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Mapping the Role of MAP7 in Axon Collateral Branching

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Review of Tymanskyj et al.

Neurons have diverse and distinctive dendritic and axonal morphologies. Proper development of these structures is vital for normal function and connectivity. Indeed, abnormal neuronal morphology has been linked to various neurobiological and psychiatric disorders (Luo and O'Leary, 2005). The development of diverse axonal morphologies depends on the ability of growing axons to respond to extracellular cues. These cues induce localized cytoskeletal remodeling for axonal turning and branching (Armijo-Weingart and Gallo, 2017).

The development of axons in DRG sensory neurons is highly stereotyped and therefore provides a good model for understanding the mechanism of axon branching. These sensory axons bifurcate upon entry into the spinal cord, and daughter axons extend either anteriorly or posteriorly. The anteriorly growing axon will ascend to transmit sensory information destined for the brain. Additionally, ascending and descending axon branches will generate interstitial axon collaterals that form off of an established axon shaft to innervate different lamina along the dorsal-ventral axis within the spinal cord (Gibson and Ma, 2011).

Recent work by Tymanskyj et al. (2017) sought to identify proteins that mediate axonal branching in DRG sensory neurons. To do so, they compared transcriptional profiles of DRG neurons before and after collateral branches formed. Because microtubule associated protein 7 (MAP7) was strongly upregulated following collateral formation, the authors next examined the effects of MAP7 overexpression and knockdown on primary sensory neuron cultures harvested at early and late embryonic stages. Overexpression of MAP7 increased in the number of interstitial branches in younger neurons that do not typically express MAP7. Conversely, knocking down MAP7 in older neurons cultured after branch formation *in vivo* resulted in fewer interstitial branches *in vitro*. These experiments demonstrated that MAP7 is both sufficient and necessary for interstitial branching in sensory neurons.

Tymanskyj et al. (2017) reasoned that a regulator of branch formation would likely localize to regions of an axon where branches emerge. Indeed, the authors found a strong preference for MAP7 at branch points. Because nascent branches are formed from filopodia that are stabilized by microtubule penetration, the timing of MAP7 localization to branch points would hint at its role governing the timing and pattern of branch formation. The authors therefore performed live imaging and determined that fluorescently tagged MAP7 was absent in pioneer filopodia, but had delayed entry into new microtubule-enriched branches. Further work found that MAP7 preferentially localized to long and stable branches. Together, these studies indicate that MAP7 is critical for branch maturation, but probably not initial branch formation.

Although these *in vitro* manipulations established a role for MAP7 in collateral branching, the authors sought to substantiate this by using a mouse mutant where the MAP7 protein is truncated at the C terminus (MAP7^{mshi}) (Turner et al., 1997). The MAP7 C terminus had previously been shown to interact with kinesin (Sung et al., 2008; Barlan et al., 2013). Thus, Tymanskyj et al. (2017) reasoned that this was the mechanism for axon branching and MAP7^{mshi} neurons would have fewer collaterals. Surprisingly, sensory neurons cultured from MAP7^{mshi} mice had a drastic increase in axon branching compared with neurons from control animals. This, combined with earlier *in vitro* findings, strongly implies that the N terminus, and not the C terminus, of MAP7 is vital for promoting axon collateral branching. Intriguingly, if the C terminus was dispensable for promoting branching, then it would be sensible to assume that MAP7^{mshi} neurons would be similar to control neurons; however, instead, the loss of the MAP7 C terminus results in a potentiation of collateral branching. One explanation is that a C-terminal domain blocks N-terminal function and loss of the C terminus leads to exuberant branching. It is also possible that C-terminal truncation produces abnormally high levels of MAP7 protein in the mutant mice promoting axon branching similar to MAP7 overexpression *in vitro*. Although Tymanskyj et al.

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(2017) used *in situ* analysis and RT-PCR to confirm the presence of the truncated mRNA in MAP7^{mshi} animals, the comparable expression levels of MAP7 are unclear. Thus, the precise regulatory role of the C terminus will require further study.

A key discovery from the MAP7^{mshi} animals came from analysis of DRG sensory neuron projections. Sensory neurons must properly innervate both peripheral target tissue, such as skin and muscle, and spinal cord by forming a number of axon collaterals in both regions for accurate sensitivity to external stimuli. Interestingly, MAP7^{mshi} mice exhibited an increased number of axon collaterals branching into the spinal cord compared with control animals, whereas the number of collaterals in the forepaw was normal. The aberrant innervation of DRG axons the spinal cord in MAP7^{mshi} mice was associated with thermal hyperalgesia, suggesting that DRG nociceptors rely on MAP7 for proper axon formation. Whether other subtypes of DRG neurons require MAP7 requires further investigation. Although Tymanskyj et al. (2017) cultured a heterogeneous population of sensory neurons from dissociated DRGs, they supplemented these cultures with nerve growth factor (NGF), which provides trophic factor for only a subset of neuronal subtypes (i.e., nociceptors) (Lallemend and Ernfors, 2012). Other DRG neuronal subtypes (e.g., proprioceptors) do not gain trophic support from NGF and were thus excluded from these cultures (Lallemend and Ernfors, 2012). Whether both nociceptors and proprioceptors rely on MAP7 is particularly interesting because they project to different targets. Notably, however, Tymanskyj et al. (2017) found that (1) only a subset of branches emerged from a MAP7-enriched region, (2) only a subset of MAP7-enriched regions along the axon produced branches, and (3) MAP7^{mshi} mice had branching abnormalities in central but not peripheral projections. Thus, it is likely that other factors govern branch formation in these axons.

Extensive work has shown that environmental cues shape axon branch formation by triggering a highly regulated sequence of cytoskeletal events, including actin patch formation, filopodial emergence, and microtubule polymerization and depolymerization (Armijo-Weingart and Gallo, 2017). Microtubule-associated proteins (MAPs) can mediate cytoskeletal changes by positively or negatively influencing microtubule stability and bundling (Armijo-Weingart and Gallo, 2017). Similar to the novel role for MAP7 in sensory branch maturation, previous work identified a role for MAP7 in Sertoli cell microtubule stability and spermatogenesis, where the absence of MAP7 in mice leads to male sterility (Komada et al., 2000; Magnan et al., 2009). Conversely, Tymanskyj and others (Tymanskyj et al., 2012; Barnat et al., 2016) have identified MAP1B as a negative regulator of axon branching and maturation in cortical and adult DRG neurons. These works shed light on the diverse protein toolbox available to the intrinsic branch maturation programs. An intriguing question is what controls the competition between positive and negative regulators to mediate branch construction or destruction.

Locally segregated branches from a single neuron may respond to permissive and/or restrictive cues. As mentioned above, Tymanskyj et al. (2017) find that MAP7^{mshi} mice have aberrant collateral branching in the spinal cord, but not the forepaw, suggesting that these diverging branches respond to different environmental cues. This apparent restriction of MAP7 function to central, but not peripheral, collateral branching mirrors the unilateral function of SAD kinases in central, but not peripheral, innervation in proprioceptive neurons, another sensory neuron subtype (Lilley et al., 2013). Lilley et al. (2013) propose a model in which SAD kinases sculpt axonal arbors through sequential long- and short-term neurotrophin exposures. Proprioceptive neurons receive tonic long-term exposure to neurotrophin-3 from peripheral targets, such as the muscle, resulting in upregulation of SAD kinase expression. This upregulation primes axons for intrinsic branch programming, but this primed pathway is only triggered following short-duration neurotrophin-3 exposure from target neurons in the spinal cord, such as motor neurons, to induce profuse branching in the ventral spinal cord (Lilley et al., 2013). In contrast, axon patterning in other neuronal subtypes can be governed by different extracellular cues. For example, the sympathetic nervous system, another neurotrophin-dependent population, responds to two different neurotrophic cues at sequential steps during axonal projection. Sympathetic axons initially grow along blood vessels, an intermediate target, which secretes a neurotrophin that promotes axon extension (Kuruvilla et al., 2004). However, upon final innervation, axon terminals are exposed to NGF that is produced by the target tissue. Target-derived NGF induces a signaling switch to halt axon growth and encourage branching to allow for extensive innervation of the target (Suo et al., 2015). Perhaps MAP7-dependent branch maturation also relies on either (1) a single axon patterning cue with temporally and locally distinct patterns or (2) different sequential cues from intermediate and final targets.

Neurological disorders may be associated with altered axon behavior and brain connectivity, contributing to their complex and multifactorial nature. Intriguingly, MAP7 is expressed in embryonic mouse brains, and fine genetic mapping studies show a significant association in MAP7 single nucleotide polymorphisms with schizophrenia (Fabre-Jonca et al., 1998; Torri et al., 2010; Venkatasubramanian, 2015). Following the discoveries in Tymanskyj et al. (2017) in the peripheral nervous system, it will be critical to investigate MAP7 regulation of axon branch maturation in the brain to elucidate any connection between MAP7 and schizophrenia. Additionally, beyond the peripheral nervous system development, axon collateral branching is required throughout the nervous system and is critical for proper wiring. Previous studies indicate that activation of intrinsic cellular programs that promote microtubule polymerization can positively regulate axon regrowth and branching after injury (Ruschel et al., 2015). Therefore, to discern potential therapeutic targets for regrowth of axons, future investigations may focus on MAP7 to reengage intrinsic developmental programming.

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