Review

Alessandro Falconieri*, Allegra Coppini and Vittoria Raffa*

Microtubules as a signal hub for axon growth in response to mechanical force

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Abstract: Microtubules are highly polar structures and are characterized by high anisotropy and stiffness. In neurons, they play a key role in the directional transport of vesicles and organelles. In the neuronal projections called axons, they form parallel bundles, mostly oriented with the plus-end towards the axonal termination. Their physico-chemical properties have recently attracted attention as a potential candidate in sensing, processing and transducing physical signals generated by mechanical forces. Here, we discuss the main evidence supporting the role of microtubules as a signal hub for axon growth in response to a traction force. Applying a tension to the axon appears to stabilize the microtubules, which, in turn, coordinate a modulation of axonal transport, local translation and their cross-talk. We speculate on the possible mechanisms modulating microtubule dynamics under tension, based on evidence collected in neuronal and non-neuronal cell types. However, the fundamental question of the causal relationship between these mechanisms is still elusive because the mechano-sensitive element in this chain has not yet been identified.

Keywords: microtubule; traction force; axon growth

1 Introduction

Mechanical force induces axon outgrowth and stimulates neuronal maturation, known as “stretch-growth” (SG) (Smith 2009; Suter and Miller 2011). Since the 1980s, many groups have demonstrated that axonal elongation is promoted in response to mechanical force in many cellular models: neuron-like cell cultures (Dennerll et al. 1989; Smith et al. 2001; Raffa et al. 2018; Wang et al. 2020), chick dorsal root ganglion (DRG) neurons (Bray 1984), chick sensory neurons (Dennerll et al. 1989; Lamoureux et al. 2010; Zheng et al. 1991), chick forebrain neurons (Chada et al. 1997; Fass and Odde 2003), rat hippocampal and cortical neurons (Abraham et al. 2018; Magdesian et al. 2016; Smith et al. 2001), rat retinal ganglion cell (RGC) neurons (Steketee et al. 2011), rat DRG neurons (Katiyar et al. 2019; Loverde and Pfister 2015; Pfister et al. 2004; Wang et al. 2020), rat spinal neurons (Katiyar et al. 2019), mouse cortical neurons (Kilinc et al. 2014), mouse hippocampal neurons (De Vincentiis et al. 2020, 2021; Falconieri et al. 2022, 2023), and neural precursor cells (Dai et al. 2019; de Vincentiis et al. 2022).

Multiple models and different methodologies used to generate the forces gave similar results, suggesting that mechanical force is a well-conserved mechanism that induces axon outgrowth and neuron maturation. AxonSeq revealed 907 differentially expressed genes between the stretched and unstretched axons. Gene ontology enrichment analysis (GOEA) highlighted that the most dysregulated processes are related to the transport of organelles (Golgi apparatus, mitochondria, endoplasmic reticulum), vesicles (lysosome, endosome, late endosome), cytoskeleton organization (cytoskeleton, the microtubule organizing centre), and synaptic remodelling (Falconieri et al. 2023). These data are in line with previous reports, showing that SG is coupled to the addition of lipids, proteins, vesicles, and organelles in the stretched axons (Chowdary et al. 2019; Falconieri et al. 2023; Lamoureux et al. 2010; Miller and Sheetz 2006).

In order to explain the mechanisms behind this process, our research group recently proposed a model by which the traction force induces the stabilization of axonal microtubules (MTs), and the resulting increase in MT density in the axon induces an accumulation of vesicles and -organelles, such as endosomes, endoplasmic reticulum and mitochondria, whose transport is MT-dependent (Falconieri et al. 2023) (Figure 1). According to this model, the local increase in concentration of vesicles and organelles promotes the formation of the translational platform (Cioni et al. 2019) and the activation of local translation (Falconieri et al. 2023).

***Corresponding authors: Alessandro Falconieri and Vittoria Raffa**, Department of Biology, Università di Pisa, Pisa, 56127, Italy,
E-mail: alessandro.falconieri@biologia.unipi.it (A. Falconieri), vittoria.raffa@unipi.it (V. Raffa). https://orcid.org/0000-0001-6166-1214 (A. Falconieri), https://orcid.org/0000-0002-4289-9937 (V. Raffa)
Allegra Coppini, Department of Biology, Università di Pisa, Pisa, 56127, Italy

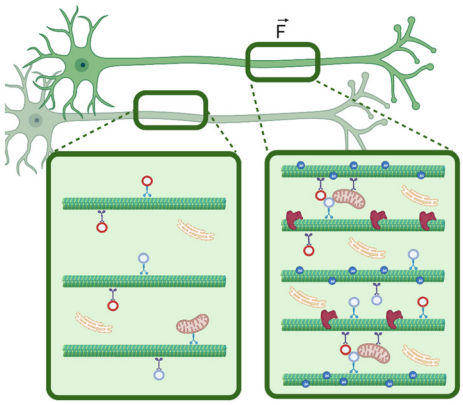

Figure 1: A traction force oriented from soma to tip induce accumulation of MTs in the axon shaft by stabilizing them (the scheme shows acetylated MTs or microtubule-binding proteins). MT-associated transport of mitochondria, endoplasmic reticulum, vesicles (among which late endosomes) facilitates the assembly of translation machinery. Local translation and axonal transport promote the process of axon elongation and synaptic maturation.

Unfortunately, the mechanism responsible for mechanosensing and signal transduction has not yet been found. Microtubules have recently been proposed as a candidate for sensing and transducing pathways activated in response to force (Hamant et al. 2019). In this review, we will overview the evidence in support of this hypothesis from *in vitro* and *in vivo* studies.

2 A brief overview of microtubule structure

MTs have a polar structure made up of αβ-tubulin heterodimers arranged in (most commonly) 13-protofilaments (Tilney et al. 1973). The interactions between adjacent dimers occur through lateral and longitudinal non-covalent bonds. The two subunits bind GTP, but only in β-tubulin is GTP hydrolysed following polymerization. The head-to-tail arrangement results in α-tubulin exposed at one end (minus end), and β-tubulin exposed at the other (plus end). Both ends show dynamic instability (i.e. alternating phases of growth and shrinkage) but the plus end typically grows faster than the minus end (Mitchison and Kirschner 1984). *In vivo*, the minus end is usually stabilized by capping proteins (Kollman et al. 2011).

The accepted model suggests that, after a new dimer has been incorporated into the growing microtubule, GTP hydrolyses after a delay that generates a "GTP cap" in the plus end that appears to be responsible for stabilization (Jánosi et al. 2002). The transition from GTP-tubulin to GDP-tubulin in the cap likely destabilizes lateral bonds, as GTP-tubulins are relatively straight, while GDP-tubulins tend to curve, thus causing plus end instability. Interestingly, in line with this model, growing MTs have blunt ends, while shortening MTs have flared ends (Mandelkow et al. 1991).

In axons, MTs are mainly localised in the shaft where they form parallel bundles. By forming polarized tracks, with the *faster-growing* plus ends preferentially pointing towards the growth cone, and the *slower-growing* minus ends pointing towards the soma (Heidemann et al. 1981). MTs also act as a structural backbone for axonal transport, including the trafficking of vesicles and organelles, powered by motor proteins (Cason and Holzbaur 2022) (Figure 1), i.e., kinesins and dynein motor proteins, whose primary role is anterograde and retrograde axonal transport (Guillaud et al. 2020). Axonal MTs are part of a highly dynamic cytoskeletal network, in which the periodic actin-spectrin network helps to maintain the axon diameter and MT stability (Krieg et al. 2017; Qu et al. 2017).

3 Microtubule-stabilizing agents

The increase in MT growth depends on MT turnover, which consists of a balance between MT polymerization and depolymerization rates, or MT stabilization. The more stable a microtubule is, the higher its flexural rigidity will be (Mickey and Howard 1995). Microtubule-stabilizing agents increase this rigidity by up to a factor of two (Mickey and Howard 1995).

MT stability and stiffness can be also increased by incorporating tubulin-GTP into the cap of growing MTs end (Vale et al. 1994), by the binding microtubule associated proteins (MAPs) such as Tau and MAP2 (Mickey and Howard 1995) or by post-translational modifications of tubulin (Bär et al. 2022; Sánchez-Huertas and Herrera 2021). For instance, MTs assembled *in vitro* in the presence of tau show minimal dynamic instability and grow along linear trajectories (Peck et al. 2011). Transglutaminase-catalysed polyamination of neuronal tubulins contributes to MT stability in axons (Song et al. 2013). Detyrosination of stabilized MTs produces disassembly-resistant protofilaments by blocking kinesin-13-mediated microtubule depolymerization (Peris et al. 2009). Some modifications (such as Lys40Ac) increase MT flexibility, preventing the loss of rigidity due to repetitive bending, thus prolonging their half-life (Xu et al. 2017) and making them more mechanically stable (Xu et al. 2017). The increase in MT stiffness generally increases the kinesin transport rate (Vale et al. 1994). There are also enzymatic processes which causes the disassembly of MTs, for example, polyglutamylation increases microtubule severing (McNally and Roll-Mecak 2018).

4 Microtubules as load-bearing elements

Studies carried out since Ingber and colleagues' seminal work in the 1980s have focused on the cytoskeleton as the key structure to maintaining a level of tension, namely "tensional homoeostasis", proposing a model referred to as tensional model or "tensegrity" (Ingber 1993, 1997; Ingber et al. 1981; Wang et al. 1993). In architecture, tensegrity asserts that opposite tension and compression elements balance each other and promote stabilization of the whole structure (Fuller 1961). In biology, tensegrity provides a model by which the cell cytoskeleton is stabilized by a tensile pre-stress that is generated and maintained through the balance between the contractile forces generated by actomyosin filaments and the resistance by compression-bearing elements, such as microtubules (Ingber 1993, 1997, 2003, 2006; Ingber et al. 1981; Ingber and Jamieson 1985).

Tensegrity provides a theoretical explanation as to why the cytoskeleton is extremely responsive to perturbations and, if force is applied to one element, the stress is re-distributed in the entire structure, without breaking. Interestingly, according to this model, mechanical disturbances can be transmitted over long distances, theoretically also from a distal point of adhesion to the nuclear envelope (Schneider et al. 2023). However, cells also bear elements to selectively propagate stress in specific directions and to block propagation through other parts, thus modulating tensional homoeostasis differentially in specific spatial domains (Blumenfeld 2006).

All these features have crucial implications for efficient signal mechanotransduction, the process by which a cell or a living organism converts a mechanical signal into a chemical, electrical or biochemical response (French 1992; Watson 1991). A crucial question in biology is which elements of the cytoskeleton are involved in converting mechanical force into biochemical signalling. Historically, the myosin motors and actin filaments have been thought to be involved in mechanosensing, force transduction and transmission (Cope et al. 1996; Lecuit et al. 2011; Pollard and Cooper 2009; Schutt and Lindberg 1992; Theriot and Mitchison 1991). However, the possible role of MTs as force sensors is also emerging.

5 There is no direct evidence that microtubules are mechanosensitive *in vivo*

Due to their physical properties, MTs would be an ideal candidate for mechanosensing as they are characterized by high stiffness and mechanical anisotropy (Hawkins et al. 2010; Kis et al. 2002; Tuszyński et al. 2005). Hamant and colleagues explored this hypothesis by building models on plant cells subjected to turgor pressure (Hamant and Haswell 2017; Hamant et al. 2019). The idea that MTs can align in the direction of force is not recent. In fact, in the 1960s it was postulated that cortical MTs are able to orient themselves in the direction of maximum stress (Green and King 1966) and this was confirmed by *in vitro* observations (Kabir et al. 2014).

Plant cells are an ideal model for this type of study, and many reports have demonstrated that their MTs are easily re-oriented in order to counteract an external stress (Colin et al. 2020; Hamant et al. 2008; Hejnowicz et al. 2000; Hervieux et al. 2016; Jacques et al. 2013; Robinson and Kuhlemeier 2018; Sampathkumar et al. 2014; Torrino et al. 2021; Verger et al. 2018; Williamson 1990; Zhao et al. 2020). The evidence that MTs align in the direction of maximum stress is in line with Ingber's tensegrity model, according to which cell cytoskeleton rapidly and adaptively remodel in response to forces (Ingber et al. 2014). Experimental observations using many different models have been collected in this direction. In fact, the increase in tensional homeostasis at the focal adhesion (FA) points in non-neuronal cells has been found to increase the MT polymerization rate (Kaverina et al. 2002; Putnam et al. 2001). Pulling the *Aplisia* growth cone, MTs were found to spend less time in depolymerization in the direction of force generation (Lee and Suter 2008). Similarly, the generation of tension at the kinetochore was found to increase the MT number (King and Nicklas 2000). However, there is still no theoretical framework explaining why force induces changes in MT dynamics.

6 Speculating about the mechanisms modulating MT dynamics under tension

Tension-dependent control of MT growth could occur through a direct mechanism that modulates the microtubule components (*cis-acting*) or an indirect mechanism, where force regulates the activity of MT-binding proteins (*trans-acting*). Evidence of a direct mechanism comes from classical micro-manipulation experiments: a traction force of 0.5–2 piconewton (pN) decreases the probability that growing MTs shrink and increases the probability that short MTs resume growth (Franck et al. 2007).

However, MTs have a Young's modulus (i.e., elastic modulus) of about 1.2 gigapascal (GPa), and a pN force can cause bending, sliding or gliding between filaments, rather than inducing a strain in the individual MT. Nevertheless,

the stress can also be stored in the MT lattice and change the MT dynamics. Interestingly, simulations provide a mechanism for load-dependent acceleration of MT assembly by facilitating lateral bond formation between GTP-tubulin dimers, thereby promoting wall closure of the protofilament (Gudimchuk et al. 2020) (Figure 2A). The maximum acceleration of MT assembly induced by a traction force was estimated to be two-fold, which is in line with the increase in elongation rate found in SG (De Vincentiis et al. 2020; Raffa et al. 2018).

Force could also indirectly influence MT stability by modulating the activity of MAPs. The application of 1 pN force to XMAP215, an enzyme that catalyses MT growth by adding tubulin dimer to the MT plus end, was found to increase the MT growth rate (Trushko et al. 2013). The authors suggest that application of the force, if directed towards the plus-end, could increase MT growth, possibly by increasing the activity of the enzyme. In fact, force likely modulates the interaction time between the MT and the MAP, increasing the probability of their association (Ciudad and Sancho 2005; Molodtsov et al. 2016) (Figure 2B). In agreement with this idea, tension appears to stabilize the attachment of Stu2 (XMAP215 family member) (Miller et al. 2016) to MTs. Another possibility is that the force induces a conformational change that stimulates enzyme activity (Gumpp et al. 2009) (Figure 2C).

Although it is not known whether force can promote MT post-translational modification, mechanical signals may be able to stabilise MT by promoting glutamine-dependent MT glutamylation (Torrino et al. 2021) or microtubule acetylation (Seetharaman et al. 2022).

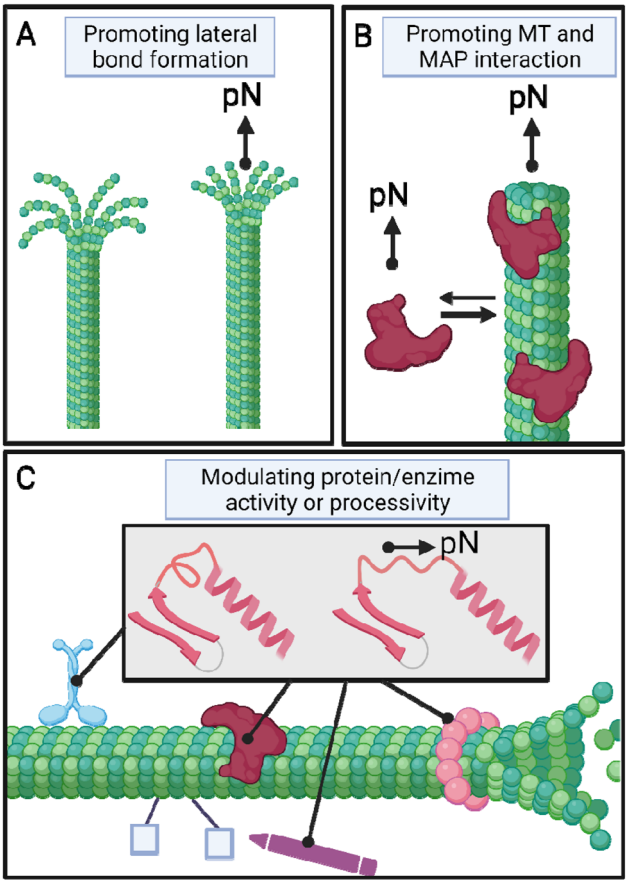

Figure 2: A traction force in the order of piconewton changes MT dynamics. (A) Force promotes the closure of the protofilament wall by promoting lateral tubulin dimer bonds. (B) Stretching applied along MTs changes the association/dissociation constants between MTs and MAPs. (C) Force acts in trans on a MT-binding protein (a motor protein, a tip protein, a MAP or enzymes responsible for writing the tubulin code), stretching a protein domain, changing the protein structure and the protein binding/activity/processivity.

7 Axon growth is force-dependent

From a biophysical perspective, axon outgrowth *in vivo* depends on the forces generated in the growth cone and in the axon shaft from cytoskeletal components (Ghose and Pullarkat 2023; Raffa 2022). In the growth cone, the acto-myosin contraction at the adhesion points, generates a contractile force that pulls the axon shaft (Lamoureux et al. 1989). Similarly, the axon shaft pulls the growth cone through a contractile force generated by the axonal actin cortex (de Rooij et al. 2018) that is organized in longitudinal trails and in cortical rings regularly spaced by spectrin. This axonal contractile force is partially counteracted by other forces, such as those generated by MT assembly or sliding. A stronger contractile force generated in the growth and a weaker contractile force generated in the axon shaft typically results in tip advance (Miller and Suter 2018).

8 Axon stretching promotes MT stabilization

When an exogenous mechanical force is applied to axons or neurites, they elongate and the density of axonal MTs increases (De Vincentiis et al. 2020; Falconieri et al. 2022, 2023). The structure of microtubules seems to be deeply involved in SG since mutants for α or β-tubulin that lack stable *in vivo* 13-protofilament MTs (but still forms 11-protofilament MTs) do not respond to the stretching (Falconieri et al. 2023).

Mechanical forces applied to axons lead to an increase in the ratio between acetylated and tyrosinated α-tubulin (Falconieri et al. 2023), which is generally associated with an increase in MT stabilization (Witte et al. 2008). Stabilization

mediated by acetylation and de-tyrosination of MTs are involved in cellular mechanotransduction. In fact, mechanotransduction mediated by MT acetylation occurs in mouse and rat cardiomyocytes (Coleman et al. 2021; Swiatlowska et al. 2020), in rat flexor digitorum brevis (FDB) fibres (Coleman et al. 2021), in rat astrocytes (Seetharaman et al. 2022), in Drosophila sensory neurons (Yan et al. 2018), in *C. elegans* sensory neurons (Teoh et al. 2022), and in mouse DRG neurons (Morley et al. 2016). On the other hand, de-tyrosination occurs in rat, mouse and human cardiomyocytes (Caporizzo et al. 2020, 2022; Chen et al. 2018, 2020; Kerr et al. 2015; Robison et al. 2016; Swiatlowska et al. 2020) and in rat FDBs (Kerr et al. 2015). MT stabilization induced by force seems to be necessary to induce stretch-growth. For instance, Zheng and colleagues found that vinblastine, a drug that disrupts MT assembly, strongly inhibits the formation of new neurites in response to force in chick DRG neurons (Zheng et al. 1993). Furthermore, electron microscopy studies have shown that when treated with vinblastine, stretched neurites presented short MTs in the axon shaft, usually not axially oriented (Zheng et al. 1993). Similarly, our group demonstrated that drug treatment with nocodazole, a MT depolymerizing agent, prevents tension from inducing axon elongation. Conversely, MT-stabilizing agents had no effects (De Vincentiis et al. 2020).

9 MT coordinates vesicle transport

MTs act as “tracks” on which vesicles can travel powered by dynein and kinesin molecular motors. The better characterised transported cargos are: the late endosomes (LEs), which are transported retrogradely by dynein (Villari et al. 2020) and anterogradely by kinesin 1 (Jongsma et al. 2020); synaptic vesicles, which are classified as synaptic vesicle precursors (SVPs) and dense-core vesicles (DCVs), which move predominantly in an anterograde direction powered by kinesin 3 (Guedes-Dias et al. 2019); signalling endosomes, which are primarily transported retrogradely by multiple dynein regulatory proteins (Schmieg et al. 2014).

Evidence collected in the last decade by several groups demonstrate that mechanical forces alter vesicular trafficking, generally leading to an accumulation of vesicle and organelles in the axon shaft. Stretch was found to increase the probability of the active motion of large DCV in Aplysia neurites (Ahmed and Saif 2014). In the same study, mechanical strain was applied *in vivo* in Drosophila neurons, leading to a global accumulation of SVPs. Interestingly, this study demonstrated that, when neurons are stretched, the activity of single molecular motors increases, giving rise to greater vesicle motion. The same *in vivo* model was used by Siechen and colleagues to demonstrate that mechanical tension induces accumulation and clustering of SVPs at presynaptic terminals (Siechen et al. 2009). The accumulation of SVPs was also found in hippocampal neurons in response to force generation (Falconieri et al. 2023).

The accumulation of vesicles promoted by mechanical force is likely to be associated with the accumulation of MTs. Several regulatory mechanisms can contribute to this modulation. First, considering that axonal MTs are the major cytoskeleton substrate for cargo transport, an increase of vesicles in the axon is very likely when the MT density increases. MT stabilization induced by tension may also play a role in this process. For instance, the preference of kinesin-1 for stable MTs may contribute to kinesin-1-mediated cargo recruitment and movement on MTs (Farías et al. 2015). In fact, MT-stabilizing modifications such as α-tubulin acetylation at Lys-40 or tubulin detyrosination increase the binding of kinesin 1 to MTs, and the motility of kinesin 1 and the transport (Konishi and Setou 2009; Reed et al. 2006), resulting in vesicle accumulation (Godena et al. 2014; Mohan et al. 2019). SG was found to induce the accumulation of synapsin SNN-1 positive SVPs and the related motor protein kinesin 3 (UNC-104) in *C. elegans* neurons. However, no difference can be detected in axon length and UNC-104 levels between control and stretched axons when neurons are treated with the MT-destabilizing agent Nocodazole (Falconieri et al. 2023). Additionally, *C. elegans* mutants harbouring the 11-protofilament MTs (rather than the more stable 13-protofilament MTs) do not show either vesicle or motor protein accumulation in response to force and do not respond to SG, thus indicating that MT stabilization is probably necessary to modulate vesicle transport in response to force (Falconieri et al. 2023).

Another potential regulatory mechanism is associated with force acting *in trans* on motor proteins, by changing their interactions with MTs (Figure 2C). Specifically, when a motor protein is manipulated with a backward force and the applied load exceeded the stall force, a backward movement is observed; this mechanical strain-dependent direction-switching behaviour has been described for many molecular motors, such as dynein (Gennerich et al. 2007; Shingyoji et al. 2015), kinesin-1 (Carter and Cross 2006) and myosin-V (Gebhardt et al. 2006). Indeed, in a stretched axon, a traction force toward the microtubule plus end can induces backward stepping by dynein that can walks backward toward the microtubule plus end, if the load is above its stall force of 7 pN (Gennerich et al. 2007). One could speculate that a force-dependent mechanism may decrease the retrograde transport, leading to vesicle accumulation in the axon.

Interestingly, we found a decrease of the retrograde component of NGF-signalling vesicles in stretched axons of mouse DRG primary neurons, resulting in NGF vesicle accumulation in the axon (Falconieri et al. 2023). Similarly, Chowdary and colleagues found a decrease of the retrograde transport of endosomes in rat DRG neurons (Chowdary et al. 2013, 2019). A decrease in motility of lipid vesicles in response to an opposing force has been observed in rat cortical neurons (Kunze et al. 2017).

10 MT coordinates organelle transport

Due to the metabolic demand, axonal trafficking of mitochondria along MTs is finely regulated by the adaptor Miro proteins, which anchor mitochondria at specific locations (López-Doménech et al. 2018), and the motor-binding TRAK proteins that can associate with both kinesin and dynein (Fenton et al. 2021). Mechanical force leads to an accumulation of mitochondria in chick sensory neurons (Lamoureux et al. 2010), in mouse hippocampal neurons (Falconieri et al. 2023), and in human neural stem cells (NSCs) (de Vincentiis et al. 2022). MTs also coordinate the axonal transport of rough endoplasmic reticulum (ER). In one mechanism, ER tubules bind to the MTs through association between the ER protein STIM1 and the MT-associated protein EB1 (Pavez et al. 2019). In another mechanisms, ER binds to stable acetylated MTs and slide using kinesin 1 and dynein motor proteins (Wozniak et al. 2009). In line with the idea of the increase in the number of MTs, MT stabilization, and MT/ER association, we found an accumulation of ER tubules in stretched mouse hippocampal neurons (De Vincentiis et al. 2020; Falconieri et al. 2022, 2023) and in human NSCs (de Vincentiis et al. 2022).

11 MT coordinates the cross-talk between axonal transport and local translation

We recently suggested that the accumulation of vesicles and organelles in stretched axons increases the probability of functional contacts that promote local translation (Falconieri et al. 2023) (Figure 1B). In 2019, Cioni and colleagues provided evidence that RNA granules and LEs associate with mitochondria, generating a platform for local translation (Cioni et al. 2019). In stretched axons, we observed that mechanical stimulation strongly promotes local translation and increases the concentration of axonal ribosomes in a stage of active translation. MTs play an important role in the localization of the components of these translational platforms by increasing the probability of associations between RNA granules and LEs, resulting in a switch of ribosomes from an inactive to an active state (Falconieri et al. 2023). However, mechanosensitivity should be at the level of MTs, while activation of local translation seems to be a downstream event. This is in line with data showing that the treatment with cycloheximide (an inhibitor of protein synthesis) blocks SG, but not the increase of MTs in stretched axons.

12 Conclusions and critical perspectives

Here, we propose a model of axon growth induced by a traction force (Figure 1), that highlights that MTs are likely to be the hub of the interactions that produce a coordinated and orchestrated response. We suggest that the force generated along the axon induces stabilization of axonal MTs. According to this model, when the axonal MTs are more stable, their turn-over decreases, and, consequently, they accumulate. Therefore, since MTs provide the main cytoskeletal “tracks” for axonal transport, their accumulation induces an enrichment of vesicles and organelles in the axon. The probability of translational platforms to form increases when the local concentration of vesicles and organelles increases. The assembly of translational platforms results in activation of local translation. This positive modulation of axonal transport and local translation can sustain the addition of the new mass required for axon to grow under stretching.

The most significant aspect that our model does not clarify is why a traction force induces MT stabilization. According to the classic idea of the tensegrity model, a traction force could lead to a purely mechanical effect: tension induces a remodelling of the axonal cytoskeleton, which is dictated by actin-MT and actin-spectrin interactions (Coles and Bradke 2015), with actin and spectrin bearing traction forces and MT bearing compression forces. MT accumulation could result from this cytoskeletal remodelling, as a consequence of MT sliding and MT translocation (Rao and Baas 2018; Reinsch et al. 1991), considering that axons behave as active fluids (Miller and Suter 2018).

However, the strong evidence that a traction force alters MT dynamics *in vitro* raises the question of whether MTs are mechanosensors or mechanotranducers in this process. A potential *cis-acting* mechanism, supported by mathematical

modelling and experimental evidence, seems to indicate that the energy transmitted by the mechanical stimulus could be stored in the lattice structure, promoting a lateral bond formation between tubulin dimers and protofilament stability (Gudimchuk et al. 2020). Alternatively, energy storage could change the MT mechanical properties, thereby increasing rigidity (Mickey and Howard 1995). This could increase MT stability by changing the interaction time between MT and MAPs (Figure 2).

Another possible mechanism, acting *in trans*, could involve an unknown mechanosensitive (MS) protein (Figure 2), which is then subjected to a conformational change under a traction force (Hu et al. 2017). This can expose cryptic domains, or alter secondary, ternary, or quaternary structures. This can modulate the affinity of the protein for its interactor or its catalytic activity or the enzyme processivity. We speculate that any MS protein involved in MT-binding, MT polymerization and in post-translational modification of tubulin may result in a change in MT stability.

In fact, the presence of a mechanosensitive element has not been yet confirmed, and questions regarding i) what the mechano-sensor could be, ii) how it could perceive the stretching forces and iii) how the signal is transduced, still need to be answered. Proving that MTs can sense the force directly or indirectly would be the first step in elucidating this mechanism. Computational studies are urgently required to corroborate experimental hypotheses. Förster resonance energy transfer (FRET) could be helpful to study molecular interactions in the MT protofilament or between the MT and an associated component. Although several FRET sensors have been developed for force sensing (Freikamp et al. 2017; Vicente et al. 2022), no specific tubulin force-sensor has been identified. However, the possibility of tagging MTs, MAPs or associated proteins would allow us to study alterations in MT dynamics and MT/MAP interaction dynamics in response to a traction force. This should then lead to the identification of novel molecular targets.

Another fascinating scenario would be to exploit nanotechnology for generating traction forces at the level of specific sub-domains or specific components, such as adhesion points (Etoc et al. 2013), cytoskeletal elements (Chen et al. 2011), vesicles (Chowdary et al. 2013, 2019; Kunze et al. 2017; Steketee et al. 2011), channel proteins (Falleroni et al. 2022), etc. Further studies on axonal transcriptome and proteome changes evoked by stretching forces are needed.

Finally, genetically modified animals fluorescently tagged and/or lacking a specific component of the MT network could be used for studying *in vivo* mechanisms of MT assembly, stabilization (e.g., tau-MT interactions), enzymatic activation (e.g., αTAT1 for MT acetylation) by two-photon microscopy, following the direct application of the mechanical stimulus.

A multidisciplinary approach is certainly required to address the complexity of this biological issue.

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