Letting Go of JuNK by Disassembly of Adhesive Complexes

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http://dx.doi.org/10.1016/j.neuron.2015.11.015

Immature neural circuits form excessive synaptic connections that are later refined through pruning of exuberant branches. In this issue, Bornstein et al. identify a role for JNK signaling in selective axon elimination through disassembly of cell adhesion complexes.

The network of synaptic connections in even a simple brain can be dizzying in its complexity. Precision in wiring neural networks is accomplished by early long-range guidance of axonal and dendritic projections to the appropriate brain regions to find synaptic partners, followed by later fine-tuning of circuit connectivity. The latter event is a key mechanism by which neurotransmission can help optimize neural circuit assembly and function. Immature neural circuits initially overwire, and axons and dendrites form excessive synaptic contacts, but these are later "pruned"—the process whereby superfluous connections are eliminated and appropriate synaptic contacts are strengthened (Luo and O'Leary, 2005). Developmental pruning can be further broken down into two distinct categories: small-scale and large-scale elimination of neuronal connections (Luo and O'Leary, 2005; Yu and Schuldiner, 2014). Small-scale pruning events occur very locally and involve retraction or phagocytic trimming of short neurites or synaptic contacts, as in the case of synaptic refinement in the mammalian visual system (Luo and O'Leary, 2005). Large-scale pruning occurs over much longer distances and involves the frank degeneration of entire neurite branches and their clearance by surrounding glia. The molecular similarities, or differences, between small- and large-scale pruning events remain unclear.

Drosophila mushroom body gamma (MBγ) neurons undergo large-scale pruning during metamorphosis and have proven an excellent model for understanding the mechanistic basis of neurite pruning. The MB contains three subsets of neurons (α/β, α′/β′, and γ) of which only γ neurons exhibit axonal and dendritic pruning (Figure 1A). During larval stages, MBγ neuron axons bifurcate and send projections to both dorsal and medial MB lobes. At metamorphosis, dendrites are removed completely, and the distal portions of axons are pruned to the base of a structure termed the peduncle. At later developmental time points, MBγ neurons re-extend axons into the medial lobe to help form the adult MB (Luo and O'Leary, 2005; Yu and Schuldiner, 2014). Activation of this pruning event requires coordination of signaling between neurons and surrounding glia, and recent studies have provided important insights into its regulation. Glial cells release TGFβ molecules that act on TGFβ receptors on MBγ neurons, which activate expression of the Ecdysone Receptor B1 (EcR-B1), thereby making MBγ neurons competent to respond to a pupal pulse of steroid hormone and initiate pruning (Yu and Schuldiner, 2014). Sox14 is a transcription factor that is downstream of EcR, which together with the ubiquitin proteasome pathway (UPS) somehow drives pruning (Figure 1C). In contrast to pruning activation, we know remarkably little about how axonal and dendritic compartments destined for elimination then drive their own destruction.

In this edition of *Neuron*, Bornstein et al. (2015) identify a role for c-Jun N-terminal Kinase (JNK) and promoting the disassembly of the axonal compartment during MBγ neuron pruning. Using an elegant forward genetic mosaic loss-of-function screen to identify novel genes required for MBγ neuron pruning in *Drosophila*, the authors discovered that loss of the *Drosophila* JNK, called *basket* (*bsk*), suppressed MBγ axon pruning. Surprisingly, *bsk* mutants showed no pruning phenotype in the MBγ dendrites, which provides direct evidence that in vivo molecular pathways governing axonal versus dendritic pruning are genetically separable. JNK is a member of the mitogen-activated protein kinase (MAPK) family and, when activated, can promote a diversity of downstream cellular responses. In the nervous system, JNK signaling has been shown to control microtubule stabilization, synaptic plasticity by regulation of dendritic spines, activate the transcription of genes involved in axon growth, as well as modulate Wallerian degeneration (Coffey, 2014; Yang et al., 2015). To explore how *bsk* regulates neurite pruning, the authors looked at the canonical downstream effectors in the dJNK signaling cascade in *bsk* mutants. There was no pruning phenotype with overexpression of the dominant negative versions of the c-Jun ortholog, Jra^{DN}, or c-Fos ortholog Kay^{DN}, suggesting *bsk* was not regulating pruning at the transcriptional level. EcR expression was normal in *bsk* clones, arguing that Bsk signaling did not act to regulate expression of EcR, as is the case for TGFβ, and reciprocal regulation of JNK by the TGFβ pathway was also ruled out. These observations argued that JNK signaling was regulating axon pruning in a non-canonical manner.

The most common approach to visualize the MB in *Drosophila* is an antibody to Fasciclin II (FasII), the fly ortholog of neural cell adhesion molecule (NCAM) (Grenningloh and Goodman, 1992; Packard et al., 2003). Fortuitously, the authors noticed that *bsk* mutant clones exhibited

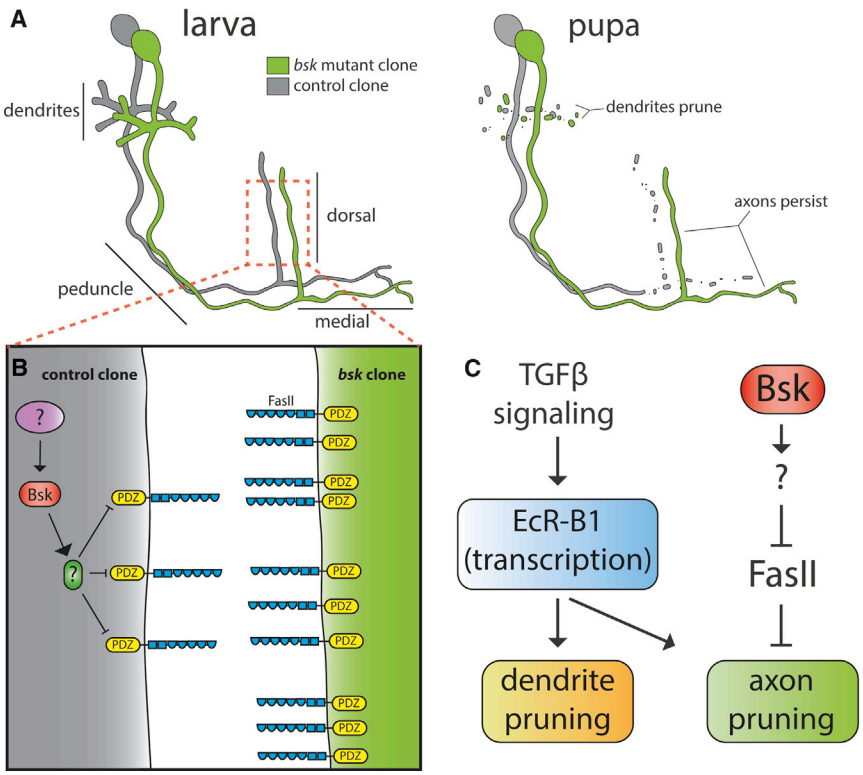

Figure 1. JNK-FasII Signaling in *Drosophila* MBγ Neuron Pruning

(A) MBγ neurons remodeling in the *Drosophila* brain during the larval and pupal stages. *bsk* mutant clones (green) fail to prune axons, but not dendrites, while control clones (gray) prune both compartments normally.

(B) Bsk negatively regulates surface expression of FasII in control cells (gray), but FasII levels and adhesion are increased in *bsk* mutant cells (green).

(C) Genetic pathways of developmental pruning in *Drosophila* MBγ neurons (see text for details).

a significant increase in FasII expression, which led them to speculate increased expression of this cell adhesion molecule could be the mechanism for suppression of pruning in *bsk* mutants. Elimination of FasII alone did not lead to defects in pruning; therefore, FasII is not required to drive axon auto-destruction during pruning. However, loss of FasII from *bsk* clones suppressed the pruning defect normally seen in *bsk* clones, and overexpression of FasII alone in control clones resulted in a significant pruning defect. Thus, elevated levels of FasII, and presumably increased cell adhesion, appeared to be sufficient to block axon pruning. Consistent with this notion, the authors found that overexpression of human NCAM or several other cell adhesion molecules were also sufficient to block MBγ neuron axon pruning, and these phenotypes were enhanced by downregulation of JNK signaling.

The precise mechanism by which JNK regulates FasII remains unclear, but a detailed structure function analysis by the authors of key FasII domains and turnover of FasII at the cell surface implies a simple model whereby a PDZ domain in the FasII intracellular domain is required for localization to the axon, and that JNK does not directly regulate FasII phosphorylation status, but rather it acts to downregulate surface FasII through an unidentified molecule (potentially a co-receptor). Future studies will be needed to define that molecule and address many additional intriguing questions. For instance, given that activated JNK (based on antibodies for phosphorylated JNK) is found through the MBγ neuron, how is the JNK-FasII pruning mechanism limited to axons, and why does the degenerative event stop at the base of the peduncle (rather than take the entire branch)? Identification of the downstream target(s) of JNK in the context of FasII downregulation seems a crucial next step to clarify how FasII is cleared from the surface of the cell. Likewise, one wonders how widespread the JNK-FasII adhesion-breaking mechanism of signaling is used throughout the *Drosophila* brain—FasII is an excellent marker for a well-defined subset of neurons in the pupal brain: are all of these pruning using this mechanism? If there are lineages that exhibit localized pruning that are FasII negative, is JNK signaling required in those cells to downregulate adhesive complexes, and if so, which adhesion complexes? Finally, since FasII is a transmembrane molecule, what roles might the extracellular environment play in regulation of axonal pruning?

That JNK-FasII signaling is required for axon, but not dendrite, pruning in MBγ neurons demonstrates that even within a single cell type, how decisions are made to eliminate axons versus dendrites is genetically complex. Axon loss is a major contributor to functional loss in patients with neurological disease. Emerging data supports the existence of unique molecular pathways driving axon degeneration during dying back neuropathy versus axotomy (Sreedharan et al., 2015), and these are distinct from developmental neurite pruning pathways (Neukomm and Freeman, 2014). The notion that there is a single neurite auto-destruction program therefore seems increasingly implausible. Would extreme diversity in neurite auto-destruction signaling be encouraging for prospects of therapeutic blockade of neurite loss in disease? High diversity in molecular pathways in different neurological diseases would necessitate disease-specific deconstruction of relevant pathways prior to therapeutic development. That would likely decelerate progress toward developing a broad toolbox of therapeutics for neurological disease. However, diversity in pathway engagement in different diseases could also be advantageous, as it would potentially provide the opportunity to selectively target key disease-relevant pathways while leaving major nervous system plasticity mechanisms unperturbed. Future exciting studies like this clarify these issues, and it seems clear much of our work is still ahead of us.

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Astrocytes, Makers of New Neurons

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http://dx.doi.org/10.1016/j.neuron.2015.11.017

Manipulating neurotransmitter release from astrocytes neighboring the developing new neurons in the course of adult hippocampal neurogenesis, Sultan et al. (2015) reveal that glial influence on neurogenesis ranges from controlling basic precursor cell function well into the establishment of functional circuitry. This extends the concept of the "neurogenic niche" and its key role into advanced stages of adult neuronal development.

Astrocytes continue to undergo dramatic changes in reputation. Once identified as the gluy filler of the gaps that the neurons leave in the brain and in more modern times at least respected as well-trained supporting staff, without whom nothing works in the brain, they increasingly make their claim for parity with the neurons. A very severe blow to the assumed nobility of the neurons has been the insight that it is astrocytes (or at least astrocyte-like cells) that are the stem cells driving much of brain development (Kriegstein and Alvarez-Buylla, 2009), so that from a neuronocentric perspective the noble neurons have not so noble origins. Astrocyte-like cells are also the stem cells in adult neurogenesis, both in the subventricular (or subependymal) zone of the lateral ventricle and in the subgranular zone (SGZ) of the hippocampal dentate gyrus (Doetsch et al., 1999; Seri et al., 2001). While these stem cells are astrocytes by many, if not all criteria, not all "astrocytes" appear to be stem cells, at least not under physiological conditions (Doetsch, 2003; Götz et al., 2015; Götz and Sommer, 2005). The distinctions blur, and what exactly qualifies at an astrocyte (or a neural stem cell for that matter) and how heterogeneous that class of cells actually is remains a not entirely open but in the end still unresolved question.

Now, Sultan and colleagues show in this issue of *Neuron* that astrocytes play an important and apparently highly interactive part in crucial steps of the development of new neurons beyond the stem or progenitor cell stage (Sultan et al., 2015). They used targeted genetic manipulation to show that blocking the vesicle release from astrocytes in the hippocampal dentate gyrus reduced synapse formation and network integration of adult-born neurons, which in turn affected neuronal survival and net neurogenesis.

This finding sheds new light on the exact mechanisms by which the new neurons become integrated into the pre-existing circuitry of the dentate gyrus, but also further highlights how tightly bound neuronal destiny is to astrocytes. The study confirms that NMDA receptor activity is indeed crucial for mediating functional maturation of new neurons (Tashiro et al., 2006) but suggests that it is astrocytes rather than only the neurons that provide the critical input at this stage. It was not, however, the glutamate that had changed. The authors rather found that NMDA receptor co-activator D-serine was reduced in the manipulated mice; restoring D-serine levels abolished the phenotype. This finding, of course, has to be seen in the context of the contribution other local neurotransmitters, especially GABA, make (Ge et al., 2006). The release of neurotransmitters from astrocytes and the extent to which they thereby might participate in signal transmission remains controversial even though Sultan et al. add a few new arguments in favor of the astrocytic contribution to that debate.

The stem cell niche is the functional unit of the stem cell itself and its immediate microenvironment, consisting of other cells, vasculature, nerve endings, and extracellular matrix. The niche both