What can Drosophila axonal development teach us about nerve regeneration?

Assembly, maintenance and repair of nervous systems rely on the precise coordination in the presentation of guidance signals and the correct reception and processing of these signals. During embryonic development, considerable progress has already been made in identifying the extracellular cues and the receptors mediating axonal guidance (Arayo and Tear, 2003). Axons are particularly vulnerable to injury and disease and axonal damage plays a central role in neurodegenerative disorders. Hence, full integration of axonal guidance information will help us understand how cells can combine extensive extracellular information to follow an unerring migration pathway. In addition, this understanding will yield clues on how to encourage axonal regeneration after injury or disease.

The core problem in central nervous system (CNS) injury and degeneration, and the cause of long-term functional impairments is the lack of an efficient regeneration. In both vertebrate and invertebrate CNS, neurons have limited capacity to grow upon damage, revealing a combination of poor regenerative capacity with a discouraging cellular environment. Therefore, knowing more about the molecular pathways that modulate axonal regeneration can yield promising targets in the treatment of human neurodegeneration and CNS injuries. A major goal in CNS regenerative research is to develop strategies to re-establish the lost innervation. Remarkably, many of the molecular players involved in regeneration also participate in axonal growth and guidance during embryonic development. This emphasizes the crucial importance of increasing the current knowledge on the molecular pathways involved in axonal growth and guidance and be able to draw parallels with axonal regeneration. To know more about axonal growth and guidance and their links to regeneration, model organisms, like the fruit fly, Drosophila melanogaster, are of foremost importance.

Sequencing of the human genome has and will accelerate the identification of many genes and the molecular processes involved in human diseases. In order to understand the molecular and cellular pathways involved in human pathologies, these can be modeled in easily manageable model organisms like Drosophila. The power of this genetic system has revealed many genetic factors involved in various pathways affected in genetic and acquired human diseases, as well as provided potential drug targets for therapeutics. Drosophila is a well-established model, ideal for forward and reverse genetics experiments and in vivo studies. The evolutionary conservation of gene function between humans and Drosophila make it an ideal model system with great applicability in the study of human pathologies. To this we must add the great and detailed knowledge gathered over the past century on the biology and genetics of this organism. Many aspects of human disease are also found in flies. These include cancer, ageing, neurodegeneration, infectious diseases, and dysfunction of neurotransmitter and endocrine systems (Lessing and Bonini, 2009). Interestingly, a number of inherited human neurodegenerative diseases have been successfully modelled in Drosophila (Lessing and Bonini, 2009) and both larvae and adult flies are good models for axonal injury and regeneration (Ayar et al., 2008). Similar to their mammalian homologues, fly CNS neurons respond to injury by transient upregulation of the stress response c-Jun N-terminal protein kinase (JNK) pathway (Leyssens et al., 2005) and by Wallianer degeneration of their axons (MacDonald et al., 2006). In addition, as in vertebrates, Drosophila nerve tracts are typically comprised of many axons, which, although lacking myelin, are ensheathed by glial cells (MacDonald et al., 2006). Finally, Drosophila axons are able to undergo new axonal growth after injury, and genetic studies indicate that conserved signalling molecules are required for this process (Ayar et al., 2008; Xiong et al., 2010).

For an axon to regenerate, it needs to go through many processes that resemble axonal embryonic development. Namely, it needs to form a growth cone, grow and extend in the appropriate direction and finally find its innervation targets. However, in human nerve regeneration, these processes mostly happen many years or even decades after the nervous system was developing. Following embryonic nervous system development, expression patterns of many axon guidance molecules are reduced, or at least changed. Others keep their embryonic expression and are still abundantly present in the mature CNS. The presence of these molecules in the adult nervous system suggests other roles for guidance cues beyond the initial phase of axonal outgrowth, growth cone navigation, and target innervation. And even though after embryogenesis, neuronal circuits become more stable, it is also important to highlight that adult nervous system connectivity does not necessarily endure all throughout adulthood in response to experience, injury, and ageing. This plasticity needs to be molecularly modulated. Because many mature neurons still express receptors for guidance cues, it has been speculated that these may continue to play a role in adulthood. Several axon guidance molecules including Semaphorins, Ephrins, Wnts, Slits, and Netrins become upregulated in the vertebrate adult CNS after injury (Giger et al., 2010). They are obvious candidates for modulating the growth of axons in the adult. Thus, these guidance factors have received much attention in regenerative studies.

Growth cones and the cytoskeleton: Leading to successful nerve regeneration, one of the first steps after physical injury of the axon is the formation of a new growth cone. This is achieved through the reorganization of the cytoskeleton at the proximal axonal stump. Growth cone formation and its consequent advancement are tightly regulated by extracellular factors and intracellular signalling molecules. In Drosophila, post-injury JNK activation has been found to initiate the microtubule arrangements that precede the formation of the new growth cone (Fang and Bonini, 2012). This triggers rapid alteration of the cytoskeleton, which implies extensive changes in actin and microtubules. In fact, these changes are at the same time so dynamic and effective that they have been shown to be able to convert a dendrite into a regenerating axon (Stone et al., 2010). In order to orchestrate an integrated response to axonal injury, JNK activity not only is necessary and sufficient for growth cone dynamics and axonal extension, but also interacts with other molecular signals including amyloid precursor protein (APP), the dual leucine zipper-containing kinase (DLK)/Wallenda (Wnd), and the CAMPPKA pathway (Fang and Bonini, 2012). Together, these pathways regulate cytoskeletal integrity, microtubule (MT) dynamics, and axonal transport, which link degeneration/injury with axonal growth and regeneration. Again, many molecular pathways involved in axonal development, also play a role in regeneration. Examples are key microtubule regulators such as the spectraklins. Spectraklins are a family of evolutionarily conserved actin-microtubule linkers that regulate microtubule dynamics. Loss of function of the mouse spectrakin AC7 or its close Drosophila homologue Short-stop (Shot) will cause severe axon shortening and microtubule disorganization (Sanchez-Soriano et al., 2009). Upon axonal injury, the MAPKKK DLK/Wnd regulates the axon injury response pathway, modulating both axonal regeneration and degeneration (Collins et al., 2006). This crucial activity of DLK after injury can be inhibited by Shot (Valakh et al., 2013). In shot mutants, the DLK/Wnd pathway is overactivated leading to an enhanced response to axonal injury that has been attributed to overall cytoskeletal destabilization. This suggests that similar molecular mechanisms operate during embryonic development and regeneration in order to stabilize the growth cone.

A major challenge to axonal injury or crush is the lack of reactivation of genes that drive growth cone reactivation and movement. In Drosophila these can be easily studied and are mediated by simple genetic techniques like the GAL4-UAS system (Brand and Perrimon, 1993). In addition, changes in the cytoskeleton can also be easily induced and their consequences tested after axonal injury.

Axonal guidance, remodeling and regeneration: Once the growth cone is formed, the growing axon needs to extend towards its target tissue. For this to occur, the axonal membrane has to grow and the damaged neuron has to be able to express guidance receptors. In addition, guidance ligands have also to be expressed appropriately in the surrounding environment. Axonal regrowth and elongation require that new membrane is added to the newly formed growth cone or the axonal membrane. Recent studies have concluded that similar molecular mechanisms are involved in membrane addition during development and regeneration (Bloom and Morgan, 2011). Key components of this process are the members of the Soluble NSF-Attachment protein Receptor (SNARE) complex and their
Embryo stained with HRP to detect all neurons and in situ. Araújo et al. (2012). Using Drosophila allows us to easily analyze this process in different genetic backgrounds. Also, using this model organism, we can change protein expression in situ and test axonal responses to injury in a variety of different conditions.

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Figure 1 The embryonic nervous system of Drosophila melanogaster. Stage 16 Drosophila embryo stained with HRP to detect all neurons and axons in the nervous system (in green) and an antibody against Fasciclin II that marks all motor axons in the lateral part of the embryo (in red).

effectors. In parallel to their roles in synaptic vesicle exocytosis SNARE proteins are also required for membrane addition during embryonic development (Cotrufo et al., 2011) and axonal regeneration (Bloom and Morgan, 2011). In Drosophila, mutants in Syx1a, a component of the SNARE complex, have defects in fasciculation (Schulze and Bellen, 1996). This suggests that the SNARE complex may also be involved in Drosophila axonal guidance and regeneration.

During regeneration, as during embryonic development, axonal extension needs to be coordinated with axonal guidance. Also in this case, INK may play an important role, because it has been shown to be involved in coordinating Netrin signalling in the developing nervous system (Qu et al., 2013). This suggests that INK signalling may help link post-injury growth cone remodeling with consequent axonal guidance.

Throughout regeneration and development, progressive and regressive events apply to the attractive and repulsive forces that guide axons. Whereas in some cases guidance molecules can exert a positive effect during regeneration, in others they are themselves responsible for the inability of axons to undergo proper post-injury repair. For therapeutic purposes it should be significant to be able to downregulate the expression of many guidance cues with known inhibitory activity, including members of the Semaphorin, Ephrin and Slit families (Giger et al., 2010). Conversely, neurotrophic factors and permissive/attractive guidance cues should be up-regulated to promote axonal growth. Despite the growing knowledge about embryonic axonal guidance in Drosophila, very little is known about remodulation of guidance receptors and their ligands after injury. It will be a challenge for the near future to learn more about how genetics and epigenetics can influence the relationship between regenerating axons and their environment.

Drosophila can be a useful model for neuroregeneration studies for many of the reasons stated above, but also because of its capacity to remodel its nervous system during metamorphosis. Remodeling includes both degenerative events like the elimination of connections and regenerative events such as regrowth and formation of new contacts between axon and target tissues. Neuronal remodeling of the Drosophila mushroom body (MB) during metamorphosis is an attractive model for studying the mechanisms of both degenerative and regenerative plasticity. It has been reported that both Highwire and DLK/Wnd are involved in regulating axon guidance after branching in the MB (Shin and DiAntonio, 2011). Furthermore, using this model, it has recently been shown that the TOR pathway is involved in development growth as well as during regeneration after injury (Yaniv et al., 2012). This further confirms the existence of common molecular mechanisms between Drosophila metamorphosis remodeling and regeneration.

Overall, we should learn about the common mechanisms involved in axonal patterning during embryonic development, remodeling during metamorphosis and post-injury mechanisms. The fact that there are also many shared mechanisms between these processes and the molecular mechanisms involved in neuronal regeneration strengthens the future use of Drosophila as a model for regeneration.

Current neural regeneration research faces many challenges. Drosophila research can help address many of the issues regarding these challenges with its unique tools and genetic advantages. The main advantage is, of course, that with Drosophila one can perform large-scale genetic analysis very fast. Another is the ability to monitor in vivo physical damage to axons and their responses by time-lapse microscopy. This technique is being applied to larvae as well as adult brain explants (Auyar et al., 2008; Ghanad-Rayazi et al., 2012). Using Drosophila allows us to easily analyze this process in different genetic backgrounds. Also, using this model organism, we can change protein expression in situ and test axonal responses to injury in a variety of different conditions.

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