, other types of structural flaws were described: for example, point defects (Fig. 1D) corresponding to missing tubulin dimers and openings between neighboring protofilaments were visualized by AFM imaging of surface-attached microtubules (Schaap et al., 2004; Wijeratne et al., 2022). Recently, cryo-electron tomography was used to show that the seam number and location can vary along individual microtubules grown *in vitro*, producing holes of one to a few subunits in size (Fig. 1E; Guyomar et al., 2022). As with protofilament transitions, mixed seam structures are less common in microtubules grown in *Xenopus* egg cytoplasmic extracts, suggesting that intracellular factors guide microtubule lattice formation. Templated microtubule nucleation, *e.g.*, by the γ-tubulin ring complex (γ-TuRC), and co-factor assisted growth (Moritz et al., 2000; Roostalu and Surrey, 2017), may also be the reason why intracellular microtubules have less polymorphic lattices in general, in spite of their faster growth compared to *in vitro*-grown microtubules.

The structures of intracellular microtubules are highly context-dependent, being governed by the specific proteome of a given cell, post-translational modifications (PTMs) of both tubulin and its regulators, and the developmental and physiological status of the cell (Janke and Magiera, 2020). Yet, recent advances in cryo-electron tomography show that intracellular microtubules do exhibit significant lattice disruptions, mostly in the form of – sometimes massive – lattice openings (Atherton et al., 2018; Chakraborty et al., 2020; Foster et al., 2022). Protofilament number transitions have also been observed in cells, although at a reduced frequency (Foster et al., 2022). Even though most imperfections in intracellular microtubules are morphologically different from the typical defects observed *in vitro*, they show that lattice defects are, in general, of physiological relevance. Accordingly, for instance, α-tubulin acetyltransferase (αTAT1; see below for details on its function) has been shown to enter the microtubule lumen through microtubule ends as well as openings or breaks in the lattice (Coombes et al., 2016). Careful *in vitro* studies varying parameters such as nucleation templates, tubulin composition (isotypes, PTMs), and regulatory proteins will in the future help us understand which factors govern the fidelity of lattice formation in cells.

4. Microtubule lattice plasticity

The variations in protofilament, helix start, and seam numbers show that the microtubule lattice can accommodate a large degree of heterogeneity (Chaaban and Brouhard, 2017; Chrétien and Wade, 1991; Guyomar et al., 2022). Furthermore, in cells, PTMs label microtubule sub-populations that often take on different tasks, creating a spatially and functionally diverse microtubule cytoskeleton (Janke and Magiera, 2020). In addition to this spatial heterogeneity, the microtubule lattice can change with time: for example, enzymes catalyzing certain PTMs such as acetylation have a higher affinity for microtubules than for soluble tubulin dimers and can thus modify fully grown microtubules over time (Janke and Magiera, 2020). Furthermore, the hydrolysis of GTP at the exchangeable site of β-tubulin, which fuels dynamic instability of the microtubule tip, is thought to induce conformational changes that lead to a longitudinal compaction of tubulin dimers after polymerization (this compaction was, however, only observed in microtubules grown from mammalian tubulin, not from yeast tubulin; Howes et al., 2017; Von Loeffelholz et al., 2017). Recent findings seem to somewhat contradict the observation that the nucleotide state is directly correlated with the compaction of tubulin. For instance, first, some MAPs have been shown to alter the compaction of the lattice, *e.g.*, kinesins can expand the lattice (Peet et al., 2018; see below). Second, since the GDP analogue GMPCP lacking a terminal phosphate (as opposed to GMPCPP) could also expand the lattice, it is now believed that the methylene group instead of the terminal phosphate in GMPCPP is responsible for the observed lattice expansion in GMPCPP-grown microtubules (Estévez-Gallego et al., 2020). Thus, this matter remains to be settled. Overall, the microtubule lattice exhibits spatial and temporal plasticity, which implies that microtubule regulation does not only occur at the tips but along the lattice as well (Cross, 2019).

5. The microtubule lattice is dynamic

In contrast to tubulin modifications and subtle conformational changes that occur after polymerization, the basic arrangement of tubulin dimers within the crystal-like microtubule lattice was long considered to be static, even though a couple of early observations challenged this view: a study based on cryo-electron microscopy showed that co-polymerizing tubulin with Taxol predominantly leads to a 12-protofilament lattice structure, whereas microtubules grown with Taxol's side-chain analogue docetaxel have mostly 13 protofilaments (Díaz et al., 1998). This induction of a ligand-specific protofilament configuration even holds when the ligand is added after microtubule polymerization, independently of the original growth conditions, inducing a surprising change in the structure of pre-formed microtubules within about a minute (Díaz et al., 1998). Even though these experiments were done in bulk and therefore have limited informative value on the single microtubule level, they suggest a certain moldability of the lattice that requires the loss and addition of protofilaments to pre-formed microtubules. Another early study reported even more direct evidence of tubulin loss and addition at the lattice: in the early 1990 s, Dye et al. grew microtubules from axonemes *in vitro* (Dye et al., 1992). Due to the high microtubule density in the assay, some microtubules annealed tip-to-tip and thus formed a single stabilized microtubule with axonemes at both ends. When they removed the soluble tubulin, the authors observed the formation of flexible regions along the lattice that eventually broke and led to microtubule disassembly from the site of breakage (Fig. 1F). Re-supplying microtubules with free tubulin before breakage reversed the formation of flexible regions, showing that the lattice can lose and incorporate tubulin far from the tips. More recently, these initial observations were followed up by an *in vitro* study using a two-color assay of fluorescently labeled tubulin, showing that tubulin dimers spontaneously leave and incorporate into the lattice (Fig. 2A; Schaedel et al., 2019). The absence of other cellular factors in these assays suggests that tubulin loss and incorporation are genuinely

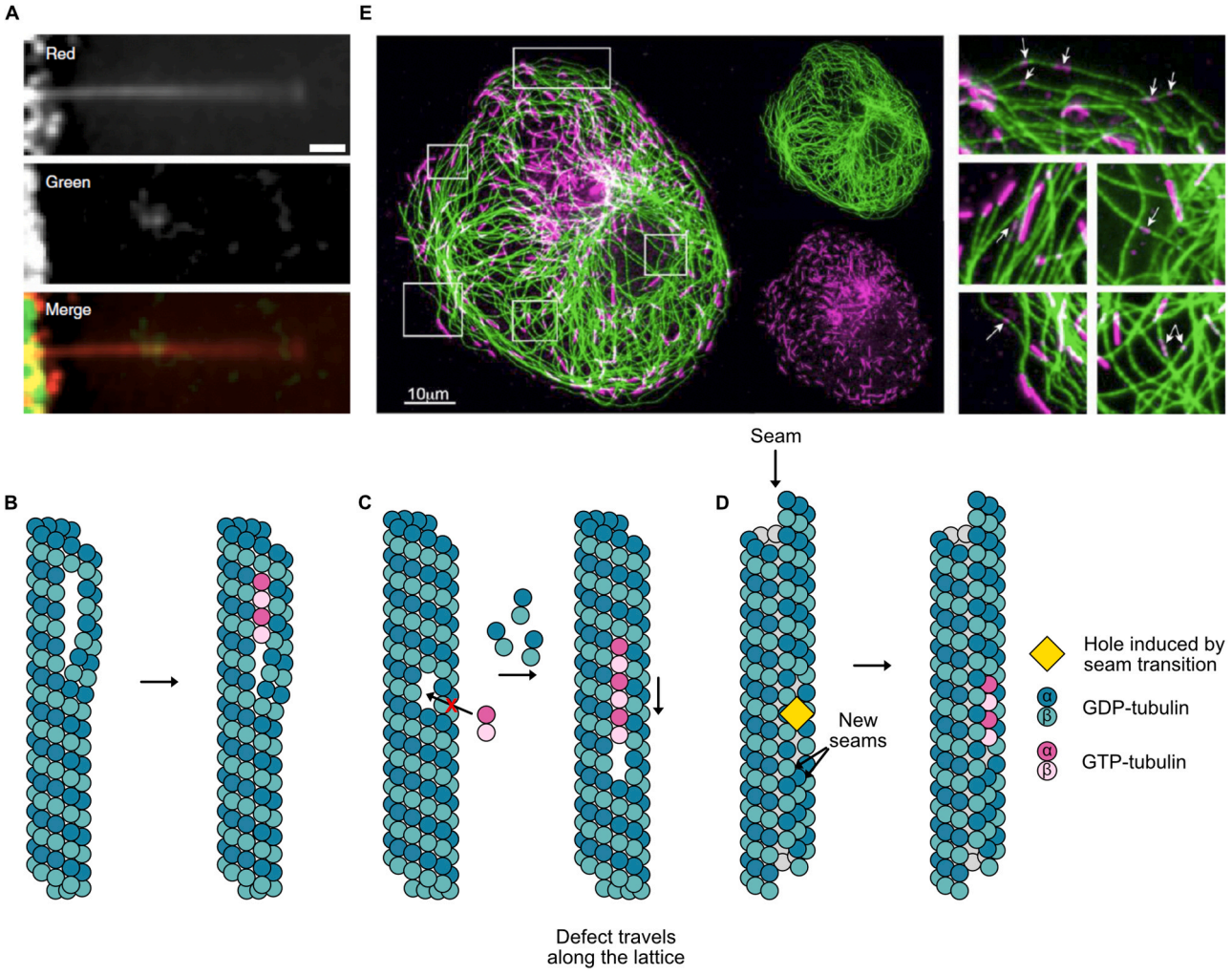

Fig. 2. The microtubule lattice is dynamic. **(A)** TIRF microscopy images of a microtubule grown in *in vitro* microtubule reconstitution assays (taken from Schaedel et al., 2019). The microtubule lattice was grown with tubulin stained with ATTO-565 (in red) and incubated with free tubulin stained with ATTO-488 (in green). After 15 min, washing out the free green tubulin revealed the incorporation spots in green along the red microtubule lattice. Scale bar: 3 µm. **(B)** A schematic representation of tubulin incorporation into the lattice of a microtubule with a protofilament transition. **(C)** A schematic representation of tubulin incorporation into the lattice of a microtubule with a point defect. Due to hypothetical steric limitations, the point defect may at first not be repaired. Because of the missing α-β-tubulin dimer, neighboring dimers are more likely to leave the lattice along the same protofilament and, hence, the defect "travels" along the lattice. When further dimers leave the lattice, there is more space in the lattice and the defect can be repaired to a certain extent. **(D)** A schematic representation of tubulin incorporation into the lattice of a microtubule with the formation of additional seams. Due to the additional A-lattice contacts in the microtubule (new seams), a hole is formed that could hypothetically be repaired by tubulin incorporation. **(E)** Confocal spinning disk microscopy images of a male rat kangaroo kidney epithelial cell expressing GFP-tubulin (green) and microinjected with tubulin stained with ATTO-565 (magenta) (taken from Gazzola et al., 2023). Images on the right correspond to insets indicated on the left. White arrows indicate sites of tubulin incorporation into the pre-existing lattices two minutes after injection.

inherent properties of the microtubule lattice. Tubulin incorporation occurs in a localized manner, in the form of individual stretches that can be several μm long. Both the length of the stretches and the frequency with which they occur depend on the concentration of soluble tubulin (Schaedel et al., 2019). These observations strengthened the evidence for the dynamic nature of the microtubule lattice. Because tubulin incorporation into the lattice happens spontaneously and in the absence of microtubule damage, we propose a shift from the term "lattice self--repair". We suggest this phenomenon to be now referred to as "lattice dynamics" in analogy to "tip dynamics".

What makes the lattice dynamic? The conformational changes induced in tubulin dimers upon GTP hydrolysis destabilize the lattice, allowing microtubules to quickly depolymerize when the stabilizing GTP cap at the tip is lost and releasing the tension that has accumulated in the lattice (Driver et al., 2017). This highlights that tubulin dimers in the lattice are in a metastable, *i.e.*, labile, state. The exact bond strengths of tubulin dimers in the lattice have, however, not been directly measured; computer simulations based on fitting parameters to experimentally determined tip dynamics report values ranging from 35 k_BT to 80 k_BT for a dimer in the fully occupied lattice (Sept et al., 2009; VanBuren et al., 2005, 2002), with longitudinal bonds being about twice as strong as lateral bonds. This anisotropy in bond strength can also be inferred from the observations of preferential tubulin loss (Dye et al., 1992; Schaedel et al., 2019) and incorporation (Schaedel et al., 2019) in the longitudinal direction: since a missing longitudinal neighbor destabilizes a dimer more than a missing lateral neighbor, tubulin loss and incorporation predominantly occur longitudinally. Recently, an elegant study reported the force required to remove tubulin dimers from the lattice to be about 30 pN using optical tweezers as well as kinesin-1 motors with DNA handles tethered to the lattice (Kuo et al., 2022). The magnitude of this force suggests that spontaneous, thermal energy-induced tubulin loss from the intact lattice is possible but relatively rare. It would lead to the occasional loss and subsequent incorporation of single tubulin dimers, which is probably of little relevance in physiological settings due to the long timescales compared to tip dynamics. It also does not explain the localized tubulin incorporations observed experimentally, since it would rather lead to a "salt-and-pepper" incorporation pattern (Schaedel et al., 2019).

So, what determines the localized pattern of tubulin incorporation? One obvious idea is that certain lattice sites may facilitate local tubulin turnover because of structural weaknesses, such as lattice defects (Fig. 1). Indeed, the frequency of tubulin incorporation correlates with the frequency of protofilament transitions (Schaedel et al., 2019). The hypothesis that tubulin incorporation occurs at protofilament transitions is supported by computer simulations showing that a passive breathing mechanism at dislocations, generating openings to allow for single protofilament elongation at the dislocations, is sufficient to induce localized tubulin turnover (Fig. 2B; Schaedel et al., 2019). However, direct evidence of tubulin incorporation at protofilament transitions, *e.g.*, *via* cryo-electron imaging of gold-labeled incorporated tubulin, would be desirable to confirm this plausible mechanism.

Apart from protofilament transitions, other types of defects may play a role in lattice dynamics. For example, vacancies of the size of single tubulin dimers ("point defects"; Fig. 2C) have been speculated to induce localized tubulin turnover based on the assumption that they cannot be immediately filled (Lecompte and John, 2022): due to the expanded conformation of GTP-tubulin dimers, it may not be sterically possible to incorporate GTP-tubulin into single vacancies within a compacted GDP-tubulin lattice. However, as soon as another dimer leaves the lattice and thus enlarges the vacancy, a GTP-dimer may partially fill the hole. In this way, the vacancy is never fully repaired, but it can diffuse along the lattice, with a preference for movement along a protofilament due to the above-mentioned anisotropy of the bond strengths (Fig. 2C). One may call this the "Lego mechanism": in a Lego wall with a single missing brick, it is sterically not possible to fill the vacancy without removing a neighboring brick first.

Additionally, switches in seam structure along a microtubule lead to defects corresponding (at least) to missing tubulin monomers (Guyomar et al., 2022), which may behave similarly to protofilament transitions (Fig. 2D). Whether or not the seam itself is a mechanically weak structure remains controversial (Harris et al., 2018; Katsuki et al., 2014), though a recent study reports that, in the GDP-tubulin lattice, the protofilaments at the seam show a slight opening that may be indicative of an intrinsically weaker interaction (Zhang et al., 2018).

Following the discovery of lattice dynamics *in vitro*, two studies directly described tubulin incorporation into intracellular microtubules, either using local photoconversion of mEOS2-labeled tubulin (Aumeier et al., 2016) or microinjection of fluorescently labeled, purified tubulin into cells followed by fixation (Fig. 2E; Gazzola et al., 2023). Interestingly, tubulin incorporations are locally more intense and occur at faster timescales compared to *in vitro* assays (the difference is almost one order of magnitude; Fig. 2E). So far, the reasons for the different morphologies and timescales with which microtubule lattice dynamics occur in cells compared to *in vitro* systems are not well understood; they are likely due to differences in the biochemical and mechanical environments. It will require a combination of *in vitro* and cell-based studies to precisely determine the parameters that govern lattice dynamics in physiological settings.

6. Microtubules self-repair when mechanically stressed

Friction at microtubule crossings (Fig. 3A) or at sites where microtubules cross synthetic obstacles and even the simple interaction with a passivated coverslip are sufficient to trigger increased tubulin turnover at the lattice (Alexandrova et al., 2022; Aumeier et al., 2016; de Forges et al., 2016).

Given the fragility of the microtubule lattice, it is surprising that microtubule breakage is a rare event (Odde et al., 1999), even in an intracellular environment where a multitude of forces produced by actomyosin, microtubule-associated molecular motors, microtubule growth against obstacles, and friction in a crowded environment are incessant sources of mechanical stress (Li et al., 2023a, 2023b; Waterman-Storer and Salmon, 1997; Wu et al., 2011). Besides the action of MAPs, part of the mechanical resilience of microtubules can be attributed to the dynamic nature of the lattice: repeated bending or localized laser irradiation damage microtubules, yet microtubules are capable of repairing themselves and restoring their mechanical properties by incorporating tubulin from the solution to patch up damages (Fig. 3B; Schaedel et al., 2015). Microtubules that exhibit a higher frequency of growth-induced lattice defects are more susceptible to weakening under mechanical load, consistent with the idea that defects are weak points in the lattice. Recently, localized lattice damage induced by an AFM tip has been shown to self-repair within a short time (Fig. 3C; Ganser and Uchihashi, 2019). Microtubules are therefore an astonishing example of ductile biological materials with self-healing properties.

Interestingly, intralumenal acetylation by αTAT1 protects microtubules from mechanical damage, both *in vitro* and in cells (Fig. 4A–C; Portran et al., 2017; Xu et al., 2017). As shown with FRET-based assays, acetylation weakens inter-protofilament interactions, which increases microtubule compliance and allows them to maintain their integrity when bent (Portran et al., 2017). Whether other PTMs also modify the response of microtubules to mechanical stress is an intriguing question that remains to be addressed.

7. The relation between lattice dynamics and tip dynamics

Understanding the dynamic instability of the microtubule tip has kept many researchers busy over the past decades. Of all dynamic instability parameters, the stochastic switching from depolymerization to growth – termed rescue – remains the least understood (Brouhard, 2015). Due to its stabilizing effect on the lattice, the theory that GTP-tubulin remnants (often termed "GTP islands") contribute to rescue

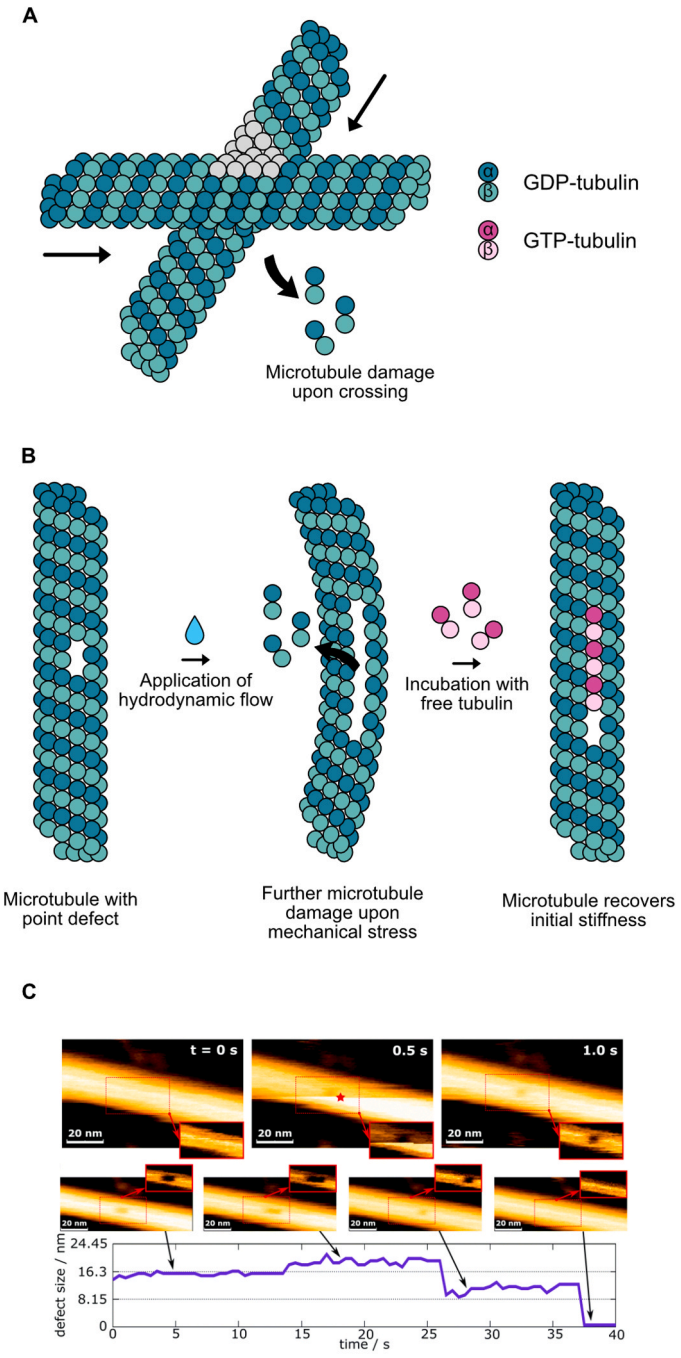

Fig. 3. The microtubule lattice self-repairs when mechanically stressed. **(A)** When two microtubules cross each other, there is friction at the overlapping point that can lead to microtubule damage, for instance in the form of missing dimers from the microtubule lattices at the cross-over site. **(B)** When microtubules with a pre-existing point defect are bent by the application of a hydrodynamic flow, further microtubule damage is produced as seen by more tubulin dimers leaving the microtubule lattice. Upon incubation with free tubulin, microtubules can heal the damage and recover their initial stiffness. **(C)** AFM images showing the creation of a microtubule defect (top images) and its healing across time (middle images) (taken from Ganser and Uchihashi, 2019). The graph (bottom image) indicates the time points at which the images in the middle section were taken (black arrows connect the time point to the corresponding images) and their respective defect sizes. The insets indicated by the red dashed rectangles represent the selected sections with increased contrast. The red star represents the time and position at which the force was applied to produce the microtubule defect.

emerged (Dimitrov et al., 2008). Indeed, islands containing slowly hydrolysable GTP analogues promote microtubule rescue *in vitro* (Tropini et al., 2012). However, it was not clear how these islands should emerge in dynamic microtubules, and why they would be exempt from hydrolysis.

Lattice dynamics may provide the missing link: since they lead to the (at least transient) presence of GTP-tubulin at incorporation sites, they may cause rescue as a side effect (Fig. 4D). Indeed, laser irradiation leads to massive lattice damage, followed by tubulin incorporation (Schaedel et al., 2015) and – often enough, and often repeatedly – by rescue and an overall increased microtubule lifetime (Aumeier et al., 2016). More subtle friction events between two crossing microtubules or between a microtubule and a synthetic obstacle can also lead to rescue and correlate with tubulin incorporation (Aumeier et al., 2016; de Forges et al., 2016).

The correlation between tubulin incorporation and rescue was also

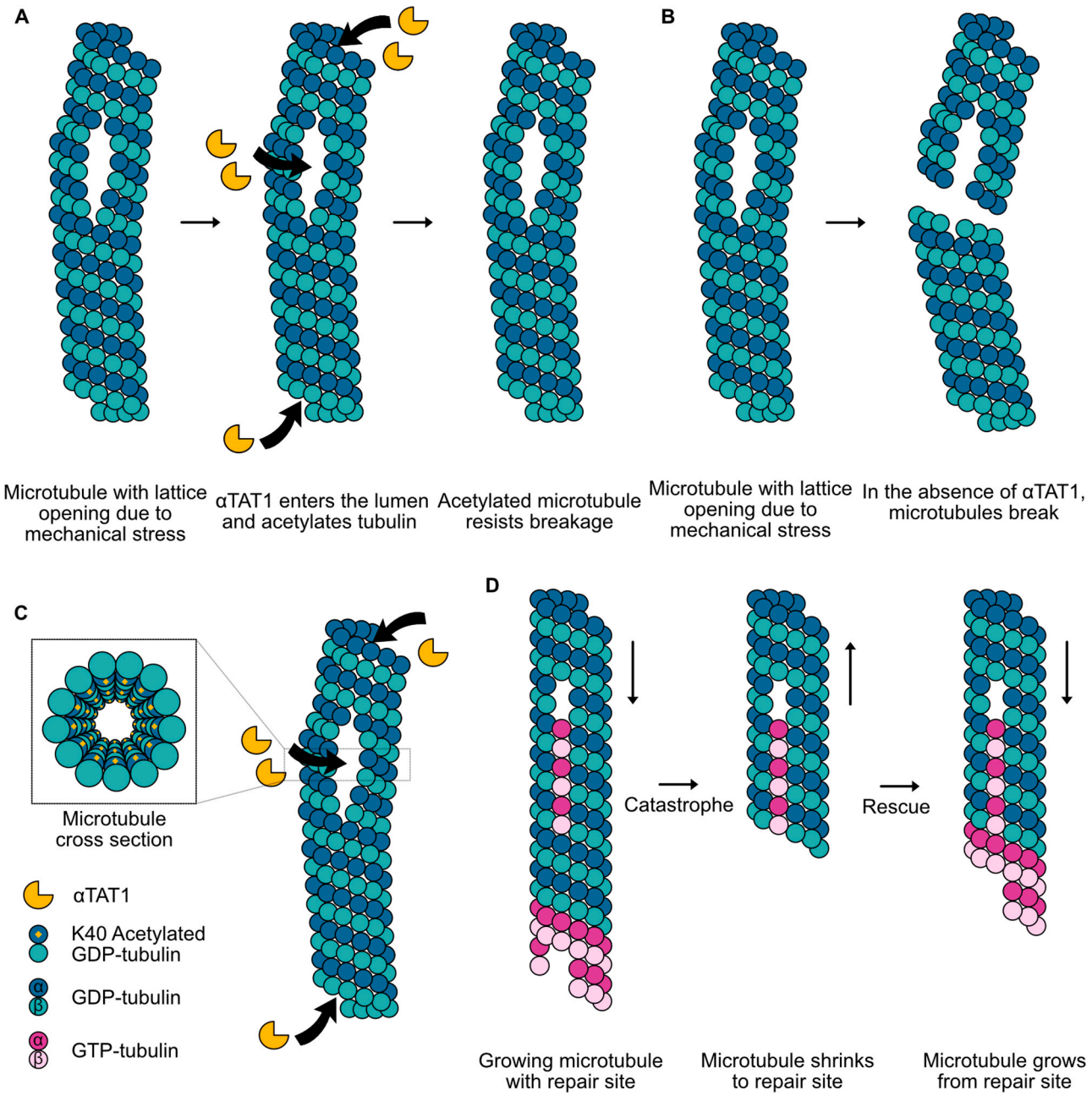

Fig. 4. Microtubules resist breakage by intraluminal acetylation and lattice repair can promote microtubule rescue. **(A)** Microtubules that have undergone mechanical stress can display openings at the lattice. αTAT1 enters the microtubule lumen through the microtubule ends and lattice openings and promotes microtubule acetylation at the K40 site of α-tubulin. This acetylation allows microtubule protofilaments to slide against each other, making the microtubule more flexible and more resistant to mechanical stress. **(B)** In the absence of αTAT1, for instance in cells expressing a small interfering RNA against *αTAT1*, microtubules do not undergo intraluminal acetylation and break when mechanically stressed. **(C)** A microtubule cross section showing the acetylation of α-tubulin in the microtubule lumen due to the action of αTAT1. **(D)** When a microtubule that contains sites of tubulin incorporation into the lattice undergoes catastrophe, it is more likely to rescue and regrow from the point of tubulin incorporation due to the presence of GTP-tubulin.

observed in cells, for laser damage as well as crossing microtubules (Aumeier et al., 2016). An early study even reported the detection of GTP islands in cells using an antibody raised against GTP-tubulin (Dimitrov et al., 2008). However, the specificity of the antibody was later called into question, since the lattice sites recognized by the antibody did not completely coincide with repair sites *in vitro*, so it probably does not recognize the presence of GTP at the lattice itself (de Forges et al., 2016; Théry and Blanchoin, 2021).

A puzzling question that remains to be addressed is: how does tubulin incorporation lead to rescue, often over prolonged periods of time, despite GTP hydrolysis? The GTP hydrolysis rate has not been measured at the lattice, but there is no apparent reason why it should be considerably different from the (relatively fast, around 0.2 s^{-1}; Roostalu et al., 2020) hydrolysis at the tip. It could be that lattice sites require constant tubulin turnover to maintain their rescue abilities. Since protofilament and seam mismatches cannot be fully repaired – unless the repair goes all the way from the mismatch site to the microtubule tip – this is entirely possible. Another possibility is the exchange of hydrolyzed GDP for fresh GTP at the exchangeable site without dissociation of the tubulin dimer from the lattice. This possibility is proposed for the

microtubule tip (Piedra et al., 2016) and, depending on the local lattice structure, the defect site may be sufficiently accessible. Last, GTP hydrolysis is triggered by the contact of an incoming dimer close to the exchangeable site of the dimer at the tip (Cleary and Hancock, 2021); hence, if no neighboring dimer is present at a microtubule defect towards the plus-end, perhaps GTP hydrolysis is not efficiently initiated. Therefore, the observation that rescue can occur several times at the same site over periods of time that go beyond the suspected time for GTP hydrolysis does not exclude the possibility that it is induced by incorporated GTP-tubulin, as has been suggested (Fees and Moore, 2019).

Beyond rescue, lattice defects are also related to switches from a growing to a depolymerizing microtubule state ("catastrophes"). The structure of the lattice close to the tip has long been suspected to influence the likelihood of a microtubule undergoing catastrophe – for instance, age-dependent catastrophe of microtubules has been associated with lagging curved protofilaments at the microtubule tips (Alexandrova et al., 2022; Duellberg et al., 2016). More recently, Rai et al. showed that microtubules with protofilament mismatches far from the tip undergo more frequent catastrophes (Rai et al., 2021): the authors consistently induced protofilament transitions by using different stabilizing agents during seed nucleation and microtubule elongation, respectively. Since these agents promote the formation of lattices with different protofilament numbers, Rai et al. could control the generation of mismatches. As catastrophes are more frequent in microtubules with protofilament mismatches, structural defects in the lattice seem to propagate over long distances until the tip and thus interconnect tip dynamics with lattice states.

Overall, the relation between lattice defects, lattice dynamics, and tip dynamics is likely complex since the presence of GTP-tubulin at a defect site may be countered by the destabilizing effect of the defect itself, which likely propagates beyond the defect site (Kim and Rice, 2019; Rai et al., 2021). These observations are in line with the idea that microtubules can function to transfer or integrate signals in a cellular context across space and time. Accordingly, microtubules that are exposed to mechanical stress are selectively stabilized over time (see above; Aumeier et al., 2016).

8. The role of MAPs in the regulation of the microtubule lattice

Microtubule properties in cells are tightly controlled by additional cellular factors, yielding highly diverse microtubule subsets whose characteristics are adapted to their particular functions. Accordingly, it is generally believed that the large family of MAPs, a heterogeneous class of proteins interacting with and structuring the microtubule cytoskeleton, is primarily responsible for regulating microtubule properties in cells, such as tip dynamics (Brouhard and Rice, 2018). Indeed, the tip-targeting MAPs XMAP215 and End-binding protein 1 (EB1; see below) can act in synergy to increase *in vitro* microtubule growth rates to physiological levels (Zanic et al., 2013).

While some MAPs regulate and selectively bind to microtubule tips, others decorate the lattice. In the context of a cell, MAPs can act in different ways to modify the microtubule lattice both under normal conditions and in response to mechanical stress (Fig. 5). First, MAPs can directly assist or inhibit tubulin incorporation into the lattice at sites of damage (Fig. 5B and D). Second, MAPs can stabilize or destabilize microtubule damage (Fig. 5B and D). Third, some MAPs can expand or compact the microtubule lattice (Fig. 5E). Fourth, MAPs can alter the network organization of microtubules, for instance, by cross-linking microtubules or promoting microtubule branching from existing filaments (Fig. 5F). Finally, MAPs can alter the microtubule lattice structure to allow microtubules to resist mechanical stress more efficiently, for instance by increasing lattice flexibility (Fig. 5G). Many of these mechanisms have been observed by using microtubule *in vitro* reconstitution assays that allow for a complete dissection of MAP function in a controlled environment. Therefore, there are well-described examples of how MAPs could perform these distinct functions. The importance of MAPs for microtubule tip dynamics has been thoroughly reviewed elsewhere (Akhmanova and Steinmetz, 2015; Goodson and Jonasson, 2018). Hence, here, we will focus on the impact of MAPs on the microtubule lattice.

8.1. Plus-end-tracking proteins at the lattice

Plus-end-tracking proteins (+TIPs) strongly accumulate at the more dynamic, growing plus tip of microtubules and usually promote microtubule growth and inhibit catastrophes (Akhmanova and Steinmetz, 2010, 2008a, 2008b). The first +TIP ever identified was Cytoplasmic LInker Protein of 170 kDa (CLIP-170), which appears as comet-like structures that are typical of +TIPs (Perez et al., 1999; Rickard and Kreis, 1990). Interestingly, the N-terminal part of the CLIP-170 protein, which contains the microtubule binding domain, was shown *in vitro* to be sufficient to promote microtubule rescue and nucleation (Arnal et al., 2004). Although the classification of CLIP-170 as a +TIP may suggest it is only present at the microtubule tips, CLIP-170 also localizes to the microtubule lattice (Bieling et al., 2008). Remarkably, it has been shown that CLIP-170 promotes microtubule rescue upon recognition of GTP islands that form at microtubule crossings following lattice repair (Fig. 5A; de Forges et al., 2016). Hence, CLIP-170 likely has an important additional function in preserving microtubules that are under mechanical stress by recognizing microtubules with sites of lattice repair and promoting their growth. As a more general rule for the description of +TIPs, this group of proteins can likely recognize GTP-tubulin or the structure of a microtubule containing GTP-tubulin, not only at the microtubule plus end but also at the lattice.

Another +TIP group that is involved in lattice dynamics is the CLIP-170-associated protein (CLASP) family. CLASPs were first described in yeast in a genetic screen for proteins that are important for spindle assembly (Pasqualone and Huffaker, 1994). Later, the CLASP family was described to have microtubule-stabilizing properties in mammalian cells (Akhmanova et al., 2001). The first microtubule *in vitro* reconstitution assays in the presence of the fission yeast CLASP revealed that this protein increases microtubule rescue frequency and decreases catastrophe frequency and microtubule disassembly rate (Al-Bassam et al., 2010), a function that is conserved across eukaryotes (Lawrence et al., 2020). More recently, the function of CLASP has been extended to the regulation of lattice dynamics. CLASP promotes tubulin incorporation into damaged microtubule lattices (Fig. 5B) and complete microtubule formation from partial protofilament structures, as shown in *in vitro* microtubule reconstitution assays (Aher et al., 2020). Furthermore, CLASP inhibits the softening of microtubules that is caused by mechanical damage following the application of a hydrodynamic flow (Aher et al., 2020). These properties of CLASP are in line with recent findings that it relocates from microtubule plus ends to the lattice upon compression of living cells and is directly involved in the mechano-stabilization of microtubules (Li et al., 2023a, 2023b). Indeed, in some cell types, such as plant cells, where the cytoplasm is squeezed between the cell membrane and the vacuole and microtubules are forced to bend around sharp cell edges, CLASP has also been suggested to play an essential role; it accumulates at highly curved cell edges and prevents microtubule catastrophe upon encounter with these structures (Ambrose et al., 2011). In summary, CLASP is a versatile microtubule stabilizer that becomes essential in the context of mechanical stress.

EB1, another +TIP, also localizes to sites of microtubule lattice repair following microtubule severing by the enzymes katanin or spastin (Vemu et al., 2018). In line with the function of CLIP-170 and CLASP in promoting microtubule rescue, EB1 localized to 74% of microtubule rescues in *in vitro* microtubule reconstitution assays in the presence of both spastin and EB1 (Vemu et al., 2018). EB3, a close homolog of EB1, was also shown to be specifically recruited to lattice repair sites, a feature that was dependent on the presence of free tubulin (Aumeier et al., 2016). In conclusion, it is likely that other +TIPs with microtubule-stabilizing characteristics will emerge as regulators or

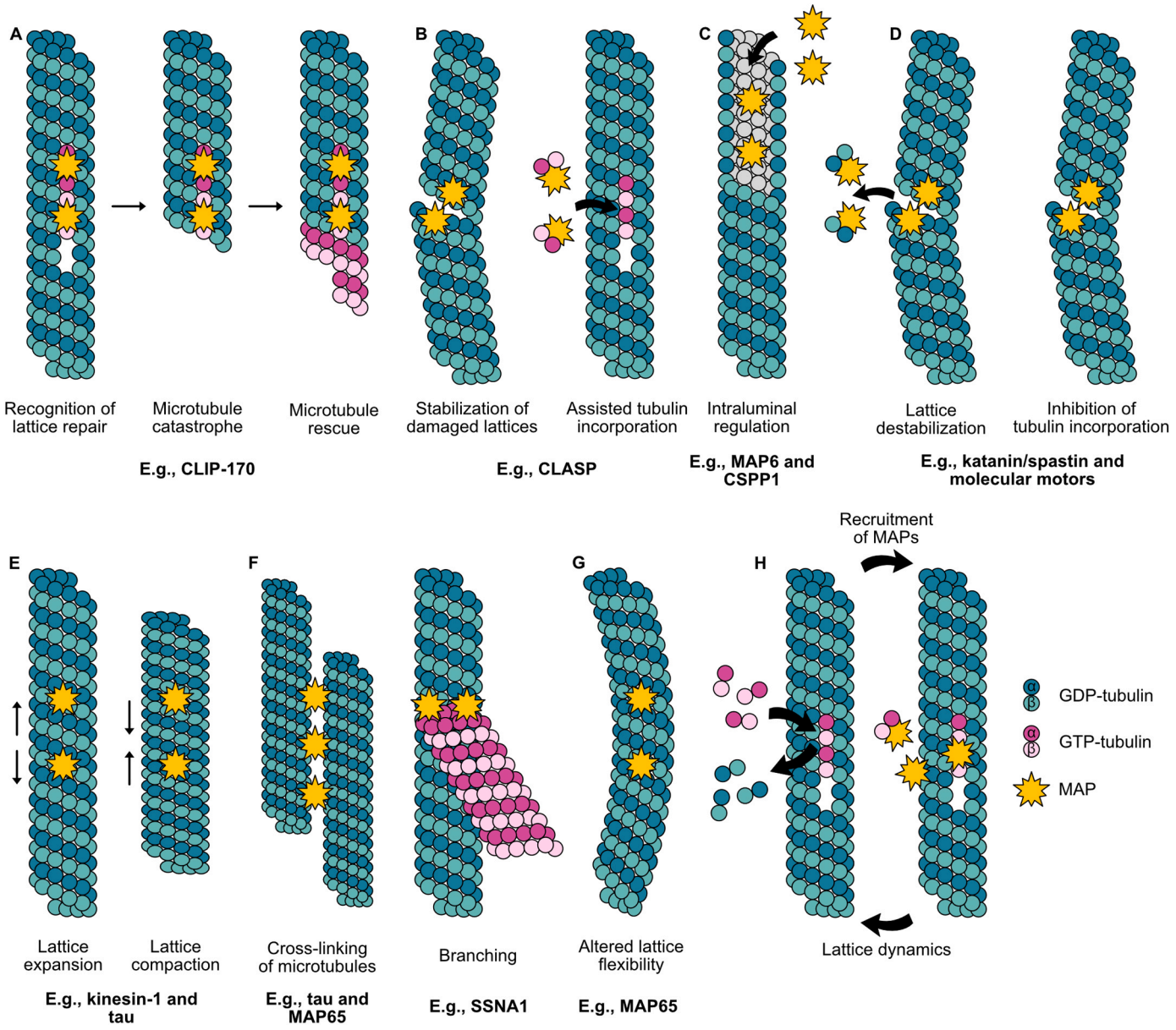

Fig. 5. Microtubule-associated proteins (MAPs) regulate the microtubule lattice in different ways. **(A)** Upon recognition of a lattice repair site, MAPs can bind and stimulate microtubule rescue from the repair site. **(B)** MAPs can have stabilizing properties on microtubule damage sites. They can stabilize microtubule damage (left) or directly assist tubulin incorporation into damage sites (right). **(C)** Some MAPs can enter the microtubule lumen and promote changes in the microtubule lattice, such as MAP6. **(D)** MAPs can have destabilizing properties on microtubule damage sites. They can destabilize microtubules by, for example, directly removing tubulin dimers (left) or inhibiting tubulin incorporation into damage sites (right). **(E)** MAPs can alter the compaction state of the lattice, either expanding it (left) or compacting it (right). **(F)** MAPs can affect microtubule network organization by, for example, promoting microtubule cross-linking (left) or branching (right) from existing filaments. **(G)** MAPs can modulate microtubule lattice flexibility, for instance, by increasing its capacity to bend. **(H)** There is presumably a two-way street between the recruitment of MAPs and lattice dynamics. Lattice dynamics recruits MAPs to sites of tubulin incorporation. MAPs can then, for example, promote further tubulin incorporation into those sites at the microtubule lattice, fueling lattice dynamics.

sensors of microtubule lattice dynamics due to the presence of GTP-tubulin along the microtubule lattice at sites of repair. However, CLIP-170, CLASP, EB1 and EB3 are the only examples described to date.

8.2. Intraluminal MAPs

Proteins that can enter the microtubule lumen, called intraluminal MAPs or microtubule inner proteins (MIPs), have also been linked to the microtubule response to mechanical stress and lattice dynamics (Fig. 5C). One example is MAP6, which has been described to confer resistance to drug- and cold-induced microtubule depolymerization (Delphin et al., 2012; Guillaud et al., 1998). Recently, MAP6 was shown to enter the microtubule lumen, provoke microtubules to coil into a left-handed helix and cause apertures in the microtubule lattice, possibly to promote the relief of mechanical stress (Cuveillier et al., 2020), a role that may be essential for the stability of neuronal microtubules. Whether the openings in the lattice generated by MAP6 get efficiently repaired by tubulin incorporation, however, is unclear.

Another intraluminal protein example is Centrosome and Spindle Pole associated Protein 1 (CSPP1), which is a vertebrate-specific MAP involved in cell cycle progression and spindle assembly (Patzke et al., 2005). Mutations in CSPP1 are associated with defects in ciliogenesis and are linked to a range of ciliopathies, such as Joubert syndrome, which is neurodevelopmental, or Meckel-Gruber syndrome, which

entails more general developmental problems (Akizu et al., 2014; Shaheen et al., 2014; Tuz et al., 2014). It has been shown in *in vitro* microtubule reconstitution assays that CSPP1 binds preferentially to microtubules that undergo growth perturbation or grow slower, resembling microtubule-stabilizing compounds such as taxanes (van den Berg et al., 2023). CSPP1 inhibits microtubule growth and shortening through two different domains and recognizes and stabilizes damaged microtubule lattices (van den Berg et al., 2023). There are good indications that CSPP1 also has a function in promoting repair of the recognized microtubule damage sites, although this has so far not been observed experimentally.

8.3. Severing enzymes

Microtubule severing enzymes, such as katanin and spastin, have also been shown to promote microtubule rescue by fostering lattice dynamics (Vemu et al., 2018). It is believed that spastin (and likely katanin as well) severs microtubules through destabilizing tubulin-tubulin interactions within the microtubule lattice by pulling the C-terminus of tubulin through its central pore and consequently generating mechanical force (Roll-Mecak and Vale, 2008). By using electron and TIRF microscopy, it has been shown that the two microtubule severing enzymes can not only sever complete microtubules, but also remove tubulin dimers out of the microtubule lattice (Fig. 5D), which is followed by incorporation of GTP-tubulin dimers at those spots and, hence, renewal of patches of the microtubule (Vemu et al., 2018). Microtubule rescues often happen at sites of severing-enzyme-dependent GTP-tubulin incorporation and severed microtubules are stable because of a high density of GTP-tubulin at their plus-ends. Therefore, katanin and spastin counterintuitively lead to microtubule amplification due to increased microtubule rescue frequency following tubulin dimer removal as well as the generation of severed microtubules with stabilized ends.

8.4. Molecular motors

Molecular motors are another class of proteins that can promote lattice dynamics. Molecular motors convert the energy from ATP hydrolysis into mechanical energy, which they use to move along microtubules by means of their motor domains and transport different cellular cargo (Gennerich and Vale, 2009). It has been suggested that molecular motors induce subtle conformational changes that reduce the energetic barrier for tubulin dimers to leave the lattice (Lecompte and John, 2022). In fact, unloaded molecular motors moving on a microtubule are sufficient to destabilize tubulin dimers within the microtubule lattice (Triclin et al., 2021). By attaching molecular motors to a cover glass in "gliding assays" or by attaching microtubules to a cover glass and allowing motors to walk on them in "motility assays", it has been shown that molecular motors can directly remove tubulin dimers out of the microtubule lattice (Fig. 5D) and rapidly cause microtubule destruction in the absence of free tubulin (Triclin et al., 2021). In the presence of free tubulin, however, these sites at the microtubule lattice are repaired and microtubules can withstand the action of molecular motors. It was later shown that knocking down kinesin-1 in cells reduces microtubule rescue frequency (Andreu-Carbó et al., 2022). Accordingly, expressing a constitutively active form of kinesin-1 increased microtubule rescue frequency and the number of microtubule lattice repair sites (Andreu-Carbó et al., 2022). Hence, similarly to severing enzymes, the destruction of microtubules by molecular motors counterintuitively allows for microtubule stabilization over time thanks to the incorporation of fresh GTP-tubulin into the lattice. This is an interesting mechanism by which cells may stabilize intensively used microtubule transport tracks and ensure their long-term mechanical stability.

The study of molecular motors in *in vitro* reconstitution assays serves as a cautionary tale for researchers that want to employ this method. First, molecular motors that are in a strong binding state and do not move on microtubules promote microtubule stabilization, as shown by a reduced shrinkage rate of microtubules that is accompanied by a lattice expansion of around 1.6% in the presence of kinesin-1 (Fig. 5E; Peet et al., 2018). Second, Taxol, a commonly used chemical in *in vitro* reconstitution assays, seems to confer some degree of mechanical resistance against molecular motors to microtubules. For instance, Taxol-stabilized microtubules in gliding assays have been shown to only split into protofilament bundles in the presence of surface-bound kinesin-1 (Vandelinder et al., 2016) and to slowly disintegrate from the ends in a kinesin-1 density- and sliding velocity-dependent manner (Dumont et al., 2015). Accordingly, Taxol-stabilized microtubules in the same assays were shown not to be destroyed by motors as opposed to dynamic microtubules (Théry and Blanchoin, 2021; Triclin et al., 2021). Taken together, these different observations show that MAPs can have multifaceted impacts on the structure of the microtubule lattice depending on the MAP and the microtubule lattice state. Hence, one should be cautious when choosing experimental parameters for *in vitro* reconstitution assays.

8.5. MAPs that are intrinsically disordered and can undergo phase separation

Intrinsically disordered MAPs have also been implicated in the regulation of the microtubule lattice (Volkov and Akhmanova, 2023). Tau, for instance, is a neuronal MAP whose alteration of function has been associated with neurodegenerative disease, including Alzheimer's (Ballatore et al., 2007). Although tau is a well-studied bundling MAP (Fig. 5F), there is an ongoing debate regarding its properties (Baas and Qiang, 2019). On the basis of *in vitro* reconstitution assays and studies conducted in non-neuronal cells, tau has been generally regarded as a microtubule stabilizer; for instance, the injection of tau in fibroblast cells, which do not normally contain the protein, leads to microtubule stabilization (Drubin and Kirschner, 1986). In neuronal cells, however, tau localizes to the more labile microtubule regions (Black et al., 1996; Kempf et al., 1996). Tau is likely not a genuine microtubule destabilizer, but rather a less strong microtubule stabilizer that can be competitively displaced by stronger microtubule-stabilizing MAPs. Thus, these observations highlight the strength as well as the limitations of *in vitro* reconstitution assays, which should ideally always be supported by data obtained in cells.

Regardless of the overall role of tau in conjunction with other MAPs in cells, *in vitro*, tau modifies the microtubule lattice in different manners. For instance, tau specifically stabilizes microtubule defects, as seen by an increase in microtubule protofilament number and the number of lattice defects in the presence of the protein (Prezel et al., 2018). Furthermore, tau forms condensates (also called envelopes) on the microtubule lattice that are selectively permeable barriers, *i.e.*, these condensates are permeable to certain proteins but not to others (Tan et al., 2019). Interestingly, such tau condensates compact the lattice structure within them (Fig. 5E), while lattice extension promotes tau condensate disassembly (Siahaan et al., 2022). Importantly, tau condensates do not form on microtubules polymerized with the slowly hydrolysable GTP analogue GMPCPP (Tan et al., 2019), perhaps because tubulin is in an expanded conformation in those lattices and tau has been shown to have a higher affinity to GDP lattices (compacted) in comparison to GMPCPP lattices (expanded) (Duan et al., 2017). MAP2, a member of the tau family, is also able to form condensates that alter tubulin spacing within the lattice (Dehmelt and Halpain, 2004; Siahaan et al., 2022). In line with these findings, tau and MAP2 restore bending stiffness of microtubules that were treated with Taxol, which increases microtubule flexibility (Dye et al., 1993). Such microtubule-compacting properties of tau and MAP2 make them perfect candidates to participate in neuronal mechanosensing, but the exact function of such lattice compaction in cells is so far not clear.

Abl family kinases are another group of proteins that are intrinsically disordered and have been associated with the regulation of the

microtubule lattice. Abl kinases phosphorylate key proteins to regulate the cytoskeleton and, with that, have an important function in cell motility, adhesion, and morphogenesis, among others (Bradley and Koleske, 2009). In agreement with Abl's function in regulating the cytoskeleton, CLASP has been identified to act downstream of Abl kinases in *Drosophila* and Abl2 has been shown to directly bind to and regulate microtubules (Hu et al., 2019; Lee et al., 2004). Recently, in *in vitro* microtubule reconstitution assays, it was then shown that Abl2 undergoes liquid-liquid phase separation, promotes microtubule nucleation, recognizes regions of microtubule lattice damage, and promotes their repair by recruiting tubulin, in addition to supporting microtubule rescue (Duan et al., 2023). It is worth mentioning that experiments with Abl2 were performed with concentrations in the micromolar range, whereas other MAPs already have a clear impact on microtubule lattice repair at lower concentrations, *e.g.*, CLASP in the nanomolar range (Aher et al., 2020). Kinases are known regulators of the cytoskeleton and it is not completely unexpected that they would act on the microtubule lattice as well. It remains to be seen whether other kinases such as Cyclin-dependent, Aurora or Polo-like kinases can also directly or indirectly regulate the microtubule lattice.

Members of the MAP65/PRC1/Ase1 family, an additional group of intrinsically disordered MAPs that function in cross-linking microtubules (Fig. 5F) and have microtubule-stabilizing properties (Burkart and Dixit, 2019; Ho et al., 2012; Sahu et al., 2023; Stoppin-Mellet et al., 2013; Walczak and Shaw, 2010), have also been found to regulate the microtubule lattice (Portran et al., 2013a, 2013b). MAP65–1 and Ase1 increase microtubule flexibility in *in vitro* microtubule reconstitution assays in both single and bundled microtubules (Fig. 5G; Portran et al., 2013a, 2013b). Interestingly, MAP65–4, one of the nine plant MAP65 homologs, is not able to increase microtubule flexibility (Portran et al., 2013a, 2013b). Therefore, except for MAP65–4, some members of the MAP65 family likely facilitate the formation of tridimensional microtubule arrays in cells by allowing them to assume more curved configurations. However, the exact mechanism by which microtubule lattice flexibility is achieved through MAP65/Ase1 function is unknown. It would also be interesting to find out why different MAP65 members have different properties in modifying microtubule mechanical properties and what is the physiological relevance of these modifications.

Interestingly, the +TIPs EB3 and CLIP-170 have recently been found to undergo phase separation and form droplets not only in solution but also along microtubule lattices, a phenomenon that concentrates tubulin, drives microtubule growth and reduces the frequency of microtubule depolymerization events (Maan et al., 2023; Meier et al., 2023; Miesch et al., 2022; Song et al., 2023). Importantly, phase separation leads to locally high concentrations of not only tubulin but also MAPs, thus creating a micro-environment with completely different biochemical characteristics (Volkov and Akhmanova, 2023). Finally, it is likely that the phase separation of +TIPs also contributes to and plays a role in the above-mentioned microtubule rescue observed upon the recognition of lattice repair sites by CLIP-170 and EB3.

8.6. Microtubule-branching MAPs

There is one known example of a peculiar MAP that can directly alter microtubule organization by branching microtubules: Sjögren's syndrome nuclear autoantigen-1 (SSNA1). This MAP is a binding partner and suggested targeting factor of spastin (Errico et al., 2004) that has been linked to disease in humans when altered (Ramos-Morales et al., 1998). SSNA1 is involved in the recognition of microtubule lattice defects, has microtubule-stabilizing properties (Lawrence et al., 2021) and promotes microtubule branching by splitting protofilaments from a main microtubule that give origin to new filaments (Basnet et al., 2018). *In vitro* microtubule reconstitution assays have shown that SSNA1 reduces growth, shrinkage and catastrophe rates and supports microtubule rescue (Lawrence et al., 2021). Additionally, SSNA1 can antagonize the severing of microtubules by spastin (Lawrence et al., 2021). Thus, SSNA1 acts as a stabilizer of dynamic microtubules and as a damage-control mechanism to contain microtubule lattice defects.

8.7. MAPs that control protofilament number

Although the microtubule protofilament number varies in cells, most of the observed microtubules have 13 protofilaments (see above). Hence, how do cells achieve this feat? Apart from the γ-tubulin ring complex that nucleates microtubules in a regular 13-protofilament arrangement, some MAPs can regulate microtubule protofilament number. One example is doublecortin, which is expressed in developing neurons and is able to nucleate microtubules *in vitro* with 13 protofilaments (Moores et al., 2004). It binds to and stabilizes microtubules with a 13-protofilament structure by having a high affinity for microtubule lattices with this configuration (Bechstedt and Brouhard, 2012). Another example is EB1, which has been shown in both humans and yeast to promote the formation of microtubules with the regular 13-protofilament configuration and a lower rate of protofilament transitions (Des Georges et al., 2008; Vitre et al., 2008). Mal3, the EB1 homolog of *S. pombe*, also promotes a high proportion of the A-lattice configuration (Des Georges et al., 2008). Therefore, there are multiple levels at which the cell can control the microtubule lattice structure.

9. Conclusion

Despite the advances that have been made (mostly) in the past years, the microtubule lattice remains underexplored compared to the tip. In particular, our understanding of the dynamic properties of the lattice is still in its infancy. The fact that it is difficult to visualize lattice dynamics experimentally is probably the reason why they have been overlooked for a long time, and, consequently, we are still far from a complete picture. A major open question is how lattice dynamics compare to tip dynamics. In contrast to the latter, so far it is not possible to visualize tubulin incorporation live; all we see are individual snapshots. To understand its time-dependence – which is rather essential for dynamic processes – we will need technical advances that facilitate live imaging. Related to this, another intriguing question is whether there is a genuine turnover of tubulin dimers – involving continuous loss and incorporation in the absence of other cellular factors – such that the entire microtubule gets renewed if one waits long enough. Furthermore, the role of different defect types in lattice dynamics remains to be explored and studying it will require high-resolution approaches, such as cryo-electron microscopy.

Similar to tip dynamics, lattice dynamics are likely a consequence of a multitude of different factors, and it is difficult to dissect these factors in complex intracellular environments. *In vitro* assays will be a valuable tool to understand the relative contributions of associated proteins, mechanical constraints, and other elements of the intracellular space. Certain intracellular microtubules may require protective mechanisms to avoid their quick turnover under mechanical stress or other challenging impacts: in particular, maintaining their tubulin code may be essential for long-lived microtubules, which could get erased by continuous tubulin loss and incorporation.

Another important aspect that deserves further attention is the interplay between lattice dynamics and tip dynamics. In particular, it will be interesting to understand how GTP hydrolysis at incorporation sites along the lattice compares to the tip, which will help shed light on the relation between lattice dynamics and rescue. In addition, long-range effects that propagate along the lattice are a promising candidate for unraveling how lattice states contribute to the initiation of catastrophes.

To comprehend the regulation of lattice morphology and lattice dynamics in cells, one of the key points will be to study the impact of MAPs on the lattice. Accordingly, recent studies highlight that MAPs have differential effects on lattice dynamics. In light of these findings, it is likely that lattice dynamics, similar to other microtubule properties,

are highly regulated by MAPs. In turn, MAPs are recruited to sites of lattice damage or GTP-tubulin incorporation (Fig. 5H). Thus, we have started to uncover a number of different ways in which MAPs influence the lattice, and it will require concerted *in vitro* and cell-based approaches to understand the feedback loop between lattice dynamics and MAP recruitment (Fig. 5H) and how it contributes to the overall regulation of the dynamics and organization of the microtubule cytoskeleton.

As a more philosophical point of view, perhaps the human mind tends to see things as homogeneous by default and, thus, to think that biological structures are likely to reach homogenization. Indeed, that is our empirical observation of the world – for instance, flower shape does not significantly vary between or within individuals of the same species. However, it is becoming increasingly clear that variability, in the form of stochasticity or heterogeneity, is the fuel for change (to generate new cell fates, cell shapes, among others). Paradoxically, variability is also the fuel for dynamic stability, which is a form of resilience. Lattice dynamics are part of a larger set of studies exemplifying how incoherent feed-forward loops generate oscillations that act as a shield against external perturbations and allow organisms to adapt quickly following changes in their environment. Thus, it is useful for a cell in ever-changing conditions to have microtubules that accommodate heterogeneity, which reflects their resilience and versatility.

Declaration of Competing Interest

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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