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Neuronal death or dismemberment mediated by Sox14

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Abstract

The pruning of unneeded axons and dendrites is crucial for circuitry maturation, but poorly understood on the molecular level. During *Drosophila* metamorphosis, the transcription factor Sox14 acts as a context-dependent mediator of death, axonal or dendritic pruning. Its transcriptional target Mical acts specifically in dendrite pruning.

Neuronal death and neurite degeneration occur in a wide variety of contexts in the developing nervous system. If excess neurons are generated or if neurons become functionally superfluous at later developmental stages, they often undergo programmed cell death. Likewise, in the final stages of neural circuit assembly, exuberant or superfluous neuronal projections are selectively eliminated while functional connections are preserved¹. This latter event is quite remarkable (functionally it is equivalent to regionally restricted cell death), somehow the cell decides to completely destroy only one compartment (the pruned axon or dendrite), but the remainder of the cell remains perfectly healthy. How specific neurons are selected for death or individual neurites are selected for auto-destruction, as well as the nature of the underlying molecular pathways that mediate these events, remains poorly defined. In this issue, Kirilly *et al.*² identify two molecules, Sox14 and Mical, that are essential for fine-tuning neural cell numbers and dendritic projection patterns.

The *Drosophila* pupa is a superb system in which to study developmentally regulated cell death and neurite pruning. Much of the larval nervous system is preserved during the larva-to-adult transition, but it needs to be rewired to integrate properly with developing adult structures. Larval neurons show a diversity of behaviors during metamorphosis; some are no longer needed and undergo programmed cell death, others prune axons and initiate new axonal growth to connect with adult-specific tissues, and still others prune their dendrites. All these events are initiated by a developmentally regulated pulse of the steroid hormone 20-hydroxyecdysone (ecdysone)^{3,4}. Ecdysone is therefore a master regulator that not only controls neuronal rewiring, but also controls remodeling of the entire body of *Drosophila* during metamorphosis.

The larval dendritic arborization (da) neurons, which are found in the peripheral nervous system, are a particularly good system in which to assay dendritic pruning and neuronal cell death. These fall into four classes (termed I–IV) on the basis of morphology. Class II and III da neurons are eliminated at metamorphosis through apoptotic cell death, whereas Class I and IV da neurons survive and exhibit near complete dendritic pruning, but have no morphological changes in the soma or axon¹.

Kirilly *et al.*² started by simply live-imaging Class IV da neuron dendrites (specifically a subset termed ddaC) as pruning was initiated. ddaC dendrites that were destined to be pruned first developed blebs that were highly dynamic, moving throughout the dendritic compartment, but not into the soma or axon. Next, a break developed in the dendrite at its base next to the soma, physically separating it from the cell body. Subsequently, ddaC dendrites underwent widespread degeneration, with no sign of directionality to the progression of degeneration. These gross changes in cellular morphology, which have also been observed by other groups in additional da neurons⁵, are similar to what is seen in fixed preparations of pruned axons in the *Drosophila* CNS and are similar to Wallerian degeneration in live preparations of mouse axons, arguing for a potential conservation of axon degenerative mechanisms in these quite different contexts.

Kirilly *et al.*² then investigated how the classes of da neurons are differentially programmed to undergo cell death or dendritic pruning. Previous studies had identified genes whose expression is responsive to ecdysone signaling during metamorphosis. On the basis of this list, the authors performed a small-scale RNA interference (RNAi) screen looking for genes whose knockdown caused severe dendrite-pruning defects. Knocking down the *sox14* gene using transgenic RNAi approaches (*sox14^{RNAi}*) was found to potently block dendritic pruning of ddaCs. Sox14 encodes a high-mobility group (HMG) transcription factor that belongs to the highly conserved Sox family of developmental regulators. To confirm a role for Sox14 in pruning, the authors next produced mutations in the *sox14* gene, generated genetic mosaic clones such that the *sox14* mutation was homozygous only in Class IV da neurons, and again found that lack of Sox14 function blocked dendritic pruning. Pruning defects observed in the *sox14* mutants were rescued by overexpression of Sox14. Moreover, overexpression of Sox14 in ddaCs was sufficient to initiate premature dendritic pruning before the ecdysone pulse. These data argue that Sox14 induction is necessary and sufficient to induce dendritic pruning.

Sox14 function is not limited to dendrite severing, but also extends to activation of programmed cell death and axon pruning. Programmed cell death in Class II and III ddaA/B/F neurons was suppressed by *sox14^{RNAi}* treatment and in *sox14* mutations. Likewise, axonal pruning of mushroom body γ neurons was suppressed by *sox14^{RNAi}*. Thus, Sox14 can initiate all three of the neuron rewiring events observed during metamorphosis: cell death and dendrite or axon pruning. How Sox14 functions in these different cells to execute each of these programs remains unclear. For example, how could Sox14 promote dendrite pruning, but not axon pruning, in peripheral da neurons, while doing apparently the opposite in central mushroom body neurons? The authors suggest that discriminating between cell death and pruning may simply entail modulating Sox14 levels; Sox14 appears to be expressed at higher levels in Class II and III da neurons that undergo death versus Class I and IV, which exhibit dendrite pruning. Clearly, testing the predictions of this model and identifying downstream targets of Sox14 could address these questions.

To identify other factors involved in pruning (including potential downstream targets of Sox14), Kirilly *et al.*² performed a forward genetic screen for mutants that suppressed ddaC pruning. One mutant line exhibited a near full suppression of ddaC pruning. However, in contrast with *sox14* mutants, apoptotic elimination of ddaA/B/F neurons remained normal,

as did pruning of mushroom body γ neuron axons. These defects mapped to *mical*, which encodes a multi-domain cytosolic protein containing an N-terminal flavoprotein monooxygenase (FM) domain, and calponin homology and LIM domains in the central region, followed by a proline-rich domain, coiled-coil domain and PDZ-binding motif at the C terminus. Overexpression of full-length Mical rescued the mutant phenotype, whereas overexpression of a truncated Mical that was missing the N-terminal FM domain did not. Thus, FM domain function appears to be essential for Mical activity in dendrite severing. Although Mical seems to only drive pruning in dendrites, antibodies to Mical were present throughout the dendrite, soma and axon. Thus, polarized activation, rather than localization, may underlie its role in selectively destroying dendrites.

Mical expression in ddaC neurons is initiated after the ecdysone pulse. Could the *mical* gene be a target of Sox14? Several lines of evidence suggest that *mical* activation is downstream of Sox14 and the receptor for ecdysone, EcR. First, overexpression of Sox14 led to an increase in Mical in ddaCs. Second, Sox14 was found to bind to the *mical* promoter in ecdysone-treated cell lines. Third, the accelerated pruning phenotype that resulted from early overexpression of Sox14 could be suppressed by blocking Mical function. Both *mical* and *sox14* also appeared to be downstream of EcR, as blocking ecdysone signaling with a dominant-negative EcR blocked induction of *mical* and *sox14* in both da neurons and mushroom body γ neurons.

Notably, Sox14 appears to be a major downstream target of EcR for nervous system reorganization during metamorphosis and Mical seems to be an important regulator of its role in ddaC dendrite pruning. For example, Sox14 overexpression rescued pruning in EcR dominant negative-treated ddaCs, as did Mical overexpression. Moreover, in *sox14* mutant ddaCs that also expressed dominant-negative EcR, overexpression of Mical could partially rescue pruning defects. Together, these data argue that Mical acts downstream of EcR, potentially directly downstream of Sox14, and is an important mediator of Sox14-dependent control of dendritic pruning (Fig. 1).

As with any exciting study, a number of new questions arise from this work. For example, what other genes are regulated by Sox14? Clearly Sox14 is a major mediator of cell death and dendritic and axonal pruning. How is the specificity of the response (death or dismemberment) of the cell encoded? How is Mical, found throughout the cell, activated only in dendrites? This protein has a number of interaction domains, some of which bind actin, but the key feature that allows for dendrite severing in a localized fashion remains undefined. One possibility is that Mical somehow converges with local caspase activation, which has been described in pruned *Drosophila* dendrites^{6,7}, to drive destruction, but this remains to be determined. Also, are dendrite severing and subsequent degeneration genetically separable events? For example, does Mical simply cause localized dendrite severing near the soma while other mechanisms drive degeneration? If so, how does Mical know where to sever the dendrite? If Mical is required for dendrite destruction, how does it drive dendrite disassembly?

This and other recent studies in the field highlight the fact that neurite degeneration is a complex process that can be initiated by a variety of molecular mechanisms. Genetic tools

have now been identified that discriminate between dendrite pruning, axon pruning and Wallerian degeneration. Is there a unifying pathway of neurite destruction into which different activating pathways feed? Are all of these processes of degradation distinct from one another? In many ways, the field is positioned similar to that of the cell death field two decades ago; we know (or strongly suspect) these events are active processes of auto-destruction, but we still need to delineate the underlying genetic pathways. In the future, clarifying precisely how Sox14 dictates the death or only dismemberment of neurons and how Mical mediates dendrite-specific localized severing and destruction should help us take important steps in this direction.

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Figure 1.

Sox14 and Mical function during developmental sensory dendrite pruning in *Drosophila*. Ecdysone initiates a host of developmental changes in the *Drosophila* pupal nervous system, including cell death, axon pruning and dendritic pruning. Ecdysone binds to a nuclear receptor heterodimer (consisting of the ECR and ultraspiracle, USP), resulting in the activation of target genes, including *sox14*. Sox14 is required for apoptosis in type II and III da neurons and for pruning in type I and IV da neurons. In type I and IV da neurons, Sox14 activates expression of dendrite pruning genes, including *mical*. Mical is found throughout the cell, but, through unknown mechanisms, it only promotes severing of the dendritic compartment. 20HE, 20-hydroxyecdysone.