Releasing the Inner Inhibition for Axon Regeneration

Michelle D. Po,1,* John A. Calarco,2,* and Mei Zhen1,3,*

1Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada M5G 1X5
2FAS Center for Systems Biology, Harvard University, Cambridge, MA 02138, USA
3Department of Molecular Genetics, Department of Physiology, Institute of Medical Science, University of Toronto, ON, Canada M5S 1A8

*Correspondence: michelle.po@utoronto.ca (M.D.P.), jcalarco@fas.harvard.edu (J.A.C.), zhen@lunenfeld.ca (M.Z.)

DOI 10.1016/j.neuron.2012.01.002

The adult mammalian central nervous system exhibits restricted regenerative potential. Chen et al. (2011) and El Beijjani and Hammarlund (2012) used Caenorhabditis elegans to uncover intrinsic factors that inhibit regeneration of axotomized mature neurons, opening avenues for potential therapeutics.

In the mature mammalian central nervous system (CNS), many axons fail to regenerate upon injury, resulting in lasting functional deficits. The inability of mature mammalian CNS neurons to regenerate contrasts the robust regenerative potential of the fish and amphibian nervous systems, mammalian PNS neurons, and even juvenile mammalian CNS neurons. Aguayo and his colleagues demonstrated that injured adult rat CNS neurons could reinitiate axon growth in PNS grafts, providing the first definitive evidence that an inhibitory environment contributes to the inability of mature CNS neurons to regrow (Richardson et al., 1980). Several extrinsic factors that potently inhibit axon regeneration in cultured neurons, including chondroitin sulfate proteoglycans and the myelin-based inhibitors MAG, Nogo, and OMgp, have since been identified (reviewed in Zheng et al., 2006). However, removing Nogo receptor (NgR) was insufficient to induce regeneration of severed mouse corticospinal axons in vivo (reviewed in Zheng et al., 2006). These studies suggest that: (1) removing NgR fails to remove all environmental inhibitory signaling, as suggested by the necessity of removal of both NgR and PirB, another myelin inhibitor receptor, for a near-complete suppression of myelin-mediated inhibition of cultured neuron regeneration (Atwal et al., 2008); (2) mature CNS neurons may also require promoting factors to initiate regeneration; and/or (3) CNS neurons have intrinsically limited regenerative potential upon maturation. The identification of both extrinsic stimulators and intrinsic inhibitors of axon regrowth upon injury would thus provide potential new targets to promote nervous system regeneration.

C. elegans is a rapidly emerging genetic model for probing axon regeneration in a mature nervous system. Its simple nervous system and transparency aids fluorescent labeling and precise severing of single axons by femtosecond (Yanik et al., 2004) or dye laser (Wu et al., 2007; Hammarlund et al., 2009) in live animals. Regenerative growth has been observed in many C. elegans neurons but has been most carefully described in the D-type GABergic motor neurons and the PLM mechanosensory neurons. Typically, severed axons undergo reproducible morphological changes over the course of several hours, starting with a retraction of the axon at the site of injury, followed by the development of a growth cone-like structure (Yanik et al., 2004). The filopodia at the leading edge of these structures extend and guide axons toward their targets over the course of several days (Wu et al., 2007). Remarkably, the regrowth of GABAergic motor axons can lead to a partial functional recovery of the motor circuit (Yanik et al., 2004; El Beijjani and Hammarlund, 2012).

Comparison of the recovery of severed axons in various C. elegans mutant backgrounds has allowed for the identification of factors that either promote or inhibit axon regeneration. For example, Dual Leucine-Zipper Kinase (DLK-1)-mediated MAPK signaling promotes axon regeneration in multiple C. elegans neurons (Hammarlund et al., 2009; Yan et al., 2009). DLK signaling also promotes Wallerian degeneration, as well as the regeneration of axotomized Drosophila olfactory receptor neurons and mouse dorsal root ganglion neurons (Miller et al., 2009; Xiong et al., 2010). Moreover, similar to vertebrate neurons, increased calcium and cyclic AMP facilitate axon regeneration in severed C. elegans neurons (Ghosh-Roy et al., 2010). Therefore, conserved machineries involved in injury repair can be discovered through the analysis of the C. elegans nervous system.

Two recent studies published in Neuron further exploit the robustness of postaxotomy regeneration of C. elegans neurons to identify novel factors that affect the regenerative capacity of a mature nervous system. Chen et al. (2011) presented the first systematic examination of genetic factors that regulate the regenerative growth of the PLM mechanosensory neuron. The regrowth of its longitudinal axon upon laser severing during the last larval stage was monitored in 654 loss- or gain-of-function mutants. A large number of genes, with roles in diverse cellular processes—signaling, cytoskeleton remodeling, adhesion, neurotransmission, and gene expression—are required for robust PLM axon regrowth in adults. By contrast, only 16 genes emerged as potent inhibitors of axon regrowth; the loss of these genes resulted in significant overgrowth of the PLM axon upon axotomy. Among them, the Exchange Factor for Arf6 EFA-6, a conserved Guanine Exchange Factor (GEF), functions in the PLM neuron to both prevent axon overextension during development and potently inhibit axon regeneration postaxotomy in late-stage larvae. Severed PLM axons exhibit proportionally more regrowth.
Hammarlund report the identification of (O’Rourke et al., 2010). Further supporting C. elegans the cell cortex in of EFA-6 decreases microtubule growth at addition to its role as a GEF, the N terminus region. Previous work showed that in but functionally poorly defined N-terminal GEF domain, but instead via a conserved induced regrowth not primarily through its EFA-6 exerts its inhibitory effect on injury-hibits axon growth reinitiation. Intriguingly, results suggest that EFA-6 likely in-

Figure 1. Cell-Autonomous Signaling Pathways that Inhibit the Regeneration of Axotomized C. elegans Neurons
Notch signaling, mediated by Notch receptor/LIN-12 and its processing enzymes ADAM10/SUP-17 and γ-secretases SEL-12 and HOP-1, inhibits growth cone initiation, an early stage of axon regeneration. The Notch Intracellular Domain (NICD) translocates to the nucleus, implying a role in transcriptional regulation of unidentified targets. An Arf Guanine nucleotide Exchange Factor, EFA-6, inhibits axon regrowth by destabilizing microtubules at the site of injury. efa-6(lf), but not the loss of Notch signaling components, partially bypasses the requirement of the DLK-1 MAPK signaling and CEBP-1-mediated local translation for axon regeneration.

during the early phase of regeneration in the absence of EFA-6. EFA-6 activity also most potently limits regrowth during the early phase of regeneration. These results suggest that EFA-6 likely inhibits axon growth reinitiation. Intriguingly, EFA-6 exerts its inhibitory effect on injury-induced regrowth not primarily through its GEF domain, but instead via a conserved but functionally poorly defined N-terminal region. Previous work showed that in addition to its role as a GEF, the N terminus of EFA-6 decreases microtubule growth at the cell cortex in C. elegans embryos (O’Rourke et al., 2010). Further supporting the involvement of microtubule remodeling in EFA-6-mediated inhibition on axon regeneration, the application of Taxol, a microtubule-stabilizing compound, partially restored the decreased regrowth of PLM axon induced by an overexpression of the N-terminal region of EFA-6. Taken together, these results suggest that EFA-6 prevents the initiation of axon regrowth by counteracting microtubule polymerization.

In this issue of Neuron, El Bejjani and Hammarlund report the identification of a new set of inhibitors of axon regeneration in mature motor neurons (El Bejjani and Hammarlund, 2012). Upon severing the commissural axons of GABAergic motor neurons, a fraction of them effectively regrow and partially restore motor deficits associated with injury, implying a partial restoration of synaptic connectivity (Yanik et al., 2004; El Bejjani and Hammarlund, 2012). These authors found that a canonical Notch signaling cascade, regulators of C. elegans vulva morphogenesis, also functions as potent intrinsic inhibitors of commissural axon regrowth and functional restoration of motor circuit activity (El Bejjani and Hammarlund, 2012). The loss of one of the C. elegans Notch receptors LIN-12 in GABAergic neurons results in accelerated growth cone initiation and regrowth of the axon. Conversely, increased LIN-12 signaling leads to reduced regeneration. Unlike the case for EFA-6 (Chen et al., 2011), Notch/LIN-12 specifically limits regeneration after axotomy, without affecting axon growth during development. The ADAM metalloproteases SUP-17 and ADM-4, and the γ-secretases/Presenilins SEL-12 and HOP-1, cleave Notch/LIN-12 and release the Notch intracellular domain (NICD). Upon its translocation into the nucleus, the NICD regulates development through modulating transcription. These authors showed that the processing of Notch/LIN-12 by SUP-17, SEL-12, and HOP-1 immediately postaxotomy is necessary for effective inhibition of axon regeneration; they were also successful in potentiating axon regeneration by injecting a γ-secretase inhibitor N-[N-(3,5-
difluorophenacetyl)-L-alanyl]S-phenylglycine t-butyl ester (DAPT) immediately after axotomy. These results indicate that Notch signaling inhibits the initiation of axon regeneration. Interestingly, the application of DAPT two hours after axotomy failed to affect regeneration, suggesting that the inhibitory Notch activity is fairly rapidly triggered upon injury.

A key issue to be addressed in future studies is how multiple intrinsic signaling events are activated upon injury and interact with each other to determine the injury response (Figure 1). Both inhibitory factors for regeneration, EFA-6 and Notch/LIN-12, are most effective during a narrow time window immediately following axotomy. Similarly, regeneration-promoting DLK-1 signaling is most critically required within two hours of the injury to enable growth cone initiation (Hammarlund et al., 2009). Upstream regulators of EFA-6 remain elusive, but signals stemming from the site of injury, such as calcium influx and an increase of cAMP, probably play a role in DLK-1 activation (Ghosh-Roy et al., 2010). In the case of Notch signaling, no single known Notch ligand was found necessary to inhibit axon regeneration (El Bejjani and Hammarlund, 2012). One ligand DSL/LAG-2 even mildly promotes regrowth (El Bejjani and Hammarlund, 2012). It is possible that multiple ligands function redundantly upon injury to activate Notch (Figure 1). These observations, however, also support a tantalizing possibility that axotomy itself is a shared trigger for multiple signaling responses, including the activation of Notch processing independently of its canonical ligands.

Despite a similar temporal requirement, DLK-1, EFA-6, and Notch signaling do not exhibit unequivocal linear genetic interactions. In efa-6; dlk-1 double mutants, severed PLM axons extend significantly
longer than in dlk-1 mutants, yet they failed to form growth cone-like structures (Chen et al., 2011). The loss of Notch signaling could not bypass the requirement of DLK-1 to reinitiate growth cones in GABAergic neurons (El Bejjani and Hammarlund, 2012), arguing against a simplistic view where DLK-1 initiates axon regeneration by suppressing inhibitory signals from EFA-6 or Notch. While the genetic interactions between the Notch signaling and EFA-6 remain to be determined, an interplay of multiple, parallel signaling events may determine the injury response in individual neurons.

These studies reinforce a notion that both common and specific factors contribute to the regeneration of different neurons. DLK-1 activity is necessary for the regrowth of both GABAergic motor neurons and PLM mechanosensory neurons. Whether EFA-6, an inhibitor of PLM axon regeneration, also affects the regeneration in GABAergic motor neurons remains to be tested. However, as observed for Notch signaling components (El Bejjani and Hammarlund, 2012), some factors that regulate regeneration are probably cell type specific or are expressed at different levels in neuronal subtypes. Comparing such differences may yield insights into the determinants of the regenerative potential of specific neuronal classes.

Another common theme emerging from these and other studies is that axon regeneration involves transcriptional and posttranscriptional regulation. The main Notch effector NICD localizes to the nucleus of injured GABAergic neurons, and the constitutive expression of NICD potently inhibits their commissural axon regeneration (El Bejjani and Hammarlund, 2012). In PLM neurons, DLK-1-mediated regrowth requires a bZip transcription factor CEBP-1 and its local translation at the severed site (Yan et al., 2009). In Drosophila neurons, DLK-mediated regeneration involves the Fos transcription factor (Xiong et al., 2010). Additional transcription factors, as well as regulators of chromatin remodeling and mRNA metabolism, influence PLM axon regeneration (Chen et al., 2011). These observations indicate that local and nuclear gene regulatory responses may contribute to different phases of regeneration. It will be important to identify and compare the downstream target(s) of these regulatory proteins.

As demonstrated in these two recent studies, the repertoire of C. elegans genetic mutants allows for both genome-wide screens and targeted investigation of factors that positively and negatively regulate axon regeneration. The factors and genetic pathways identified by these studies, however, probably represent only the tip of the iceberg. Recently identified intrinsic inhibitors for adult mouse retinal ganglion cell axon regeneration include more transcriptional regulators, such as the Krüppel-like factors, repressors of mTOR-mediated protein translation PTEN and TSC1, as well as SOCS3, a negative regulator of JAK/STAT signaling (reviewed in Liu et al., 2011). The dual deletion of PTEN and SOCS3 results in significantly more sustained axon regeneration than either single gene deletion (Sun et al., 2011), further supporting the view that the interplay of multiple regeneration-promoting factors determines the regenerative ability of neurons. Given that the cellular response to injuries inflicted by various forms of axotomy and neurological trauma may differ, assessing the effect of multiple factors in different neurons, injury paradigms, and animal models is critical for revealing general and specific targets for nervous system repair. Results from Chen et al. (2011) and El Bejjani and Hammarlund (2012) provide exciting starting points for testing the role of orthologous proteins in other animal and injury models for axon regeneration.

REFERENCES


