JCB Journal of Cell Biology

SPOTLIGHT

Expecto Patronin for slow and persistent minus end microtubule growth in dendrites

Heather T. Broihier

Microtubule plus ends are highly dynamic in neurons, while minus ends are often capped and stable. In this issue, Feng et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201810155) demonstrate that in dendrites, free minus ends undergo slow and processive growth mediated by the minus end–binding protein Patronin.

A famous young wizard with a lightning bolt scar uses the Patronus charm (Expecto Patronum) to summon his magical Patronus for protection from harm. In a decidedly more cell biological context, the microtubule-binding protein Patronin is known to shield microtubule minus ends from catastrophe. Patronin was originally identified as *Drosophila* ssp4, a short spindle mutant, and renamed Patronin when it was found to bind microtubule minus ends and protect them from kinesin-13–mediated depolymerization in cultured cells (1).

A microtubule-organizing center, usually the centrosome, anchors and stabilizes microtubule minus ends. However, some cell types, including neurons, can lack centrosomes, underscoring the importance of alternative minus end–stabilizing mechanisms in these cells (2). Perhaps not surprisingly, then, Patronin homologues are conserved regulators of neuronal microtubules. In cultured hippocampal neurons, the Patronin family member CAMSAP2 stabilizes noncentrosomal microtubules and regulates axon specification and dendrite branching (3). *Caenorhabditis elegans* Patronin is similarly required for neuronal morphology and microtubule stability (4, 5) as well as axon regeneration (6). In this issue, Feng et al. (7) now demonstrate a novel requirement for Patronin in promoting sustained minus end microtubule growth in dendrites.

EB-GFP fusion proteins bind microtubule ends and are widely used to probe microtubule organization because dynamic plus ends marked with EB-GFP appear as comets in live cells. All axonal EB-GFP comets move away from the cell body, indicating that microtubules are oriented with uniform plus-end-out polarity in axons (2). Dendritic EB-GFP comets, on the other hand, contain minus-end-out microtubules. *Drosophila* dendritic arborization (da) neurons are a powerful system for characterizing microtubule organization and dynamics in vivo. These sensory neurons elaborate highly stereotyped two-dimensional dendritic arbors immediately underneath the body wall, making them ideal for live imaging. In third-instar da neuron dendrites, >90% of EB1-GFP comets move toward the soma, indicating that microtubules are overwhelmingly minus-end-out at this stage.

In the current study, Feng et al. uncover a previously undescribed population of slow-moving EB1-GFP puncta (7). These puncta are readily distinguishable from well-characterized plus end comets. In addition to moving much more slowly, their movement is highly processive and directed away from the soma. Arguing that the slow-moving puncta reflect a common feature of neuronal microtubule organization, the authors also document them in zebrafish sensory neurons. The authors hypothesized that these slow-moving puncta represent growing microtubule minus ends. If so, they might be labeled by the minus end–binding protein Patronin. Consistent with their hypothesis, they find that EB1 and Patronin mark the same growing microtubule minus ends. Hence, the slow-moving puncta are indeed mobile minus ends. While individual free microtubule ends have been shown to undergo periods of slow growth in cultured cells (8), this is the first evidence they do so in neurons.

Feng et al. next tested if Patronin regulates minus end microtubule dynamics. They found that Patronin knockdown results in a striking fourfold decrease in average minus end run time as well as an increase in polymerization speed. Thus, Patronin is required for slow, sustained growth of microtubule minus ends in dendrites. Does this change in minus end dynamics have implications for overall microtubule polarity in Patronin mutants? Indeed, loss of Patronin results in a loss of minus-end-out microtubules, demonstrating a requirement for Patronin in establishing normal dendritic microtubule polarity (Fig. 1). Before delving further into Patronin function, the authors investigated whether the observed phenotypes are specifically attributable to loss of Patronin or whether they might be secondary consequences of a neuronal stress response. This was an important concern because altering microtubule dynamics by a number of different means induces DLK stress signaling in these cells (9). While the authors find that Patronin loss does activate DLK signaling, inhibiting DLK does not suppress either the minus end growth or microtubule polarity phenotypes in Patronin knockdown neurons. Therefore, these phenotypes are not explained by DLK activation.

Thus far, Feng et al. (7) have focused on Patronin's function in microtubule dynamics in proximal dendrite branches of da

Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH.

Correspondence to Heather T. Broihier: heather.broihier@case.edu.

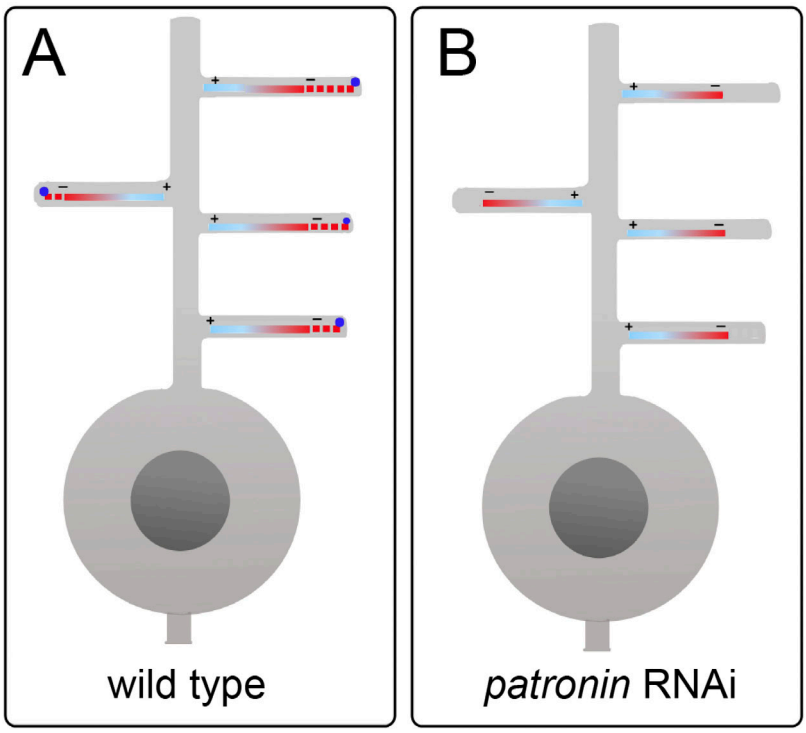

Figure 1. **Model illustrating the function of Patronin in minus end microtubule growth in dendrites. (A)** In wild-type dendrites, Patronin (represented by a blue dot) enables slow and sustained growth of minus end microtubules. **(B)** In *patronin* RNAi dendrites, minus end growth is impaired, leading to a reduction in overall minus-end-out microtubules.

neurons at the third-instar stage. They next turned to three distinct situations where Patronin function might be predicted to be especially important. First, they tested whether Patronin promotes minus-end-out microtubules in terminal branches. Local nucleation of microtubules at dendrite branch points is a key mechanism to populate dendrites with microtubule minus ends (10). But what about dendrite branches distal to all branch points? How do minus ends get into these branches? Feng et al. tested whether Patronin is required for the presence of minus ends in terminal branches and found a reduction in minus-end-out microtubules upon depletion of Patronin. Moreover, Patronin overexpression results in a roughly twofold increase in minus-end-out microtubules in these branches, arguing that Patronin is both necessary and sufficient for addition of minus-end-out microtubules to terminal branches. Second, the authors investigated whether Patronin is required for establishing minus-end-out microtubules earlier in dendrite development. Microtubule polarity is mixed during early stages of dendrite development and subsequently resolves to predominantly minus-end-out. Feng et al. found that loss of Patronin impairs this transition so that dendritic microtubule polarity remains mixed until later developmental stages (7).

Lastly, the authors assessed the function of Patronin in dendrite regeneration following injury. The Rolls laboratory previously demonstrated that da neurons can elaborate an entirely new arbor after dendrite removal and reasoned that Patronin might be involved. Indeed, loss of Patronin results in a marked reduction in the regeneration capacity of da neurons as well as a loss of minus-end-out microtubules in the new arbors. The defect in dendrite growth following injury is particularly noteworthy, as the authors did not find a role for Patronin in dendrite morphogenesis during development. Thus, Patronin-mediated minus end growth is particularly important in the context of dendrite regeneration.

Until recently, the microtubule minus end has been viewed as a relatively static structure in neurons. The current study alters this view significantly by demonstrating that in dendrites, minus ends undergo slow and sustained microtubule growth, which is required for normal microtubule polarity and dendrite regeneration following injury. In the future, it will be important to determine how Patronin-mediated minus end growth is balanced with the activity of microtubule nucleators present at branch points as well as how Patronin's growth-promoting activity is harnessed.

Acknowledgments

H.T. Broihier is supported by National Institutes of Health grant NS095895.

The author declares no competing financial interests.

1. Goodwin, S.S., and R.D. Vale. 2010. *Cell.* https://doi.org/10.1016/j.cell.2010.09.022
2. Akhmanova, A., and M.O. Steinmetz. 2015. *Nat. Rev. Mol. Cell Biol.* https://doi.org/10.1038/nrm4084
3. Yau, K.W., et al. 2014. *Neuron.* https://doi.org/10.1016/j.neuron.2014.04.019
4. Marcette, J.D., et al. 2014. *eLife.* https://doi.org/10.7554/eLife.01637
5. Richardson, C.E., et al. 2014. *eLife.* https://doi.org/10.7554/eLife.01498
6. Chuang, M., et al. 2014. *Cell Reports.* https://doi.org/10.1016/j.celrep.2014.09.054
7. Feng, C., et al. 2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201810155
8. Jiang, K., et al. 2014. *Dev. Cell.* https://doi.org/10.1016/j.devcel.2014.01.001
9. Chen, L., et al. 2012. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.1121180109
10. Ori-McKenney, K.M., et al. 2012. *Neuron.* https://doi.org/10.1016/j.neuron.2012.10.008