Molecular mechanisms for organizing the neuronal cytoskeleton

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Summary

Neurofilaments and microtubules are important components of the neuronal cytoskeleton. In axons or dendrites, these filaments are aligned in parallel arrays, and separated from one another by nonrandom distances. This distinctive organization has been attributed to cross bridges formed by NF side arms or microtubule-associated proteins. We recently proposed a polymer-brush-based mechanism for regulating interactions between neurofilaments and between microtubules. In this model, the side arms of neurofilaments and the projection domains of microtubule-associated proteins are highly unstructured and exert long-range repulsive forces that are largely entropic in origin; these forces then act to organize the cytoskeleton in axons and dendrites. Here, we review the biochemical, biophysical, genetic and cell biological data for the polymer-brush and cross-bridging models. We explore how the data traditionally used to support cross bridging may be reconciled with a polymer-brush mechanism and compare the implications of recent experimental insights into axonal transport and physiology for each model. ***BioEssays*** **26:1017–1025, 2004. © 2004 Wiley Periodicals, Inc.**

Introduction

The notion of a cytoplasmic network with a defined three-dimensional architecture emerged more than 60 years ago.[1,2] High-voltage electron microscopy imaging of whole cells led Porter and colleagues to propose that the interior of a cell is composed of a "microtrabecular lattice".[3] In this conceptualization of the cytoskeleton, fine cross links connect organelles to one another and separate the cell interior into protein-rich and water-rich phases. Key elements of this framework have since been extensively validated in many eukaryotic systems; most notably, actin filaments form cross-linked networks and bundles under control of regulatory proteins such as Arp2/3, α-actinin and ABP-120.[4] Intermediate filaments and microtubules (MTs) can also form a variety of organized structures within a cell that can act as structural scaffolds,[5] an idea that has been extended to the cytoskeleton of axons and dendrites (collectively neurites). NFs and MTs are both present and oriented along the length of axons, while dendrites lack NFs but are relatively rich in MTs.[6,7] Cross sections through these processes show that NFs and MTs are non-randomly distributed, with a large interfilament spacing (Fig. 1A,B). For microtubules with MAPs bound (MT-MAPs), the spacing between adjacent filaments is approximately 65 nm in dendrites and 25 nm in axons,[8] while NFs have an interfilament spacing of 33-49 nm.[9] Interfilament "cross-bridges" have been proposed to account for this distinctive organization.[10–13] While this view has gained widespread recognition, there remains significant evidence for models that do not invoke cross-bridging. In one recently advanced model, NF and MT organization arises from entropic, repulsive forces between the filaments.[14,15] Here we review the evidence for this "polymer-brush" model, and contrast it with evidence for the cross-bridge model. We begin by introducing the problem of how neuronal cytoskeletal elements organize themselves in the neurites and describe the leading hypotheses for this organization. We then examine the data that support both models, including evidence from axonal preparations, structural and biophysical studies with reconstituted filaments, and biochemical and genetic studies. Finally, we explore how each hypothesis might be incorporated into current paradigms for the regulation of axonal caliber and transport as well as the role of aberrant cytoskeletal interactions in neurodegenerative diseases.

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DOI 10.1002/bies.20088
Published online in Wiley InterScience (www.interscience.wiley.com).

Abbreviations: ABP, actin binding protein; AFM, atomic force microscopy; BPAG, bullous pemphigoid antigen; EM, electron microscopy; MAP, microtubule-associated protein; MT, microtubule; NF, neurofilament; NF-H, neurofilament protein heavy; NF-L, neurofilament protein light; NF-M, neurofilament protein medium, QFDE-EM, quick-freeze deep-etch electron microscopy.

Models for NF and microtubule organization in neurites

The overall morphology of NFs and MT-MAPs is conspicuously similar in some regards. Isolated NFs and MT-MAPs are

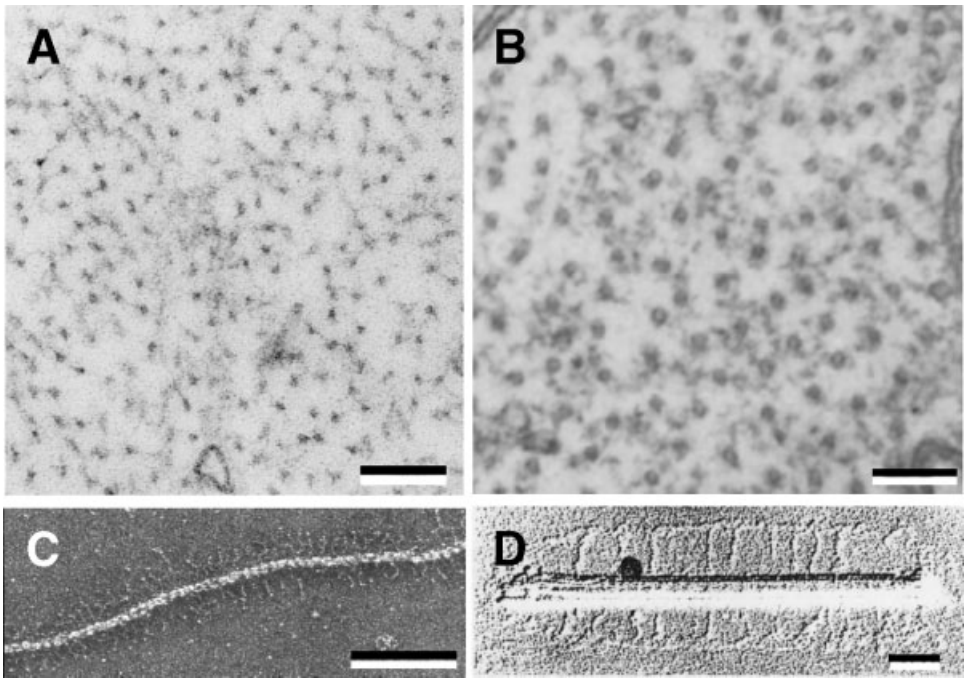


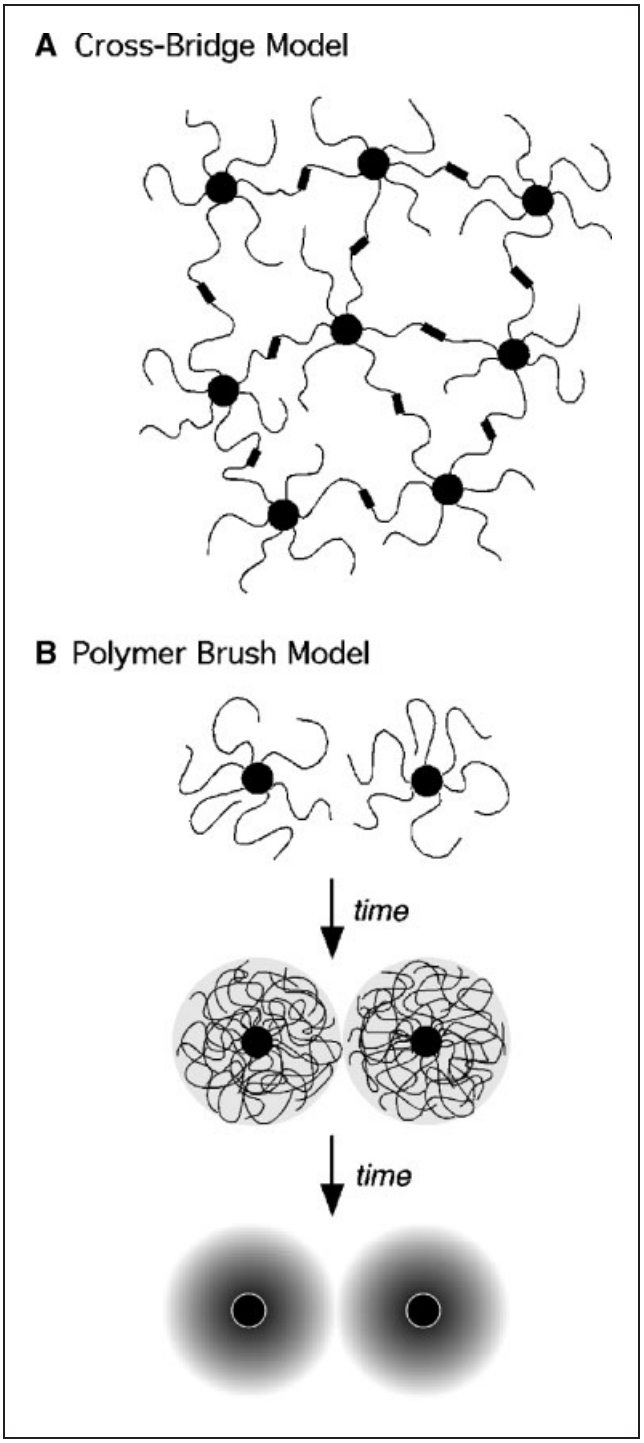
Figure 1. Electron micrographs of **A:** an axon and **B:** a dendrite in cross section. The NFs in the axon and microtubules in the dendrite exhibit a non-random liquid-crystal-like organization with a large interfilament spacing. Rotary shadow EM of **C:** NFs and **D:** MT-MAPs show the bottle-brush-like appearance produced by the NF side arms and MAP projection domains, respectively. All scale bars are 100 nm. B is reproduced from Potter, HD. 1971. J Comp Neurol 143:385–409 with permission from Wiley Intersciences, C is courtesy U. Aebi, and D is reproduced from Voter, WA, Erickson, HP. 1982. J Ultrastruc Res 80:374–382 with permission from Elsevier.

both long filamentous structures that display protrusions extending away from their surfaces, giving them a bottle-brush appearance (Fig. 1C,D).[16–18] These protrusions are the primary candidates for controlling the interactions between NFs and microtubules. In neuronal microtubules, the protruding structure is formed by MAPs; principally MAP2 in dendrites[19] and tau in axons.[20] MAP2 and tau share the same general organization: a C-terminal microtubule-binding domain and an N-terminal "projection" domain that form the protrusions.[4] Mammalian NFs are composed of three polypeptides, classified as light (NF-L, 61 kDa in humans), medium (NF-M, 90 kDa) and heavy (NF-H, 110 kDa).[21–23] The amino terminus of each subunit is composed of a rod domain that associates with the rod domains of the other two subunits to form the filament core. The C termini of NF-M and NF-H have long "side arms" of more than 300 and 600 residues, respectively, which form the protrusions.

In one view of the neuronal cytoskeleton, NFs and MTs are held together by cross-bridges. The molecular details of these cross-bridge interactions are not known, but for NFs the side arms are thought to form a non-convalent "bridge" between two adjacent neurofilaments (Fig. 2A). In published schematics, the side arms are often drawn as struts emerging from one filament and extending towards a neighbor.[24–26] Similarly, MAP projection domains are thought to connect microtubules by forming cross-bridges; there are also interactions between NFs and microtubules.[12,27] In some cases, accessory proteins, including plakins such as bullous pemphigoid antigen 1 (BPAG1), have been proposed to participate in the cross-bridge formation.[28,29]

Models based on repulsive interactions between NFs and MTs have also been proposed. One repulsive interaction hypothesis developed from the observation that NF side arms are highly phosphorylated, suggesting that filament spacing is controlled by electrostatic interactions.[30,31] Recently another hypothesis involving repulsive interactions has been proposed, where the NF side arms and MAP projection domains are highly unstructured; by virtue of being unstructured they exert long-range repulsive forces that maintain spacing between NFs and MT-MAPs (Fig. 2B).[14,15,32–34] The repulsive forces here originate from the thermally driven (Brownian) motion of the polypeptide chain, which moves rapidly to explore a very large number of conformations within some characteristic volume. When this volume is compromised by the approach of another molecule, the number of conformational states available to the polypeptide is reduced and the conformational entropy of the polypeptide decreases. As a result, a long-range repulsive force of entropic

origin is exerted on the approaching molecule. This type of behavior is often referred to as steric repulsion or an excluded volume effect.[35] When unstructured polymers of this type are tethered to a surface at high density, they tend to stretch away from the surface to avoid one another. Such structures are frequently referred to as polymer brushes, or less frequently, entropic brushes.[35,36] Thus, in this model, the NF side arms and MAP projection domains form polymer brushes on their respective filaments and control interfilament spacing.



Ultrastructure of NFs and MT-MAPs

The cross-bridge model was first proposed in the early 1980s as a mechanism to explain the large interfilament spacing between NFs and MTs in neurites. Hirokawa reported the presence of cross-bridges between NFs, MTs and membranous organelles in quick-freeze deep-etch electron micrographs (QFDE-EM) of fixed and unfixed frog axons.[11] The cross-bridges between NFs were fibrous features 4–6 nm in diameter that connected adjacent NF backbones; they varied in length from 20 to 50 nm (Fig. 3A). Cross-bridges between MTs were also 4–6 nm in diameter, but shorter than their NF counterparts at about 20 nm in length. These cross-bridges remained after chemical extraction and physical rupture of the axon, leading to the conclusion that the cross-bridges were bona fide structures in the axon.[11] Subsequently these types of cross-bridges were reported in other systems.[37,38]

Decoration using antibodies to the three NF polypeptides, in combination with QFDE-EM, was used to identify the composition of the cross-bridge.[39] Antibodies to NF-L decorated the NF core uniformly but not cross-bridges; antibodies to NF-M also decorated the core but less uniformly and were sometimes found located at the bases of cross-bridges. NF-H antibodies primarily decorated the cross-bridges between NFs, suggesting that NF-H was a component of cross-bridges. The nature of the NF cross-bridge was also

Figure 2. Schematic representations of two models for neurofilament or microtubule interactions. **A:** In the cross-bridge model, filaments are linked by non-covalent interactions involving surface projections to form an interconnected network; for microtubules, this interaction is mediated by the projection domain of MAP2 or tau while, in neurofilaments, the interaction is mediated by the side arms of the NF-M or NF-H subunits. Accessory proteins such as BPAG1 may also be involved. The filaments are shown in cross section as round circles, the connecting elements are shown as squiggly lines and the link is shown as a small rectangle. Few details about the structure of the connecting elements or the link between them are known, and this depiction is intended as a conceptual representation. **B:** The central feature of the polymer-brush model is that the surface projections from microtubules or neurofilaments are highly unstructured, in rapid Brownian motion and form a so-called polymer brush. In relatively short times, on the order of nanoseconds, these projections adopt a very large number of conformations, essentially filling some characteristic space. Proteins entering this space tend to be excluded based on entropic considerations; thus as two filaments are brought together, the polymer brush gives rise to a repulsive interaction between the filaments.

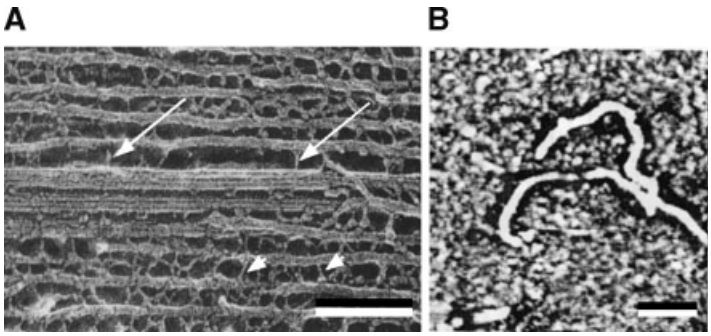


Figure 3. Micrographs illustrating central features of the cross-bridge model and polymer-brush model. **A:** Quick-freeze deep-etch electron micrograph of a frog axon. Cross bridges are visible between neurofilaments (arrowhead) and between microtubules and neurofilaments (long arrows). Scale bar is 100 nm. Reproduced from Hirokawa, N. 1982. J Cell Biol 94:129–142 with permission of the Rockefeller University Press. **B:** AFM image of a bovine NF.[14] A clear zone where adsorbates are excluded surrounds the NF core for about 100 nm on either side, suggesting the presence of a long-range repulsive force exerted by the NF side arms. Scale bar is 500 nm.

explored using the baculovirus expression system.[40] Cells expressing NF-L and NF-H formed parallel arrays of 10 nm filaments with frequent 30–40 nm cross-bridges between filaments as determined by QFDE-EM. A series of deletion mutants lacking varying portions of the NF-H tail were used to delineate the role of the tail domain. The results suggested that the last 191 amino acids of the side arms participate in cross-bridge formation.

For MTs, it has been proposed that MAPs serve a role similar to the NF side arms and act to cross-bridge MTs. MT-MAPs purified from *C. elegans* and reconstituted in vitro formed a cross-bridged network.[10] The cross-bridges were periodic, with a frequency of one interfilament connection every 7.7 nm along the protofilament length. The MAPs could be extracted with 0.4 M NaCl, resulting in the loss of cross-bridges. In another QFDE-EM study, tau purified from porcine brain was observed to form periodic protrusions (18.7 ± 4.8 nm long) from the microtubule surface. Most of these tau projections formed cross-bridges between adjacent microtubules.[41] Hirokawa and colleagues used the baculovirus expression system to examine the role of MAP2 and tau in organizing microtubules.[8] In this system, MAP2 and tau were both observed by QFDE-EM to form cross-bridges between adjacent microtubules. MAP2 produced spacing between microtubules similar to that seen in dendrites, while tau produced microtubular spacing similar to that seen in axons. This suggested that the characteristic interfilament spacing was related to the length of the projection domains of MAP2 ($\sim$1,400 amino acids) and tau ($\sim$242 amino acids). Heterologous cross-bridges between MTs and NFs have also been observed; 20–50 nm cross-bridges between microtubules and NFs were described in frog axons by QFDE-EM.[11] Immunogold labeling and in vitro reconstitution showed that MAP2 was a component of these cross-bridges.[27]

While QFDE-EM has provided important insight into the location and distributions of MT-MAPs and NFs, a significant criticism of QFDE-EM is that the structures seen by this technique may not reflect bona fide cross-bridges. It has been suggested that cross-bridges may be artifacts of the QFDE-EM preparation method.[42] In the polymer-brush model, the unstructured protrusions (i.e. NF side arms or MAP projection domains) are in rapid Brownian motion and would not be expected to withstand EM preparation methods. The chains would either collapse onto the filament backbone or entangle with protrusions from adjacent filaments thereby forming a structure that grossly resembles a cross-bridge. This would explain why the number of protrusions seen in QFDE-EM is much smaller than the number known to be present from other methods.

The spatial arrangement of NFs relative to each other as seen in cross-sections of axons provides insight into the nature of interfilament interactions. Katz and colleagues analyzed the distribution of NFs in thin section electron micrographs of axons and found a Poisson distribution.[42] Such a distribution, they concluded, was only possible if NF organization was primarily due to non-specific stochastic forces acting to distribute the NFs, with no significant NF–NF binding. In a subsequent study, NF–NF nearest neighbor spacings were also compared with simulated distributions of highly ordered and randomly positioned particles.[9] From the results of this study, the authors concluded that NF distributions lie intermediate between completely random and highly ordered. Finally, this general approach was extended in a study of the distribution of NFs in mouse sciatic nerve cross sections, using radial distribution functions and occupancy probability distributions to characterize interfilament interactions.[33,34] These statistical metrics showed the NF organization to be best described in terms of pair-wise repulsive interactions. Monte Carlo simulations were used to compare the various models of rigid cross-bridges, soft cross-bridges and long-range repulsive forces. For the models tested, the long-range repulsive force model was most consistent with the experimental observations.

Physical properties of the NFs and MAPs

NFs and MT-MAPs form gels in vitro, and the physical characteristics of these gels have been studied to gain insight into interfilament interactions. Morris and Lasek initially showed

that axoplasm from squid giant axon retained its shape after extrusion, where the chief difference between the sheathed axoplasm and the extruded one was the latter was wider and shorter.[43] This suggested that the cytoskeleton of the giant squid axoplasm was held together, providing support for the cross-bridging idea. However, a later study on extruded axoplasm found that the volume increased continuously after release from the confines of the plasma membrane.[44] This result suggested that Brownian forces were sufficient to separate NFs, and that interactions between NFs were either repulsive or very weak.

Rheological studies of isolated NFs have shown that these filaments can form gels with parallel filaments organized in bundles that under some conditions behave as though they are cross linked.[45] This cross linking can be modulated by addition of peptides containing the sequence KSP, which mimics a set of repeats found in the side arms of NF-H. This led to the proposal that NF cross linking is mediated through variable antiparallel overlap of the phosphorylable KSP domains of NF side arms on adjacent NFs.[24] However, this cross-linked behavior is most prominent at high (>6 mM) Mg^{2+} concentrations. At Mg^{2+} concentrations close to those found inside neurons (0.5 mM) there is little or no cross linking.[46,47]

Centrifugation of MTs in the presence or absence of MAPs produces dramatically different pellets; MTs without MAPs form a small, tight pellet, while MT-MAPs form a highly hydrated and gelatinous pellet.[48] Analysis of MT-MAP pellets showed that MAPs increased the specific volumes of microtubule pellets more than tenfold while the protein mass only increased ~30%. The size of the pellet was linearly dependent on pH, without changes in the number of MAPs bound to microtubules. Further, rheological measurements suggested that gels were not cross linked. These observations do not support a model where microtubule–MAP gels are cross linked; they are consistent with the polymer-brush-based mechanism. A natively unstructured protein has a specific volume much larger than a folded protein, and an unstructured projection domain would produce the changes in specific volume as seen in these experiments. The pH dependence arises from the changes in the charge on the projection domain. Treating the projection domain as an unstructured polyelectrolyte, reducing the charge would cause the specific volume (and radius of gyration) to decrease. This would produce a smooth dependence of interfilament spacing on pH. The idea that the MAP2 is unstructured is also supported by NMR and hydrodynamic measurements.[49,50]

Analysis of protein sequences has generated additional insight into the potential properties of NFs and MAPs. Both the MAP projection domain and the NF side arms are composed of so-called "low complexity" sequences.[51] Low sequence complexity has been correlated with proteins that are highly unstructured.[52] A neural network predictor, developed to recognize long disordered regions from protein databases, identified the murine NF-H side-arm as the sixth highest scoring sequence in the Swiss-Prot database.[53] Further, a broad analysis of unstructured proteins identified MAP2 as one such protein with a characteristic combination of low overall hydrophobicity and excess net charge.[54]

Additional support for repulsive interactions between MT-MAPs comes from X-ray scattering experiments on microtubule gels.[55] In this work, the authors found that MAPs hindered the parallel packing of MTs during sedimentation while MTs without MAPs packed efficiently. Further, as the concentration of MAPs increased, the sedimentation force required for the efficient packing of MTs also increased. Finally, these investigators demonstrated the inhibitory effect of MAPs on microtubule packing increased with the level of phosphorylation. The authors concluded that these data support a role for MAPs as repulsive spacers that maintain MT–MT separation distances rather than attractive cross linkers that recruit and organize MTs into bundles.

Atomic force microscopy (AFM) has enabled the direct measurement of forces near neurofilaments and MAPs. These experiments revealed long-range (>50 nm) repulsive forces on isolated bovine NFs that are consistent with a polymer-brush model (Fig. 3B).[14] Similar measurements on neuronal MAPs showed a repulsive interaction that extends >100 nm, and the MAP polymer-brush height scales with ionic strength as expected for an unstructured polyelectrolyte.[15] These measurements are not readily reconciled with an electrostatic model or a cross-bridge model.

Biochemical and genetic characterization of NFs and MAPs

If NFs and MTs are cross-bridged by side arms or projection domains, it should be possible to biochemically demonstrate interactions through quantitative binding assays. To our knowledge, there are no demonstrations of specific interactions between NF side arms, nor has binding of side arms on one filament to the NF core or another been shown. To the contrary, binding measurements of purified NF-M and NF-H sides arms showed no propensity of these molecules to interact,[56,57] and NF-M and NF-H have been shown to sediment as monomers.[58] Similarly, when MT–MAPs were treated with chymotrypsin under conditions that sever the projection domain from the MT-binding domain, only the MT-binding domain sedimented with the MTs, suggesting that the projection domain does not bind directly to the microtubule.[59] However, there are cases in which biochemical interactions between NFs and microtubules have been reported. Using a sedimentation binding assay, Hisanaga and Hirokawa measured the interactions between dephosphorylated NF side arm and bare microtubules and found dissociation constants of 1×10^{-7} M and 3.8×10^{-8} M.[60] Further, a study of MAP–NF interactions found a specific, saturable, and reversible binding of MAPs to NF-L with a K_d of 2×10^{-7}.[61] Thus there is

some biochemical evidence for a weak MAP–NF interaction, but no MT-MAP–MT-MAP or NF–NF interactions.

This lack of an unambiguous demonstration of biochemical binding in purified NF or MT-MAP preparations could be explained by the need for accessory proteins. One possible class of such proteins are the plakins, which have been shown to anchor the constituents of the cytoskeleton to each other and to membrane junctions.[62] One member of the plakin family is bullous pemphigoid antigen (BPAG1); its neuronal isoforms BPAG1n1 and BPAG1n2 are thought to cross link NF with actin cytoskeleton,[63] while a third splice form, BPAG1n3, was found cross link NFs to microtubules.[64] These results imply that BPAG1 is an integral component of the neuronal cytoskeleton, capable of linking the three main cytoskeletal networks. However, subsequent work on BPAG1 suggested that it does not interact directly with neurofilaments and as such would not be capable cross linking MTs to NFs.[65]

There have also been efforts to use mouse genetics to probe the role of neurofilaments in determining axonal properties and contributing to neurologic disease. All three NF subunits have been knocked out and overexpressed individually and in various combinations.[21,66] While this has provided insight into the importance of the NF cytoskeleton to axonal dimensions and overall neuronal physiology, these approaches have not substantially helped to distinguish between different models for NF organization. However, recent results where the tails of NF-M and NF-H are deleted are consistent with the polymer-brush model.[67] Deletion of the NF-M tail results in a reduction of the interfilament spacing, and deletion of both the NF-M and NF-H tails produces an even smaller interfilament spacing. Deleting the NF-M side arms would reduce the density of the polymer brush and thereby reduce the repulsive interaction force; deleting both NF-M and NF-H side arms would eliminate the brush and the associated forces.

Functional considerations

The mechanisms that organize the NF and MT cytoskeleton must be consistent with the functional properties of the filaments themselves and of the neuronal processes in which they participate. We now discuss the cross-bridge model and polymer-brush model in the context of some of these properties.

Modulating interfilament spacing

Nearest neighbor NF spacing varies significantly in different cellular locations, and under different physiological conditions. NF–NF spacing varies continuously or with very small increments over the entire observed range, with almost any distance between the extremes allowed. The spacing is also known to correlate with phosphorylation, where higher phosphorylation is associated with larger interfilament spacing, which implicates phosphorylation in the control mechanism.[68,69] For a well-folded protein, it is difficult to imagine how interfilament spacing might be modulated continuously, since this would require a gradual conformational change over a very large distance. Although, it has been proposed that antiparallel overlap of NF side arms could account for a variable length cross link.[24]

The polymer-brush model provides a simple and intuitive mechanism for modulating interfilament spacing. Treating the side arms or projection domains as unstructured polyelectrolytes, the magnitude of the repulsive interaction that an approaching (neutral) object experiences is closely related to the charge of the polymer.[70] Increasing the charge along the polymer, such as by phosphorylation, produces intramolecular electrostatic repulsion and causes the polymer to effectively swell (Fig. 4). Because of the large number of phosphates in NF side arms and MAPs, such a mechanism would allow for a very smooth modulation of interfilament distance. It should be noted that this mechanism is different from the electrostatic repulsion model, where electrostatics between adjacent NFs are thought to control spacing.[31] The polymer-brush model also allows for interfilament electrostatic interactions, but the dominant effect of electrostatics is to determine the excluded volume of individual filaments. One consequence of this model is that hyperphosphorylation of NF-M could compensate for the loss of NF-H, which is consistent with results from the experimental deletion of the NF-H tail.[26] Evidence for a similar control mechanism in MTs comes from Mandelkow and colleagues who have demonstrated that tau protein is a highly elastic molecule that can stretch or contract by $>$300%, depending on phosphorylation state.[71]

Is axonal caliber determined, modified or maintained by NFs?

A number of studies have established a clear association between axonal caliber and neurofilaments.[72,73] However, the nature or functional consequences of this relationship remains unclear. NF-L knockout mice lack neurofilaments yet still form axons properly, albeit with a smaller bore.[74] Thus, NFs are not essential for the formation of a functional axon, although they appear to be involved in modifying axonal caliber during development. NFs may also act to maintain axonal patency and integrity.

The polymer-brush model offers a mechanism to provide mechanical protection of axons and dendrites. Compression of an axon can result in collapse of the bore, and loss of electrical conduction and material transport.[75,76] Unstructured side arms (or projection domains) would act as entropic or Brownian springs that resist compression by virtue of the thermally driven motion in the polypeptide chain.[32] By acting as springs, the NF side arms in effect radially stiffen the axon so the process can resist mechanical compression. Cell mechanical properties could be further modulated using phosphorylation to control interfilament forces. Interestingly,

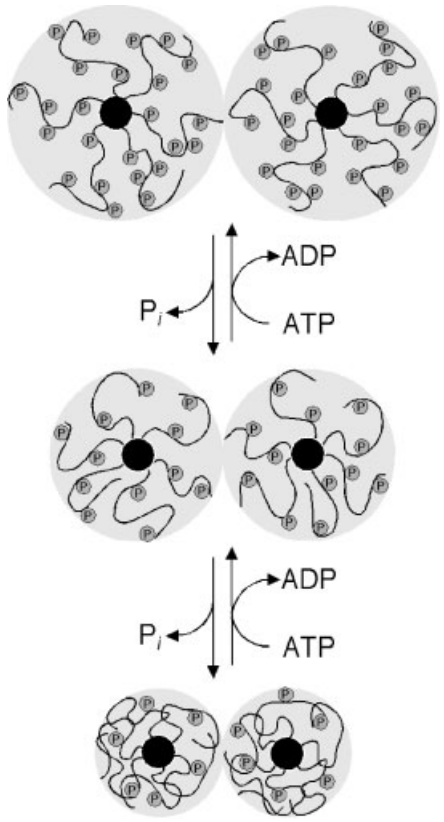


Figure 4. Schematic representation of a mechanism for modulating interfilament interactions by phosphorylation. If the MAP projection domains or the NF side arms are treated as unstructured polyelectrolytes, then phosphorylation can be used to control interfilament interactions by primarily controlling intramolecular interactions. The volume of the characteristic space occupied by an unstructured polyelectrolyte depends in large part on electrostatic interactions between different segments of the polymer; as the overall net charge increases, the volume increases due to intramolecular repulsion between segments. Thus, in the polymer-brush model, increasing phosphorylation would tend to increase interfilament repulsion. This increase in repulsion would tend to increase the interfilament spacing or increase the order of NFs (as seen in cross sections). Because of the large number of phosphates, relatively small changes in repulsion can be achieved by adding or removing a small number of phosphates at any one time.

double NF-M/NF-H knockout mice have been shown to recover more slowly than wild-type mice from neuronal crush injuries.[77] Such a result could be reconciled with changes in mechanical properties such that a given crush produces more significant damage in knockouts than in wild-type animals, rather than altering the maturation dynamics. It is also interesting to note that, in the polymer-brush model, MT-MAPs can act to functionally complement NFs. The MAP projection domain serves as an entropic spring similar to the NF side arms, and could similarly affect cell mechanics. This is consistent with the observation that the number of microtubules often increases when the NF number is reduced.[78,79] It further suggests that MAPs may play an important mechanical role in dendrites.

Transport

The cytoskeleton plays an important role in transport through neurites. One issue with a highly cross-bridged cytoskeleton is that it a priori would seem to hinder transport, and that large numbers of cross-bridges would have to be actively broken and reformed as material is moved along an axonal or dendritic process. A polymer brush would pose a barrier to objects entering a neurite; this obstacle can be overcome stochastically (i.e. trying many times to enter) and would slow but not prevent entry. Once an object has entered a process, there is little or no barrier to movement; neuronal transport is a low Reynolds number process and the viscosity of axoplasm is only slightly higher than water.[80] Consistent with this idea, Mandelkow and colleagues have shown that overexpression of MAPs on microtubules decreases the rate of attachment and detachment of motor proteins, but does not affect the run length or speed of the motors.[81] We also note that, in the microtubule motor kinesin, the motor and cargo domains are separated by an 80 nm stalk,[82] which would allow the cargo to move at the periphery of the MAP polymer brush.

Pathological conditions

One pathologic hallmark of a number of neurodegenerative diseases is the aggregation of and accumulation of neurofilaments.[83] In the polymer-brush model, NF side arms may act to stabilize solutions of NFs similar to the way other colloidal suspensions are stabilized. Grafting of unstructured polymers is widely used in the production of cosmetics, foodstuffs and pharmaceutical to keep particles from aggregating.[36] A well-studied biological example is casein stabilization of milk micelles.[84] In these systems, a failure of the polymer brush results in particle aggregation. Thus one might speculate failure of the side arms to maintain interfilament spacing could lead to neurofilament aggregation and subsequent pathologies.

Conclusions

We have reviewed the evidence supporting two leading models of NF and MT organization in neurites: cross-bridging and polymer-brush-based repulsion. It is interesting to note that, while entropic repulsion has only recently been formally articulated as a hypothesis for cytoskeletal organization, elements of this model have been sporadically invoked throughout the literature. For example: MAPs bound to microtubules have been described as "fuzzy coats";[85] Weiss and Mayr described neurofilament and microtubule

distribution in axons as "liquid crystals of heterogeneous composition";[72] Heidemann referred to the idea that "...MAPs determine bundle spacing, possibly by steric hindrance";[86] and Grant and Pant described the role of NFs as "space filling",[21] where the phosphorylation of the KSP repeats in the NF-H and NF-M side arms contribute to space filling. In a study of the phosphorylation sites in squid NF-220, Jaffe et al note that "...the sidearms continue to unfold as they are phosphorylated".[87]

In the end, the question remains: which model for NF and MT organization is more important in vivo? While cross-bridging has been promoted as the primary or even sole structural basis for NF and MT organization in axons and dendrites, the evidence for this is inconclusive at best. This model has its foundation in electron microscopic observations, but has not been unequivocally supported by subsequent biochemical, biophysical or genetic studies. There are many instances where a model in which NF–NF and MT–MT interactions are controlled by entropic repulsive forces more consistently explains the data and more easily integrates into current thinking about the role of cytoskeletal filaments in neuronal physiology. This is not to say that the two models are mutually exclusive; the polymer-brush-based model does not exclude the possibility of cross linking or other attractive interactions. NF and MT organization in vivo is almost surely controlled by a complex equilibrium between attractive and repulsive forces; determining the origin of the forces and what controls this balance represents an important problem in the study of the neuronal cytoskeleton.

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