



HAL
open science

A dual role for β II-spectrin in axons

Christophe Leterrier

► **To cite this version:**

Christophe Leterrier. A dual role for β II-spectrin in axons. Proceedings of the National Academy of Sciences of the United States of America, 2019, pp.201909789. 10.1073/pnas.1909789116. hal-02179640

HAL Id: hal-02179640

<https://amu.hal.science/hal-02179640>

Submitted on 11 Jul 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



A dual role for β II-spectrin in axons

Christophe Leterrier^{a,1}

Spectrins have been known for a long time as submembrane structural proteins, but a study from Lorenzo et al. (1) demonstrates an unexpected role for a neuronal spectrin in axonal transport. Actin and spectrins form specialized submembrane scaffolds important for the morphogenesis, compartmentation, and mechanical properties in a range of differentiated cell types (2, 3). Spectrin assembles the submembrane scaffold that shapes red blood cells—in fact, it was named from the membranous ghosts (specter) of erythrocytes where it was first discovered (4). In these cells, spectrins arrange in a hexagonal pattern, connecting short actin nodes (5–7), generating the toroidal shape of erythrocytes. Spectrins are tetramers made of 2 α - and 2 β -spectrin subunits (α I and β I in erythrocytes) that can stretch between 60 and 200 nm in length (8). The actin/spectrin scaffold thus provides flexibility and mechanical resistance to the large deformations that erythrocytes undergo along small capillaries (9).

In neurons, a protein resembling spectrin had been identified and initially named fodrin (10). Fodrin was later found to be the general spectrin present in most cells (11), and later shown to consist of α II- and β II-spectrin tetramers (12). These spectrins assemble under the plasma membrane in cultured cells (10, 13) and in neurons (10, 14), but their precise arrangement remained unknown until recently. In 2013, superresolution optical microscopy images showed that spectrins assemble axially along the axon and parts of dendrites, with tetramers forming a succession of cylinders connecting actin rings (15). Due to the 190-nm length of the neuronal spectrin tetramer (16, 17), this results in a scaffold containing submembrane actin rings regularly spaced every 190 nm along the axon by spectrins (15, 18). This unique membrane-associated periodic scaffold (MPS) of actin rings connected by spectrins is present along the axons of all types of neurons and organisms studied so far (19, 20), and is thought to endow axons with both flexibility and resistance to stress from body movements (21, 22).

In parallel, a more intracellular role of nonerythrocyte spectrins has been suggested ever since their first

description (10). Fodrin was shown to be slowly transported along the axon, progressing together with cytoskeletal and cytoplasmic proteins by slow axonal transport (10). Furthermore, α II- and β II-spectrin link transported vesicles to microtubule motors: the dynein/dynactin complex (23) and the kinesin KIF3 (24, 25). Importantly, interaction of spectrin with transport vesicles can occur by direct interaction with acidic phospholipids (26, 27). In vivo, disease-relevant mutants of the single β -spectrin in *Drosophila* neurons results in axonal transport defects (28).

Deciphering the role of β II-spectrin in mammalian neurons is hindered by the early embryonic lethality of the full knockout (29). Lorenzo et al. (1) thus used a mouse line with floxed β II-spectrin alleles crossed with a Nestin-Cre line (25), resulting in the ablation of β II-spectrin from neuronal progenitors and neurons derived from them (“ β II-spectrin neuronal knockout”). These mice survive up to 15 to 45 d after birth, and the authors characterized their phenotype, zooming in from brain connectivity to molecular defects within axons. Altogether, the study makes a compelling case for a dual role of axonal spectrins as both structural and trafficking proteins that ultimately support axon growth and maintenance for proper brain wiring (Fig. 1) (1). The in vivo experiments use diffusion tensor imaging to highlight a defect of long-range projections in the brain of β II-spectrin neuronal knockout mice, notably along the corpus callosum. Electron microscopy shows that axons forming the corpus callosum are larger, with thinner myelin sheets and signs of degeneration. This is reminiscent of the results from neuron-specific ablation of α II-spectrin, the other subunit present in axonal spectrin tetramers (30).

To understand the molecular basis of these brain-wide defects, Lorenzo et al. (1) turn to neuronal cultures from β II-spectrin neuronal knockout mice. Neurons lacking β II-spectrin have shorter axons after 5 and 11 d in culture; the length of their axon initial segment is unaffected, which differs from previous in vivo results (25). What could cause this defect in axonal

^aNeuroCyto, Institute of Neurophysiopathology UMR 7051, Aix Marseille Université, CNRS, 13385 Marseille, France

Author contributions: C.L. wrote the paper.

The author declares no conflict of interest.

Published under the PNAS license.

See companion article 10.1073/pnas.1820649116.

¹Email: christophe.leterrier@univ-amu.fr.

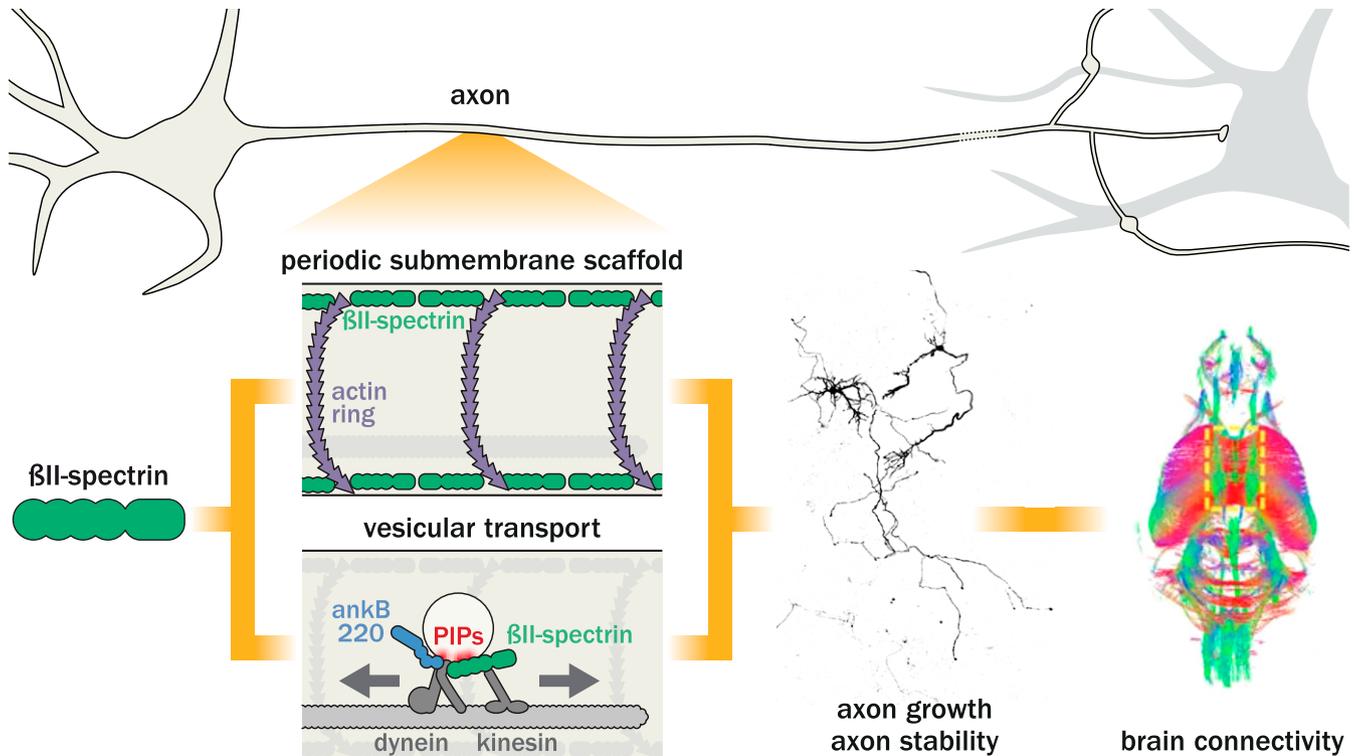


Fig. 1. The dual role of β II-spectrin in axons. Along the axon of a neuron (Top), β II-spectrin (Bottom Left, green) participates both in the assembly of the periodic submembrane scaffold by linking actin rings (purple) and in the bidirectional transport of vesicles via an interaction with dynein and kinesins (gray), together with the 220-kDa form of ankyrin-B (ankB 220, blue). These 2 functions result in β II-spectrin supporting axonal growth in vitro and neuronal connectivity in the brain (Bottom Right). Adapted with permission from ref. 1.

growth? β II-spectrin is a key component of the MPS (15), and its knockdown results in MPS disorganization (31, 32). In neurons genetically ablated for β II-spectrin, the MPS is, indeed, barely detectable, although it seems to be partially restored in more mature neurons.

Given earlier hints of a role for β II-spectrin in supporting vesicular trafficking, Lorenzo et al. (1) then tested whether axonal transport is affected in β II-spectrin knockout neurons. Axonal transport of both synaptophysin (a presynaptic protein) and LAMP1 (a late endosome marker) is markedly impaired in both directions after 7 d in culture. This role of β II-spectrin for axonal transport is further supported by its interaction with molecular motors: p150^{Glued} (part of the dynein/dynactin complex) and the KIF3 (24), KIF1A, and KIF5B kinesins. Furthermore, association of these motors with intracellular vesicles is impaired in β II-spectrin knockout neurons, showing that β II-spectrin helps the association of motors with vesicles. The roles of β II-spectrin in assembling the MPS and in driving vesicular transport are likely independent: Firstly, defects in transport do not seem to result from microtubules disorganization by the absence of the MPS. Secondly, immature neurons where the MPS is only present along the proximal axon show similar transport characteristics in the proximal and distal axon, with an equal impairment along the whole axon in β II-spectrin ablated neurons.

Lorenzo et al. (1) also examine the possible connection between transport defects induced by β II-spectrin ablation and those induced by ankyrin-B depletion (33), given that these 2 proteins interact at the MPS. The effects of ankyrin-B and β II-spectrin on vesicular trafficking are independent and additive, with a near-complete abolition of axonal transport along the axon of neurons genetically

depleted for both proteins. This surprising result points to a separate but general role of ankyrin-B and β II-spectrin as axonal transport adaptors. Independence of ankyrin-B and β II-spectrin in supporting vesicular transport is confirmed by rescue experiments: Expression of an ankyrin-binding-deficient mutant of β II-spectrin can rescue defects of axonal transport and growth in β II-spectrin knockout neurons. Furthermore, another rescue experiment suggests direct binding of β II-spectrin to vesicles via an interaction between the spectrin pleckstrin homology domain and acidic phospholipids (26, 27): A lipid-binding-deficient mutant of β II-spectrin is not able to rescue axonal transport and growth defects.

Overall, this study by Lorenzo et al. (1) sheds light on neuronal spectrins, unveiling a function in axonal transport (Fig. 1). It is a striking example of how a protein can evolve different roles, even in the same cellular compartment, via differential localization and interaction with distinct partners. Both MPS assembly and axonal transport seem to contribute to axonal growth and stability, and it would be very interesting to tease apart the respective role of each of these β II-spectrin-mediated processes. Axonal transport and growth share a similar dependence on β II-spectrin binding to phospholipid, but not to ankyrin-B, suggesting a primary contribution from transport. Testing these binding partners requirements in MPS assembly would be useful for resolving this question, as would be a genetic way of perturbing the MPS independently from β II-spectrin itself. Another intriguing possibility is that different β II-spectrin-containing tetramers are used in the MPS and on transported vesicles. As spectrins are obligatory tetramers, all tetramers likely contain the neuronal α II-spectrin subunit, but different isoforms of either α II- or β II-spectrin could distinguish MPS and vesicular spectrin tetramers. Future work will

hopefully address these important open questions, and decipher what balances the dual functions of axonal spectrins. In our era of immediate access and acceleration of knowledge production, it is

an interesting twist to see this paradigm-shifting study having its roots in the sagacious observations made almost 40 y ago by the pioneers of neuronal cell biology (10).

- 1 D. N. Lorenzo *et al.*, β II-spectrin promotes mouse brain connectivity through stabilizing axonal plasma membranes and enabling axonal organelle transport. *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.1820649116 (2019).
- 2 B. Machnicka *et al.*, Spectrins: A structural platform for stabilization and activation of membrane channels, receptors and transporters. *Biochim. Biophys. Acta* **1838**, 620–634 (2014).
- 3 V. Bennett, D. N. Lorenzo, An adaptable spectrin/ankyrin-based mechanism for long-range organization of plasma membranes in vertebrate tissues. *Curr. Top. Membr.* **77**, 143–184 (2016).
- 4 V. T. Marchesi, E. Steers Jr, Selective solubilization of a protein component of the red cell membrane. *Science* **159**, 203–204 (1968).
- 5 T. J. Byers, D. Branton, Visualization of the protein associations in the erythrocyte membrane skeleton. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6153–6157 (1985).
- 6 S. C. Liu, L. H. Derick, J. Palek, Visualization of the hexagonal lattice in the erythrocyte membrane skeleton. *J. Cell Biol.* **104**, 527–536 (1987).
- 7 L. Pan, R. Yan, W. Li, K. Xu, Super-resolution microscopy reveals the native ultrastructure of the erythrocyte cytoskeleton. *Cell Reports* **22**, 1151–1158 (2018).
- 8 J. W. Brown *et al.*, The physiological molecular shape of spectrin: A compact supercoil resembling a Chinese finger trap. *PLoS Comput. Biol.* **11**, e1004302 (2015).
- 9 S. E. Lux 4th, Anatomy of the red cell membrane skeleton: Unanswered questions. *Blood* **127**, 187–199 (2016).
- 10 J. Levine, M. Willard, Fodrin: Axonally transported polypeptides associated with the internal periphery of many cells. *J. Cell Biol.* **90**, 631–642 (1981).
- 11 J. R. Glenney Jr, P. Glenney, Fodrin is the general spectrin-like protein found in most cells whereas spectrin and the TW protein have a restricted distribution. *Cell* **34**, 503–512 (1983).
- 12 J. C. Winkelmann, B. G. Forget, Erythroid and nonerythroid spectrins. *Blood* **81**, 3173–3185 (1993).
- 13 K. Burridge, T. Kelly, P. Mangeat, Nonerythrocyte spectrins: Actin-membrane attachment proteins occurring in many cell types. *J. Cell Biol.* **95**, 478–486 (1982).
- 14 B. M. Riederer, I. S. Zagon, S. R. Goodman, Brain spectrin(240/235) and brain spectrin(240/235E): Two distinct spectrin subtypes with different locations within mammalian neural cells. *J. Cell Biol.* **102**, 2088–2097 (1986).
- 15 K. Xu, G. Zhong, X. Zhuang, Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons. *Science* **339**, 452–456 (2013).
- 16 V. Bennett, J. Davis, W. E. Fowler, Brain spectrin, a membrane-associated protein related in structure and function to erythrocyte spectrin. *Nature* **299**, 126–131 (1982).
- 17 J. R. Glenney Jr, P. Glenney, K. Weber, F-actin-binding and cross-linking properties of porcine brain fodrin, a spectrin-related molecule. *J. Biol. Chem.* **257**, 9781–9787 (1982).
- 18 M.-J. Papandréou, C. Leterrier, The functional architecture of axonal actin. *Mol. Cell. Neurosci.* **91**, 151–159 (2018).
- 19 J. He *et al.*, Prevalent presence of periodic actin-spectrin-based membrane skeleton in a broad range of neuronal cell types and animal species. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 6029–6034 (2016).
- 20 E. D'Este *et al.*, Subcortical cytoskeleton periodicity throughout the nervous system. *Sci. Rep.* **6**, 22741 (2016).
- 21 M. Hammarlund, E. M. Jorgensen, M. J. Bastiani, Axons break in animals lacking β -spectrin. *J. Cell Biol.* **176**, 269–275 (2007).
- 22 M. Krieg, A. R. Dunn, M. B. Goodman, Mechanical control of the sense of touch by β -spectrin. *Nat. Cell Biol.* **16**, 224–233 (2014).
- 23 E. A. Holleran, M. K. Tokito, S. Karki, E. L. Holzbaur, Centractin (ARP1) associates with spectrin revealing a potential mechanism to link dynactin to intracellular organelles. *J. Cell Biol.* **135**, 1815–1829 (1996).
- 24 S. Takeda *et al.*, Kinesin superfamily protein 3 (KIF3) motor transports fodrin-associating vesicles important for neurite building. *J. Cell Biol.* **148**, 1255–1265 (2000).
- 25 M. R. Galiano *et al.*, A distal axonal cytoskeleton forms an intra-axonal boundary that controls axon initial segment assembly. *Cell* **149**, 1125–1139 (2012).
- 26 M. Hyvönen *et al.*, Structure of the binding site for inositol phosphates in a PH domain. *EMBO J.* **14**, 4676–4685 (1995).
- 27 V. Muresan *et al.*, Dynactin-dependent, dynein-driven vesicle transport in the absence of membrane proteins: A role for spectrin and acidic phospholipids. *Mol. Cell* **7**, 173–183 (2001).
- 28 D. N. Lorenzo *et al.*, Spectrin mutations that cause spinocerebellar ataxia type 5 impair axonal transport and induce neurodegeneration in *Drosophila*. *J. Cell Biol.* **189**, 143–158 (2010).
- 29 Y. Tang *et al.*, Disruption of transforming growth factor- β signaling in ELF β -spectrin-deficient mice. *Science* **299**, 574–577 (2003).
- 30 C. Y.-M. Huang, C. Zhang, D. R. Zollinger, C. Leterrier, M. N. Rasband, An all spectrin-based cytoskeleton protects large-diameter myelinated axons from degeneration. *J. Neurosci.* **37**, 11323–11334 (2017).
- 31 G. Zhong *et al.*, Developmental mechanism of the periodic membrane skeleton in axons. *eLife* **3**, 194 (2014).
- 32 B. Han, R. Zhou, C. Xia, X. Zhuang, Structural organization of the actin-spectrin-based membrane skeleton in dendrites and soma of neurons. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E6678–E6685 (2017).
- 33 D. N. Lorenzo *et al.*, A PIK3C3-ankyrin-B-dynactin pathway promotes axonal growth and multiorganelle transport. *J. Cell Biol.* **207**, 735–752 (2014).