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REVIEW ARTICLE

The cytoskeleton as a modulator of tension driven axon elongation

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Abstract

Throughout development, neurons are capable of integrating external and internal signals leading to the morphological changes required for neuronal polarization and axon growth. The first phase of axon elongation occurs during neuronal polarization. At this stage, membrane remodeling and cytoskeleton dynamics are crucial for the growth cone to advance and guide axon elongation. When a target is recognized, the growth cone collapses to form the presynaptic terminal. Once a synapse is established, the growth of the organism results in an increased distance between the neuronal cell bodies and their targets. In this second phase of axon elongation, growth cone-independent molecular mechanisms and cytoskeleton changes must occur to enable axon growth to accompany the increase in body size. While the field has mainly focused on growth-cone mediated axon elongation during development, tension driven axon growth remains largely unexplored. In this review, we will discuss in a critical perspective the current knowledge on the mechanisms guiding axon growth following synaptogenesis, with a particular focus on the putative role played by the axonal cytoskeleton.

KEYWORDS

axon elongation, axon stretch growth, axonal cytoskeleton, axonal microtubules, axonal tension

1 | INTRODUCTION

One common aspect behind all the adaptations that neurons must undergo throughout their lifetime—embryonic development, synaptic formation, or mechanical adaptations to the environment—is the immediate response and fine-tuning capability of the neuronal cytoskeleton (Bezanilla, Gladfelter, Kovar, & Lee, 2015; Fletcher & Mullins, 2010). The axonal cytoskeleton is a polymer fiber-based scaffold, composed of actin, microtubules, and intermediate filaments—neurofilaments—that is responsible for the spatial organization, architecture and morphology of the neuron. Rather than being a stiff structure as the word skeleton may imply, the cytoskeleton is highly dynamic and its dynamics is essential for cell survival. In association with their own set of regulatory proteins, the different cytoskeleton components play an unique role in numerous neuronal functions (Leterrier, Dubey, & Roy, 2017). Despite having specific roles, it is their interplay and interdependency that allow the cytoskeleton to be an adaptive network, enabling neurons to integrate intracellular signals, as well as adjust to changes in the microenvironment by perceiving extracellular cues.

During development, axons must integrate their intrinsic growth program with extrinsic cues to be capable of growing until their synaptic targets are reached. At this stage, many of the morphological changes culminating in axon elongation are powered by the growth cone (Gomez & Letourneau, 2014). After this sensory motile structure touches its target and a synapse is formed, axon elongation still needs to proceed to accommodate the growth of the organism. Of note, following synapse formation, central nervous system axons are mostly unable to reactivate a proregenerative program and mount a competent growth cone; in contrast, in the peripheral nervous system axon regeneration is still possible (Silver, 2009). The

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field of axon growth and regeneration has been widely focused in understanding developmental growth cone-powered axon elongation and as a result, many molecules and mechanisms regulating this process have been identified (Gomez & Letourneau, 2014; Jiang & Rao, 2005; Kim, Hur, Snider, & Zhou, 2011; Neukirchen & Bradke, 2011). However, the mechanisms controlling tension-driven axon elongation after synaptogenesis, are still essentially unknown. In this Review, we will discuss the current knowledge in tension-driven axon elongation, emphasizing the possible function of the neuronal cytoskeleton, in particular of microtubules and actin, as tension modulators. Given that this fascinating field has been widely understudied, the general lack of experimental details still fails to provide a clear indication on the mechanisms that might be relevant to stretching during organismic growth. Therefore, we here provide a hypothesis exposition rather than a revision of a mature field**.**

2 | FROM GROWTH CONE-POWERED AXON GROWTH TO TENSION-DRIVEN AXON ELONGATION

Neuronal development is one of the most fascinating processes of cellular polarization. It is accomplished through the intrinsic coordination of biosynthesis, membrane dynamics (Dai & Sheetz, 1995), intracellular transport (Gumy & Hoogenraad, 2018), cytoskeleton changes and mechanosensing (Koser et al., 2016) that orchestrate complex changes in neuronal morphology. Cytoskeleton-dependent mechanisms driving neuronal polarization and axon growth during development have been extensively covered in previous reviews (Bentley & Banker, 2016; Flynn, 2013; Neukirchen & Bradke, 2011; Polleux & Snider, 2010; Stiess & Bradke, 2010). In particular, local actin instability (Bradke & Dotti, 1999) together with microtubule stabilization (Witte, Neukirchen, & Bradke, 2008), and the formation of uniform microtubule bundles (van Beuningen & Hoogenraad, 2016), are crucial to axon formation. In the axon tip, the highly dynamic structure of the growth cone integrates intrinsic and extrinsic signals being essential for the establishment of neuronal polarity, functioning as the pulling force that drives axon elongation (Kerstein, Nichol, & Gomez, 2015; Tahirovic & Bradke, 2009). Moreover, for axon growth to take place, neuronal membrane addition occurs mainly at the axon tip, through the incorporation of plasmalemmal precursor vesicles (Pfenninger, 2009). Interestingly, there are intrinsic differences regarding growth rates between central and peripheral nervous system neurons (Goldberg, 2003). The latter are able to grow at much faster rates than central nervous system neurons, which is probably related to the distance of their final targets (Davies, 1989). Moreover, growth cones of peripheral nervous system neurons are capable of exerting higher forces than central nervous system neurons (Koch, Rosoff, Jiang, Geller, & Urbach, 2012), making them suitable to sustain and migrate through a stiffer cellular environment. At their final destination, once a contact is established, the axon growing terminal interacts with the target cell forming intercellular contacts. The stabilization of the newly formed contacts, and the rearrangement of the cytoskeleton transforms the growth cone into a functional presynaptic terminal. Once synaptogenesis maturation occurs, the neuron is capable of transducing synaptic signals to its postsynaptic cell.

Upon synapse establishment and formation of a neuronal network, axon growth is known to depend mainly on mechanical tension from the surrounding environment (Franze, Janmey, & Guck, 2013; Harrison, 1935; Weiss, 1941), a process also known as axon "stretch growth" (Bray, 1984) or "towed growth" (Heidemann, Lamoureux, & Buxbaum, 1995; Weiss, 1941). As an organism grows, the distance between the neuronal cell body and its target increases. In the human body, a motor neuron innervating the lower leg may be 1 m long in an adult, but only 1 cm in an 8 week-old embryo, when the formation of synaptic terminals occurs. Throughout human development there are two main peaks of growth: (a) the fifth month of gestation where growth can reach a rate of 11 cm/month, and (b) the growth spurt in adolescents (11–15 years old) that reaches around 8–10 cm/year (Bray, 1984; Soliman, De Sanctis, Elalaily, & Bedair, 2014; Tanaka, Suwa, Yokoya, & Hibi, 1988). In nature, one of the most striking examples of extreme growth is the spinal cord development of the largest mammal, the blue whale. In this extreme scenario, axons that go from the brainstem to the end of the spinal cord can reach around 30 m in length, displaying growth rates higher than 3 cm/day (Smith, 2009). In addition to the nervous system, another striking example that is highly influenced by mechanical forces is the bone. In this case, the interaction between loading and longitudinal growth takes place as an adaptation to tension (Turner, 1998). Interestingly, a tight molecular and mechanical interplay occurs between bone and the peripheral nervous system (Gkiatas et al., 2017).

During adolescence, the growth spurt is mediated by several factors including growth hormones. One key hormone for adolescent development is insulin growth factor-1 (IGF-1), which slowly increases during childhood until it reaches a peak during puberty, correlating with the adolescence growth spurt (Lundberg, Kristrom, Jonsson, Albertsson-Wikland, & On Behalf of the Study Group, 2015). Of note, IGF-1 enhances axon growth and regeneration (Beck, Powell-Braxton, Widmer, Valverde, & Hefti, 1995; Caroni & Grandes, 1990; Fernyhough, Willars, Lindsay, & Tomlinson, 1993; Gao et al., 1999; Recio-Pinto, Rechler, & Ishii, 1986). In addition to IGF-1, alterations in thyroid function occur during puberty (Fleury, Melle, Woringer, Gaillard, & Portmann, 2001),

including a transient increase in circulating thyroid hormones (T_4 and T_3) (Michaud et al., 1991). It is noteworthy that T_3 exerts a trophic action on sensory neuron survival and neurite outgrowth (Walter, 1996). In the specific case of DRG neurons, thyroid hormone receptors are expressed throughout their lifetime playing a continuous functional role in this cell type (Papakostas & Macheras, 2013). As such, although there is no direct evidence of hormonal regulation specifically in the process of axon stretch growth, this possible regulatory mechanism should certainly be explored in the future.

In general, biophysical constraints provided by the environment are key players in modulating intracellular pathways and their regulatory mechanisms, and conditioning cellular shape (Shivashankar, Sheetz, & Matsudaira, 2015). These mechanical forces affect not only the cellular size and shape, but can also modify gene expression, the localization of molecules within the cell, and vary with cell and tissue properties including elasticity (Hernandez-Hernandez, Rueda, Caballero, Alvarez-Buylla, & Benitez, 2014). Nonphysiological mechanical strain can also result in tissue injury. This is particularly relevant in the nervous system, where mechanical deformation can cause loss of function (Shi & Pryor, 2002). In the specific case of traumatic brain injury (that may occur as a consequence of concussion) depending on the acceleration, axonal injury may arise (Holbourn, 1943; Smith, Meaney, & Shull, 2003). Of note, several models of axon stretch injury have been reported (Ahmadzadeh, Smith, & Shenoy, 2014; Smith et al., 2003; Wang, Wang, Li, Hao, & Wang, 2016) and the knowledge gained on the mechanisms involved in pathological stretch conditions is deeper than the one available for physiological axon stretch growth. The current knowledge on axon stretch growth and axon stretch injury is further discussed below.

3 | MODELS OF STRETCH DURING AXON GROWTH

Mechanosensitive pathways are responsible for integrating cues allowing cytoskeleton adaptation and membrane addition, ultimately giving rise to a very dynamic system (Schwarz, 2017; Wyatt, Baum, & Charras, 2016). In the 1980s, pioneer studies using dorsal root ganglia from 10 to 12 days-old chick embryos cultured in glass coverslips showed that mechanical tension promoted by a microelectrode tip can lead to axon formation and elongation (Bray, 1984). Similarly, in neurons from the telencephalon of 7–8 day old chick embryos, axons could also be initiated when mechanical tension is applied by calibrated glass needles mounted on a micromanipulator (Chada, Lamoureux, Buxbaum, & Heidemann, 1997). Using neurons from rat embryonic hippocampal neurons (E18–19) and by applying tension in minor neurites from stage 2–3 cultured neurons, axon specification occurred (Lamoureux, Ruthel, Buxbaum, & Heidemann, 2002). Overall, regardless of the neuronal type, all neurons grow when subjected to external tension, with the elongation rate being directly proportional to the magnitude of applied tension (Heidemann & Bray, 2015). This proportionality is a characteristic of a Newtonian fluid-mechanical element: a mechanical stimuli produces a pulling force that makes the axon to accommodate, dissipating the force by further elongating. The extent of the resulting axon elongation will be dictated by several parameters: tension, the viscoelastic properties of the axon, and its surrounding microenvironment (Figure 1) (Heidemann & Bray, 2015; O'Toole, Lamoureux, & Miller, 2008). Intrinsically, different neuron types have different viscoelastic responses, which leads to a variation in strain rate response. Motor axons display a very slow rate during stretch growth (0.1–0.3 mm/day) as higher values will lead to rupture. However, sensory neurons such as DRG neurons are capable of withstanding rates of 1 mm/day (Katiyar, Struzyna, Das, & Cullen, 2019). In embryonic (E15) rat dorsal root ganglion plated onto overlapping membranes, tension is able to produce axon elongation that reaches rates of approximately 300 μm/h (i.e, 8 mm/day), leading to an axonal length of about 10 cm in 2 weeks (Pfister, Iwata, Meaney, & Smith, 2004). This extreme tension-driven axon growth has an almost 10-fold increased rate than that observed for growth-cone extension during axon regeneration, which is of approximately 1 mm/day (Pfister

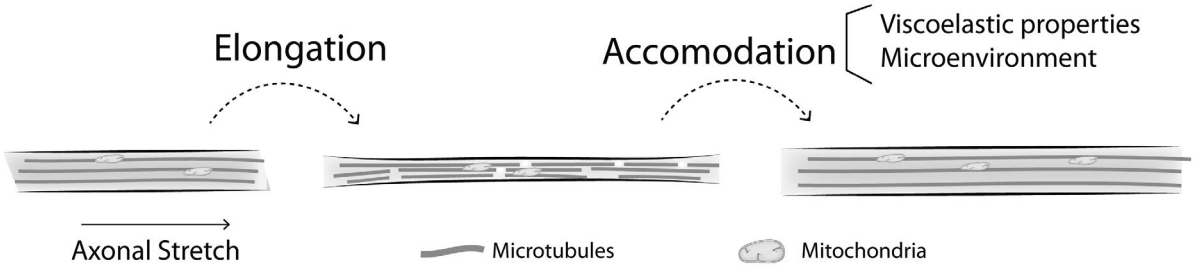

FIGURE 1 Schematic representation of axonal accommodation upon mechanical stretch. After a mechanical force, depending on the tension applied, axon elongation occurs, leading to a reduced axonal diameter, which promotes microtubule breakage. Viscoelastic properties, as well as microenvironment resistance are key factors in the accommodation period when axon diameter is restored. During the accommodation period, microtubule rearrangements are restored promoting a normal axonal transport. Adapted from (Heidemann & Bray, 2015; O'Toole et al., 2008)

et al., 2011). The above data provided the first evidence that mechanical stimuli can induce extreme stretch growth of integrated axon tracts, far exceeding any previously observed limits of axon growth. Evaluation of axonal ultrastructure by transmission electron microscopy from fixed elongated axons (5 cm stretch during 14 days) demonstrated that microtubule density was similar between controls and stretched axons, and neurofilament organization and mitochondria morphology and number also remained unchanged between experimental settings (Pfister et al., 2004). Regarding membrane addition, by pulling growth cones of attached neurites, membrane can be added throughout the neurite, whereas in growth cone-mediated axon elongation membrane addition occurs mainly at the distal tip (Zheng et al., 1991).

In relation to their function, neurons subjected to stretch growth retain their capacity to transmit active electric signals. Using whole cell patch clamp and functional calcium imaging, no alterations were found on the activation and inactivation of sodium and potassium channels, and action potentials were found to be generated normally (Loverde & Pfister, 2015; Pfister, Bonislawski, Smith, & Cohen, 2006). However, recent data obtained by stretching F11 cells (hybrid of rat neuroblastoma and primary DRG neurons) showed that stretch leads to their hyperpolarization, and that depolarization only occurs after stretch release (Bianchi et al., 2019). Despite maintaining their ability to fire action potentials, the initial current needed to originate them was significantly higher in stretched cells than in control ones. This points toward the possibility that ion channels might be damaged by tension, affecting neuronal electrophysiological activity (Bianchi et al., 2019). Another key element in axonal conduction, is the regulation of myelin sheath length (Waxman, 1980). The regulation of axon length and myelination during body growth must be an interdependent process as nerve conduction velocity needs to be maintained during and after this period. In fact, it has been demonstrated in zebrafish that after an initial period of a very dynamic growth, myelin sheaths continue to grow and adapt their length to compensate for body growth (Auer, Vagionitis, & Czopka, 2018). The authors hypothesized that myelin sheath length might be regulated both by intrinsic and extrinsic factors. Interestingly, in vivo studies using a rabbit limb lengthening model (at a rate of 0.7 mm per day), demonstrated that the increased length of the tibial nerve is accompanied by increased intermodal length in proportion to mechanical strain (Simpson et al., 2013). Yet, axons do not alter their diameter or myelin thickness, suggesting axonal accommodation, with more axoplasm and myelin being produced as a response to the elongation stimulus (Simpson et al., 2013). Conduction velocity is also unaltered by limb lengthening. It remains, however, unknown for how long myelin sheath extension can occur to compensate for body and axon elongation, as high lengths would result in failure of action potential initiation (Davis, Lambert, & Bennett, 1996). One possibility is that a new sheath is formed at a node of Ranvier by splitting the node into two (Auer et al., 2018).

4 | THE NEURONAL CYTOSKELETON AS A TENSION MODULATOR DURING AXON STRETCH GROWTH

Transmission of tension is coupled with the cortical cytoskeleton, which is composed by several proteins sensitive to mechanical cues (Haswell, Phillips, & Rees, 2011). Axons must, therefore, adapt their cytoskeleton organization to tension. *Drosophila* motor neurons behave like viscoelastic solids in response to stretch, displaying a linear force-distortion response, followed by relaxation back to steady state when tension is released (Rajagopalan, Tofangchi, & Saif, 2010). Accommodation to stress has also been suggested by imaging mitochondria in rat dorsal root ganglia neurons (Chetta, Kye, & Shah, 2010). By evaluating the distance of consecutive stationary mitochondria, the behavior of the axonal cytoskeleton was inferred as being capable of responding to stretch rapidly and locally (Chetta et al., 2010). Axons thin when rapidly stretched and axonal diameter is restored when tension is released, supporting the need of structural adaptations (Lamoureux, Heidemann, Martzke, & Miller, 2010). It has been hypothesized that as tension produces axon thinning, it will force cytoskeleton components together and reduce the available space for axonal transport (Heidemann & Bray, 2015). In fact, as the authors point out, compaction of microtubules and neurofilaments has been previously detected in stretched axons (Ochs, Pourmand, Jersild, & Friedman, 1997) and tension may cause these cytoskeleton components to break (Tang-Schomer, Johnson, Baas, Stewart, & Smith, 2012). As a consequence, transported microtubule and neurofilament segments will tend to accumulate in the thinned area of the axon, and then, incorporate its cytoskeleton such that normal axonal diameter is recovered allowing to restore normal transport rates (Heidemann & Bray, 2015). However, this model still lacks experimental validation and the mechanisms by which integration into existing cytoskeleton networks and membrane structures may occur remains to be discovered. One should also note that axon elongation is generally considered to be limited by the rate of the slow component of axonal transport (approximately 1 mm/day), that is responsible for the transport of cytoskeletal proteins such as tubulin and neurofilaments (Roy, 2014). This transport rate is clearly insufficient to support extreme stretch growth, like the one present in the blue whale spinal cord axons. Local protein synthesis may be a possible mechanism to overcome the challenges of long-range transport and respond to the need of rapid adaptation to the stress imposed by stretch. Recently, a mathematical model for axon stretch

growth was developed where the rate of protein production required for growth was dependent on membrane tension (Purohit & Smith, 2016). The model is capable of predicting the maximum stretching rate that an axon can sustain without disconnection, being thereby useful to support the design of axon stretch growth protocols.

Given their crucial role in cells, microtubules may be one of the key players in maintaining the directionality of axon growth upon mechanical tension (Hamant, Inoue, Bouchez, Dumais, & Mjolsness, 2019). Supporting this perspective, in keratocytes, mechanical tension leads to microtubule outgrowth in lamellipodia (Kaverina et al., 2002). In vitro studies using isolated microtubules, demonstrate that they soften upon continuous cycles of microtubule bending. In this case, the microtubule lattice areas subjected to tension are self-repaired by the incorporation of tubulin dimers along the lattice, recovering their stiffness (Schaedel et al., 2015). Of note, applying tension to growing microtubules attached to kinetochores results in faster microtubule growth (Akiyoshi et al., 2010). How can tension induce increased microtubule growth rates? A possible explanation is that mechanical tension in the microtubule end can lead to alterations in microtubule conformation (Brouhard & Rice, 2018). Could axonal mechanical tension lead to a faster microtubule growth? Interestingly, by stretching peripheral nerves, mTOR activation and increased protein synthesis have been recently detected in response to axonal tension (Love et al., 2017). Of note, mTOR is capable of regulating several neuronal processes that involve cytoskeleton rearrangement, and potentially induce microtubule alterations (LiCausi & Hartman, 2018). Previous studies using either theoretical models or purified proteins, have also demonstrated the effect of mechanical tension on the actin cytoskeleton showing that it increases actin polymerization rate, mediated by several actin-binding proteins (Courtemanche, Lee, Pollard, & Greene, 2013; Jegou, Carlier, & Romet-Lemonne, 2013; Kozlov & Bershadsky, 2004). In further support of the importance of actin during stretch adaptation, mechanical stretch of *Drosophila* wing disk was shown to induce myosin II polarization, regulating tissue elasticity and stiffness to prevent fractures and injuries (Duda et al., 2019). In lung cells (the lung is a dynamic organ due to cyclic respiratory patterns) cyclic stretch leads to the reorganization of microtubule and actin networks (Geiger, Taylor, Glucksberg, & Dean, 2006). Using a laser-based optical stretcher coupled with microfluidics (Lincoln, Wottawah, Schinkinger, Ebert, & Guck, 2007) in a breast cancer cell line (MCF-7), the effect of stretch in the uncoupling of actin and microtubules has also been addressed. At low stretch rates, actin filaments alone seem to determine cell mechanics. However, by applying high stretch rates, disrupting actin filaments resulted in a significant increase in cell deformation, and interfering with microtubule stability promoted an increased cellular plasticity. Thus, at high stretch rates, both actin filaments and microtubules seem to be necessary to maintain cellular integrity (Kubitschke et al., 2017).

The intrinsic correlation between alterations in plasma membrane and cytoskeleton components may also be a central player during mechanical tension adaptations. In the course of axon stretch growth, membrane remodeling may involve an increase in plasmalemmal vesicles that will integrate the axonal membrane and accommodate elongation. In this respect, a study addressing the contribution of plasma membrane lipids to reshaping of red blood cells demonstrated that membrane lipids act as stabilizers upon deformation, and as platforms for Ca^{2+} efflux during shape restoration (Leonard et al., 2017). It is widely established that both actin and microtubules are highly sensitive to alterations in calcium levels. In fact, during embryonic development calcium controls growth cone advance (Gasperini et al., 2017). Assuming a similar model, alterations in membrane lipids during axon stretch growth could lead to an increase in neuronal calcium levels, activating signaling cascades that might culminate in cytoskeleton rearrangement.

5 | WHAT CAN WE LEARN FROM AXON STRETCH INJURY?

Mechanical strain can be a major cause of several axonal pathologies. In the peripheral nervous system, stretch injury can lead to severe disability (Bareyre, 2008); whereas in the central nervous system, the cerebral cortex is particularly affected in traumatic brain injury (Ahmadzadeh et al., 2014; Smith et al., 2003; Wang et al., 2016). Axonal injury is dependent on strain and on the rate of strain during brain trauma. Strain is defined as the ratio of the total deformation of a given object in relation to its original length, whereas the strain rate, is the rate at which a specific strain is delivered (Magou et al., 2011). Interestingly, whereas membrane permeability is not compromised during uniaxial strain, high biaxial strain results in high calcium influx (Geddes-Klein, Schiffman, & Meaney, 2006; Hemphill, Dauth, Yu, Dabiri, & Parker, 2015; Sherman et al., 2016). Moreover, different strain rates in cortical neurons lead to calcium peaks proportional to the strain rate applied (Geddes-Klein et al., 2006). These data suggest that either low strain rates or uniaxial strain may alter cellular physiology but not to the extent of generating severe injury signals. Calcium influx following strain initiates the activation of specific proteases resulting in cytoskeleton alterations (Smith et al., 2003), including microtubule disassembly in cortical neurons (Tang-Schomer, Patel, Baas, & Smith, 2010; Yap, Dickson, King, Breadmore, & Guijt, 2014), which was also determined by mathematical modelling of the axonal cytoskeleton (Ahmadzadeh et al., 2014). This will result in disrupted axonal transport

which culminates in axon degeneration (Tang-Schomer et al., 2010). In organotypic cerebellar slices, a biaxial strain of 30% increases axonal amyloid precursor protein accumulation, which supports disrupted axonal transport, and the formation of axonal swellings is observed (Chierto et al., 2019). Of note, the microtubule-stabilizing drug epothilone D is capable of reducing axon fragmentation after stretch injury in cortical neurons (Yap et al., 2017). In addition to disrupting axonal physiology, axon stretch injury can cause the formation of periodic swellings in dendrites, which could also be the consequence of microtubule rupture and impaired axonal transport (Monnerie et al., 2010).

In summary, in the case of axon stretch injury several models (mainly using primary cortical neurons) have been developed mostly focused in understanding the pathology underlying traumatic brain injury. As detailed above, these models have already unraveled a number of crucial cellular mechanisms altered by stretch injury. Building on this knowledge, analogous studies should be performed to understand axon stretch during physiological axon growth, bearing in mind that different neuronal cells can withstand different strains (Goldberg, 2003; Katiyar et al., 2019).

6 | CONCLUSION AND FUTURE PERSPECTIVES

From their initial development led by the growth cone, to tension-driven axon elongation after synapse formation, neurons must adapt to respond to external chemical and physical cues. Axon elongation during neuronal development has been widely addressed to gain a better understanding of growth mechanisms with the ultimate hope to provide insight into potential regenerative therapies. Understanding the molecular players and mechanisms powering axon stretch growth may also provide insight into molecules and mechanisms enhancing axon growth that may find application in conditions where axon regeneration is needed. These mechanisms may also prove to be important to understand peripheral neuropathies that arise or show a specific decline when axon growth rates increase, such as during adolescence (Macleod & Appleton, 2007). It is possible that the inability of the diseased peripheral nervous system to adapt to the increased growth rates at this specific stage may contribute to the worsening of pathology.

Overall, tension-driven axon elongation remains poorly understood. Under axonal tension, similar cytoskeleton-dependent mechanisms as the ones described in this Review for other cellular systems may be in place (Figure 2). Meanwhile, many questions remain to be answered. What are the key molecules that are locally expressed or transported as a consequence of axon stretch? How is axonal cytoskeleton dynamics and stability affected during axonal tension? How does the axon membrane periodic skeleton (Xu, Zhong, & Zhuang, 2013) adapt to stretch? How does axonal transport of organelles, synaptic vesicles and proteins behave? What are the mechanisms underlying the addition of novel membrane? How is axonal retrograde signaling affected by stretch? The combination of innovative stretching platforms mimicking a circuit of postsynaptic

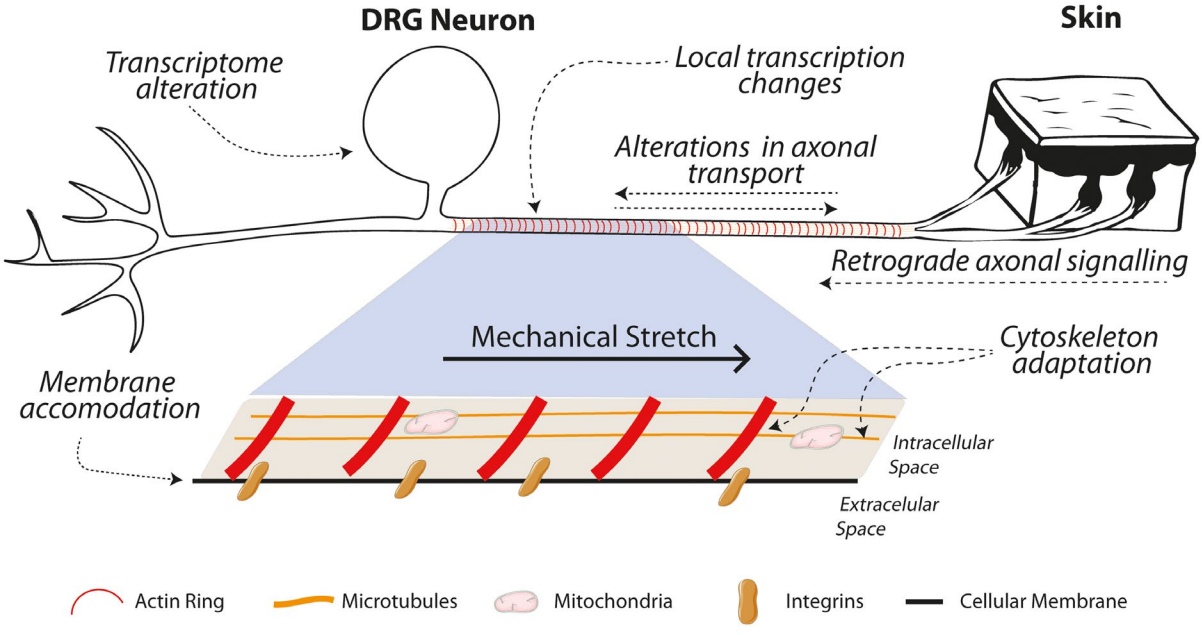

FIGURE 2 Schematic representation of possible mechanisms altered after mechanical stretch. Mechanotransduction will occur upon stretch potentially leading to several molecular and cellular alterations, including in the actin and microtubule cytoskeletons, axonal transport, retrograde axonal signaling, among others

neurons, controlled stretch rates, and high resolution live imaging of the axonal cytoskeleton and transport, together with the current knowledge available will certainly enrich the field.

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