

Force generation by cytoskeletal motor proteins as a regulator of axonal elongation and retraction

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Axons elongate and retract in response to environmental signals during the development of the nervous system. There is broad agreement that these signals must affect the cytoskeleton to elicit bouts of elongation or retraction. Most contemporary studies have speculated that bouts of elongation involve polymerization of the cytoskeleton whereas bouts of retraction involve depolymerization of the cytoskeleton. Here we present an alternative view, namely that molecular motor proteins generate forces on the cytoskeletal polymers that can affect their distribution and configuration. In this view, bouts of axonal elongation involve net forward movement of cytoskeletal elements whereas bouts of retraction involve net backward movements. We propose that environmental cues elicit bouts of elongation or retraction via biochemical pathways that modulate the activities of relevant motors.

Axons can extend over exceedingly long distances to reach their target tissues during the differentiation of the nervous system. The development of the axon involves bouts of elongation and retraction, in addition to the formation of branches that can also elongate and retract^{1–6}. The parent axon and its branches are tipped by highly motile structures called growth cones, which forage through the embryo to find specific targets for innervation. The movement of these growth cones is highly sensitive to environmental factors such as cell-surface molecules and chemotactic signals. Some factors attract axons and promote their elongation whereas other factors repel axons or cause retraction^{7–14}. Despite substantial work on the visualization of such responses and characterization of attractive and repulsive factors, surprisingly little is known about how these factors cause an axon to elongate or retract. Most articles on this topic have noted that axonal elongation and retraction must involve alterations in the cytoskeletal arrays that provide architectural support for the axon (see, for example, Refs 15–17). Dense arrays of cytoskeletal polymers are contained within the axon and are necessary for its extension and maintenance. The prevailing view is quite simple – that axonal

growth involves the assembly of new cytoskeleton and axonal retraction involves the disassembly of existing cytoskeleton.

This view is based mainly on the results of pharmacological studies that show that wholesale microtubule depolymerization can cause axons to bead-up along their lengths, become thinner, wither, and retract^{18–21}. Interestingly, however, there is almost no evidence to support the idea that axons physiologically elongate and retract by building and tearing down cytoskeletal polymers in such a wholesale fashion. Certain repulsive factors have been shown to produce local microfilament disassembly in growth cones²², but there is no evidence of widespread microfilament disassembly or any kind of dramatic microtubule disassembly during physiological axonal retraction. In fact, experimental studies have shown that axons can develop and generate branches fairly normally under conditions of suppressed microtubule assembly and disassembly^{23,24}. In recent years a great deal of progress has been made in elucidating the biochemical pathways that transduce environmental signals into molecular changes within the growth cone and the axon^{11,15–17,25,26}, but it remains unclear exactly how these changes alter the cytoskeleton. For example, it has been proposed that changes in the activity of small GTPases and kinases can cause retraction by inducing disassembly of microfilaments²⁷, but paradoxically, pharmacological studies have shown that disassembly of microfilaments prevents axons from retracting rather than causing them to retract^{18,19}.

This article aims to explore a mechanism that has received relatively little attention as a potential means by which environmental factors might alter the cytoskeleton to elicit elongation or retraction of the axon. In this new model, cytoskeletal elements are subjected to forces generated by molecular motor proteins. The modulation of these forces determines whether there is a net forward or backward movement of the cytoskeletal elements (as opposed to wholesale assembly and disassembly of the cytoskeletal arrays) and these movements, in turn, determine whether the axon elongates or retracts.

Molecular motors transport, organize and integrate cytoskeletal polymers

Since the earliest days of cytoskeletal research there have been two schools of thought on how the cytoskeleton is configured and remodeled in living cells. Microtubules and microfilaments are dynamic polymers that exchange subunits with soluble tubulin and actin pools respectively. One school of thought maintains that cytoskeletal remodeling occurs exclusively or mainly through the dynamic assembly, disassembly and

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reassembly of these polymers. The other school of thought maintains that cytoskeletal remodeling occurs not only by these dynamics but also by forces that can push and pull on the cytoskeletal polymers and thereby configure them. There has been strong opposition to this latter view, with opponents arguing that molecular motors only move small vesicular organelles along cytoskeletal polymers and do not transport or configure the polymers themselves^{28,29}. As much as we all relish a good controversy, it seems that there is now little left upon which to base this extreme point of view. It is now known that cytoplasm is rich in molecular motor proteins that hydrolyze ATP to generate forces that move the motor along the surface of a microtubule or a microfilament³⁰. Assuming that the motor interacts with a structure with less resistance to movement than the cytoskeletal polymer, this structure will indeed move along the polymer. However, if the motor protein interacts with a structure with greater resistance to movement than the cytoskeletal element, then it is the cytoskeletal element that will move. This is well documented by *in vitro* experiments that demonstrate the movement of microtubules along motors tethered to glass. In recent years, microtubules, microfilaments and intermediate filaments have also been directly observed to move within living cells^{31–33}. If the motor interacts with two cytoskeletal polymers (rather than a polymer and a small vesicular organelle or an immobile substrate), then the forces generated by the motor would move and configure the polymers relative to one another. It is provocative to speculate that motor-based force generation could be the principal means by which cytoskeletal elements are organized and distributed within the cytoplasm.

There is now precedent for motors acting in this fashion. The best studied example is the mitotic spindle, the most fundamental of all cytoskeletal arrays in eukaryotic cells. It is now known that both the formation and functioning of the mitotic spindle require motor-driven forces that push and pull on the microtubules to configure them into a bipolar spindle and then to separate the half-spindles during anaphase^{34–36}. Some of these motor-driven forces are generated between microtubules and other microtubules, and other motor-driven forces are generated between microtubules and the dense array of microfilaments concentrated in the cell cortex. Recent studies have shown that there are multiple motor-driven forces acting simultaneously, some complementing one another and others acting antagonistically^{37,38}. Even when there is no apparent motion in the spindle, these forces are nevertheless at play, but perfectly balanced. Dramatic movement of microtubules (such as during the transition from metaphase to anaphase) occurs when there is a shift in one or more of the motor-driven forces. Of course, dynamic assembly and disassembly events occur as well. The cellular and environmental cues that

regulate mitosis do so by impinging on factors that affect motor-driven forces, cytoskeletal dynamics, or both.

Older views of the axonal cytoskeleton

Historically, the axon was among the first systems for which it was strongly argued that microtubules and microfilaments are under forces that move them through the cytoplasm. This early model pre-dated the so-called ‘motor revolution’ of the 1980s, and hence the relevant forces were simply termed ‘transport machinery’. In this early model, the cytoskeletal polymers were proposed to move anterogradely down the axon³⁹. Although not discussed extensively, the idea was suggested that microtubules might be moved by forces generated against the microfilaments, and that the microfilaments might be moved by forces generated against cytoskeletal components and substrate attachment points associated with the plasma-membrane⁴⁰. The model posited that attachment of the axon to the substrate must be strong enough to bear all of these forces to drive the cytoskeleton anterogradely down growing axons, and if not, then these same forces would have the opposite effect, causing the axon to retract^{39,41}. The precise mechanics by which this would occur were not considered in great detail before studies on this model came to an abrupt halt. For the next few years this model was put aside in favor of a more structural model for how the cytoskeleton is remodeled during axonal growth and retraction. In this model, sometimes referred to as the tensegrity model, the microtubules and microfilaments are likened to the tensile and compression-bearing components of a static tensegrity structure such as a geodesic dome⁴². According to this model, the microfilament array is under structural tension, much like a stretched rubber-band, while the microtubule array bears this tension and hence is said to be under compression. When microtubules are removed, the tensile forces have no structures to act as compressive elements and hence the axon retracts. This model is consistent with the observation that axons retract in response to microtubule depolymerization but do not retract if the microfilaments are also depleted experimentally^{18,19}. The earlier transport-based model is harder to reconcile with the results of these pharmacological studies.

Nevertheless, we find the tensegrity model (at least in its purest sense) unattractive for several reasons. First, living cells are highly dynamic and, as such, are more comparable to a machine with moving parts than to a static architectural structure. Second, as noted above, there is really no good evidence for the kind of wholesale depolymerization of microtubules that would have to occur under a tensegrity model in order for an axon to retract physiologically. Finally, as explained above, living cells normally regulate their cytoskeletal arrays not by dynamics or architectural

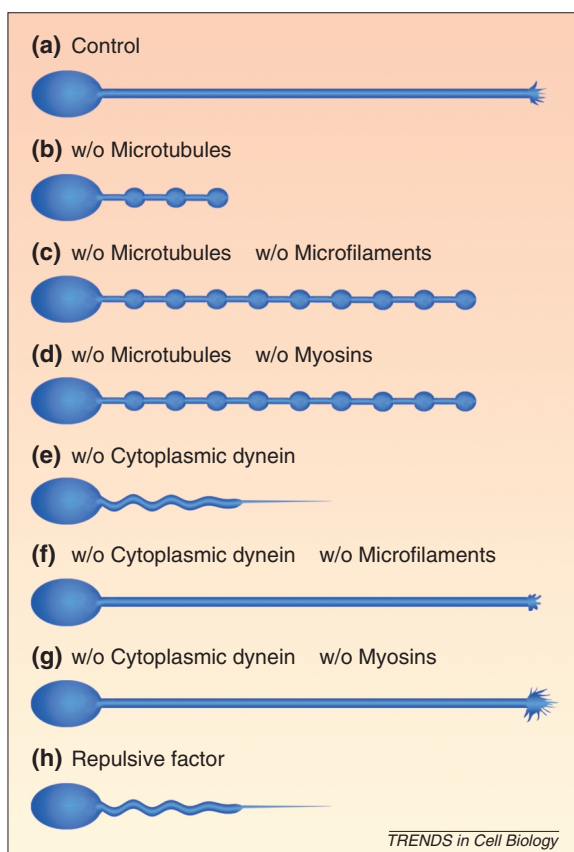


Fig. 1. Morphological features of axons induced to retract by manipulations of the cytoskeleton, motor-driven forces or repulsive factors. (a) Control axon. (b) When microtubules are pharmacologically depleted, the axon becomes thinner, develops bead-like swellings, and retracts. (c) When microfilaments and microtubules are both pharmacologically depleted, the axon becomes thinner and develops bead-like swellings but does not retract. (d) When microtubules are depleted and myosins are inhibited, the axon becomes thinner and develops bead-like swellings but does not retract. (e) When cytoplasmic dynein is inhibited, the axon retracts but does not become thinner or display bead-like swellings. Instead, it shows sinusoidal curves and a trailing streak-like remnant. (f) When cytoplasmic dynein is inhibited at the same time as microfilaments are depleted, the axon does not retract. (g) When cytoplasmic dynein and myosins are inhibited simultaneously, the axon does not retract (see Ref. 45 for more details). (h) When a typical repulsive factor is applied, axonal retraction ensues with morphological characteristics much more similar to those that accompany retraction induced by the inhibition of cytoplasmic dynein than retraction induced by microtubule depletion.

principles alone but also through the use of motor-driven forces. The mitotic spindle is clearly a machine, full of moving parts, regulated by energy-dependent motors, and capable of getting work done. We believe that the axonal cytoskeleton is also a machine with comparable properties.

New studies implicate motor-driven forces in axonal elongation and retraction

The underlying principle of our model arose from studies that implicated cytoplasmic dynein as the motor protein that transports microtubules anterogradely down the axon⁴³. These studies suggested that the cargo-domain of the motor is associated in some way with the actin cytomatrix, leaving the motor-domain available to transport

microtubules. In this way, the microtubule and microfilament systems are linked through motor-driven force generation by cytoplasmic dynein. Experimental inhibition of dynein-driven forces curtails microtubule transport and prevents axons from growing⁴⁴. Very interestingly, if neurons are cultured on a poorly adhesive substrate, inhibition of dynein-driven forces results in dramatic retraction of the axon⁴⁵. No detectable microtubule depolymerization accompanies this retraction, but instead the microtubules slide retrogradely. These observations led us to believe that it is not the mere presence of microtubules as architectural elements that opposes the tendency of the axon to retract but rather it is the motor-driven forces that integrate the microtubule and microfilament systems.

Pursuing this further, we demonstrated that this kind of retraction is abolished under experimental conditions in which microfilaments are depleted (reminiscent of the earlier drug studies). Notably, retraction induced by either dynein inhibition or microtubule depolymerization was also abolished when microfilaments were left intact, but myosin-driven forces were curtailed. This result strongly indicates that it is not some sort of intrinsic tension in the microfilaments themselves that causes axons to retract, but instead axonal retraction results from actomyosin-based contractility. A key point to be drawn from these studies (summarized in Fig. 1) is that retraction phenomena similar to those originally reported for the depolymerizing drugs can be obtained when the cytoskeletal polymers are left intact, and motor-driven forces are instead manipulated. Although the precise nature of the motor interactions remains to be elucidated, we have speculated that the contractility of the actomyosin is normally strong enough to cause axons to retract but that these forces are attenuated by the forces generated by cytoplasmic dynein between the microtubule and microfilament arrays. Thus, when either the microtubules or the dynein-driven forces are removed, the contractility becomes sufficiently strong to induce retraction.

When axons retract in response to experimental microtubule depolymerization, they become thinner and beaded along their length. The 'beads' are regions of the axon that are particularly depleted in microtubules and hence tend to round-up into spherical blebs⁴⁶. By contrast, axons that are induced to retract by experimental inhibition of cytoplasmic dynein do not show such a beading effect. Instead, these axons tend to show sinusoidal curves along their length, and consistently leave behind a very thin trailing streak-like remnant as they shorten⁴⁵. The curves, the trailing remnant and the lack of beading are far more reminiscent of the appearance of axons retracting in response to physiological factors compared with the appearance of axons retracting in response to drug-induced microtubule depolymerization (see, for example, axons retracting

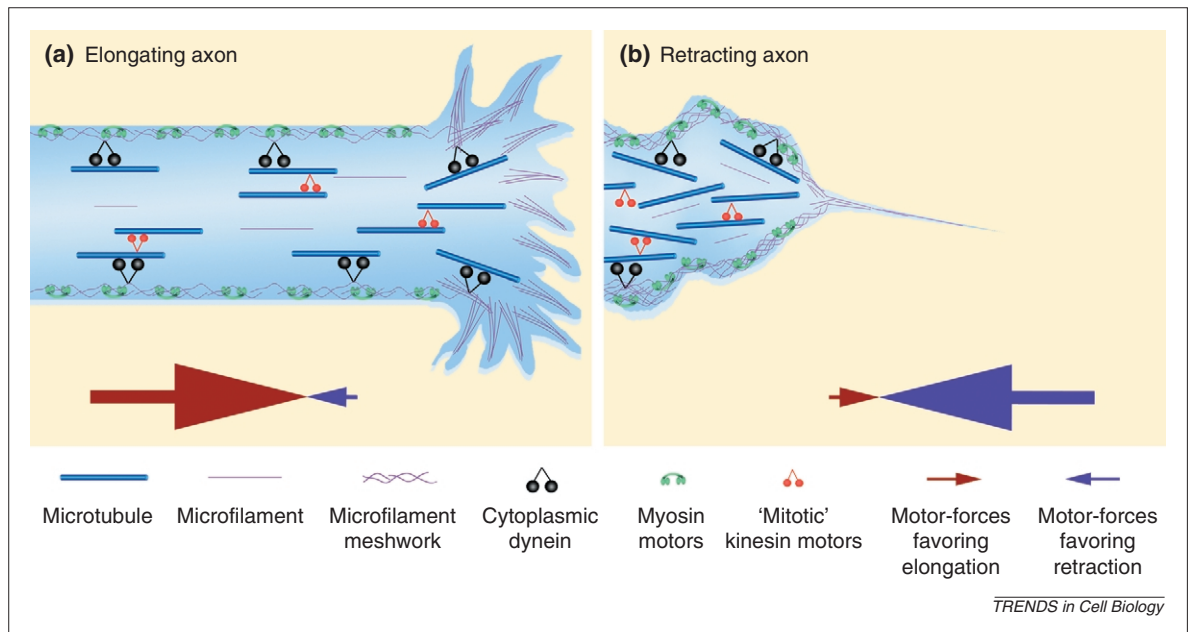


Fig. 2. Model for the regulation of axonal elongation and retraction by motor-driven forces on cytoskeletal elements, showing (a) an elongating axon and (b) a retracting axon. In the model, forces are generated on the microtubules and microfilaments by molecular motor proteins. Some of these forces favor the elongation of the axon whereas others favor the retraction of the axon. Microtubules are transported anterogradely down the axon by forces generated by cytoplasmic dynein against the microfilament meshwork. In addition to moving the microtubules forward during axonal elongation, these forces attenuate myosin-driven contractility in the microfilament meshwork. This contractility favors axonal retraction. 'Mitotic' kinesin-related motors generate additional forces between microtubules that can contribute to their forward or backward movement, thus favoring either axonal elongation or retraction respectively. All of these various forces serve to integrate the axonal cytoskeleton into a machine that can be regulated by physiological factors. Such factors can induce axons to either elongate or retract by increasing or decreasing one or more of the relevant motor-driven forces.

in Refs 47–51; see also Fig. 1). In fact, it is possible that the trailing remnant is very important for 'guiding' the axon when it shifts back from a bout of retraction to a bout of elongation. Thus the morphological features of axonal retraction are another strong indication that cytoskeletal redistribution by motor proteins is a more likely mechanism than wholesale depolymerization for the normal means by which axons retract.

Complementary and antagonistic motor-driven forces in the axon

Based on the observations described above, we have suggested the following working model. Microfilaments oriented paraxially are transported actively down the axon by one or more members of the myosin family; however, a portion of the microfilaments is arranged into a meshwork such that the myosin-driven forces would create contractility rather than anterograde filament transport within this population of filaments. Cytoplasmic dynein moves microtubules anterogradely down the axon and does so by generating forces against the actomyosin system. In some way, these forces attenuate the contractility of the actomyosin system.

The mechanism by which this attenuation occurs is unknown, but a simple way of thinking about it might be that the extra forces imposed by the dynein-based microtubule movements create a kind of 'drag' on the actomyosin movements, thereby making the contractility less robust. In any event, as a result of all of this, the cytoskeleton undergoes a net anterograde movement and the axon grows. However, alterations in motor driven forces (increases in myosin forces or decreases in dynein forces) can cause an increase in actomyosin contractility and hence induce the axon to retract. As a result, the microtubules slide backwards. These effects can be global but more often are local, resulting in short bouts of retraction in response to specific environmental molecules. Evidence already exists for biochemical pathways that can lead to enhanced phosphorylation of myosin light-chain, thus leading to increases in contractility^{52–54}. New studies suggest that certain repulsive factors might cause a redistribution of microfilaments (presumably by motors) rather than their disassembly⁵⁵ and there is good evidence that myosin motors can induce dramatic movements of microfilaments in growth cones⁵⁶. Such myosin-driven movements might very well be regulated by phosphorylation events and additional pathways undoubtedly exist to regulate myosin and dynein functions. The scenario we propose, if correct, would provide the axon with enormous flexibility to regulate its morphology by recrafting the underlying cytoskeleton through dynamic changes in the motor-driven forces that transport, organize and configure microtubules and microfilaments. This scenario, shown schematically in Fig. 2, would not be mutually exclusive from the idea that local assembly or disassembly events might also contribute to remodeling the axonal cytoskeleton.

It is our intuition that this scenario only scratches the surface of how motor-driven forces might regulate the distribution of cytoskeletal elements underlying axonal elongation and retraction. The mitotic spindle,

which we believe shares a common blueprint with the neuronal cytoskeleton⁵⁷, is now known to use a vast array of complementary and antagonistic motor-driven forces^{37,38}. We suspect that the same is true of the axonal cytoskeleton. Specific kinesin-related proteins have been identified in the mitotic spindle as being designed not for vesicle transport but for force generation between microtubules and other cytoskeletal elements. These kinesin-related proteins were initially thought to be mitosis-specific because they appeared to be inactive during interphase. However, we have found that these motors continue to be expressed in terminally postmitotic neurons where they are prominent components of the microtubule arrays of axons or dendrites. The best studied example is CHO1/MKLP1, originally discovered in the midzonal region of the mitotic spindle where microtubules of opposite polarity orientation overlap⁵⁸. We have found this motor to be necessary for the transport of a population of microtubules into dendrites oriented oppositely to those found in the axon⁵⁹. CHO1/MKLP1 seems to generate forces between microtubules of opposite orientation, which would decrease the efficiency of their transport. This could be a significant reason why dendrites remain short compared to axons⁶⁰. There is no detectable CHO1/MKLP1 in the axon, but we have demonstrated that axons contain another mitotic motor protein, known as Eg5, which can also generate strong forces on microtubules⁶¹. Using a polyclonal antibody, we detected a dramatic enrichment of Eg5 in the distal tips of axons and at newly-forming branch points. Ongoing work is aimed at determining whether other so-called mitotic motors such as CHO2 and KIF15 are present in axons.

We are fascinated by the possibility that motors such as Eg5, CHO2 and KIF15 might generate forces on microtubules in different regions of the axon. These forces could help to drive microtubules forward or backward, or could potentially zipper together microtubule bundles or splay apart already-formed bundles. These forces might also further integrate microtubules with other cytoskeletal elements such as the microfilament system in different regions of the axon. Modulation of these forces could then induce local changes in the distribution of cytoskeletal

elements which could favor axonal elongation or retraction (see Fig. 2). Microtubules can be driven backwards by the contractility of the actomyosin system, and they can also be driven backwards by specific kinesins, probably pushing one microtubule against another. It is conceivable that cytoplasmic dynein could also generate backward movements by moving microtubules against other microtubules rather than microfilaments. Microtubules would mainly be driven anterogradely by dynein-driven forces, but kinesins could facilitate these forward movements. If these ideas are essentially correct, the axonal cytoskeleton would indeed be similar to the mitotic spindle, with a vast array of different motor-based forces working in tandem. These forces could then be altered by various biochemical cascades to regulate features of axonal elongation and retraction. In addition, the regulation of such forces could result in more subtle changes in the cytoskeleton important for such processes as growth cone navigation and interstitial branch formation.

Concluding remarks

The purpose of this article is to present a new way of thinking about how the cytoskeleton might be remodeled in response to signals that cause axons to elongate or retract. The contemporary literature abounds with superb studies on factors that can repel or attract axons, but studies and hypotheses on how these factors reconfigure the cytoskeleton to underlie such changes in axonal morphology are sorely lacking. In some kinds of axons, such as those that comprise the callosal tracks, the elongation and retraction of the axon and its branches can be quite dramatic, with huge lengths of axon retracting or sprouting rapidly^{62,63}. We find very provocative the view that motor proteins including myosins, cytoplasmic dynein, and certain kinesin-related proteins generate a host of forces that can be regulated in such a way as to elicit profound effects on the microtubule and microfilament arrays. We propose that this mechanism, rather than wholesale polymerization and depolymerization of the cytoskeleton, is the principal means by which the cytoskeleton is reconfigured during bouts of axonal elongation and retraction.

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Articles can present new models or hypotheses, speculate on the interpretation of new data, or discuss controversial and developing issues.

We encourage you to send in ideas for this section and take advantage of this opportunity to stimulate debate.

Please send a short outline
of your proposal to the Editor at: tcb@current-trends.com.