Di-rectifying Tau

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In this issue of *The EMBO Journal*, Li *et al* (2011) show that the axon initial segment (AIS) functions as a retrograde trafficking barrier, or axonal rectifier, to exclude axonal Tau from re-entry into the somatodendritic compartment. They elucidate the molecular basis of this rectification by showing that hyperphosphorylation of Tau permits it to detach from microtubules (MTs) and bypass the retrograde barrier.

Neurons are highly polarized cells with distinct axo-dendritic polarity that is central to proper nervous system function. Polarity is reflected by the restricted localization of membrane and cytosolic proteins to dendritic or axonal compartments. For example, Map2 and Tau are MT-associated proteins (MAPs) that in mature neurons sort to somatodendritic or axonal compartments, respectively. What determines the selective localization and retention of these proteins within the axon, or within the dendrite? During early development, sorting of proteins to distinct domains can occur through multiple mechanisms. For example, direct and selective mobilization of cargoes by motor proteins such as

kinesins or dyneins along MTs can restrict proteins to unique compartments (Hirokawa and Takemura, 2005). Proteins can also be stabilized, anchored, or tethered within specific compartments, or polarity may result from the assembly of molecular fences or physical barriers. In neurons, the AIS is an excellent example of a barrier that limits mixing of somatodendritic and axonal proteins, organelles, and even lipids (Rasband, 2010). The AIS is located at the base of the axon hillock and defines the transition from somatodendritic to axonal domains (Figure 1A). It is enriched with the cytoskeletal scaffolding proteins ankyrinG and β IV spectrin, which together cluster Na^+ and K^+ channels and link them to the actin cytoskeleton (Figure 1B). These ion channel clusters initiate and modulate action potentials at the AIS, and function as a physiological bridge between somatodendritic input and axonal output.

What do we know about the AIS barrier? The AIS barrier impedes the lateral mobility of membrane proteins in mature neurons and consists of detergent insoluble protein complexes (Winckler *et al*, 1999). The AIS barrier was also

Figure 1 (A) A cultured hippocampal neuron immunostained for β IV spectrin (green) to label the axon initial segment (AIS), Map2 to label the somatodendritic compartment and Tau (red) to label the axonal compartment. (B) The AIS is characterized by the presence of dense ion channel clusters anchored to the actin-based cytoskeleton through the scaffolding proteins ankyrinG and β IV spectrin. An actin-based barrier has previously been reported for membrane proteins. The AIS also consists of fasciculated microtubules that are exclusive to this region of the axon. Li *et al* (2011) describe a microtubule-based retrograde barrier for Tau at the AIS, which becomes leaky when Tau is hyperphosphorylated and detached from microtubules.

reported to selectively exclude fluorescent dextrans depending on their different sizes (Song *et al*, 2009). These functions of the AIS are disrupted when neurons are treated with drugs that depolymerize actin, suggesting that actin is an important component of the barrier (Figure 1B). As further support for the existence of an anterograde barrier, MT-dependent transport vesicles carrying dendritic cargoes are excluded from entry into axons (Song *et al*, 2009). Together, these observations all point to the important role of the cytoskeleton at the AIS as the molecular basis of the filter. Intriguingly, it is worth noting that the microtubular structure of the AIS is unique and exclusive to this region. Specifically, electron micrographs show fascicles of MTs connected by cytoplasmic bridges (Palay *et al*, 1968). In spite of the above insights, the AIS barrier and other sorting mechanisms that determine protein compartmentalization remain poorly understood.

In the current issue of *The EMBO Journal*, Li and colleagues study sorting of Tau, an axonally restricted MAP. Tau regulates MT stability and motor protein-dependent transport along MTs (Stamer *et al*, 2002). In Alzheimer's disease (AD), hyperphosphorylation of Tau, aggregation into neurofibrillary tangles, and missorting into the somatodendritic compartment are major pathological markers of the disease. Missorting of Tau is thought to be an early indicator of Tauopathies including AD. Several mechanisms could contribute to the axonal compartmentalization of Tau. For example, Tau could bind more tightly to MTs in axons than in dendrites, or Tau might get selectively targeted to axons and/or removed from dendritic compartments (Hirokawa *et al*, 1996). Previously, the Mandelkow laboratory used GFP-tagged Tau and FRAP to show that Tau is highly dynamic, rapidly diffuses into axons, and that Tau can be co-transported with MTs (Konzack *et al*, 2007). Here, they have extended these observations by using a Dendra2-tagged version of Tau; Dendra2 is a variant of GFP that fluoresces green to red upon photoconversion. With this new tool, the authors were able to accurately determine the diffusion and mobility of Tau in real time and in multiple neuronal compartments. Upon photoconversion in the somatodendritic compartment, Tau-Dendra2 spread throughout the cell and filled both

dendrites and axons. However, when Tau-Dendra2 was photoconverted in the axonal compartment near the AIS, it was only able to move into more distal regions of the axon and not into the somatodendritic compartment. This observation revealed a rectifying barrier that permits the anterograde, but not retrograde passage of Tau (Figure 1B). This new mechanism may partially explain the long-standing question for how Tau is restricted to axons.

What is the molecular basis of this Tau rectifier? In contrast to previous studies of the AIS barrier, disrupting actin had no effect on the retrograde barrier. Instead, depolymerizing MTs permitted Tau to move from the axonal into the somatodendritic compartment. Similarly, hyperphosphorylation of Tau, which inhibits its binding to MTs, permitted Tau re-entry into soma and dendrites (Figure 1B). It is possible that the unique MT organization of the AIS itself may discriminate between anterograde and retrograde movement of Tau and its phosphorylation states. Since Tau can move to dendrites only when hyperphosphorylated, Li *et al* propose that a disruption in the balance of kinases and phosphatases makes the AIS retrograde barrier leaky. This may explain Tau accumulation in dendrites in AD and other neurodegenerative Tauopathies. Consistent with this idea, previous studies showed that phosphatases in the somatodendritic compartment dephosphorylate Tau less efficiently than in axonal compartments (Bertrand *et al*, 2010). However, where and under what conditions hyperphosphorylation of Tau occurs remains unknown. Interestingly, several kinases have been reported to be enriched at the AIS including CK2 and CAMKII (Bréchet *et al*, 2008; Hund *et al*, 2010), but so far no phosphatase has been reported at the AIS.

Schafer *et al* (2009) recently showed that neuronal injury leads to irreversible loss of the AIS and neuronal polarity. It will be interesting to determine if the retrograde AIS barrier is also vulnerable to disruption during cell stress, or after exposure to amyloid- β peptide in AD, and whether this also contributes to aberrant localization of Tau. In summary, the results of Li *et al* reveal the existence of a retrograde diffusion barrier at the AIS, and emphasize the emerging role of the AIS in nervous system diseases and injuries.

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