Title: Special Issue on 'Cytoskeletal proteins in health and neurodegenerative disease'

Author: Roland Brandt

PII: S0361-9230(16)30172-1
DOI: http://dx.doi.org/doi:10.1016/j.brainresbull.2016.08.003
Reference: BRB 9066

To appear in: *Brain Research Bulletin*

Author: Jürgen Götz

PII: S0361-9230(16)30172-1
DOI: http://dx.doi.org/doi:10.1016/j.brainresbull.2016.08.003
Reference: BRB 9066

To appear in: *Brain Research Bulletin*

Please cite this article as: Jürgen Götz, Special Issue on 'Cytoskeletal proteins in health and neurodegenerative disease', Brain Research Bulletin http://dx.doi.org/10.1016/j.brainresbull.2016.08.003

Editorial

Special Issue on 'Cytoskeletal proteins in health and neurodegenerative disease'

Roland Brandt[1,*] & Jürgen Götz[2,*]

[1]Neurobiologie der Universität Osnabrück, Barbarastrasse 11, D-49076 Osnabrück Germany, [2]Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, St Lucia Campus, Brisbane, QLD 4072, Australia

[*] Send correspondence to RB (brandt@biologie.uni-osnabrueck.de) or JG (j.goetz@uq.edu.au)

Abstract

The cytoskeleton is the major intracellular structure that determines the morphology of neurons and maintains their structural integrity. It is therefore not surprising that a disturbance of cytoskeletal structure and function underlies many neurodegenerative diseases. This special issue brings together current information on the three major neuronal cytoskeletal filament systems, microtubules, microfilaments and neurofilaments, and aims to provide a comprehensive overview of the role of the key components of these three systems under both physiological and pathological conditions. It therefore also addresses the role of microtubule-associated proteins (with a focus on tau) and motor proteins (with a focus on kinesin).

Main text

The cytoskeleton is a key component of every eukaryotic cell. It consists of protein polymers and a collection of associated factors that are organized in a network of filamentous arrays that interact in a dynamic manner. The cytoskeleton provides a structure that, on the one hand, is rigid, and on the other hand is responsive to a range of cues which allow it to be sufficiently plastic to facilitate a change in cell shape and, in some cases, cell movement. The cytoskeletal elements also interact with motor proteins that provide both the force and directionality that are needed for intracellular transport and mobility (Tuszynski et al., 2003).

In neurons, the cytoskeleton is tailored to meet the specific needs of this specialized cell type, which consists of a cell soma and two principal types of processes, the axons and the dendrites, which can extend a remarkable distance from the soma and develop a complex branching pattern depending on the type of neuron. The axon is separated from the soma by the axon initial segment, which provides an effective diffusion barrier. Dendrites are further compartmentalized based on the presence of dendritic spines that represent the post-synaptic compartment of most excitatory input. The dendrites and the soma comprise a relay station where incoming signals are integrated. Neurons maintain their peculiar structure and compartmentalization with the help of an internal dynamic cytoskeleton made up of different filament systems. The three main neuronal cytoskeletal structures discussed in this Special Issue are actin filaments (Nixon, Gallo), neurofilaments (Vickers) and microtubules (Baas, Gallo, Prokop). Associated with microtubules, and binding to them, are microtubule-associated proteins (MAPs), including the axonal protein tau (Arendt), and motor proteins, such as kinesin, which provides anterograde transport, and dynein, a retrograde transporter (Kins). Prominent proteins that interact with actin filaments and are considered to regulate microfilament structure are the tropomyosins (Fath) and drebrin (Gordon-Weeks).

A plethora of articles are available on selective aspects of cytoskeletal proteins but what has been lacking from the literature is a collection of review articles focusing on all of these major cytoskeletal elements and the proteins with which they are associated, as well as their function in health and disease. This Special Issue brings together nine review articles that present information on the role of the neuronal cytoskeleton under both physiological and pathological conditions, its interactions and its regulation, as detailed below.

(1) Microtubules serve as structural elements to allow axons and dendrites to obtain their specialized morphology. They also function as long-distance railways for

the anterograde and retrograde transport of proteins and organelles. Peter Baas and Andrew Matamoros discuss the intricacies of microtubule dynamics and outline how the nucleation of microtubules occurs as well as the way in which the three forms of tubulin (α, β, and γ) differ (Baas). Microtubules are intrinsically polar structures, with β-tubulin being located at the plus and α-tubulin at the minus end; the motor dynein moves towards the minus end of the microtubule and most kinesins move towards the plus end. Facilitating microtubule organization in neurons, microtubule-interacting proteins are enriched in specific compartments, such as TRIM46 in the axon initial segment. The authors also discuss the puzzle regarding the source of neuronal microtubule stability. Although tau and other MAPs are traditionally seen as microtubule-stabilizing compounds, it is suggested (factoring in the millisecond on and off rate of these proteins) that they may be more important in regulating rather than conferring stability. Attention is also given to microtubule end-targeting proteins (such as EB3) and microtubule-severing proteins (katanin and spastin). Regarding motor proteins, the concept of a specific tubulin code, which seems to mark microtubules for defined interactions, is discussed. The final section of this review then discusses microtubule dysfunction in the context of disease (Alzheimer's disease, amyotrophic lateral sclerosis and Huntington's disease). The authors suggest that, rather than exploring the potential of microtubule-stabilizing drugs, inhibiting microtubule-severing proteins might be the better approach for therapeutic interventions targeting microtubules.

(2) Andreas Prokop and colleagues specifically discuss the dynamics of microtubules, dissecting the cytoskeletal machinery into its conceptual sub-machineries and then assessing how these interface with each other (Prokop). The first section of this review addresses the role of microtubule dynamics in development and how this dynamic feature persists into adulthood. The second section provides an overview of the physical and biochemical properties of tubulin and how microtubules, the stiffest of all cytoskeletal polymers, form lattices. The various mechanisms regulating the dynamics of microtubules at the plus end are discussed and summarized in an integrating figure, highlighting the importance of how tubulin pools are controlled in axons. The article discusses this issue, delving into the transcriptional control of tubulin, the different modes of transport, microtubule stability and controlled degradation pathways. Up to a few hundred EB proteins can bind to the microtubule plus end with a very short dwell time, i.e. in a highly dynamic manner with a constant turn-over. These interactions are discussed, together with the various functions of the plus end. Finally, a section also addresses the influence of lattice-based mechanisms on the polymerization and depolymerization of microtubules, and reports on the post-translational modifications of microtubules that are often taken as indicators of microtubule stability, with detyrosination and acetylation correlating with stable fractions. As is also discussed by Matamoros and Baas (Baas), the mechanism by which MAPs act on microtubules is poorly understood. Prokop and colleagues present a model in which microtubules in mature axons are constantly renewed through steady state de/polymerization processes.

(3) Thomas Arendt and colleagues present a comprehensive overview of the 'neuronal' MAP tau, broadly covering its cell biology and central role in tauopathies including Alzheimer's disease (Arendt). The cell biology section covers tau haplotypes and the regulation of gene expression of this multiple isoform protein, providing structural insight into tau and its domains, its post-translational modification, with a major focus on phosphorylation, and its cellular functions (canonical in binding microtubules, non-canonical, and even non-neuronal). The second half of the review

discusses all facets of tau pathology, initially focusing on Alzheimer's disease as 'the' tauopathy prototype, and then on tauopathies more generally, in which the tau pathology is not confined to neurons, but is also present in glial cells. The review contains 12 figures that are suitable for didactic purposes and four detailed tables, listing tau phosphorylation sites, the various tauopathies, a classification of tauopathies based on the predominant tau isoform, and the predominant cell-type-specific tau pathology in different tauopathies. In their conclusion, Arendt and colleagues point out that our appreciation of the role of tau in 'non-typical' compartments, such as the nucleus and spines, is only slowly unfolding. Furthermore, as they point out, how tau causes neurodegeneration is still not well understood. Another intriguing question is the biological significance of the large heterogeneity of tau isoforms, with tau being an apparently promiscuous molecule. Finally, determining how the two key lesions of Alzheimer's disease, tau and amyloid β (Aβ), interact, is important for developing treatment strategies, yet there is a still a lack of basic understanding in relation to this issue.

(4) In his review, Phillip Gordon-Weeks discusses the filamentous (F)-actin side-binding and bundling protein drebrin, which couples actin filaments to dynamic microtubules (Gordon-Weeks). This interaction has a critical developmental role during neuronal growth cone formation, as well as in the dendritic spines of mature neurons. As elaborated in the article, drebrin comes in two forms, an 'embryonic' drebrin E and an 'adult' drebrin A; however, their distinctive roles have not been fully dissected. The author suggests the use of super-resolution microscopy to better understand how drebrin is distributed in neurons and what governs its localization. He further discusses the various binding partners of drebrin, together with the functional domains in the protein that govern these interactions. A preferred partner of drebrin is EB3 that localizes to the growing plus end of dynamic microtubules. Evidence from cell biological studies and structural analyses are presented that indicate that drebrin stabilizes F-actin. In the case of spines, it is well known that the dynamic behaviour of F-actin and microtubules dominates dendritic spine changes in response to synaptic activity. Here, drebrin has an important role, influencing dendritic spine morphology by regulating F-actin, facilitating the activity-dependent accumulation of the scaffolding protein PSD-95 in the post-synaptic density, and distributing NMDA receptors. An interesting discussion is presented that focuses on activity-driven microtubule capture and insertion into dendritic spines. Whereas normally only approximately 1% of dendritic protrusions contain a microtubule at any time, the frequency of invasion, as well as the dwell time, are enhanced by activation, with EB3 being localized to the tip of these microtubules. In a disease context, drebrin loss, concomitant with an increase in the actin-severing factor cofilin, has been shown to precede synaptic loss in Alzheimer's disease. Drebrin loss can also be induced *in vitro* by incubation with Aβ oligomers. Cofilin activity is negatively regulated by the activity of LIM kinase that, interestingly, protects against Aβ-induced drebrin loss. As suggested, more work needs to be undertaken to investigate the drebrin/EB3 pathway, or any other pathway, that links dynamic microtubules and F-actin under physiological or pathological conditions.

(5) Next, Gianluca Gallo and Ammudena Pacheco review the literature covering recent advances in our understanding of cytoskeletal interactions in neurons by focusing on the initiation of processes from neuronal cell bodies and the collateral branching of axons (Gallo). The authors come to the conclusion that the appreciation of the neuron as an integrated system remains a frontier of investigation. The review discusses differences in nucleation mechanisms that are more restricted for

microtubules than for actin, with the majority of work being done *in vitro*. The authors discuss the evidence for cross-talk and interactions between actin filaments and microtubules in the growth cone and in axon branching and initiation, presenting the data in a historical overview. There is a section on MAPs, with the different forms of MAP2 and MAP1b all binding to both microtubules and actin. The fourth section discusses microtubule plus end-associated proteins (EB proteins, drebrin, CLIP-170, cytoplasmic linker protein-associated proteins (CLASPs), spectraplakins, neuron navigator 1 (NAV1) and adenomatous polyposis coli (APC)), after which additional regulators of cytoskeletal cross-talk and organization (septins, collapsin response mediator proteins (CRMPs) and doublecortin) are considered. The review then finishes with a mechanistic section on how microtubules are targeted into filopodia. The authors conclude that, in order to fully understand the complexity of cytoskeletal cross-regulation, the full spectrum of interactions and interactors needs to be addressed in space and time during the multiple steps in the formation of processes and collateral branches. Given that the cytoskeleton underlies neuronal biomechanics, they also suggest that pioneering studies are required to address the biomechanics of axon initiation and branching.

(6) Thomas Fath and colleagues write about the role of tropomyosins in the healthy and diseased nervous system (Fath). Their review first provides a general overview of tropomyosins, for which more than 40 isoforms are known. Tropomyosins are a family of actin-associated proteins, initially identified in muscle, that play a major role in the regulation of the actin cytoskeleton. The major neuronal isoforms are Tpm1, Tpm3 and Tpm4, although their function is not fully understood. For each major isoform, multiple variants are known, adding to the complexity of this protein family. The authors then discuss the role of tropomyosins in neurite outgrowth and at neuronal synapses. There is a cross-talk of different actin regulating proteins; for example, members of the actin filament-severing protein family ADF/cofilin compete with tropomyosins for actin binding, whereas tropomyosin sorting is regulated by formins, a class of potent actin filament nucleators. A section in the review is dedicated to the role of tropomyosins in nervous system disorders, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and schizophrenia, as well as in spinal cord injury and traumatic brain injury. The review concludes by discussing tropomyosins as potential mediators and targets in neuronal repair. The authors suggest that because the interaction of tropomyosins with actin-binding proteins and actin itself is isoform-dependent, these isoforms represent excellent specific targets for therapeutic intervention.

(7) James Vickers and colleagues discuss the third major group of neuronal cytoskeletal structures, neurofilaments, which have not been demonstrated to play a role in the initiation or branching of axons (Vickers). This review is mainly focused on disease. Acknowledging that abnormalities of the neuronal cytoskeleton are frequently a key feature of neurodegenerative diseases, in particular Alzheimer's disease, the authors identify several knowledge gaps, such as those related to the biochemical and histological characterization of changes that have been identified ultrastructurally. They present a historical discourse on the development of staining techniques for the cytoskeleton, with the silver impregnation methods of Bielschowsky and Bodian having the greatest affinity for the neurofilament triplet NFL, NFM and NFH, whereas the closely related Gallyas and Campbell-Switzer methods are routinely used to visualize the two Alzheimer's lesions, tau-containing neurofibrillary tangles and Aβ-containing amyloid plaques. Interestingly, tangles were originally, but incorrectly, thought to be composed of neurofilaments; however, as discussed, some neurofilament

epitopes may lie buried within the filament structure of the tangles. Vickers and colleagues discuss the structure and polymerization of neurofilaments, and expand on the interaction of these proteins with other cytoskeletal elements, including actin, myosin and plectins, as well as MAPs and motor proteins. A section has been included to discuss the susceptibility of neuronal subsets in neurofibrillary pathology and the associated involvement of neurofilaments, together with the role of Aβ in inducing dystrophic changes. This leads the authors to conclude that neurofilaments have an important role in the susceptibility of neurons to key cytoskeletal changes in Alzheimer's disease.

(8) Ralph Nixon and Aidong Yuan also focus on neurofilaments, in particular their role in synapses, where neurofilaments form distinctive assemblies (Nixon). They refer to data showing that the triplet proteins and the neurofilament protein α-internexin are all abundant in fractions obtained from the post-synaptic density, and that they interact with various synaptic proteins, including SAPAP and spinophilin, as well as tau. A section is dedicated to the neurofilament subunit-specific modulation of synaptic functions and behaviours. Here, NFL has been shown to interact not only with NMDA receptors, but also with the adaptor protein 14-3-3 and protein phosphatase 1 (PP1). NFM, in contrast, specifically interacts with dopamine receptors, as shown for synaptic boutons, whereas NFH has a role in long-term potentiation. Ralph Nixon and Aidong Yuan move on to discuss alterations of neurofilament proteins in psychiatric disorders, starting with schizophrenia and bipolar disorder, and then discussing the role of distinct neurofilament proteins in drug addiction. In their discussion of Alzheimer's disease, they make the point that neurofibrillary tangles contain not only tau, but also neurofilaments, vimentin and different forms of MAPs, with a variable degree of phosphorylation. They finish their review by introducing an only recently described disease, neurofilament inclusion disease (NFID), that clinically resembles frontotemporal dementia. They conclude that, although synaptic dysfunction characterizes major neuropsychiatric disorders, the dynamics of cytoskeletal proteins at synaptic terminals is not really understood, particularly in the case of neurofilaments. These intermediate filaments have unique space-filling properties that facilitate their well-established role in calibre expansion of myelinated axons, a critical feature of effective nerve conduction. However, as also discussed in this review, there is growing evidence that neurofilaments in the central nervous system have important functions that go beyond calibre expansion.

(9) In the final article of this series, Stefan Kins, Gerardo Morfini and colleagues focus on conventional kinesin, the most abundant kinesin-related motor protein in the mature nervous system (Kins). After introducing axonal transport in general, and the role of microtubules more specifically, they introduce the kinesin superfamily of motor proteins (KIFs) which is classified into 15 subfamilies. A presentation of the subunit composition, structural diversity and domain structure of conventional kinesin is followed by a section on the mode of kinesin attachment to membrane-bound organelle cargoes. Although no 'bona fide' receptors have been identified, and the mode of attachment of conventional kinesin to its cargoes therefore remains a mystery, several adaptors are known to bind either the heavy or the light chain of the kinesin heterotetrameric complex. This is discussed, followed by an assessment of the role of the different kinesin variants. The authors imply the existence of regulatory mechanisms for the localized delivery of cargoes, considering that there can be up to thousands of presynaptic terminals as 'delivery addresses'. The final section of the article addresses the role of impaired axonal transport in neurodegenerative disease, with a role of pathogenic forms of tau and Aβ known to inhibit conventional kinesin-

based axonal transport. Here, specific kinases have been found to have a role; however, as the authors state, it remains an open question whether kinase-mediated axonal transport deficits represent a primary event in the course of neurodegenerative disease or, alternatively, an epiphenomenon.

Outlook

Gurel and colleagues (Gurel et al., 2014) recently captured what is needed in this rapidly advancing field, writing that 'a tendency in cell biology is to divide and conquer. For example, decades of painstaking work have led to an understanding of endoplasmic reticulum and Golgi structure, dynamics, and transport. In parallel, cytoskeletal researchers have revealed a fantastic diversity of structure and cellular function in both actin and microtubules. Increasingly, these areas overlap, necessitating an understanding of both organelle and cytoskeletal biology.'
Not surprisingly, several of the nine review articles in this Special Issue address the interaction of cytoskeletal proteins, most prominently actin and microtubules, in mediating cellular functions. With the unique features of neurons and their compartmentalization, there will undoubtedly be an increased interest in understanding the role of the cytoskeleton at the site at which action potentials are initiated, as well as at the synapse where neurons communicate. With the advent of super-resolution microscopy techniques, it should now be possible to obtain a deeper understanding of where and how the different cytoskeletal elements interact, the nature of their dynamics in different compartments and how they respond to both internal and external stimuli, be it under physiological or pathological conditions. Finally, obtaining a better basic appreciation of the function of the cytoskeleton will assist in developing tailored strategies to combat disorders of the human nervous system.

We hope that this Special Issue will help to generate a unified picture of the different aspects of cytoskeletal structure and function in neurons, and will aid in developing integrative concepts to understand and modulate cytoskeletal function and malfunction in health and disease.

References

Gurel, P.S., Hatch, A.L., and Higgs, H.N. (2014). Connecting the cytoskeleton to the endoplasmic reticulum and Golgi. Curr Biol *24*, R660-672.

Tuszynski, J.A., Brown, J.A., and Sept, D. (2003). Models of the collective behavior of proteins in cells: tubulin, actin and motor proteins. J Biol Phys *29*, 401-428.

Zhong, G., He, J., Zhou, R., Lorenzo, D., Babcock, H.P., Bennett, V., and Zhuang, X. (2014). Developmental mechanism of the periodic membrane skeleton in axons. Elife *3*.

(references of 9 reviews need to be added by journal)