A dual role for βII-spectrin in axons

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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 (αI and βI 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 αII- and βII-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, αII- and βII-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 βII-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 βII-spectrin alleles crossed with a Nestin-Cre line (25), resulting in the ablation of βII-spectrin from neuronal progenitors and neurons derived from them ("βII-spectrin neuronal knockout"). These mice survive up to 15 to 45 d after birth, and the authors characterized their phenotype, zooming on 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 βII-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 αII-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 βII-spectrin neuronal knockout mice. Neurons lacking βII-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

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Author contributions: C.L. wrote the paper.

The author declares no conflict of interest.

See companion article 10.1073/pnas.1820649116.

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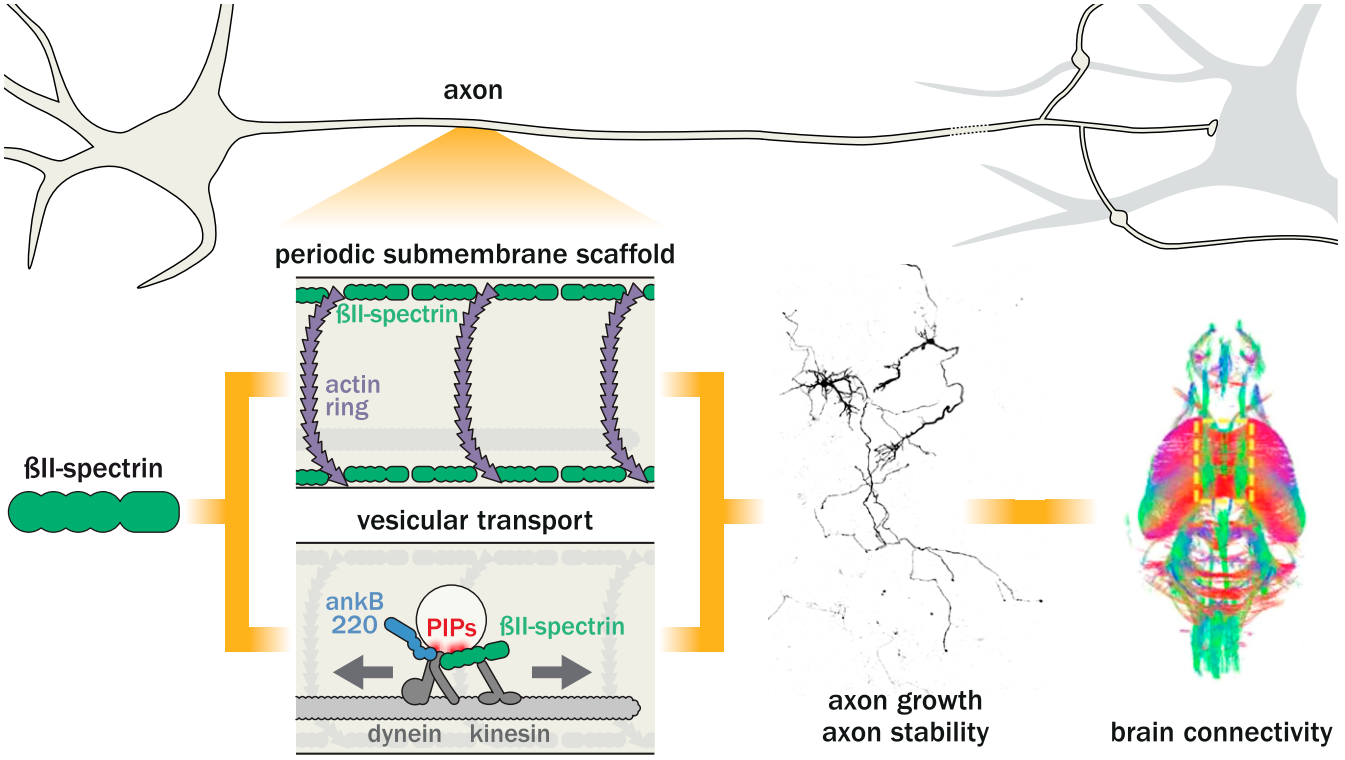

Fig. 1. The dual role of βII-spectrin in axons. Along the axon of a neuron (*Top*), βII-spectrin (*Bottom Left*, green) participates both in the assembly of the periodic submembrane scaffold by linking actin rings (purple) and in the bidirectional transport of vesicles via an interaction with dynein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

growth? βII-spectrin is a key component of the MPS (15), and its knockdown results in MPS disorganization (31, 32). In neurons genetically ablated for βII-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 βII-spectrin in supporting vesicular trafficking, Lorenzo et al. (1) then tested whether axonal transport is affected in βII-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 βII-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 βII-spectrin knockout neurons, showing that βII-spectrin helps the association of motors with vesicles. The roles of βII-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 βII-spectrin ablated neurons.

Lorenzo et al. (1) also examine the possible connection between transport defects induced by βII-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 βII-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 βII-spectrin as axonal transport adaptors. Independence of ankyrin-B and βII-spectrin in supporting vesicular transport is confirmed by rescue experiments: Expression of an ankyrin-binding–deficient mutant of βII-spectrin can rescue defects of axonal transport and growth in βII-spectrin knockout neurons. Furthermore, another rescue experiment suggests direct binding of βII-spectrin to vesicles via an interaction between the spectrin pleckstrin homology domain and acidic phospholipids (26, 27): A lipid-binding–deficient mutant of βII-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 βII-spectrin–mediated process. Axonal transport and growth share a similar dependence on βII-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 βII-spectrin itself. Another intriguing possibility is that different βII-spectrin–containing tetramers are used in the MPS and on transported vesicles. As spectrins are obligatory tetramers, all tetramers likely contain the neuronal αII-spectrin subunit, but different isoforms of either αII- or βII-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).

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