Stabilization of neuronal connections and the axonal cytoskeleton

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Stabilization of axonal connections is an underappreciated, but critical, element in development and maintenance of neuronal functions. The ability to maintain the overall architecture of the brain for decades is essential for our ability to process sensory information efficiently, coordinate motor activity, and retain memories for a lifetime. While the importance of the neuronal cytoskeleton in this process is acknowledged, little has been known about specializations of the axonal cytoskeleton needed to stabilize neuronal architectures. A novel post-translational modification of tubulin that stabilizes normally dynamic microtubules in axons has now been identified. Polyamination appears to be enriched in axons and is developmentally regulated with a time course that correlates with increased microtubule stabilization. Identifying one of the molecular mechanisms for maintaining neuronal connections creates new research avenues for understanding the role of stabilizing neuronal architecture in neuronal function and in neuropathology.

Building and maintaining a nervous system represents a constantly changing balance between the need for stability of overall neuronal architecture and functional plasticity. During development and maturation of the nervous system, processes are extended and connections established. Once formed, these connections need to be refined in order to create an appropriate match between neurons and target cells. For example, the visual system relies on this refinement in connectivity to produce a precise map of visual space, but once established that map needs to

be maintained for decades with little or no change. Other parts of the brain may continue to exhibit a significant degree of plasticity at the synaptic level while retaining their overall cytoarchitecture and relationship between neurons in different parts of the brain. For example, hippocampal learning and memory requires plasticity in forming new synaptic contacts and generation of new neurons, but if we hope to retain those memories for a lifetime, the appropriate neuronal connections must be stabilized.

While mechanisms for altering synaptic contacts and function have been studied extensively, less attention has been paid to mechanisms for stabilization of the axonal processes that support those synapses. Given that axons in humans may be a meter or more in length and need to be maintained for many decades, neurons face a daunting logistical problem: how to maintain and renew the microtubules and neurofilaments that comprise the axonal cytoskeleton without affecting the connections they support.

Increases in the number and composition of axonal neurofilaments have long been thought to contribute to axonal stability, because the most dramatic changes in neurofilament composition and number are associated with maturation of the neuron and myelination.¹ Although neurofilaments can affect axonal diameters,^{2,3} the metabolic stability of these cytoskeletal structures limits their contribution to axonal plasticity. Indeed, during development and regeneration of axons, neurofilaments are downregulated⁴ and neurofilament proteins are not transported into regenerating fibers until the fibers begin to

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mature.⁵ Changes in the neurofilament cytoskeleton do not seem to be sufficient to explain changes in plasticity.

Microtubules appear to be well suited for mediating axonal plasticity, given the evidence that microtubules exhibit dynamic instability in many cell types⁶ and dynamic microtubules are critical for both neurite elongation and growth cone function.⁷ However, mechanisms for stabilization of microtubules were not as well characterized. Originally, microtubule associated proteins (MAPs) were thought to be the primary mechanism for stabilization of microtubules⁸ and the unique MAPs of dendrites (MAP2) and axons (tau) were suggested to provide specialization of microtubules in these compartments.⁹ However, mice lacking tau protein are viable and exhibit only minor changes in small caliber axons.¹⁰ Alternatively, post-translational modifications of tubulins^{11,12} were proposed to stabilize microtubules. Elevated levels of tubulin acetylation and detyrosination in the brain^{11,13} were used to suggest that these modifications were associated with stabilization of microtubules in the neuron, but such modifications fail to alter microtubule dynamics *in vitro*.¹⁴ Some combination of post-translational modification and MAPs might be invoked as a way to stabilize MTs, but even this option is difficult to reconcile with the mild phenotype of the tau null mouse.

To explain stabilization of the axonal cytoskeleton, we need to recognize that axonal microtubules differ from microtubules in other cell types in several fundamental ways. First, axonal microtubules may be hundreds of microns long.¹⁵ Second, axonal microtubules appear to be maintained for however long it takes for their arrival at the presynaptic terminal.¹⁶ Third, neuronal microtubules lack a connection with the microtubule organizing center (MTOC), unlike most non-neuronal microtubules. In neurons, microtubules are released after nucleation and transported into axons or dendrites,^{17,18} where they may undergo dynamic exchanges of tubulin subunits, but at the same time keep their integrity as

polymerized microtubules. This implies the existence of stable domains that serve both to nucleate microtubules in the distal axon and to stabilize microtubules in order to maintain neuronal structures.

As a result, the balance between microtubule stability and dynamics is especially important for neuronal axons. Previous work established that a significant fraction of axonal tubulin remains stably polymerized after treatment with depolymerizing treatments, such as cold temperature, millimolar levels of calcium and anti-mitotic drugs like vincristine.¹⁹ This particular fraction was given the name of cold stable tubulin (CST), which represents a fraction of tubulin in axonal microtubules with a stabilizing modification.¹⁷ This fraction was found to be increased during maturation and aging of the nervous system.^{20,21} However, the biochemical characteristics and underlying mechanisms for production of CST were largely unknown.

Recent studies showed that difluoromethylornithine (DFMO), an irreversible inhibitor for polyamine synthesis, reduced microtubule stability *in vivo*, and that tubulin in the rat optic nerve covalently incorporated radioactive polyamines from intraocular injection.²² This strongly suggested that polyamines were involved in microtubule stabilization. A specific biochemical mechanism was provided by evidence that transglutaminase (TG), a family of enzymes that catalyze a transamidation/deamination reaction, can stabilize substrates by catalyzing a covalent bond between the primary amine group of the polyamines and a glutamine residue of the proteins.²³ Although the role of transglutaminase and polyamines in stabilizing neuronal tubulins had not been studied previously, both polyamines^{24,25} and multiple TG isoforms (including TG1–3 and 6, with TG2 as the major neuronal transglutaminase)²⁶ are widely expressed in neurons.

The effects of transglutaminase-mediated addition of polyamines to tubulin on microtubule stability and microtubule dynamics were characterized both *in vitro* and *in vivo*, demonstrating that

polyamination was able to generate stable microtubules.²² This tubulin modification appears to occur preferentially in the nervous system and to be enriched in axonal tubulin fractions, consistent with a mechanism for stabilizing the axonal cytoskeleton. Significantly, the expressions of transglutaminase and polyamines are developmentally regulated and continue to increase during maturation and aging of the nervous system,²² the times when microtubule stability is increasing. Levels of CST are dramatically affected by myelination,^{20,21} which is correlated with increases in transglutaminase activity (unpublished observations, Y Song and ST Brady).

These observations make a compelling case for a role of transglutaminase and polyamines in stabilization of neuronal architecture, particularly axonal connections. The implication is that this pathway may be an essential modulator of functional plasticity in the nervous system. If so, then this pathway may prove to be a key to solving two longstanding puzzles: (1) How can the balance between stability and plasticity in axonal connections be modulated during different stages of development, maturation and aging of the nervous system? and (2) What are the functional implications for cell-specific modifications of tubulin? Understanding how this novel post-translational modification of the axonal cytoskeleton affects connectivity in the brain in normal maturation and aging, as well as in pathological conditions such as traumatic brain and spinal cord injury, stroke and neurodegeneration is an important next step in defining the molecular basis for neuronal plasticity and stabilization.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

- Brady ST, Witt AS, Kirkpatrick LL, de Waegh SM, Readhead C, Tu P-H, Lee VM. Formation of compact myelin is required for maturation of the axonal cytoskeleton. *J Neurosci* 1999; 19:7278-88; PMID:10460234
- de Waegh SM, Lee VM-Y, Brady ST. Local modulation of neurofilament phosphorylation, axonal caliber, and slow axonal transport by myelinating Schwann cells. *Cell* 1992; 68:451-63; PMID:1371237; [http://dx.doi.org/10.1016/0092-8674\(92\)90183-D](http://dx.doi.org/10.1016/0092-8674(92)90183-D)
- Xu Z, Marszalek JR, Lee MK, Wong PC, Folmer J, Crawford TO, Hsieh ST, Griffin JW, Cleveland DW. Subunit composition of neurofilaments specifies axonal diameter. *J Cell Biol* 1996; 133:1061-9; PMID:8655579; <http://dx.doi.org/10.1083/jcb.133.5.1061>
- Oblinger MM, Lasek RJ. Axotomy-induced alterations in the synthesis and transport of neurofilaments and microtubules in dorsal root ganglion cells. *J Neurosci* 1988; 8:1747-58; PMID:3130470
- McQuarrie IG, Lasek RJ. Transport of cytoskeletal elements from parent axons into regenerating daughter axons. *J Neurosci* 1989; 9:436-46; PMID:2493076
- Kueh HY, Mitchison TJ. Structural plasticity in actin and tubulin polymer dynamics. *Science* 2009; 325:960-3; PMID:19696342; <http://dx.doi.org/10.1126/science.1168823>
- Gordon-Weeks PR. Microtubules and growth cone function. *J Neurobiol* 2004; 58:70-83; PMID:14598371; <http://dx.doi.org/10.1002/neu.10266>
- Amos LA, Schlieper D. Microtubules and maps. *Adv Protein Chem* 2005; 71:257-98; PMID:16230114; [http://dx.doi.org/10.1016/S0065-3233\(04\)71007-4](http://dx.doi.org/10.1016/S0065-3233(04)71007-4)
- Dehmelt L, Halpain S. Actin and microtubules in neurite initiation: are MAPs the missing link? *J Neurobiol* 2004; 58:18-33; PMID:14598367; <http://dx.doi.org/10.1002/neu.10284>
- Harada A, Oguchi K, Okabe S, Kuno J, Terada S, Ohshima T, Sato-Yoshitake R, Takei Y, Noda T, Hirokawa N. Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature* 1994; 369:488-91; PMID:8202139; <http://dx.doi.org/10.1038/369488a0>
- Hammond JW, Cai D, Verhey KJ. Tubulin modifications and their cellular functions. *Curr Opin Cell Biol* 2008; 20:71-6; PMID:18226514; <http://dx.doi.org/10.1016/j.cceb.2007.11.010>
- Janke C, Kneussel M. Tubulin post-translational modifications: encoding functions on the neuronal microtubule cytoskeleton. *Trends Neurosci* 2010; 33:362-72; PMID:20541813; <http://dx.doi.org/10.1016/j.tins.2010.05.001>
- Fukushima N, Furuta D, Hidaka Y, Moriyama R, Tsujiuchi T. Post-translational modifications of tubulin in the nervous system. *J Neurochem* 2009; 109:683-93; PMID:19250341; <http://dx.doi.org/10.1111/j.1471-4159.2009.06013.x>
- Maruta H, Greer K, Rosenbaum JL. The acetylation of alpha-tubulin and its relationship to the assembly and disassembly of microtubules. *J Cell Biol* 1986; 103:571-9; PMID:3733880; <http://dx.doi.org/10.1083/jcb.103.2.571>
- Bray D, Bunge MB. Serial analysis of microtubules in cultured rat sensory axons. *J Neurocytol* 1981; 10:589-605; PMID:7310467; <http://dx.doi.org/10.1007/BF01262592>
- Paggi P, Lasek RJ. Axonal transport of cytoskeletal proteins in oculomotor axons and their residence times in the axon terminals. *J Neurosci* 1987; 7:2397-411; PMID:2441008
- Brady ST. Increases in Cold-Insoluble Tubulin during Aging. *Soc Neurosci Abstr* 1984; 10:273
- Ahmad FJ, Baas PW. Microtubules released from the neuronal centrosome are transported into the axon. *J Cell Sci* 1995; 108:2761-9; PMID:7593317
- Sahenk Z, Brady ST. Axonal tubulin and microtubules: morphologic evidence for stable regions on axonal microtubules. *Cell Motil Cytoskeleton* 1987; 8:155-64; PMID:2891447; <http://dx.doi.org/10.1002/cm.970080207>
- Kirkpatrick LL, Brady ST. Modulation of the axonal microtubule cytoskeleton by myelinating Schwann cells. *J Neurosci* 1994; 14:7440-50; PMID:7996186
- Kirkpatrick LL, Witt AS, Payne HR, Shine HD, Brady ST. Changes in microtubule stability and density in myelin-deficient shiverer mouse CNS axons. *J Neurosci* 2001; 21:2288-97; PMID:11264304
- Song Y, Kirkpatrick LL, Schilling AB, Helseth DL, Chabot N, Keillor JW, Johnson GV, Brady ST. Transglutaminase and polyamination of tubulin: posttranslational modification for stabilizing axonal microtubules. *Neuron* 2013; 78:109-23; PMID:23583110; <http://dx.doi.org/10.1016/j.neuron.2013.01.036>
- Griffin M, Casadio R, Bergamini CM. Transglutaminases: nature's biological glues. *Biochem J* 2002; 368:377-96; PMID:12366374; <http://dx.doi.org/10.1042/BJ20021234>
- Gilad GM, Gilad VH. Overview of the brain polyamine-stress-response: regulation, development, and modulation by lithium and role in cell survival. *Cell Mol Neurobiol* 2003; 23:637-49; PMID:14514021; <http://dx.doi.org/10.1023/A:1025036532672>
- Hand D, Perry MJ, Haynes LW. Cellular transglutaminases in neural development. *Int J Dev Neurosci* 1993; 11:709-20; PMID:7907835; [http://dx.doi.org/10.1016/0736-5748\(93\)90060-Q](http://dx.doi.org/10.1016/0736-5748(93)90060-Q)
- De Vivo G, Di Lorenzo R, Ricotta M, Gentile V. Role of the transglutaminase enzymes in the nervous system and their possible involvement in neurodegenerative diseases. *Curr Med Chem* 2009; 16:4767-73; PMID:19929789; <http://dx.doi.org/10.2174/092986709789909594>