The Genetics of Axon Guidance and Axon Regeneration in *Caenorhabditis elegans*

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ABSTRACT The correct wiring of neuronal circuits depends on outgrowth and guidance of neuronal processes during development. In the past two decades, great progress has been made in understanding the molecular basis of axon outgrowth and guidance. Genetic analysis in *Caenorhabditis elegans* has played a key role in elucidating conserved pathways regulating axon guidance, including Netrin signaling, the slit Slit/Robo pathway, Wnt signaling, and others. Axon guidance factors were first identified by screens for mutations affecting animal behavior, and by direct visual screens for axon guidance defects. Genetic analysis of these pathways has revealed the complex and combinatorial nature of guidance cues, and has delineated how cues guide growth cones via receptor activity and cytoskeletal rearrangement. Several axon guidance pathways also affect directed migrations of non-neuronal cells in *C. elegans*, with implications for normal and pathological cell migrations in situations such as tumor metastasis. The small number of neurons and highly stereotyped axonal architecture of the *C. elegans* nervous system allow analysis of axon guidance at the level of single identified axons, and permit *in vivo* tests of prevailing models of axon guidance. *C. elegans* axons also have a robust capacity to undergo regenerative regrowth after precise laser injury (axotomy). Although such axon regrowth shares some similarities with developmental axon outgrowth, screens for regrowth mutants have revealed regeneration-specific pathways and factors that were not identified in developmental screens. Several areas remain poorly understood, including how major axon tracts are formed in the embryo, and the function of axon regeneration in the natural environment.

KEYWORDS netrin; semaphorin; ephrin; Wnt; Slit; Robo; fasciculation; DLK; growth cone; actin; microtubule; WormBook

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IN development, many cells migrate or extend processes to specific locations to make connections with the appropriate partner cells. For this to happen, cells must recognize their extracellular environment, and direct the outgrowth activity in the appropriate direction. One of the most striking examples of directed cell outgrowth occurs during the extension of a process from a neuronal cell. Neurons are typically polarized, and extend two different types of processes or neurites: an axon, which represents the output side of neuronal signals, and a larger number of dendrites, providing input. In the case of axons, a specialized structure, the growth cone, forms at the tips of neuronal extensions. The growth cone acts as a navigation center, integrating information from the extracellular environment, and executing changes in the direction of outgrowth by modulating the cytoskeleton. Growth cones can navigate considerable distances by traveling along specific pathways to find their target cell(s). To form a properly functional nervous system, neurons must make precise connections with synaptic partners, and form intricate networks.

Defects in axonal navigation lead to the disruption of functional neuronal circuits, and serious neurological defects.

The process of directed outgrowth of neurites can be divided into consecutive steps. First, the neuron has to initiate formation of a new process. Process identity as axon or dendrite has to be established. This is typically coupled to the overall polarization of the neuron. The axon or dendrite has to navigate toward the target, which usually requires a number of guidance decisions on the way. The navigation process is much more complex for axons, which frequently extend over large distances, whereas dendrites are often branched and cover a volume closer to the cell body. Neurite outgrowth stops in the target area, usually at well-defined positions. When neurites are branched, the position and number of branches has to be controlled. For dendrites, which cover a volume with a highly branched dendritic tree structure, a mechanism of self-avoidance ensures that dendritic branches are spaced optimally to cover the volume. Finally, synaptic partners have to be identified, and synapse formation

has to be coordinated between pre and postsynaptic neurons. The best understood aspect of neurite outgrowth is the targeted navigation step. This review therefore focuses largely on pathways and cues that directly influence axon navigation, and will not exhaustively cover other aspects of axon outgrowth such as cell type specification or developmental timing.

Most axons in *C. elegans* navigate to their targets during embryonic or early larval stages, where the overall architecture of the nervous system is laid down. In addition to navigation-based outgrowth, axons elongate while maintaining circuit architecture while the animal grows fourfold in length during larval and early adult life. Little is known of mechanisms underlying this proportional growth of axons, in any organism. Collateral branching also occurs after axon growth; as this involves formation of growth cones from existing axons, it may be mechanistically relevant to regenerative axon growth. The architecture of the *C. elegans* nervous system is complete by late larval stages and does not display overt changes or plasticity in early adult life, although several studies have characterized age-dependent sprouting (Tank *et al.* 2011; Toth *et al.* 2012). After their initial outgrowth, axons possess remarkable capacities to regenerate after injury. Regenerative axon regrowth can take many forms, among which a typical mechanism involves reformation of a growth-cone-like structure at the severed end of a damaged axon. This regenerative growth cone resembles developmental growth cones, but typically undergoes error-prone navigation to its original targets.

History of *C. elegans* as a Model for Process Outgrowth

C. elegans was chosen as a model organism because of its unique advantages for studying the development and function of the nervous system. In 1986, a landmark paper, “The structure of the nervous system of the nematode *Caenorhabditis elegans*,” was published (White *et al.* 1986). This paper described for the first time the complete axonal morphology and synaptic connectivity (the *wiring diagram* or *connectome*) of a nervous system. More recently, the wiring diagram controlling mating behavior in an adult male was elucidated (Jarrell *et al.* 2012). By the early 1980s the entire cell lineage of the animal had also been determined. Thus the developmental history and connectivity of every neuron was known. Development of *C. elegans* is highly invariant, in that every individual has almost exactly the same number of cells, and the same arrangement of neuronal processes. This is a key advantage for detecting developmental defects, which can be subtle and incompletely penetrant due to redundancies.

These systematic descriptions of the wiring and cell lineage of the animal set the stage for genetic research that could reveal, in unprecedented detail, how genes affect the development and structure of a nervous system. Seminal studies by Hedgecock *et al.* (1990) revealed how three genes, *unc-5*, *unc-6*, and *unc-40* coordinately control directionality of circumferential cell migrations and axon guidance. Subsequently, it was found that *unc-6* encodes a secreted extracellular protein (Ishii *et al.* 1992), and that *unc-5* and *unc-40*

encode receptors for *UNC-6* (Leung-Hagesteijn *et al.* 1992; Chan *et al.* 1996). An independent biochemical approach isolated vertebrate *UNC-6* orthologs, named Netrins, as key axon outgrowth-promoting factors (Kennedy *et al.* 1994; Serafini *et al.* 1994). This is of historical significance because the existence of extracellular guidance cues of this type had long been proposed but had remained unknown. Moreover, it was unclear whether invertebrates were useful models for developmental mechanisms in the vertebrate nervous system. Yet these results indicated that, despite the 600 million years of evolution that separates nematodes from humans, there is a remarkable conservation in the basic molecular machinery that controls the development of nervous systems (Chisholm and Tessier-Lavigne 1999). Since the *UNC-6*/Netrin receptors were already identified in *C. elegans* in the same genetic screen that identified *unc-6*, vertebrate researchers could quickly identify vertebrate Netrin receptors by cloning the vertebrate homologs of *unc-5* and *unc-40* (Keino-Masu *et al.* 1996; Leonardo *et al.* 1997).

Initial studies on *C. elegans* nervous system development came largely from serial section electron microscopy; a limited number of mutants isolated from behavioral screening were examined, and some defects in axonal outgrowth or guidance characterized. However, this was a very time-consuming strategy, and the ability to analyze axon guidance was greatly enhanced by development of tools to visualize identified neurons using light microscopy. Among these was the use of lipophilic dyes to stain exposed sensory axons; in fact axon guidance defects associated with mutations in *unc-6* (which was mistakenly referred to as a new gene, *unc-106*) were first revealed using this method (Hedgecock *et al.* 1985). Immunostaining for specific neuronal antigens was another technique used early on to characterize mutant axonal morphology (Siddiqui *et al.* 1989; Siddiqui and Culotti 1991; McIntire *et al.* 1992; Hekimi and Kershaw 1993; Wightman *et al.* 1997). Also, the discovery of the optically enhanced *clr-1* mutant allowed axonal morphology to be discerned using differential interference contrast microscopy. The early studies of axon morphology and cell migration mutants were summarized in an insightful review (Hedgecock *et al.* 1987). A major advance was germline transformation and the ability to express reporter genes in *C. elegans*. This allowed the visualization of neurons in fixed animals using stains such as β -galactosidase, and in live animals after the advent of GFP and other genetically encoded fluorescent proteins (Figure 1A). As a consequence, many screens have been done directly using axon morphology phenotypes, without relying on behavioral outcomes (selected examples listed in Table 1). Other productive approaches have involved screening for enhancers or suppressors of axon guidance phenotypes (Table 1).

Structure of the *C. elegans* Nervous System

The nervous system of the mature hermaphrodite comprises 302 neurons. Despite this small and invariant number,

Figure 1 Overall architecture of axon tracts in *C. elegans*. (A) Adult animal, side view, showing major ganglia, process bundles (dorsal and ventral nerve cords, DNC and VNC), and lateral neurons ALM, AVM, BDU, and SDQ. (B) Head region, ventral view, showing the left and right bundles of the VNC. (C) Midbody region, dorsal view, showing motor commissures, the DNC and dorsal sublateral tracts. Images are of the pan neural marker *Prgef-1-GFP(ev111)*; Bars, 50 μ m (A), 20 μ m (B, C).

C. elegans neurons exhibit high structural and functional diversity, and can be classified into at least 118 classes based on morphology (White *et al.* 1986). Embryonic neurons are positioned individually or in clusters (ganglia) on the basal surface of the epidermis. While most neurons are born during embryonic development, division of epidermal cells creates new postembryonic neurons during the larval stages that are interspersed with the embryonic neurons. Most *C. elegans* neurons have a simple morphology, being monopolar or bipolar with mostly unbranched processes. Whereas some processes appear to function as an axon or dendrite, others appear to have mixed functions, capable of receiving both inputs and sending outputs (Altun and Hall 2011). Among individual animals, the processes follow nearly identical pathways. Some motor neurons make simple branches at their target muscle, while sensory neurons typically extend a single axon to target neurons, and have dendritic processes positioned underneath the epidermis. The most striking dendritic branching patterns are created by the PVD neurons, which form extensive candelabra-like structures that traverse around the animal. Our current understanding of the molecular basis of dendritic branching and arbor formation has recently been reviewed (Dong *et al.* 2015).

The nerve ring, longitudinal, and circumferential nerves are formed by axons. There are longitudinal nerve tracts that transverse along the bodywall at the ventral, subventral, lateral, subdorsal, and dorsal positions (Figure 2A). Most longitudinal axons are in the ventral nerve cord (VNC) (Figure 1A and Figure 2C), followed by the dorsal nerve cord (DNC). The remaining longitudinal axon tracts contain only a small number of axons. Circumferential tracts, referred to as commissures, are created by neurons that extend processes either dorsally or ventrally to the longitudinal tracts. More than 40 individual commissures are formed by the extension of processes to the dorsal midline from motor neurons, which are located along the ventral midline. Commissures generally extend individually and do not form bundles, although there are exceptions, such as the amphid commissures. Together, the tracts connect the neuron cell bodies to major neuropils. The nerve ring comprises the largest neuropil, containing

about 180 processes. In addition to the somatic nervous system, the pharynx contains 20 neurons whose axon guidance has been the subject of a limited number of studies (Pilon 2014).

Neurons are in contact with the plasma membrane and the basement membrane of the epidermal cells, which provides a substrate for the outgrowth of axons (Figure 2B). Some guidance cues such as *UNC-6* are thought to be localized in the basement membrane. Many outgrowing processes are also in direct contact with epidermal cells, which could also provide navigational cues on their surface. When outgrowing commissures pass lateral cell bodies on their way to the dorsal cord, they invariably leave the basement membrane and stay in contact with the epidermal cells (Durbin 1987). This suggests that epidermal cells can provide a substrate for outgrowth as well. In places where the muscles attach to the epidermis, the axons extend between the muscle and the epidermis. Specialized glia cells provide additional support for some developing axons, particularly around the nerve ring, and along dendrites of sensory neurons (Wadsworth *et al.* 1996; Yoshimura *et al.* 2008; Shaham 2015). These glia are also a potential source for positional information relevant for axonal navigation and synapse formation. Synapses between neurons and between muscles and neurons are made *en passant*, *i.e.*, reproducibly along the neuronal processes in similar positions. This is similar to central nervous system synapses in vertebrates, where, in a recent reconstruction of a part of the neocortex in mouse, >70% of the synapses were found to be made *en passant* as well (Kasthuri *et al.* 2015).

Since direct contact is required for synapses to form *en passant*, axons not only have to be in the correct axon bundle, they have to be in a specific position *within* an axon bundle. It has long been known from electron microscopy (EM) reconstructions that axon location within bundles is highly stereotyped, defining axon neighborhoods (White 1985). Most likely this is mediated by selective adhesion, even though the mechanism of localization of an axon within an axon bundle is currently not understood at the molecular level. Individual axon diameters are in the range of 200 nm, at

Table 1 Forward genetic screens for axonal outgrowth and guidance

Screen and marker	Selected genes isolated	References
Uncoordinated locomotion (Unc)	<i>unc-5,-6,-40 unc-33,-44,-73,-76</i>	Brenner (1974)
Dye filling of exposed sensory neurons	<i>unc-33, -44, -51,-76</i>	Hedgecock <i>et al.</i> (1985)
Suppressor of ectopic UNC-5 (Seu)	<i>unc-129, seu-1</i>	Colavita and Culotti (1998)
Sensory axon guidance (Sax, <i>Pceh-23</i> -GFP)	<i>sax-3, sax-7</i>	Zallen <i>et al.</i> (1999)
Axon position (pan-neural, <i>Punc-119</i> -GFP)	<i>nid-1</i>	Kim and Wadsworth (2000)
Motor axon guidance (Max, <i>Punc-25</i> -GFP)	<i>max-1, max-2, unc-71</i>	Huang <i>et al.</i> (2002)
Interneuron guidance (<i>Pglr-1</i> -GFP)	<i>zag-1, ast-1, fmi-1</i>	Hutter <i>et al.</i> (2005); Wacker <i>et al.</i> (2003)
PVQ guidance (<i>Psra-6</i> -GFP)	<i>zag-1</i>	Clark and Chiu (2003)
Touch neuron guidance (<i>Pmec-4</i> -GFP)	<i>vps-38</i>	Prasad and Clark (2006)
Genome-wide RNAi (Pan neural, <i>Punc-119</i> -GFP)	<i>ced-1, unc-101, pry-1</i>	Schmitz <i>et al.</i> (2007)
Enhancement of <i>unc-40</i> ventral axon defects	<i>eva-1</i>	Fujisawa <i>et al.</i> (2007)
Suppression of <i>unc-6(lf)</i>	<i>clec-38, rpm-1, etc.</i>	Kulkarni <i>et al.</i> (2008)
AVG development	<i>plr-1, klp-7</i>	Moffat <i>et al.</i> (2014)
Enhancer of AVG defects in <i>nid-1</i>	<i>aex-3</i>	Bhat and Hutter (2016)

Selected examples of forward screens that yielded genes involved in axon guidance. Many genes were isolated in multiple screens, or from screens for related phenotypes (cell migration, muscle arm guidance).

or below the resolution limit of light microscopy. Disorganized VNC axon bundles have been seen using EM reconstructions of mutants, such as *unc-3* [cited in (Prasad *et al.* 1998)], or after ablation of the pioneer neuron AVG (Durbin 1987). Dye filling revealed that mutations in *mec-1* and *mec-8* disrupt the organization of amphid neurons, which form a cilia-based sensory system at the anterior end of the animal (Ward *et al.* 1975; Ware *et al.* 1975; Perkins *et al.* 1986). However, the difficulty of diagnosing fasciculation defects within a bundle by light microscopy has so far precluded intensive investigation of mechanisms.

Growth cones are found at the tips of elongating axons. Growth cones interact with the local extracellular environment, and are able to produce directed outgrowth in response to features of the environment. In *C. elegans*, the most extensive growth cones are seen at the growing tips of commissural (circumferentially directed) motor neurons. Imaging of these growth cones migrating along the body wall in larvae has revealed rapid and dynamic movements and shape changes (Knobel *et al.* 1999). Embryonic growth cones have been described from EM reconstructions as “flattened lamellar structures that also have thin filopodial extensions,” and are 2–5 μm in size (Durbin 1987). Axon outgrowth in the embryo coincides with elongation from approximately the comma to the threefold stage (Durbin 1987). Until recently, embryo movements have impeded imaging of axon outgrowth. However, recent advances in light-sheet microscopy have allowed time-lapse observation of ALA neurite outgrowth in elongating embryos (Christensen *et al.* 2015).

Initial observations of axon outgrowth in the developing embryo using serial section EM indicated that VNC axons grow out in a well-defined sequence, with the AVG neuron being the first to extend an axon pioneering the right VNC tract (Durbin 1987). At that time, the nerve ring, the main neuropil in the head, already contains the majority of its processes; the order of process outgrowth in the nerve ring remains unknown. In the VNC, the next neurons to extend axons are the DD motor neurons, whose processes are also located in the right axon tract. Commissures from all classes of motor neurons start to extend

toward the DNC immediately afterward. DA/DB processes then grow into the right VNC axon tract, and, eventually, interneuron axons extend from the nerve ring into the VNC. The left VNC axon tract is pioneered by the PVPR axon, which is followed closely by the PVQL axon (Durbin 1987). Laser ablation studies have been used in *C. elegans* to identify cell interactions in the developing embryo, and ablation experiments in the nervous system have established the importance of pioneers. Ablation of the VNC pioneer AVG leads to a disorganized VNC, with axons crossing the midline, and extending in the left axon tract (Durbin 1987). Later studies found that defects are variable and not completely penetrant. A substantial fraction of animals where the AVG pioneer was ablated did not show defects in the major classes of interneurons and motor neurons, suggesting that later outgrowing axons can navigate in principle without the pioneer (Hutter 2003). Ablation of the pioneer for the left VNC, PVPR, leads to a failure of this axon tract to form, suggesting that no other neuron can pioneer this axon tract (Durbin 1987). More recently, mutants have been identified where one of the follower axons, AVKR, is found in the left axon tract in animals where the PVPR axon is found in the right axon tract, indicating that AVKR might be able to navigate independently of the pioneer (Steimel *et al.* 2010).

Signaling Pathways Controlling Axon Outgrowth and Guidance

Proteins that provide instructional information for outgrowing axons are either secreted and found in the extracellular environment, or are presented on cell surfaces along the path of an axon. Given the large number of different axon trajectories in the developing nervous system, the number of identified guidance cues is rather small. Below, we review the major proteins known to affect axon outgrowth.

Basement membrane proteins

Basement membranes are important substrates for outgrowing axons, and some guidance cues are thought to be

Figure 2 Schematic drawing of the nervous system and dorso-ventral navigation. (A) Cross-section of the mid body showing positions of longitudinal axon tracts relative to the epidermis and muscle. (B) Axonal trajectories along the dorso-ventral axis and idealized representation of gradients of guidance cues. (C) Schematic of the VNC from a dorsal viewpoint, showing the relative locations of cell bodies, left and right bundles, commissures, and axons projecting into the VNC.

embedded in the basement membrane, even though the actual ultrastructural localization of guidance cues in *C. elegans* is currently unknown. Specific components of the extracellular matrix are known to modulate guidance cue signaling. Collagen IV and laminin are the major structural components of basement membranes (Figure 3). The *C. elegans* genome contains one $\alpha 1$ and one $\alpha 2$ collagen IV (*EMB-9* and *LET-2*, respectively), and two α (*EPI-1* and *LAM-3*), one β (*LAM-1*), and one γ subunit (*LAM-2*) of laminin (Kramer 2005). Mutations in these structural components lead to misassembled and/or broken basement membranes, resulting in severe defects in tissue organization, and embryonic or larval lethality. This indirectly affects cell and axonal migrations as well. Interestingly, partial loss-of-function alleles of *epi-1* have been isolated that affect axonal migrations without compromising the structural integrity of basement membranes (Forrester and Garriga 1997; C. Huang *et al.* 2003). This raises the possibility that certain laminin mutations might affect the localization of guidance cues, or the ability of cells to interact with basement membrane components. In laminin mutants, basement membrane structures are found surrounding individual misguided axons and mispositioned axon bundles (C. Huang *et al.* 2003; Kao *et al.* 2006). In mutants where

axons are misguided, laminin localizes to the mispositioned axons rather than the normal pathways of the nerves. Further, laminin is associated with cell surfaces before the reported expression of other basement membrane components, and the laminin α subunits have unique expression patterns; in particular, *LAM-3* is associated with the nerve ring, ventral nerve cord, and sublateral nerves (C. Huang *et al.* 2003). Together, this suggests that axons have specific laminin α receptors, and actively direct the assembly of an extracellular matrix.

Nidogen (*NID-1* in *C. elegans*) can bind to both collagen IV and laminin, and was thought to act as essential cross-linker for these major basement membrane components. However, mutations in *nid-1* (Kang and Kramer 2000; Kim and Wadsworth 2000), and also in the two mouse homologs of *nid-1* (Murshed *et al.* 2000; Schymeinsky *et al.* 2002; Bader *et al.* 2005), do not cause major disruptions of basement membranes, indicating that nidogens are not essential for basement membrane assembly and structure. *nid-1* mutants, however, do have specific defects in longitudinal axonal tracts (Kim and Wadsworth 2000). In the VNC, *nid-1* mutant animals show a disorganization of the left and right fascicle, with many classes of axons extending in the wrong axon tract, and axons inappropriately crossing the ventral midline

Extracellular	Basement membrane		HSPGs		Guidance cues	
	Laminins: EPI-1, LAM-1,2,3 Collagen IV: EMB-9, LET-2 Nidogen: NID-1		UNC-52 (Perlecan) CLE-1 (Collagen XVIII) KAL-1 (anosmin-1)		UNC-6 (Netrin) SLT-1 (Slit) UNC-129 (TGF- β) MAB-20 (Sema) Wnts: LIN-44, EGL-20, CWN-2 MADD-4	
Cell surface	IgCAMs		Cadherins		HSPGs	
	SAX-7 (L1CAM) LAD-2 (L1CAM) WRK-1 RIG-6		HMR-1 CDH-4 FMI-1 (CELSR)		LON-2 (Glypican) GPN-1 (Glypican) SDN-1 (Syndecan)	
Intracellular	Receptors		Other			
	UNC-5 (UNC5) UNC-40 (DCC) SAX-3 (Robo) VAB-1 (EphR) LIN-17 (Frizzled) EVA-1 Integrins: INA-1, PAT-2, PAT-3		SMP-1 SMP-2 VAB-2, EFN-2 EFN-3, EFN-4 CLEC-38			
	Arp2/3 activators		Kinesins		GTPases & regulators	
	WSP-1 (WASP) WAVE complex GEX-2 (Sra-1/CYFIP) GEX-3 (HEM2/NAPI/Kette) WVE-1 (WAVE) ABI-1		VAB-8 UNC-104		CED-10 (Rac) CDC-42 MIG-2 (RhoG) UNC-73 (Trio) TIAM-1 CED-5 (Doc180)	
	Effectors					
	UNC-34 (Ena/VASP) AGE-1 (PI3K) DAF-18 (PTEN) MADD-2 (TRIM) MIG-10 (RIAM/Lpd) MIG-15 (NIK) UNC-51 (ATG1) ANI-1 (anillin)		UNC-53 (Nav2) RPM-1 (PHR) UNC-44 (ankyrin) UNC-33 (CRMP) ERM-1 (ezrin/radixin/moesin) UNC-115 (abLIM) MAX-1 MAX-2 (PAK)			

Figure 3 Important proteins controlling axonal navigation. This summarizes major pathways focusing on those more directly implicated in growth cone guidance.

(Kim and Wadsworth 2000). The DNC, which normally is a tight fascicle of motor neuron axons, is frequently split in *nid-1* mutants. The dorsal sublateral tracts are often shifted in their position toward the dorsal side. Axonal migrations along the dorso-ventral axis are not affected. *NID-1* is concentrated along the longitudinal axon tracts that are affected in *nid-1* mutants (Kang and Kramer 2000), suggesting that either itself, or another component bound to it, is required for proper extension along longitudinal axon tracts.

cle-1 is the single *C. elegans* ortholog of vertebrate collagen type XV and type XVIII. Similar to *NID-1*, *CLE-1* is associated with longitudinal axon tracts, although its distribution is uneven, and enriched near synapses (Ackley *et al.* 2003). *cle-1* mutants have defects in the organization and function of synapses but only a few minor defects in axonal navigation have been described (Ackley *et al.* 2001, 2003). Mutations in a putative receptor for collagens, *ddr-2*, cause navigation defects in the VNC and other longitudinal axon tracts (Unsoeld *et al.* 2013), implying a role for one or more collagens in axon navigation. None of the characterized collagens share the *ddr-2* defects, so the ligand *DDR-2* remains unclear. Indirect evidence for an involvement of collagens in axon guidance comes from the observation that mutations in a collagen processing enzyme, *DPY-18*, a prolyl 4-hydroxylase, cause VNC midline crossing defects (Torpe and Pocock 2014).

unc-52 encodes the *C. elegans* homolog of perlecan (Rogalski *et al.* 1993). *UNC-52* is associated with body wall

muscle basement membranes, and is required for the proper organization of dense bodies connecting body wall muscle cells to the basement membrane. Complete loss-of-function mutations in *unc-52* are embryonic lethal, arresting at the twofold stage (Rogalski *et al.* 1993). Viable alleles of *UNC-52* have no defects in cell or axonal migration on their own, but can alter the responses of migrating cells and axons to the guidance cue *UNC-6*. *unc-52* mutations enhance distal tip cells migration defects in *unc-5* mutants (Merz *et al.* 2003). This enhancement of defects can be suppressed by mutations in various growth factors (*UNC-129*, *EGL-20*, *DBL-1*), raising the possibility that *UNC-52* modulates growth factor signaling in this context (Merz *et al.* 2003). It is important to note the potential role for *UNC-52* and growth factors in DTC migration only becomes apparent in a compromised (*unc-5* mutant) background, indicating that while these genes do not seem to contribute to the migration in an otherwise wild type background, they have the potential to do so. In HSN neurons, *UNC-52* modulates the response to *UNC-6* by affecting the distribution of *UNC-40* on the cell surface, and thereby the direction of initial axon outgrowth (Tang and Wadsworth 2014; Yang *et al.* 2014).

Integrin receptors

Integrins are heterodimeric receptors for several basement membrane molecules. *C. elegans* has only two integrins, formed from two α subunits, *ina-1* and *pat-2*, and one β

subunit, *pat-3*. Complete loss-of-function alleles in integrin subunits are lethal (Williams and Waterston 1994; Baum and Garriga 1997), but partial loss-of-function alleles of *ina-1* have been isolated in screens for neuronal migration defects (Baum and Garriga 1997). Axons generally extend to their normal targets in *ina-1* mutants, suggesting that *ina-1* does not play a major role in axon pathfinding (Baum and Garriga 1997). Minor defects have been found in D-type motor-neuron axons, which show fasciculation defects in the VNC (Baum and Garriga 1997), partial defects in the directional outgrowth of commissures (Forrester and Garriga 1997; Poinat *et al.* 2002), and minor defects in commissure navigation toward the DNC (Poinat *et al.* 2002). The early embryonic arrest phenotype of *pat-2* and *pat-3* mutants precludes an analysis of a potential role of these integrin subunits in axonal guidance.

Heparan sulfate proteoglycans

Heparan sulfate proteoglycans (HSPGs) are extracellular or membrane-bound proteins carrying complex modified sugar side chains. HSPGs in *C. elegans* that are implicated in axon guidance include *UNC-52* (Perlecan) (see above), *SDN-1* (Syndecan), the glypicans *GPN-1* and *LON-2*, and *CLE-1* (collagen XV/XVIII) (Figure 3). Enzymes required for the synthesis of the sugar side chains also show axonal defects, suggesting that sugar modifications are important for HSPG function. This, together with data from other organisms, has led to the idea of a “sugar code” for HSPG function (Díaz-Balzac *et al.* 2014; Poulain and Yost 2015).

hst-2, *hst-6*, and *hse-5* encode three major heparan sulfate (HS)-modifying enzymes: two O-sulfotransferases (*hst-2* and *hst-6*) and a C5-epimerase (*hse-5*). Mutations in these genes cause overlapping and unique defects in navigation of axons at the ventral midline (Bülow and Hobert 2004), most notably midline crossing defects of PVP and PVQ axons, with some commissures growing on the wrong side (left vs. right), and a few commissures not reaching the DNC (Bülow *et al.* 2008). Genetic interaction data and expression data suggest that these enzymes affect at least two different substrates in epidermal and neuronal tissue (Bülow and Hobert 2004; Bülow *et al.* 2008). *SDN-1* (syndecan) is the only HS core protein showing commissural defects similar to mutations in HS-modifying enzymes, suggesting it might be the main substrate here. Overall axon outgrowth in *sdn-1* mutants is mildly affected (Rhiner *et al.* 2005), whereas axon regeneration is dramatically reduced (Edwards and Hammarlund 2014) (see below). The glypican *LON-2* is another potential substrate in the context of directional outgrowth of commissures, since *lon-2* mutations can suppress guidance defects induced by ectopic expression of HS-modifying enzymes (Bülow *et al.* 2008). Ultimately HS-modifications are thought to modulate cellular responses to guidance cues such as *SLT-1* (Bülow *et al.* 2008).

Two HS core proteins, *SDN-1* (syndecan) and *LON-2* (glypican) act in parallel to help guide D-type motor neuron

commissures toward the dorsal cord. Mutations in *hst-2*, *hst-6*, and *hse-5* enhance *sdn-1* but not *lon-2* defects, suggesting that sugar modification on *lon-2* (but potentially not on *sdn-1*) are important (Gysi *et al.* 2013). A function of the core protein *LON-2* independent of sugar modifications was also observed during ventral migration of the AVM axons. *lon-2* mutants alone show no defects, but enhance defects in *sdn-1* mutants in this case (Blanchette *et al.* 2015). Genetic interaction data and binding studies in cell culture indicate that *LON-2* modulates responses to *UNC-6* (netrin) by interacting directly or indirectly with the *UNC-6* receptor *UNC-40* (DCC) (Blanchette *et al.* 2015). *kai-1* encodes the *C. elegans* ortholog of the Kallmann syndrome protein Anosmin-1. Overexpression of *KAL-1* causes ectopic branching in AIY and AFD neurons (Bülow *et al.* 2002), and *KAL-1*/Anosmin-1 function is required for branch formation in HSN neurons (Díaz-Balzac *et al.* 2015). *KAL-1* is not itself a proteoglycan, but interacts with multiple proteoglycans in axon branching and in embryonic morphogenesis (Bülow *et al.* 2002; Hudson *et al.* 2006). *KAL-1* also acts as an autocrine cofactor with *EGL-17* (FGF) through a receptor complex of the fibroblast growth factor receptor *EGL-15* (FGFR) and *SAX-7* (L1CAM) (Díaz-Balzac *et al.* 2015).

Compared to mutations in guidance cues such as *UNC-6*, mutations in major HS-modifying enzymes, or their HSPG substrates, cause only mild defects in axon navigation. There is evidence that at least some of these defects are due to a modulation of responses to guidance cues such as *SLT-1* and *UNC-6*, and that HS-core proteins interact with receptors for these cues. Some functions of the HS-core proteins are independent of sugar modifications, further complicating the picture.

Tropic guidance cues

Tropic guidance cues are molecules that, through various assays, were found to provide directionality by acting as either attractants or repellents. Historically, genes involved in axon outgrowth were revealed through genetic screens for mutations that affect the ability of either circumferentially or longitudinally directed axons to reach their targets. In general, defects in dorso-ventral navigation along the body wall are somewhat easier to interpret. Defects in genes affecting anterior-posterior migration typically do not lead to a complete loss of axonal orientation, since axons in such mutants still broadly extend along the anterior-posterior axis, but are either in the wrong axon tract or deviate from a straight trajectory. Furthermore, the penetrance of defects in mutants affecting anterior-posterior navigation tends to be substantially lower compared to mutants affecting dorso-ventral navigation, which are highly penetrant in mutants in guidance cues such as *UNC-6*. One reason for this difference could be the fact that axons navigating along the dorso-ventral axis navigate in isolation, *i.e.*, they are all pioneers and have to find their way independently. By contrast, the major longitudinal axon tracts contain a larger number of axons, so that at least later outgrowing axons have pre-existing axon tracts for additional orientation. In addition, the guidance system

along the anterior–posterior (a–p) axis could be organized in a more redundant fashion with a larger number of cues. Muscle cells are organized in four longitudinal rows flanking the main axon tracts. Since muscle cells in the embryo have established tight attachments and connections through the basement membrane to the epidermal cells, muscle quadrants likely form physical barriers that restrict the possibility of axons to stray too much from a longitudinal trajectory. In fact, navigation defects within the VNC, which is flanked by two muscle quadrants, typically involve axons switching back and forth between left and right axon tracts (midline crossing), but rarely if ever leaving the VNC itself. Live imaging of motor commissure outgrowth in larvae (Knobel *et al.* 1999) shows that growth cones stall and pause when they encounter dorsal muscle cells, indicating that the muscle cells indeed form a physical barrier for growth cones.

Because of these differences, the genes involved in dorso-ventral navigation appear to be better known and understood. Although a number of genes have been identified to control navigation in longitudinal axon tracts, most notably the VNC, these genes encode adhesion molecules, putative receptors components of signal transduction pathways or cytoskeletal regulators, but not clearly defined tropic guidance cues. As mentioned above, the first tropic guidance cue to be identified in *C. elegans* was *UNC-6*/Netrin, which is essential for dorsally and ventrally directed axon guidance. The role of *UNC-6* in axon navigation has been reviewed previously (Wadsworth 2002; Killeen and Sybingco 2008), and will be discussed only briefly here. *UNC-6* is produced by ventral cells, and is thought to form a gradient with its highest concentration around the ventral midline (Figure 2B) (Wadsworth *et al.* 1996; Wadsworth and Hedgecock 1996). Axons that express the receptor *UNC-40* (DCC) are attracted to *UNC-6* and grow ventrally. Axons that express the receptor *UNC-5* (UNC5), alone or in combination with *UNC-40*, are repelled by *UNC-6* and will grow dorsally. Forced misexpression of *UNC-5* can reorient axons from ventral to dorsal outgrowth, indicating *UNC-6* elicits a directional response from the neuron that is dependent on the repertoire of receptors, and that netrin signaling instructs guidance choices (Hamelin *et al.* 1993). *UNC-129* encodes a member of the TGF- β family, and modulates the response to *UNC-6*. In *unc-129* mutants motor neuron commissures fail to navigate to the dorsal nerve cord. *UNC-129* is secreted from dorsal body wall muscle cells, and is thought to form a dorsal-to-ventral gradient (Figure 2B). Misexpression of *UNC-129* on the ventral side causes a variety of migration defects along the dorso-ventral axis, suggesting that localized expression of *UNC-129* is essential for function (Colavita *et al.* 1998). Single and double mutant analyses indicate that *UNC-129* does not positively or negatively regulate the only type II canonical TGF-beta receptor (*DAF-4*) encoded by the *C. elegans* genome, and therefore likely signals through an unknown noncanonical receptor mechanism (Nash *et al.* 2000). *UNC-129* can bind to *UNC-5* and may enhance *UNC-5*+*UNC-40* signaling at the expense of *UNC-5*, only signaling at low concentrations of

UNC-6 (MacNeil *et al.* 2009), thus promoting dorso-ventral navigation in the dorsal part of the animal.

A third guidance cue affecting dorso-ventral navigation is the secreted protein, *SLT-1* (Slit), which is expressed predominantly on the dorsal side, and affects navigation of axons toward the ventral midline (Figure 2B). *SAX-3* encodes a major receptor for *SLT-1*, but also has *SLT-1* independent functions, since *sax-3* mutants have midline crossing defects in the VNC, and defects positioning of the nerve ring not found in *slt-1* mutants (Hao *et al.* 2001). *SLT-1* acts in parallel to *UNC-6* for ventral-directed axon navigation (Hao *et al.* 2001). *EVA-1* encodes a potential coreceptor for *SAX-3* that is required for *SLT-1* signaling (Fujisawa *et al.* 2007). Genetic interaction data suggest that, in the absence of *EVA-1*, *SAX-3* negatively impacts *UNC-6* signaling to further enhance ventral navigation defects (Fujisawa *et al.* 2007). Therefore, in the absence of *SLT-1* or *EVA-1*, or both, *SAX-3* may negatively regulate *UNC-6* function. Recently, the *EBAX-1* E3 ligase and Hsp90 were shown to buffer axon guidance and *SAX-3* function in developing neurons in conditions of temperature variation (Wang *et al.* 2013).

Muscle arms extend from the cell bodies of body wall muscle cells to the dorsal and ventral nerve cords to establish neuromuscular junctions. Screens for muscle arm guidance defects identified the secreted protein *MADD-4*, homologous to vertebrate ADAMTSL-1/Punctin-1 and ADAMTSL-3/Punctin-2, which acts as an attractant for muscle arms (Seetharaman *et al.* 2011). *madd-4* mutants alone do not show axonal navigation defects, but they enhance ventral navigation defects of the AVM axon in *unc-6* and *slt-1* mutants (Seetharaman *et al.* 2011), establishing an additional attractive cue for some axons extending toward the ventral midline. Ectopic expression of *MADD-4* can reroute AVM axons, an effect that requires the receptors *UNC-40* (Seetharaman *et al.* 2011) and *EVA-1* (Chan *et al.* 2014). *MADD-4* physically interacts with an *EVA-1*/*UNC-40* complex (Chan *et al.* 2014). Evidence suggests a model (Chan *et al.* 2014) in which *EVA-1* enhances an *UNC-40*-mediated attractive response toward the *MADD-4* source. This enhancement may occur, in part, by *EVA-1* counteracting the inhibition of *MADD-4* and *UNC-40* interaction by *UNC-6*. Interestingly, the model predicts that AVM axon attraction toward *MADD-4*-expressing sources occurs through distinct pathways that act simultaneously to mediate attraction (Chan *et al.* 2014). How multiple attractive guidance pathways, which use common components, are synchronized is not understood.

There are five Wnt genes, *mom-2*, *lin-44*, *egl-20*, *cwn-1*, and *cwn-2*, and four Frizzled receptors, *mom-5*, *lin-17*, *mig-1*, and *cfz-2*, which play pleiotropic roles in *C. elegans* development (Sawa and Korswagen 2013). Wnts control the a–p polarity of some neurons, most notably ALM, PLM, and AVG (Hilliard and Bargmann 2006; Prasad and Clark 2006; Moffat *et al.* 2014; Bhat *et al.* 2015). However, Wnt mutants show limited defects in process outgrowth and navigation along the a–p axis. AVM and PVM processes, which normally extend from lateral cell bodies to the VNC before turning

anterior, show a variety of defects in *cwn-1*; *egl-20* double mutants including premature stop of outgrowth and extending in a posterior direction (Pan *et al.* 2006). *egl-20*, which is expressed in the posterior of the animal, seems to act as repellent in this case (Pan *et al.* 2006). The Wnts LIN-44 and EGL-20 control the termination point of the DD6 axon in the dorsal nerve cord, which overextend in *lin-44* single and *lin-44*; *egl-20* double mutants (Maro *et al.* 2009). For the PQR dendrite, LIN-44 acts as attractive cue, as ectopic expression of *lin-44* leads to a reorientation of the dendrite toward the source of expression (Kirszenblat *et al.* 2011). CWN-2 controls the outgrowth of dorsal processes of the RMED/V neurons. *cwn-2* mutants completely lack these processes, suggesting *cwn-2* is required to initiate process outgrowth (Song *et al.* 2010). In addition, ectopic expression of *cwn-2* can partially reroute these processes, indicating that *cwn-2* also acts as attractive cue (Song *et al.* 2010). Finally, Wnts control the position of the nerve ring as *cwn-2* mutants have anteriorly displaced nerve rings (Kennerdell *et al.* 2009). CWN-2 seems to act as a permissive rather than instructive cue here, since the site of expression is not crucial for activity (Kennerdell *et al.* 2009). Recently, a minor redundant function for netrin and Wnt signaling has been discovered for a-p outgrowth of CAN axons (Levy-Strumpf and Culotti 2014). In *unc-5*; *egl-20* double mutants (but not single mutants) CAN axons show premature termination of outgrowth, excessive branching, or reversal of the direction of outgrowth with a penetrance of all defects in the range of 9–17% depending on the allele combination used (Levy-Strumpf and Culotti 2014). Distal tip cells, which migrate along the a-p and d-v axis, show much more penetrant synergistic defects when Netrin and Wnt signaling is compromised (Levy-Strumpf and Culotti 2014), suggesting that migrating cells and axons have at least different sensitivities to guidance cues along the a-p axis, and that Netrin and Wnt signaling may function redundantly for both a-p and d-v guidance. Taken together, while Wnts play an important role in cell migration along the a-p axis, they do not appear to act as major tropic guidance cue for neuronal processes. This is in contrast to the major tropic guidance cue for migration along the d-v axis (UNC-6), which is equally important for cell and axon migration. Wnt mutants show defects mainly in initiation and termination of process outgrowth in a small number of neurons. Axon navigation defects are sometimes found in addition to process outgrowth defects, but limited to an even smaller number of neurons. The role of Wnts in axonal navigation in *C. elegans* contrasts with the apparently more substantial function of Wnts in a-p navigation of axons in both *Drosophila* and vertebrates (Yam and Charron 2013; Onishi *et al.* 2014).

Some members of the semaphorin family play an important role in axon guidance in insects and vertebrates (Kolodkin 1998; Roy *et al.* 2000). The *C. elegans* genome contains one secreted semaphorin, *mab-20*, and two transmembrane semaphorins, *smp-1* and *smp-2*. *mab-20* mutants have a number of morphogenetic defects including aberrant epidermal cell migrations and fusion of male sensilla (Baird *et al.* 1991; Roy

et al. 2000; Hahn and Emmons 2003). *mab-20* has a limited role in axon guidance, affecting the navigation of SMD and SDQ axons (Wang *et al.* 2008). *smp-1* and *smp-2* are important for the correct positioning of male sensilla (Ginzburg *et al.* 2002), but have no documented role in axonal navigation. This suggests that, in contrast to vertebrates, Semaphorins play a more limited role as guidance cues in *C. elegans*.

Receptors and adhesion molecules

Receptors for guidance cues, and cell adhesion molecules, are located at the surface of outgrowing neuronal processes. Cadherins and IgCAMs (immunoglobulin family cell adhesion molecules) are the largest families of adhesion molecules. The *C. elegans* genome contains 12 cadherin genes (Hill *et al.* 2001), and 17 IgCAMs (Hutter *et al.* 2000; Aurelio *et al.* 2002)—defined here as cell surface proteins with immunoglobulin domains either alone or in combination with fibronectin III repeats. There is only one classical cadherin, *hmr-1*, in the *C. elegans* genome. *hmr-1* produces a splice variant expressed in two types of motor neurons, AS, and D. It is required for D-type commissure migration and fasciculation of AS axons in the VNC (Broadbent and Pettitt 2002), but even those defects are incompletely penetrant in a loss-of-function mutant, suggesting that *hmr-1* does not play a major role in axon navigation. *cdh-4* encodes a Fat-like cadherin, and *cdh-4* mutants show a variety of defects in neuronal and non-neuronal tissues. The most prominent axonal defects are fasciculation defects of various classes of neurons in the DNC and VNC (Schmitz *et al.* 2007). *fmi-1* encodes a Flamingo-type cadherin. *fmi-1* mutants show ventral cord navigation defects in several classes of interneurons and motor neurons (Steimel *et al.* 2010). Furthermore, in *fmi-1* mutants, the navigation of the pioneer PVP axons and the follower PVQ axons is uncoupled. This suggests that *fmi-1* mediates adhesion between pioneer and follower axons. The remaining members of the cadherin family are either uncharacterized, or have no documented role in axon navigation (*cdh-3*, *casy-1*). Intracellular downstream effectors of cadherins in axon guidance are currently unknown.

Members of the IgCAM family include some of the known receptors for guidance cues, such as UNC-40 and SAX-3. Deletion mutants of eight members of the IgCAM family revealed that the majority of these genes has no major role in axon navigation; analysis of an octuple mutant suggests this is not due to redundancy among the IgCAMs tested (Schwarz *et al.* 2009), suggesting that a substantial fraction of this highly conserved family has no major role in axon guidance. Other IgCAMs with a documented function in axon guidance include *wrk-1*, *sax-7* (L1CAM), *lad-2* (L1CAM), and *rig-6*. Mutations in *wrk-1* cause mild midline crossing defects of interneuron axons in the VNC (Boulin *et al.* 2006; Schwarz *et al.* 2009). *wrk-1* acts nonautonomously in motor neurons, whose cell bodies are located at the ventral midline. *wrk-1* is proposed to mediate a repulsive interaction with axons to prevent them from crossing the midline (Boulin *et al.* 2006). *lad-2* is required for dorsal migration of SDQ axons, and

longitudinal migration of SMD and PLN axons (Wang *et al.* 2008). In SDQ navigation, **LAD-2** acts as coreceptor for **MAB-20** (Sema2) (Wang *et al.* 2008). More recently, **LAD-2** has been found to also act as receptor for the ephrin **EFN-4** (Dong *et al.* 2015). Defects in **rig-6** lead to truncations of ALM and PLM axons, and mild defects in D-type motor neuron axon navigation (Katidou *et al.* 2013). **SAX-7** (L1CAM) is required to maintain the positions of certain neuronal cell bodies in response to mechanical forces when the animal is moving (Zallen *et al.* 1999; Sasakura *et al.* 2005; Wang *et al.* 2005). **SAX-7**/L1CAM is also required for correct positioning of the nerve ring (Zallen *et al.* 1999), maintenance of axon position in the VNC (Bénard *et al.* 2012), and navigation of D-type commissures (Wang *et al.* 2005). **SAX-7** is also involved in patterning of the highly branched PVD dendrites (Salzberg *et al.* 2013), illustrating its pleiotropic functions in the nervous system.

Taken together, with the exception of the receptors for **UNC-6** (Netrin) and **SLT-1** (Slit), mutations in IgCAMs have limited defects in axonal navigation of subsets of neurons. In most cases, IgCAMs seem to act as receptors rather than adhesion molecules, but ligands or binding partners for many IgCAM are currently unknown. For defects in the ventral nerve cord, it is difficult to determine whether the primary defect is an adhesion defect leading to defasciculation of axons, or a failure to respond to a guidance cue(s). In both cases, the consequence is likely that axons in the VNC would cross the midline to extend in the contralateral axon tract. Therefore, based on this phenotype alone, it is not clear whether a missing IgCAM acts as an adhesion molecule or as a receptor.

ADAMs (**a** disintegrin and **metal**loproteinase) are a family of transmembrane proteins that can proteolytically cleave the ectodomain of various cell surface receptors. Some members of this family also act as adhesion molecules (Edwards *et al.* 2008). **UNC-71**/ADM-1 is one of four ADAMs in *C. elegans*, the others being **ADM-2**, **ADM-4**, and **SUP-17**. **UNC-71** acts nonautonomously in sex myoblast migration, and controls several aspects of D-type motor neuron navigation (X. Huang *et al.* 2003). **UNC-71** is thought to act as an adhesion molecule rather than as a protease (X. Huang *et al.* 2003). The other members of the ADAM family so far are not implicated in axonal navigation.

Ephrins are important cell-surface-bound axon guidance cues in vertebrates, with major roles in the formation of retinotopic projections (Lemke and Reber 2005; Cang and Feldheim 2013). *C. elegans* has four ephrins, **vab-2/efn-1**, **efn-2**, **efn-3**, **efn-4**, and one ephrin receptor, **vab-1** (George *et al.* 1998; Chin-Sang *et al.* 1999; Wang *et al.* 1999). Eph signaling has pleiotropic functions in *C. elegans* development, including (but not limited to) epidermal morphogenesis (George *et al.* 1998; Chin-Sang *et al.* 1999; Wang *et al.* 1999) and muscle cell migration (Tucker and Han 2008; Viveiros *et al.* 2011). Ephrins also have several documented functions in axonal navigation. At the ventral midline, three ephrins (**vab-2/efn-1**, **efn-2**, **efn-3**) act redundantly via the

canonical ephrin receptor **vab-1**, to prevent inappropriate midline crossing of axons in the VNC (Boulin *et al.* 2006). The IgCAM **wrk-1** potentially acts as coligand for **vab-1** here, or might affect the subcellular localization of ephrins. Curiously, an earlier study reported no significant VNC midline crossing defects in **vab-1** mutants (Hutter 2003). **VAB-1** function in axon guidance is also regulated by hypoxia via the **HIF-1** pathway (Pocock and Hobert 2008). PLM and CAN axons overextend in **vab-1** mutants (Mohamed and Chin-Sang 2006), pointing to an additional role for ephrins in determining the stopping point of certain axons. **efn-2**, **efn-3**, and **efn-4** also act redundantly in PLM extension (Mohamed and Chin-Sang 2006). **EFN-1** functions to guide amphid axons ventrally through the amphid commissure (Zallen *et al.* 1999; Grossman *et al.* 2013); amphid axon ventral guidance also involves partly redundant **SAX-3** and netrin signals. **EFN-4** also signals via noncanonical mechanisms that do not involve **VAB-1** (Chin-Sang *et al.* 2002), and interacts with the L1CAM **LAD-2** to provide a cue for the dorsal migration of the SDQ axons (Dong *et al.* 2016). **EFN-4** is also required for AIY and D-type motorneuron axon outgrowth, as these axons stop prematurely in **efn-4** mutants (Schwieterman *et al.* 2016).

Regulation of the Actin Cytoskeleton in Axon Outgrowth

Actin filaments provide the mechanical support that allows force to generate movement. Actin filaments also provide tracks for the movement of intracellular molecules. Directed outgrowth in response to extracellular cues requires remodeling of the actin cytoskeleton. Remodeling occurs through the cyclical polymerization and depolymerization of actin filaments. Over 100 accessory proteins are known to regulate the pool of actin monomers, polymerization, filament length, cross-linking, and the disassembly of filament networks (Pollard and Cooper 2009). The roles of many proteins involved in this remodeling have been studied using *C. elegans*.

Ena/VASP proteins are actin-associated proteins that regulate filament elongation (Krause *et al.* 2004), and the *C. elegans* homolog, **UNC-34**, has been implicated in controlling directional outgrowth in response to extracellular cues, including **UNC-6**, Wnts (**CWN-1**, **CWN-2**, and **EGL-20**), and **SLT-1** (Yu *et al.* 2002; Gitai *et al.* 2003; Quinn *et al.* 2006; Fleming *et al.* 2010). **UNC-34** functions together with other proteins known to regulate actin dynamics, including **MIG-10** (RIAM/Lpd), a protein implicated in promoting actin polymerization (Chang *et al.* 2006; Quinn *et al.* 2006). Genetic evidence suggests **UNC-34** acts in parallel to the actin-binding protein **UNC-115** (abLIM) and Arp2/3—a complex that nucleates actin filaments and helps to control growth cone filopodia formation (Lundquist *et al.* 1998; Struckhoff and Lundquist 2003; Norris *et al.* 2009). Activation of the Arp2/3 complex by upstream signals occurs through WASP and WAVE/SCAR, families of actin-associated scaffolding proteins (Takenawa and Suetsugu 2007). WASP and

WAVE/Scar are themselves regulated by plasma membrane-associated complexes that include *GEX-2*, a homolog of the Rac1-interacting Sra-1/PIR121 protein, and *GEX-3*, a homolog of Rac1-interacting HEM2/NAP1/Kette protein (Soto *et al.* 2002; Shakir *et al.* 2008). In axon termination, *VAB-1* inhibits an interaction between *UNC-34*/Ena and the conserved adaptor *NCK-1*/Nck (Mohamed *et al.* 2012).

An outgrowth response requires interactions between surface receptors and the actin cytoskeleton. *UNC-70* (Beta-Spectrin) mediates actin binding to the intracellular side of the plasma membrane (Hammarlund *et al.* 2000), and *UNC-44* (ankyrin) mediates the attachment of membrane proteins to the spectrin-actin cytoskeleton (Otsuka *et al.* 1995). *UNC-44* has been shown to be important for correct localization of axonal proteins. For example, *UNC-44* is involved in the localization of *SAX-7*/L1CAM (Zhou *et al.* 2008), and of *UNC-33* (CRMP). Both *UNC-44* and *UNC-33* help to organize the microtubule (MT) cytoskeleton, which is required for the polarized distribution of various proteins (Maniar *et al.* 2012). Interestingly, *UNC-33* regulates transport mediated by the kinesins *VAB-8* and *UNC-104* (Maniar *et al.* 2012; Meng *et al.* 2016). In other systems, CRMP proteins like *UNC-33* are implicated in the kinesin-mediated transport of WASP and WAVE complexes (Kawano *et al.* 2005). *UNC-33* might also directly facilitate actin cytoskeleton remodeling through its interaction with *FLN-1*, an actin-binding protein (Nakamura *et al.* 2014). Similar to *UNC-44*, *ERM-1*, a member of the ezrin/radixin/moesin family of proteins implicated in linking plasma membrane receptors to the actin cytoskeleton is required in axon guidance, and may be involved in guidance receptor signaling (Teulière *et al.* 2011).

A complex network of signaling pathways impinges on the regulation of actin dynamics. Important components of these pathways are the GTPases and their regulators, including *CED-10* (Rac), *CDC-42*, *MIG-2* (RhoG), *UNC-73* (Trio), *TIAM-1*, and *CED-5* (Dock180) (Lundquist *et al.* 2001; Gitai *et al.* 2003; Quinn *et al.* 2008; Shakir *et al.* 2008; Demarco and Lundquist 2010; Bernadskaya *et al.* 2012; Demarco *et al.* 2012). In addition to the pathways already noted, a wide variety of other effectors has been implicated in axon guidance, including *MIG-15* (NIK) (Poinat *et al.* 2002; Shakir *et al.* 2006; Teulière *et al.* 2011), *AGE-1* (PI3K) (Chang *et al.* 2006), *MAX-1* (Huang *et al.* 2002), *MAX-2* (PAK) (Lucanic *et al.* 2006), *UNC-51* (ATG1) (Lai and Garriga 2004), and *ANI-1* (anillin) (Tian *et al.* 2015), as well as other phosphatases and kinases.

Effective axon outgrowth must involve considerable cytoskeletal remodeling. First the surface receptor and its signaling components must be trafficked to the cell surface. Effectors are then recruited to the site of receptor activation. These effectors then facilitate further remodeling of the cytoskeleton. In order to produce movement, this remodeling needs to alter the cell's structural scaffold, the cell's adhesive properties, and it needs to assemble the machinery that produces force. The cytoskeleton is integrally involved in each event, and regulating the assembly and disassembly of actin filaments

is intricate, involving the integration of signals from multiple pathways (Pollard and Borisy 2003). With this in mind, molecules that regulate actin dynamics might play multiple roles in determining outgrowth. The role that a particular molecule is observed to play in outgrowth may depend on the context under which the system is observed. For example, guidance receptor accumulation and distribution appear to be affected by *MIG-2* (RhoG) and *UNC-73* (TRIO) activity in some neurons (Levy-Strumpf and Culotti 2007; Watari-Goshima *et al.* 2007), but not in others (Norris *et al.* 2014).

Axon outgrowth occurs at growth cones, which are located at the tips of neuronal processes. The lamellipodial and filopodial of growth cones are actin-based structures that protrude dynamically in the direction of migration. Growth cones migrating circumferentially across the epidermis are highly dynamic in morphology, as determined using time-lapse imaging (Knobel *et al.* 1999). Upon contact with lateral nerves, growth cones of the VD motoneurons stall and spread out before extending further. At sites where the muscles attach to the epidermis, the growth cones stall and extend processes through the muscle/epidermis extracellular matrix. Once a process extends through this matrix, a new growth cone develops at the end of the process, and the original growth cone at the other side collapses. These observations highlight the dynamic responses that growth cones have to their environment.

In VD neurons, the protrusive activity of migrating growth cones is regulated by *UNC-6*, *UNC-40*, and *UNC-5* (Norris and Lundquist 2011). *UNC-5* inhibits protrusion, and increases the bias for protrusion away from the *UNC-6* source, whereas *UNC-40* drives protrusion. Correspondingly, the distribution of F-actin in these growth cones is biased away from the *UNC-6* source. Further evidence indicates that these growth cone protrusions are also controlled by *UNC-73*, *CED-10*, *MIG-2*, *UNC-44*, and *UNC-33* activity (Norris *et al.* 2014).

Orienting Outgrowth Activity to the Extracellular Environment

The extracellular cues and signaling pathways required for axon initiation and extension have been studied using a number of different *in vivo* and *in vitro* systems (Polleux and Snider 2010). Despite knowledge of many pathways and their components, it is still not well understood how extracellular cues control signaling pathways and produce directed outgrowth. A key feature of the initial response is the specification of neuronal polarity. Experimental evidence reveals that *in vivo* extracellular guidance cues can polarize protrusive activity within a neuron (Adler *et al.* 2006; Quinn *et al.* 2006). In particular, the *UNC-6* (netrin) guidance cue promotes axon formation by causing the *UNC-40* (DCC) receptor to localization to the side of the neuron closest to the source of the cue (Adler *et al.* 2006). It has been observed that *MIG-10* (RIAM/Lpd) becomes localized to the site where *UNC-40* asymmetrically localizes, and that this localization requires *AGE-1* (PI3K), *DAF-18* (PTEN), (Adler *et al.* 2006),

and MADD-2 (TRIM) (Alexander *et al.* 2010; Hao *et al.* 2010). This suggests that the asymmetric localization of receptors causes asymmetric signaling which, in turn, causes the asymmetric recruitment of effectors that promote actin-based protrusive activity (Quinn and Wadsworth 2008).

It is commonly understood that a growth cone turns away from the point where it makes contact with a cue because the response causes the disassembly of the actin superstructure and the disruption of actomyosin contraction, whereas a growth cone moves toward the point of contact with a cue because its response causes the asymmetrical incorporation of actin on the side of the growth cone closest to the cue (Dent *et al.* 2011). This is described as a repulsive or attractive response, respectively. The function of a guidance cue is often classified as repulsive or attractive depending on whether loss of function prevents an axon from extending away from or toward the source of the cue. Some cues clearly play a dominant role in guiding outgrowth toward or away from the source of that cue. For example, different neurons extend outgrowth either toward or away from ventral sources of UNC-6. Conceivably the UNC-6 cue strongly influences the actin superstructure along the surface of these neurons; whether a repulsive or attractive response is elicited by the cue depends on the repertoire of proteins expressed by the neuron.

Conceptually, any adhesion event that asymmetrically alters cytoskeleton dynamics within the neuron could alter outgrowth occurring at one side of a neuron or growth cone. Indeed, there are several cases where extracellular matrix molecules, and their receptors, act with guidance cues to direct migrations. These include basement membrane components such as UNC-52 (perlecan), NID-1 (nidogen), and CLE-1 (type XVIII collagen); and receptors such as DDR-1 and DDR-2 (discoidin domain receptors), DGN-1 (dystroglycan), and UNC-71 (ADAM) (Kim and Wadsworth 2000; Ackley *et al.* 2001; X. Huang *et al.* 2003; Johnson and Kramer 2012; Unsoeld *et al.* 2013). These molecules can regulate axonal outgrowth activity when there are contact-specific extracellular matrices, tissues, or cells, thereby changing outgrowth behavior at specific locations. For example, at the muscle/epidermis extracellular matrix, the probability of UNC-40-mediated outgrowth activity occurring from different surfaces of HSN is controlled in part by UNC-52 (perlecan), a basement membrane component, and the INA-1 (α integrin) receptor (Tang and Wadsworth 2014).

Several families of secreted molecules are known to affect cytoskeletal dynamics. Molecules such as members of the TGF β and Wnt families can be distributed asymmetrically in the extracellular environment, and could thereby asymmetrically affect the cytoskeleton within neurons. UNC-129 (TGF β) and the Wnts (CWN-1, CWN-2, EGL-20, LIN-44) are all known to play roles in determining the direction of outgrowth (Hilliard and Bargmann 2006; Pan *et al.* 2006; Prasad and Clark 2006; Maro *et al.* 2009; Song *et al.* 2010; Kirszenblat *et al.* 2011; Bhat *et al.* 2015).

The Wnts and UNC-6/netrin are thought to have a graded distribution along the anterior-posterior and dorso-ventral axes, respectively. Correspondingly, disrupting UNC-6 signaling or Wnt signaling affects migrations along these axes (Killeen and Sybingco 2008; Levy-Strumpf 2016). The outgrowth of axons from neurons such as AVM, PVM and HSN are normally directed toward the ventral UNC-6 source; however, with loss of UNC-6 or UNC-40 the axons are instead directed primarily anteriorly, and this bias requires EGL-20 (Wnt) (Hedgecock *et al.* 1990; Kulkarni *et al.* 2013). EGL-20 is expressed by posterior sources (Pan *et al.* 2006). Possibly, UNC-6 and Wnt signaling act independently such that the direction of outgrowth is always determined by one or the other cue. For AVM, PVM, and HSN, outgrowth UNC-6 signaling could mask the effect of the Wnt signal. However, there are several examples in which components of Wnt signaling are involved in directing migrations along the dorso-ventral axis, and components of UNC-6 signaling are involved in directing migrations along the a-p axis (Levy-Strumpf and Culotti 2007, 2014; Watari-Goshima *et al.* 2007; Levy-Strumpf 2016).

The emerging picture is of interdependence between the signaling pathways that determine the direction of outgrowth. This is underscored by the roles that UNC-5 has been observed to play. UNC-5 has long been considered a repulsive receptor that causes dorsal movement away from UNC-6 sources; however, recent studies now indicate that UNC-5 also plays a role in orienting outgrowth toward UNC-6 sources, as well as perpendicular to the source along the a-p axis. Whereas the ventral direction of AVM and PVM outgrowth is only slightly impaired in either *unc-5* or *egl-20* mutants, in double mutants there is a much greater penetrance, suggesting a synergistic interaction between UNC-5 and EGL-20 (Levy-Strumpf and Culotti 2014). In HSN, both UNC-5 and EGL-20 affect the surface to which UNC-40 will localize, and they correspondingly affect the direction of outgrowth (Kulkarni *et al.* 2013). Finally, in different genetic backgrounds UNC-5 has been observed to affect outgrowth along the a-p directions (Levy-Strumpf and Culotti 2007, 2014; Watari-Goshima *et al.* 2007; Li *et al.* 2008; Levy-Strumpf *et al.* 2015; Bhat and Hutter 2016).

Models of Axon Guidance

An intricate network of connections is formed between neurons. During development, neuronal growth cones navigate along specific pathways to find their correct targets. This requires precise patterns of outgrowth to occur. The trajectories of axons are determined by the distribution of cues that the axons encounter as they transverse their environment, and by the expression of receptors on the surface to the neuron that enables the axon to respond in the appropriate manner to the environmental cues. Therefore, the expression of environmental cues and of neuronal receptors must be precisely orchestrated in order for proper connections to be established. A major area of study, which is not

covered in this review, is the temporal and spatial control of these expression patterns.

The patterning of axon tracts begins as the growth cones from pioneer neurons are guided across the ectoderm and a scaffold of longitudinal and circumferential tracts develops (Durbin 1987). There is bilateral symmetry; however, some axons cross over midline boundaries to make connections on the opposite side. Following the initial migrations, most later axons will adhere (fasciculate) with the established axons, although some will pioneer new tracts. Development of the embryonic axon scaffold is dynamic as the neuronal cell bodies become positioned, the first tracts are created, and groups of axons are spatially ordered into the major nerves. Eliminating pioneer or guidepost neurons leads to disorganized axon bundles, with axons joining inappropriate bundles (Durbin 1987; Garriga *et al.* 1993; Ren *et al.* 1999; Hutter 2003). The expression of guidance cues reflects these complex changes. For example, the pattern of *UNC-6* expression changes temporally and spatially, providing a series of global and local cues to guide migrations throughout development (Wadsworth *et al.* 1996; Wadsworth and Hedgecock 1996; Asakura *et al.* 2007). The development and sorting of axons into specific tracts is achieved by the outgrowth responses that pioneer and follower axons have to the combination of cues expressed by the surrounding environment and by each other (Hutter 2003). At present only a few molecules are known to be involved in this sorting process, including *NID-1* (nidogen), *FMI-1* (flamingo), *LIN-17* (Frizzled), and the discoidin domain receptors, *DDR-1* and *DDR-2* (Steimel *et al.* 2010; Unsoeld *et al.* 2013).

A prevailing model for guidance is that cues are chemo-attractive or chemorepulsive (Tessier-Lavigne and Goodman 1996; Kolodkin and Tessier-Lavigne 2011). In this model, cues associated with intermediate or final target cells elicit responses from the growth cone, and direct outgrowth toward or away from the targets (Figure 4A). This is a particularly useful model for explaining trajectories toward or away from the source of a prevailing cue. In some cases, however, axons develop more complex trajectories, such as extending toward, and then away from, an intermediate target. The attraction-repulsion model predicts that axons must be able switch their response to cues at such choice points (Figure 4C) (Dickson and Zou 2010).

Chemoattraction and chemorepulsion are movements that occur in response to the relative concentration of a chemical in the environment. The outgrowth movement of the neuron is the result of outward force that is generated by the neuron because of a response to a cue at the plasma membrane. The movement is not a direct response to the source of the cue itself. That is, outgrowth movement is not produced by *forces of attraction* or *forces of repulsion* between the growth cone and the source. There is no intrinsic property of the system that specifies that the outgrowth activity must occur toward or away from the source of the cue. Although coincidentally outgrowth *in vivo* is often directed toward or away from the source of one cue because of the manner in which cues are

distributed, in truth, axons are not guided by an attraction or repulsion between the neuron and a source. Therefore, attraction and repulsion are not intrinsic properties of the guidance system. This nuance is important because the function of a gene in guidance is often assessed by the effect it has on movement toward, or away from, a source of a cue. Gene function is placed into attraction or repulsion genetic pathways. However, there may be no attractive or repulsive mechanisms encoded by neurons.

The results of an ectopic expression experiment show how the direction of outgrowth activity *in vivo* is not innately determined by the relative positions of the neuron and the cue source. In wildtype, the SDQR axon is directed away from the ventral *UNC-6* source, and in *unc-6* null animals it is directed ventrally. However, the axon is directed toward an ectopic dorsal source of *UNC-6* in *unc-6* null mutants (Kim *et al.* 1999). Only the location of the source relative to the neuron is changed in this experiment, and, presumably, neither the properties of *UNC-6* nor of the neuron are altered. It is thought that other factors found primarily at the dorsal surface of the neuron will modify the neuron's response to *UNC-6* so that the axon will extend dorsally outward. It follows that, if these factors were moved to a different surface, they would alter the response to *UNC-6* at that surface, and produce a different direction of outgrowth.

Guidance by attraction and repulsion is a very useful model. It provides a clear conceptual picture of how major guidance decisions are made, and it brings a much better understanding of the relationships between the function of different genes. However, another model of guidance has been proposed that predicts directed outgrowth is caused by a self-organizing mechanism that orients the outgrowth machinery to a surface of the neuron. This mechanism establishes a site for outgrowth without any external reference; the direction of outgrowth is determined stochastically. Impinging on this mechanism are the neuronal responses to cues at its surface. These cues together orient the outgrowth machinery to a specific surface, thereby establishing directionality.

This model was proposed from genetic evidence that indicates that conformational changes to the *UNC-40* receptor can trigger independent signals (Xu *et al.* 2009). One signal causes the *UNC-40* receptor to polarize to one side of the neuron, whereas another acts to orient the localization relative to the gradient of the *UNC-6* ligand. The results reveal that the receptor and the outgrowth activity it organizes can become polarized within the cell independently of any spatial information that could be provided by the external asymmetric distribution of *UNC-6*. That is, the neuron has the intrinsic ability to orient outgrowth activity in one direction. This might be accomplished by a process that is similar to that proposed for chemotaxis of eukaryotic cells such as neutrophils and *Dictyostelium*. For these cells, there is a polarization response that is self-organizing (Wang 2009).

UNC-40 self-organizing polarization is a stochastic process (Xu *et al.* 2009; Kulkarni *et al.* 2013). The *UNC-40* receptor will asymmetrically localize to one surface with some

A neuronal response to guidance cues

1. attractive and repulsive

2. self-organizing polarization

B random walk movement

C guidance models

1. attraction and repulsion

2. self-organizing polarization and random walks

Figure 4 Axon guidance models. (A) In the attraction-repulsion model, extracellular cues act as attractants or repellents. Here, the neuron only responds to the attractant ligand and the receptor mediates outgrowth activity that is directed toward the source of the attractant. In the self-organizing polarization model, a signal triggers feedback loops that asymmetrically localizes receptor signaling. The response to the cues increases or decreases the probability of where the receptor will mediate outgrowth activity. (B) Because the direction of outgrowth stochastically fluctuates at any instant of time, the movement of outgrowth can be considered as a succession of random steps. Depicted here are different objects (any mass) on a line, and 50 random walks of 50 steps from each point according to the probability distribution below. A probability distribution for the location of the object after the walk is given by the endpoints of the lines. The displacement of an object varies according to the degree of fluctuation. Because the outgrowth activity along the surface of an axon fluctuates, the moments of the membrane (a unit of mass) would move according to the same properties. (C) To illustrate how cues control the direction of outgrowth according to each model, an axon is depicted that approaches and crosses over an intermediate target, which expresses two different cues. In the attraction-repulsion model, the axon must switch its response from attraction to repulsion. In the self-organizing polarization model, the cues continue to promote outgrowth activity, but there is a change in the degree to which the direction of outgrowth fluctuates. The random walk models illustrate that the direction of outgrowth does not change, although the displacement does.

probability. Once the self-organizing polarization is initialized, the role of *UNC-6* is to increase the probability of *UNC-40* asymmetric localization at the site of interaction. However, the site of asymmetric localization does not require the *UNC-6* ligand; *UNC-40* will localize stochastically at different sites if the self-organizing polarization is triggered and there is no *UNC-6*. Since *UNC-40* signaling at the surface orients the outgrowth activity, the direction of *UNC-40*-mediated outgrowth activity is also determined stochastically. In the simplest case, the *UNC-6* cue could set a very high probability

of outgrowth activity at the side of the neuron toward the source of *UNC-6*, and the outgrowth activity will be oriented relative to the source. In this case, the system behaves as predicted by an attraction-repulsion model. However, with the self-organizing model there is always a probability associated with outgrowth in every other direction, so that if the probability of outgrowth is low at the surface toward the source, then the probability of outgrowth activity in other directions will be high. Therefore, if one cue increases or decreases the probability of outgrowth in one direction, it

must affect the probability of outgrowth in other direction(s) as well. Because there are different cues acting on a neuron *in vivo*, the influence that one cue has on the direction of outgrowth will depend on the effects that every other cue has on the probabilities.

The self-organizing model has implications regarding the directional effect that cues can have on movement (Kulkarni *et al.* 2013). There is always a probability distribution for outgrowth in each direction, and movement occurs over time through the fluctuations of outgrowth activity. Random walk models describe the path made by a succession of randomly directed movement, and they can suggest nonintuitive behaviors (Figure 4B). For example, if the probability of outgrowth in the anterior direction is 0.4, in the posterior direction is 0.4, and in the ventral direction is 0.2, then the direction of movement over time will be in the ventral direction. This predicts that a cue that has a strong effect on outgrowth, at either the anterior or posterior surface, will not direct outgrowth along the a–p axis; instead, outgrowth will be directed by the weaker effect of the cue at the ventral surface. Movement will fluctuate back and forth in the anterior and posterior directions, but will consistently move ahead in the ventral direction. The effect of random walk movement might not be directly observed. The random walk movement could occur at the microscale, *i.e.*, set up by fluctuating forces acting on the plasma membrane across the surface of the neuron. At the macroscale, the movement over time would be observed to be ventral. Further, depending on the degree to which the direction of outgrowth fluctuates, the displacement of the membrane will vary (Figure 4B). Because of these properties, the predicted effect that a cue *in vivo* might have on the direction and strength of guidance differs from the attraction and repulsion model (Figure 4C).

Little is known about the molecular mechanisms that would underlie a self-organizing model. Evidence suggests that the ability to self-organize UNC-40 polarization in the *C. elegans* neuron is regulated by SAX-3/Robo and UNC-53/Nav2 (Kulkarni *et al.* 2013; Tang and Wadsworth 2014). In *sax-3* mutants, robust UNC-40 localization is not seen and the receptor remains uniformly distributed across the surface of the HSN neuron. When UNC-6 is absent, the ability of UNC-40-mediated outgrowth activity to polarize is suppressed by UNC-53/Nav2 activity (Kulkarni *et al.* 2013). UNC-53 is a scaffold protein that interacts with ABI-1—a member of the WAVE complex that regulates the Arp2/3 complex—and ABL-1 is known to interact with MIG-10 (Schmidt *et al.* 2009; Stringham and Schmidt 2009; McShea *et al.* 2013). UNC-53 may also be tied to the Wnt response. For AVG axon outgrowth along the a–p axis, loss of UNC-53 function can suppress the a–p polarity defect caused by loss of PLR-1 function (Bhat *et al.* 2015). PLR-1 is a putative E3 ubiquitin ligase expressed in neurons that can lower the levels of Wnt receptors from the surface of the AVG neuron (Moffat *et al.* 2014). Loss of PLR-1 function also causes HSN axon guidance defects (Bhat *et al.* 2015).

It has been proposed that the self-organizing polarization process might be a general means by which cells can orient intracellular activity in response to the surrounding environment (Kulkarni *et al.* 2013; Yang *et al.* 2014). In general, triggering UNC-40 self-organizing polarity allows external factors to determine the probability of UNC-40 asymmetrically localizing at a surface of the cell. The outcome of this process is to tightly localize UNC-40 signaling to specific locations, which, in turn, could dramatically increase the effect of UNC-40-mediated signaling activity. Besides outgrowth, in neurons UNC-40-mediated signaling plays an important role in orienting synapse assembly, axon arborization, and extra-synaptic neurosecretory terminals (Colon-Ramos *et al.* 2007; Poon *et al.* 2008; Park *et al.* 2011; Stavoe and Colón-Ramos 2012; Stavoe *et al.* 2012; Nelson and Colón-Ramos 2013; Tu *et al.* 2015). Like outgrowth, these events require an activity to be asymmetrically oriented in response to local environmental cues. Consistent with these different events being linked, several proteins are known to affect multiple events. For example, the intracellular protein, SYD-1, regulates synaptogenesis and the active transport of organelles to the synaptic region of axons (Hallam *et al.* 2002; Dai *et al.* 2006; Patel *et al.* 2006; Edwards *et al.* 2015a,b). SYD-1 also plays a role in UNC-40-mediated axon guidance (Xu *et al.* 2015). Another example is CLEC-38—a transmembrane protein that negatively regulates UNC-40-mediated axon outgrowth and is required for synaptic assembly (Kulkarni *et al.* 2008). The precise molecular mechanisms through which different proteins orient UNC-40 activity remain to be determined. However, due to the UNC-40 self-organizing polarization mechanism, a protein might influence seemingly diverse developmental events, such as axon guidance and synaptogenesis, because its activity can alter the probability of UNC-40 asymmetrically localizing at a particular surface. Depending on the state of the cell, UNC-40 localization may influence the localization of components required for axon outgrowth, synaptogenesis, etc.

RPM-1 (PHR), is a cytoplasmic protein that coordinates and integrates different signaling events within neurons (Grill *et al.* 2016). Several studies indicate that RPM-1 regulates both synapse formation and axon termination (Schaefer *et al.* 2000; Zhen *et al.* 2000). In *rpm-1* mutants, the number of synapses is reduced, and the architecture of those present is disorganized; in addition, many axons fail to stop outgrowth when they should, and instead extend further than normal (Schaefer *et al.* 2000; Zhen *et al.* 2000; Nakata *et al.* 2005). Genetic evidence indicates that RPM-1 also regulates SAX-3 and UNC-40 activity for axon guidance (Li *et al.* 2008). Consistent with an involvement in regulating both the outgrowth and synaptogenesis processes, RPM-1 localizes to both the tip of mature axons and to the presynaptic terminal (Opperman and Grill 2014). The effect that RPM-1 has in determining axon length is sensitive to SAX-3, UNC-5, and UNC-40 (in the absence of CLEC-38) (Kulkarni *et al.* 2008; Li *et al.* 2008). A hypothesis is that RPM-1 is part of a mechanism that allows the self-organizing polarization process to

modulate different cellular activities in response to specific extracellular cue. Interestingly, *RPM-1* function might be regulated by Wnt signaling via *ANC-1* (nesprin) and *BAR-1* (β -catenin) (Tulgren *et al.* 2014), suggesting that *RPM-1* activity may be modulated by Wnt signaling and possibly by β -catenin-mediated adhesion activity.

Although in some cases *UNC-6* appears to trigger *UNC-40* self-organizing polarization, the process does not require the *UNC-6* ligand, and *UNC-40* asymmetric localization does not require *UNC-6* (Xu *et al.* 2009; Kulkarni *et al.* 2013). *UNC-40*-mediated signaling is also required for Q neuroblast migration, muscle arm extension, postsynaptic organization, anchor cell membrane extension, and the movement of intestinal cells (Honigberg and Kenyon 2000; Alexander *et al.* 2009; Tu *et al.* 2015; Asan *et al.* 2016). In all cases, *UNC-40* is regulating a morphological change that requires some activity to become polarized within a developing cell. These activities must be oriented with respect to local environmental cues. This is consistent with the findings that *UNC-40* conformational changes trigger independent signals to regulate self-organizing polarization and the orientation activity (Xu *et al.* 2009). Cells may have developed strategies to differentially regulate the signals.

Molecular and Functional Parallels Between the Directed Movement of Axons and Cells

Axon outgrowth is a specialized type of cell movement. During the development of *C. elegans*, many different cell types move to occupy new spaces. These movements share many common features, including navigation over cell surfaces and through extracellular matrixes. One of the remarkable conclusions from the original description of *unc-5*, *unc-6*, and *unc-40* mutant phenotypes is that the guidance of many different cell types, of both ectodermal and mesodermal origin, are affected by these genes (Hedgecock *et al.* 1990). Subsequent analyses in different organisms, and under different *in vitro* experimental conditions, has substantiated the view that the directed movement of different cell types are controlled by many of the same molecules. Further, it appears that many of the molecular mechanisms underlying the directed movement of diverse cell types are similar. Besides their role in normal development, axon guidance molecules are known to play key roles in processes important for cancer, including cell survival, cell growth, and angiogenesis (Chedotal *et al.* 2005; Mehlen *et al.* 2011). Because the migration and invasion into surrounding tissues is a common trait of both metastatic tumor cells and of neuronal extension, it is perhaps not surprising that molecules can have shared functions in both cell types.

Because there are many similarities between the directed movement of neurons and other cell types, studies using the movement of different cell types have greatly enhanced the understanding of neuronal outgrowth, and *vice versa*. In this review, we have noted several examples, including distal tip cell (DTC) migration, anchor cell (AC) invadopodia

extension, and muscle arm extension. These, and other cell types movements, offer several advantages. Here we briefly describe the DTC and AC models to emphasize the benefits of studying directed movement of different cell types within *C. elegans*.

During larval development, the DTCs migrate in three phases, which are distinguished by the direction of migration, and which are coordinated with developmental stages (Hall and Altun 2008). The DTCs are easily seen in living animals and, in fact, misguided cells cause phenotypes that can be visualized under a dissection microscope. Screens for DTC migration defects have uncovered guidance genes, and gene interactions whose function may be difficult to discern by their effects on axon guidance. These include genes for basement membrane components, metalloproteases, and integrins (Gettner *et al.* 1995; Baum and Garriga 1997; Belloch *et al.* 1999; Nishiwaki *et al.* 2000; Lee *et al.* 2001; Merz *et al.* 2003; Kubota *et al.* 2004; Meighan and Schwarzbauer 2007; Tamai and Nishiwaki 2007; Kawano *et al.* 2009), as well as guidance cues, such as *UNC-129* (TGF β), *UNC-6* (netrin), the Wnts, and their receptors (Hedgecock *et al.* 1990; Colavita *et al.* 1998; Merz *et al.* 2001; MacNeil *et al.* 2009; Levy-Strumpf and Culotti 2014).

The anchor cell is a specialized cell of the gonad that induces vulval formation during larval development. The cell establishes contact between the uterine and vulval epithelium by extending through a basement membrane (Sherwood and Sternberg 2003). This invasion process provides a model for studying how surrounding cells and extracellular matrix temporally and spatially control outgrowth. In comparison to neuronal growth cones, the larger size and less dynamic shape changes of the AC makes it more amenable to studies quantifying temporal and spatial changes. The AC has been particularly useful for exploring the mechanisms and dynamics associated with basement membrane remodeling during cell extension (Sherwood and Sternberg 2003; Hagedorn *et al.* 2009, 2013; Ziel *et al.* 2009; Hagedorn and Sherwood 2011; Morrissey *et al.* 2016). Moreover, live-cell imaging of the AC revealed the dynamic nature of *UNC-40* self-organizing polarization (Wang *et al.* 2014). The imaging shows that clusters of *UNC-40* form, disassemble, and reform along the membrane in the absence of *UNC-6*. Imaging has also revealed that *MADD-2* and F-actin colocalize with the *UNC-40* clusters, and that *UNC-6* orients and stabilizes the clustering toward the *UNC-6* source.

Outlook and Future Directions in *C. elegans* Axon Guidance

Redundancy and differential effects of mutations in guidance genes

Axonal navigation defects in null alleles of axon guidance genes are typically not completely penetrant, often with a penetrance well below 50%. This suggests a high degree of redundancy in guidance systems and signaling pathways. In

many cases, the redundancy is not simply due to gene duplications resulting in additional family members with overlapping functions. Major guidance cues and many signaling pathway components in *C. elegans* are small families, often with only one family member, suggesting redundant action of nonrelated proteins. Basic aspects of anatomy might impact the penetrance of defects. At the time of axon outgrowth, the embryo is well into the morphogenesis phase, with all tissues in place and differentiating. In particular, the rows of muscle cells provide barriers along the a–p axis that limit where misguided axons can grow. Furthermore multiple tissues flanking axon tracts could provide redundant information for navigating axons.

Axons from different types of neurons often show differences in the penetrance of defects, even if they have the same trajectory or extend in the same axon tracts. For example, commissures of D-type motor neurons typically show more penetrant defects than commissures of DA and DB motor neurons. Some attempts have been made to uncover a molecular basis for the differences (Yee *et al.* 2011), but, at present, this cell type specificity is not well understood. In the VNC, pairs of neurons with one axon in each of the two axon tracts (PVP, PVQ, HSN, and AVK) also show consistent left–right differences in the penetrance of defects, with axons in the left axon tract being more affected than those in the right axon tract. The difference in this case might be due to the larger number of axons in the right tract, which can provide additional (adhesive) cues for axons extending along the VNC. Differential use of guidance cues of axons extending in the same axon bundle also comes from the observation that the penetrance of defects in the absence of the pioneer (AVG) is very different in different classes of follower neurons, suggesting that certain classes of neurons depend more on cues from the pioneer than others (Hutter 2003). These observations indicate that studying the navigation of selected classes of neurons (as typically done) is unlikely to reveal all guidance cues and signaling pathways involved in axonal pathfinding.

Axon guidance defects should lead to wiring defects in the nervous system with negative consequences for the functioning of the nervous system. The major output of the nervous system of *C. elegans* is controlled movement of the animal in response to environmental stimuli. As a consequence, major wiring defects should result in movement defects. Mutations affecting the dorsal navigation of motor neuron commissures indeed result in an uncoordinated (*Unc*) phenotype with animals no longer able to move in a normal fashion. However, many defects in the VNC, which contains major components of the motor circuit, do not lead to obvious movement defects. In part, this is due to the incomplete penetrance and variability of the navigation defects, but it is probably also an indication that a certain degree of miswiring in a neuronal circuit can be tolerated and compensated for. Studies correlating axonal defects in an individual with potentially subtle movement defects are required to shed more light on the

connection between axonal navigation defects and nervous system function.

Missing players

Genetic screens have been very successful in the identification of key genes controlling axonal navigation. However, simple screens for mutants causing axonal defects have two limitations. If there is a high degree of redundancy, so that the penetrance of the defects in a single mutant is low, the corresponding gene might not be identified. If axonal defects are present only in a compromised background, it becomes impossible to identify mutations. For example, mutations in *madd-4* enhance certain axonal defects in the background of *unc-6* or *slt-1* mutations, but there are no axonal defects in *madd-4* single mutants. Enhancer screens are one possibility to uncover such redundantly acting genes. The use of strains that ectopically express a guidance gene of interest may also be useful, since resulting phenotypes could be more dependent on genes that function in the same or in parallel pathways.

Essential or pleiotropic gene functions also pose a challenge for genetic screens, as axon navigation occurs in late embryonic or larval development. For example, mutations in the α integrin *ina-1* cause axonal navigation defects. This suggests that mutations in the only β integrin, *pat-3*, should have similar defects. However, known alleles of *pat-3* arrest at a stage before axons grow out, so that this idea currently cannot be tested. While large-scale screens can identify partial loss-of-function alleles (as in the case of *ina-1* itself), such mutations tend to be rare, and require a substantial screening effort. Many axon guidance genes play multiple roles in embryonic tissue morphogenesis; for example, loss-of-function mutations in *vab-1*/Eph receptor were identified based on epidermal morphogenesis defects, not guidance defects (George *et al.* 1998). Tissue-specific RNAi screens targeting early embryonic lethal genes might allow us to circumvent this problem. Tissue specific knockouts using Cre recombinase or CRISPR/Cas9 have recently been used to address the functions of essential genes in neurons (Chen *et al.* 2015; Tian *et al.* 2015).

Understanding the dynamics of axon outgrowth

The decisions of a growth cone during outgrowth are typically inferred indirectly by evaluating the final trajectory of the axon often in the adult animal. In most cases, the axon trajectory does not change after outgrowth is completed. However, axons in the left VNC can end up in the right VNC in certain mutants due to a failure to maintain the axon position in response to mechanical stress when the animal begins to move (Hobert and Bülow 2003). This can lead to a misinterpretation of such maintenance defects as navigation defects. For a better understanding of the dynamics of axonal outgrowth, is it desirable to observe growth cones directly during outgrowth. In *C. elegans*, this is currently only possible for neurons that extend their axons postembryonically, such as VD, AVM, PVM, and HSN. Studying changes in receptor localization during outgrowth of the HSN axon has led to new insights and models for the regulation of the direction

of outgrowth (Xu *et al.* 2009; Kulkarni *et al.* 2013; Tang and Wadsworth 2014; Yang *et al.* 2014).

The major tools to study axon guidance genes are mutations that eliminate gene function. For proteins that are part of a short-term dynamic process, *e.g.*, proteins controlling actin dynamics in response to guidance cues, these are rather crude tools. To better understand such processes, we need to develop modified versions of such proteins where the activity of the protein can be manipulated in more subtle ways. Temperature-sensitive mutations are one way to transiently inactivate proteins. Engineered mutations are another possibility. As yet, for most axon guidance genes such tools are currently not available.

Axon Regeneration After Injury

Neural regeneration is widespread in the animal kingdom, and there is increasing interest in understanding the basic biology of regeneration in regeneration-competent animal groups, in part to understand why regeneration is relatively minimal in the mature human central nervous system (CNS). Neural regeneration can mean any of several processes: neurogenesis in response to injury, dedifferentiation and redifferentiation of existing cells, or axonogenesis, meaning the regrowth of new axons from existing but damaged axons (Bradke *et al.* 2012). Yanik *et al.* (2004) reported previously that *C. elegans* axons displayed regenerative regrowth after laser surgery (axotomy) (Figure 5). Since then, *C. elegans* has emerged as a tractable model for genetic, pharmacological, and cellular analysis of axon regeneration.

The ability to visualize identified axons in live transgenic animals with genetically encoded fluorescent markers allows axon regrowth to be studied at single-axon resolution. Work in *C. elegans* has elucidated how injury-generated signals trigger reformation of a motile growth cone from a mature axon. Although some outgrowth and guidance pathways defined from developmental studies also function in regenerative regrowth, their precise role often differs. Several groups have exploited the tractability of *C. elegans* for genetics or RNAi to screen for genes affecting regenerative axon regrowth. While some genes identified from developmental studies have roles in regeneration, these studies have uncovered many pathways specifically involved in axon regrowth after injury.

In mammals, the adult CNS is conspicuously deficient in its ability to regenerate after injury, accounting for the incurable nature of traumas such as spinal cord injuries. A major impetus in studying models such as *C. elegans* is to understand the processes underlying successful regeneration so as to better understand its failure in the mammalian CNS. In part, the poor regenerative capacity in mammalian CNS results from inhibitory effects of myelin and other signals in the CNS environment. *C. elegans* lacks myelin, so it is expected that the microenvironment of axons should be more permissive to regeneration. Moreover, many studies have underscored the importance of cell-intrinsic pathways in regulating axonal regrowth capacity in mammals; such pathways may be more

widely conserved than vertebrate specializations such as myelin. As $>1/3$ of *C. elegans* genes have ortholog relationships to human genes (Shaye and Greenwald 2011), *C. elegans* should provide a rich source of new genetic factors relevant to biomedical studies of axon regeneration.

Since 2004, over 40 research or methods articles have been published on *C. elegans* axon regeneration biology. *C. elegans* axon regeneration has been reviewed several times, and readers are referred to these for additional discussion (El Bejjani and Hammarlund 2012; Hammarlund and Jin 2014). For a general review of intrinsic pathways in axon regeneration, see He and Jin (2016); for reviews of the roles of guidance pathways and extrinsic factors in regeneration, see Giger *et al.* (2010) and Geoffroy and Zheng (2014).

Axon Regeneration in the Wild Type: Effects of Cell Type, Life Stage, and Location of Injury

To date, most studies of *C. elegans* axon regeneration have focused on mechanosensory or motor neurons, which have long, well-separated processes. Mechanosensory neurons have large diameter axons that extend half the length of the animal, and which undergo robust regeneration in larval and adult stages, although it remains unclear whether such regeneration restores circuit function. Motor neurons innervating body wall muscles are also highly tractable models for regrowth. The circumferential commissures of body motor neurons extend from the ventral to the dorsal midline (see above), and can be severed at mid-lateral positions. Both cholinergic and GABAergic motor neurons exhibit comparable regenerative responses; the axonal regrowth, although error-prone, can restore behavioral function (Yanik *et al.* 2004).

Several other neuron classes have been tested for regenerative capacity, and not all are proficient in regrowth, although the reasons for these differences are not well understood (Wu *et al.* 2007; Gabel *et al.* 2008). Some chemosensory neurons, such as ASJ, exhibit regenerative axon regrowth, whereas others are unable to regrow axons (Chung *et al.* 2006). Such chemosensory axons extend within a fasciculated bundle, the amphid commissure; it has not been established whether their differential regrowth reflects differences in intrinsic growth capacity, or in the microenvironment of axons within the bundle.

Several studies have explored the effects of varying the location or stage of axon injury (Gabel *et al.* 2008). Severing of an axon very close to the neuronal cell body can trigger a variety of responses, including cell death or sprouting of new neurites (AVM). In contrast, severing of motor commissures or mechanosensory axons 50–100 μm from the soma typically results in formation of a new growth cone from the severed end of the proximal axon. Regrowth responses diminish distally from the soma. Some chemosensory dendrites exhibit regenerative responses after dendrite injury (dendrotomy) (Wu *et al.* 2007); moreover, a recent report suggests that dendrotomy alters the axonal response to injury in ASJ neurons (Chung *et al.* 2016) (see below).

Figure 5 Axon regeneration in touch neurons and motor neurons. (A) Cartoons of morphological stages in regrowth after laser injury, based on studies of mechanosensory neurons (Wu *et al.* 2007). (B) Images from time lapse movie of PLM axotomy and regeneration. PLM soma is to the right. At 2 hr post axotomy the proximal and distal severed ends have retracted a few microns. By 3 hr, 50 min, the proximal end is extending filopodia; by 4 hr, 30 min, a morphologically distinct growth cone has reformed, which begins to extend anteriorly by 6 hr. (C) Images of PLM regrowth in the wild type, regeneration defective mutants (*dlk-1*, *unc-75*), and regeneration-enhanced mutants (*efa-6*). Images are 24 hr post axotomy; Bar, 10 μ m; transgene *Pmec-4-GFP(zdIs5)* or *Pmec-7-GFP(muls32)*. (D) Confocal images of GABAergic motor neuron regrowth in wild type and in regeneration-defective mutant (*unc-75*). Marker, *Punc-25-GFP(juls76)*.

Axons regenerate whether severed in larval or adult stages, with regenerative capacity declining gradually in larval or adult stages depending on the neuron. Regrowth in early larval stages is less error prone, consistent with higher growth capacity and more accurate interpretation of cues. However, the mechanisms of injury response and growth cone reformation in juvenile vs. mature neurons are likely to be similar.

Overview of the Response to Axon Injury and Stages of Regrowth

After injury, axons undergo a sequence of responses over varying timescales leading to repair and regeneration (Figure

5A). Here, we present an overview of the phenomenology of *C. elegans* axon regeneration, based mostly on studies in larval motor and sensory axons.

Immediate responses to axon injury

After laser severing, the ends of the remaining axon fragments retract several microns over the next few minutes. *C. elegans* axons generally tolerate local laser injury, and degeneration or death of the damaged neuron is rare if the injury is distal from the soma. It is assumed that, concomitant with retraction, the damaged axonal plasma membrane is sealed, although this has not been observed directly. Subsequent release of microvesicles from axons may represent elimination of damaged membrane (Nix *et al.* 2014).

As in other organisms, axonal injury triggers a rapid rise in intracellular Ca^{2+} , starting at the injury site and spreading as a wave along the axon to the soma within seconds, as visualized with genetically encoded Ca^{2+} indicators such as GCaMP (Ghosh-Roy *et al.* 2010; Sun *et al.* 2014). The injury-triggered Ca^{2+} influx has multiple origins: entry through the damaged membrane, opening of VGCCs such as EGL-19, and Ca^{2+} -induced Ca^{2+} release from internal stores. Genetic and pharmacological manipulations indicate that the amplitude of the Ca^{2+} rise affects the extent of subsequent regeneration. In part, this reflects sustained Ca^{2+} elevation in the axon and soma, which requires the ER Ca channel UNC-68; optogenetic elevation of Ca^{2+} can enhance axon regeneration (Sun *et al.* 2014). In addition to Ca^{2+} , other early injury-triggered signals likely include cAMP, although this has not been directly visualized in *C. elegans*. Genetic or pharmacological manipulation of cAMP levels indicates that cAMP signaling promotes regeneration (Ghosh-Roy *et al.* 2010).

Injury also triggers rapid reorganization of the axonal cytoskeleton (Chisholm 2013). Whereas analysis of cytoskeletal regulators in developmental guidance has focused on actin (see above), the MT cytoskeleton appears to play a prominent role in regenerative axon regrowth, and has been a focus of intensive analysis. Within seconds of injury, axonal MTs become depolymerized locally at the injury site, as visualized by MT plus end binding protein markers (Chen *et al.* 2015). Over the next 2–3 hr, MTs undergo a series of changes, ultimately leading to an upregulation of dynamic MTs relative to the more stable MTs of the intact mature axon (Ghosh-Roy *et al.* 2012). Studies in vertebrate axon regeneration indicate that MT stability is a major determinant of axon regrowth, and genetic or pharmacological modulation of MT dynamics has major effects on axon regeneration, as discussed below.

Growth cone reformation, axon extension, and navigation

Following initial retraction and membrane repair, the damaged axon is overtly quiescent for several hours. The proximal severed end becomes swollen: the so-called retraction bulb. Beginning 2–4 hr after injury, the severed end begins to sprout filopodia, followed by formation of a morphologically distinct regenerative growth cone. Compared to the developmental growth cones discussed above (Knobel *et al.* 1999), regenerative growth cones are typically smaller, slower-moving, and more erratic in guidance. Nevertheless, these growth cones can be motile for up to 2–3 days post injury. Notably, regrowth of an axon in late larval or early adult stages requires navigation over significantly larger distances than faced by developmental growth cones. The regenerative growth cones of motor neuron axons extend at rates of 2–3 $\mu\text{m/hr}$, whereas larval touch neuron (ALM or PLM) axons extend at rates between 4 and 7 $\mu\text{m/hr}$ (Wu *et al.* 2007); regrowth rates decline in early adult stages. In general, regenerative axon extension is slower than developmental axon growth (e.g., VD growth cones have been observed to extend at up to 60 $\mu\text{m/hr}$), although the discontinuous

nature of axon growth makes exact comparisons difficult. The navigation of regenerating growth cones becomes increasingly error-prone in larval development. Regenerating axons also typically extend numerous branches, some of which are pruned over time, often leaving one major process by 24 hr after injury (Wu *et al.* 2007).

Although axon regeneration most often involves growth-cone-based navigation, there are examples of axon regrowth without formation of a classical growth cone (e.g., ALM in *dlk-1 rsk-1* double mutants). These observations suggest that the extension and navigation roles of growth cones may be genetically separable in certain contexts.

Lastly, successful regeneration requires recognition of targets, reformation of synaptic contacts, and reintegration into a functional circuit. Severed motor axon commissures are frequently able to navigate to their target areas in the dorsal nerve cord (Yanik *et al.* 2004; Wu *et al.* 2007; Nix *et al.* 2011), resulting in restored locomotor function. In the case of motor neurons with dorsal neuromuscular junctions, such as the DD neurons, functional regeneration involves reformation of synapses with dorsal body wall muscles. It is not known whether muscle arms display plasticity during motor axon regeneration. In contrast, neurons with postsynaptic dorsal processes (VD, etc.) must restore dorsal dendritic contacts.

Axonal degeneration and axonal fusion

Complete axotomy generates two cell fragments: a proximal axon fragment connected to the soma, and a distal, anucleate fragment. The distal fragment can exhibit transient filopodial sprouting but slowly degenerates (Wu *et al.* 2007), and is phagocytosed by the surrounding epidermis (Nichols *et al.* 2016). Degeneration and phagocytosis of axonal fragments occur rapidly in early larval stages, but are slower in adults. The phagocytic engulfment of axon fragments involves pathways defined from studies of engulfment of apoptotic cell corpses, and suggests that the anucleate axonal fragments are recognized as dying cells (Nichols *et al.* 2016).

In initial studies of touch neuron axon regeneration, it was found that a significant minority of regrowing axons grew back to their distal axonal fragments, leading to apparent fusion of the two axonal fragments, and preventing degeneration of the distal fragment (Yanik *et al.* 2006). Ultrastructural analysis showed that the axon fragments had physically fused, dependent on the membrane fusogen EFF-1 (Ghosh-Roy *et al.* 2010), previously known to function in muscle and epithelial cell fusions (Mohler *et al.* 2002), and in dendrite morphogenesis (Oren-Suissa *et al.* 2010). The fused fragments can form a continuous cytoplasmic compartment (Neumann *et al.* 2011). Fusion of axotomized axon fragments has been observed occasionally in other organisms, but its mechanistic basis had not been explored. In *C. elegans*, axon–axon fusion appears to involve active participation of the distal fragment, as regrowing axons target to their distal fragments with high specificity. Axon fragments display membrane phosphatidylserine (PS), required for the fusion pathway (Neumann *et al.* 2015). Despite acting as an “eat-me”

signal for dying cells or axon fragments, axonal PS may also act as a “save-me” signal, leading to degeneration and engulfment or fusion depending on the context (Nichols *et al.* 2016). Axon–axon fusion has also been observed in the *unc-70* fragile axon mutant, where it can occur between neighboring axons at a low frequency (Neumann *et al.* 2015).

Regeneration Screens: Methods and Metrics

A key rationale for developing *C. elegans* as a model for axon regeneration is the need for axon regeneration models suitable for large-scale genetic, RNAi, or chemical genetic screens. Laser axotomy allows precisely controlled and reproducible injury of single axons. The surgery can be performed with femtosecond near-infrared or UV lasers, and axonal responses to these kinds of damage appear broadly similar (Wu *et al.* 2007; Williams *et al.* 2011). Using femtosecond laser axotomy, a medium-scale screen of mutants affecting >650 candidate genes has been reported (Chen *et al.* 2011); this has since expanded to >1300 genes (A. D. Chisholm and Yishi Jin, unpublished results). A focused screen of small molecule inhibitors in axon regrowth has also been reported (Samara *et al.* 2010).

Laser axotomy and measurement of regrowth using traditional microscopy (worms immobilized on slides using anesthetics) is labor intensive, precluding very large-scale screening. Significant effort has been put into developing microfluidic methods for worm handling, facilitating larger scale approaches (Guo *et al.* 2008). A fully automated platform for laser axotomy has been reported (Gokce *et al.* 2014), and it will be interesting to see the results from these approaches.

A key attribute of *C. elegans* is that regrowing axons can be imaged *in vivo* using live or time-lapse microscopy, allowing detailed analysis of axon regrowth rate and growth cone dynamics (Rohde and Yanik 2011). However, for routine use or screening, less labor intensive metrics of axon regeneration have been used. The length of the regenerated axon segment is readily measured in longitudinal axons such as PLM and ALM (Chen *et al.* 2011). For circumferential processes, such as motor neuron commissures, a simpler metric is the fraction of axons with regenerative responses (% regeneration), or the fraction that reach dorsal target areas within a certain period (% DNC regeneration). Presence of a growth cone *per se* does not correlate strongly with axon extension, as growth cones are often most prominent in stalled axons. Metrics of axon guidance have also been developed (Wu *et al.* 2007; Gabel *et al.* 2008).

Because of the labor-intensive nature of laser axotomy, there has also been interest in mutants that undergo spontaneous axon breakage, potentially precluding the need for laser surgery. *unc-70* β -spectrin mutants were discovered to have fragile axons (Hammarlund *et al.* 2007). Mechanical stress generated by locomotion in larval development causes certain axons to break, triggering formation of new growth cones from broken axons. The new growth cones regrow in

an error-prone manner, and the regenerated axon undergoes more cycles of breakage and regrowth, with less regrowth at each cycle. Although the chronic defects in axon integrity mean that regrowth phenotypes are more complex compared to those after laser surgery, this natural axotomy model has great advantages for large scale screening (Nix *et al.* 2014).

Direct forward screens for mutants affecting regeneration remain technically challenging. However screens for modifiers of other phenotypes associated with regeneration mutants potentially allow such genes to be identified. A successful example has been the screen for suppressors of *vhp-1* (*svh*). The VHP-1 phosphatase negatively regulates two key regeneration-promoting MAP kinases, PMK-3/p38 and KGB-1/JNK (Mizuno *et al.* 2004). Loss of function in *vhp-1* results in larval lethality due to constitutive activation of the MAPK pathways, and this lethality is efficiently suppressed by loss of function in either cascade. A genome-wide RNAi screen identified several additional *svh* genes that define new regulators of MAPK signaling (Li *et al.* 2012), including the HGF-MSP-plasminogen related growth factor SVH-1 and its receptor SVH-2 (discussed below).

Genes and Pathways Regulating Axon Regeneration

Overview of genetic landscape of regeneration

Several themes emerge from the published large-scale genetic and RNAi screens. Regenerative regrowth is impaired in a wide variety of mutants or RNAi treatments. In a mutant-based screen of 654 genes for PLM regeneration (Chen *et al.* 2011), ~10% of mutants tested displayed significantly reduced regrowth; about 1–2% of mutants displayed enhanced regrowth. An RNAi screen of >3750 genes for effects on motor axon regrowth in the *unc-70* background identified 70 candidates (Nix *et al.* 2014); after retesting, 50 new regrowth genes were identified. Such genes span many functional and structural classes, some of which are described below. Most seem to have little to no role in developmental axon outgrowth; some may be involved in injury sensing. Regenerative axon growth may also be generally more sensitive to mutant backgrounds than development. Overall, these screens have greatly expanded the genetic landscape of axon regeneration, setting the stage for mechanistic studies currently underway. Axon regeneration is highly multifactorial, and no single manipulation is sufficient to restore perfect regeneration. Nevertheless, certain pathways and signals have emerged as playing major roles, and an outline of the events and pathways active in the minutes and hours after injury is emerging (Figure 6).

Injury-triggered signals: second messengers and kinase cascades

As discussed above, two injury-triggered second messengers are Ca^{2+} and cAMP signaling. These appear to act upstream of two main stress-responsive MAPK cascades: the DLK-1 and MLK-1 pathways. The roles and regulation of these

injury-triggered MAPK cascades have been reviewed recently in detail (Pastuhov *et al.* 2015; Andrusiak and Jin 2016).

A critical role for the dual leucine zipper kinase *dlk-1* in regeneration was revealed in both the *unc-70* RNAi screen (Hammarlund *et al.* 2009), and in PLM regeneration (Yan *et al.* 2009). *dlk-1* null mutants have essentially normal axon outgrowth in development but display an almost total block in the response to axon injury and are unable to reform regenerative growth cones. *dlk-1* encodes a MAPKKK acting at the first step of a MAPK cascade that includes *MKK-4*, *PMK-3*, and *MAK-2*. *dlk-1* and its downstream kinases had previously been identified in screens for suppressors of a synaptic overgrowth mutant, *rpm-1* (Nakata *et al.* 2005), and the entire *DLK-1* cascade is required for axon regeneration. *DLK-1* signaling acts cell autonomously and at the time of injury (Hammarlund *et al.* 2009; Yan *et al.* 2009). Moreover, overexpression of *DLK-1* is sufficient to enhance axon regrowth in larval stages, and to restore larval regrowth levels to adult axons. These and other findings have led to the model that *DLK-1* activity is a key determinant of intrinsic regenerative capacity.

A second MAPK cascade, the *MLK-1/MEK-1/KGB-1* (JNK) pathway, was also identified as required for motor axon regeneration (Nix *et al.* 2011). Loss of function in *mlk-1* or in its downstream kinases impairs regeneration, although not as severely as a *dlk-1* mutant; like *DLK-1*, overexpression of *MLK-1* can enhance regeneration. The *DLK-1* and *MLK-1* cascades appear to function in parallel, in that overexpression of one upstream kinase (e.g., *DLK-1*) can compensate for loss of function in the other pathway, suggesting that they converge on common targets. The *VHP-1* phosphatase mentioned above acts as a negative regulator of both pathways at the level of p38 and JNK.

The requirement for *DLK-1* and *MLK-1* at the time of regrowth suggests these pathways may respond to injury. A systematic analysis of *dlk-1* alleles revealed the presence of an endogenous inhibitory isoform *DLK-1S* (Yan and Jin 2012), leading to the model that in the steady state *DLK-1* is present mostly as inactive heterodimers. Ca^{2+} influx after injury triggers dissociation of these inactive heterodimers and formation of active *DLK-1L* homodimers. Several lines of evidence from *C. elegans* and other organisms have suggested that *DLK* activity is also sensitive to the polymerization state of axonal MTs, by an unknown mechanism (Bounoutas *et al.* 2011). Finally, *DLK-1* and *MLK-1* levels are also under negative regulation by the *RPM-1* ubiquitin ligase. *RPM-1* affects baseline levels of *DLK-1* and *MLK-1*, and is not itself injury-regulated (Nix *et al.* 2011).

Activators of the *MLK-1* kinase were discovered in the *svh* screen, and have been characterized primarily in the context of motor commissure regrowth. The *SVH-2* HGFR acts cell autonomously to promote regeneration upstream of *MLK-1*. Interestingly, its ligand *SVH-1* is expressed by a distant pair of exposed chemosensory neurons, ADL. Ablation experiments suggest *svh-1* is constitutively expressed by ADL, and acts cell nonautonomously. Insight into how *SVH-1/2* signaling is

Figure 6 Pathways and genes involved in axon regrowth. Overview of stages and selected regulators in *C. elegans* axon regeneration. Growth promoting factors are in green, and growth-inhibiting factors in red. See text for details.

regulated by injury came from analysis of the *ETS-4* transcription factor, identified as *svh-5* (Li *et al.* 2015). *ETS-4* is activated by injury-induced cAMP signaling, and induces transcription of *svh-2* in injured neurons. *ETS-4* and *CEBP-1* are coordinately required for injury-triggered transcription of *SVH-2* in injured neurons. Thus the *DLK-1* and *MLK-1* cascades could act sequentially to ensure sustained regeneration.

Identification of *svh-3* as the fatty acid amide hydrolase *FAAH-1* revealed an additional layer of regulation of *MLK-1*. *svh-3/faah-1* mutants display reduced regeneration due to excessive accumulation of the endocannabinoid anandamide (Pastuhov *et al.* 2012). Elevated levels of endocannabinoids suppress *MLK-1* activity via the GPCRs *NPR-19* and *NPR-32* (Pastuhov *et al.* 2016), which act through a G-protein-coupled receptor cascade and *TPA-1/PKC*. There is also evidence that endocannabinoids may be locally induced by injury, resulting in repulsion of growth cones away from the injury site (Pastuhov *et al.* 2016).

The *DLK-1* pathway has at least two main outputs in regeneration: it regulates gene expression via the bZip protein *CEBP-1* (Yan *et al.* 2009), and regulates the axonal MT cytoskeleton independent of *CEBP-1* (Ghosh-Roy *et al.* 2012). *CEBP-1* was identified in the screen for *rpm-1* suppressors, and is required for axon regeneration. *cebp-1* mRNA is localized to axons, and undergoes local translation within axons after injury (Yan *et al.* 2009). *CEBP-1* may be subsequently transported to the nucleus to regulate genomic targets; apart from *svh-2* mentioned above, the targets of *CEBP-1* in regeneration are not yet known. *DLK-1* and *PMK-3* may also undergo retrograde transport after injury to influence downstream targets. *DLK-1* pathway activity can also affect the MT cytoskeleton independent of *CEBP-1*. One potential target of *DLK-1* is the MT depolymerase kinesin-13, as well as enzymes regulating MT post-translational modifications (Ghosh-Roy *et al.* 2012). However the mechanisms by which *DLK* regulates MT dynamics remain poorly understood. Interestingly, *DLK-1* is also required for developmental modulation of axonal MT dynamics during GABAergic synaptic remodeling, independent of *CEBP-1* (Kurup *et al.* 2015). As

noted above, the **DLK-1** pathway itself is also regulated by MT dynamics, suggesting it may participate in a feedback mechanism monitoring axonal MT integrity both in development and regeneration.

Other pathways regulating axon regeneration

In addition to the cAMP, Ca^{2+} /**DLK-1**/p38 and **MLK-1**/JNK cascades, several other pathways have been implicated in axon regrowth. The mechanistic relationships of these pathways to the MAPK cascades have yet to be fully determined.

Loss of function in the ribosomal S6 kinase **rsks-1** strongly enhances regeneration in touch neurons, likely acting via the metabolic sensor AMP kinase **AAK-2** (Hubert *et al.* 2014). **RSKS-1** may partly act in parallel to the **DLK-1** cascade, as **rsks-1** loss of function partly suppressed ALM regrowth defects of **dlk-1** null mutants. Interestingly, in **dlk-1 rsks-1** animals, ALM axons regrew without forming morphologically distinct growth cones (Hubert *et al.* 2014).

The **DLK-1** pathway is critical for regrowth multiple neuron types, including PLM mechanosensory neurons, amphid sensory neurons, and GABAergic motor neurons. However, in neurons such as ALM, **dlk-1** null mutants display partial regrowth after axotomy, indicating the existence of **DLK-1**-independent regrowth pathways. Moreover, axotomized chemosensory neurons such as ASJ display regenerative regrowth in response to dendrotomy that is independent of the **DLK-1** and **KGB-1**/JNK cascades (Chung *et al.* 2016). In these neurons, inhibition of neuronal activity by a variety of methods, including mutation of Ca^{2+} channels, altered membrane potential, or sensory transduction, can promote **DLK-1**-independent regeneration after axotomy. **DLK-1**-independent regenerative regrowth is delayed, is slower than **DLK-1**-dependent axon regrowth, and appears to be mechanistically related to the formation of ectopic neurites in mutants with diminished neuronal activity (Peckol *et al.* 1999). The **DLK-1**-independent regrowth observed in *C. elegans* may also provide insight into conditioning lesion effects in which dendrotomy promotes axon regrowth, long studied in vertebrate neurons (Neumann and Woolf 1999).

The core apoptotic proteins **CED-3**/caspase and **CED-4**/Apaf-1, but not other regulators of cell death, are required for efficient regenerative regrowth of the ALM neuron (Pinan-Lucarre *et al.* 2012). Injury may trigger **CED-4** activation by Ca^{2+} signaling, mediated by the calreticulin **CRT-1**. Genetic double mutant analysis suggests that **CED-3** may also act upstream of **DLK-1**. This study revealed a novel protective role for parts of the apoptotic cascade. Interestingly, in injured mouse neurons, **DLK** triggers transcriptional programs promoting apoptosis and axon regeneration, although, in this case, apoptotic factors promote cell death (Watkins *et al.* 2013). Nevertheless, there may be additional links between **DLK** signaling and caspase activity.

An unexpected finding from large-scale screens was that several genes with classical roles in neurotransmitter synthesis are also required for axon regrowth (Chen *et al.* 2011; Nix *et al.* 2011). These include the 5-hydroxytryptamine (5-HT)

biosynthetic enzyme tryptophan hydroxylase (**tph-1**), even though neither motor neurons nor touch neurons are thought to be serotonergic. Recently, it was found that, after injury, motor neurons activate transcription of **tph-1** (Alam *et al.* 2016), a rare example of apparent neurotransmitter plasticity in *C. elegans*. Injury-triggered 5-HT expression promotes axon regrowth via the **SER-7** GPCR and a series of downstream factors (**GPA-12**, **RHGF-1**), ultimately acting via **MLK-1**. Induction of **tph-1** expression after injury requires the **HIF-1** hypoxia-inducible factor. It is not yet known how **HIF-1** is regulated by axon injury, although its activation appears to be independent of classical hypoxia-sensing pathways. **HIF-1 α** is also required for axonal regeneration in mammals (Cho *et al.* 2015).

The axonal cytoskeleton: a central role for MTs

MTs are emerging as important intrinsic determinants of axon regenerative capacity in many organisms (Chisholm 2013; Tang and Chisholm 2016). As mentioned above, in mature neurons axonal MTs are relatively stable. To convert a mature axon into a dynamic regrowing axon requires a multistep reorganization of the axonal cytoskeleton. In *C. elegans*, one output of the **DLK-1** pathway is to upregulate axonal MT dynamics. **DLK-1** may directly or indirectly regulate activity of the depolymerizing kinesin-13/**KLP-7**, and may regulate expression or function of enzymes involved in post-translational modification of MTs (Ghosh-Roy *et al.* 2012).

Several other components or regulators of MTs have been implicated in regenerative regrowth. Regrowth initiation is impaired in animals with a gain of function mutation in **mec-7**/ β -tubulin (Kirszenblat *et al.* 2013). Axon regeneration is also dependent on function of the MT minus end binding protein **PTRN-1**, a member of the Patronin/CAMSAP family (Chuang *et al.* 2014). **ptrn-1** null mutants display minor abnormalities in axon development (Marcette *et al.* 2014; Richardson *et al.* 2014), but are severely impaired in regeneration. It is generally thought that, during neuronal differentiation, the axonal MT cytoskeleton transitions from a centrosomal to a noncentrosomal organization, as **PTRN-1** appears to function specifically in noncentrosomal MT minus ends, regenerative axon regrowth may be specifically dependent on noncentrosomal MT pathways.

Loss of function in **EFA-6** results in dramatically enhanced regeneration (Chen *et al.* 2011). Although clearly related to other EFA6 family Arf6 GEFs, studies in *C. elegans* embryos revealed **EFA-6** also functions as a MT destabilizing factor (O'Rourke *et al.* 2010). Extensive analysis of **EFA-6** in neurons supports the model that it restrains regeneration predominantly via inhibiting axonal MT dynamics (Chen *et al.* 2015). Loss of function in **EFA-6** results in elevated numbers of dynamic axonal MTs, and prevents the injury-triggered downregulation in MT dynamics. Conversely, overexpression of **EFA-6** blocks MT dynamics, an activity that can be localized to the intrinsically disordered N-terminus of **EFA-6**. Screens for **EFA-6** interactors identified the MT-associated proteins **TAC-1**/TACC and **ZYG-8**/DCLK, each of which is

required for axon regrowth (Chen *et al.* 2015). Moreover, EFA-6 activity is dynamically regulated: after injury, EFA-6 translocates from the cell cortex to axonal puncta that contain the minus end marker PTRN-1. The mechanisms by which injury regulates EFA-6 dynamics and function remain to be fully elucidated.

Guidance pathways and the extracellular matrix in regeneration

Regenerating axons likely navigate an environment in which developmental axon guidance cues, if still expressed, are distributed over a larger spatial scale than they occupy during development. For efficient regeneration, regrowing axons might upregulate guidance receptors to become more sensitive to long-range cues. Alternatively, regenerative regrowth may be more dependent on short-range cues or guidepost cells. While several studies indicate that the developmental guidance cues discussed above are important in regrowth, their effects may be smaller, larger or even the opposite of their developmental effects. For example, Slit/Robo signaling promotes PLM outgrowth in development, yet strongly inhibits PLM regeneration (Chen *et al.* 2011).

Dorsoventral guidance systems such as netrin or TGF β signaling also play distinct roles in regeneration. The AVM process extends ventrally in development, dependent on partly redundant attractive and repulsive cues. Guidance of regenerating AVM axons in young adults requires UNC-6/netrin, but did not require the netrin receptor UNC-40 (Gabel *et al.* 2008). Regenerating AVM axons were sensitive to repulsion by Slit/SLT-1, but not by UNC-129/TGF β . AVM regeneration was also dependent on guidance adaptors such as CED-10, UNC-34, and MIG-10, though these play relatively minor roles in AVM developmental outgrowth (Gabel *et al.* 2008). Other guidance adaptors with major roles in PLM regrowth include MAX-2 and UNC-115 (Chen *et al.* 2011).

PLM axon regeneration is also strongly dependent on the cell surface receptors SAX-7/L1CAM and SDN-1/Syndecan (Chen *et al.* 2011). As described above, *sax-7* has pleiotropic roles in neuron or tissue adhesion, and promotes dendrite morphogenesis, but is not generally involved in developmental axonal outgrowth. Likewise, *sdn-1* mutants display variable defects in axon undershooting and overshooting (Rhiner *et al.* 2005), but are strongly defective in axon regrowth in touch neurons and motor neurons (Chen *et al.* 2011; Edwards and Hammarlund 2014).

The roles of the extracellular matrix in axon regeneration have not been analyzed in depth, but analyses of partial loss of function mutants suggest it may contain permissive and inhibitory signals. Partial loss of function in the basement membrane components SPON-1/F-spondin and PXN-2/peroxidasin resulted in significantly enhanced axon regrowth (Gotenstein *et al.* 2010).

Axonal injury and gene expression

Axonal injury has profound effects on neuronal gene expression, inducing the expression of regeneration-associated

genes (RAGs), and repressing other genes (Tedeschi 2011). In *C. elegans*, many transcription factors have been implicated in axon regeneration in large-scale screening, including the bZip protein CEBP-1, a key target of the DLK-1 pathway (Yan *et al.* 2009), and the cAMP-regulated factor ETS-4. CEBP-1 and ETS-4 can form a complex that induces transcription of the SVH-2 receptor (Li *et al.* 2015). These observations have led to the model that injury initially triggers parallel Ca²⁺/DLK-1 and cAMP/PKA cascades, thus upregulating SVH-2 and activating its downstream MLK/JNK pathway (Figure 6B). Overexpression of SVH-2 was insufficient to suppress axon regeneration defects of *cebp-1* mutants, again consistent with the DLK-1/CEBP-1 cascade regulating additional targets required for regeneration.

In *Drosophila*, the nuclear hormone receptor UNF is required for regrowth after stereotyped axon pruning of mushroom body gamma axons, acting via the Tor/S6K pathway. Although not injury-triggered, such developmental remodeling may share mechanistic similarities with regeneration, and indeed the *C. elegans* UNF ortholog *fax-1* is required for axon regeneration in chemosensory neurons (Yaniv *et al.* 2012).

RNA processing and regeneration

Large-scale screens have also identified regulators of gene expression likely acting at the levels of mRNA or translation. *unc-75* mutants display strong regeneration defects (Figure 5B) (Chen *et al.* 2011), but essentially normal axon outgrowth in development (Loria *et al.* 2003). *unc-75* encodes a member of the CELF family of RNA-binding proteins, generally implicated in mRNA splicing or stability. Analysis of UNC-75-interacting targets in neurons has linked *unc-75* to a set of synaptic transmission proteins that are also required for axon regeneration (Chen *et al.* 2011).

RNA processing by the RtcB RNA ligase has also been implicated in axon regeneration (Kosmaczewski *et al.* 2015). Loss of function in *rtcb-1* enhances motor neuron regeneration, independent of known catalytic roles of RtcB in ligation of tRNAs or splicing of the mRNA for the unfolded protein response sensor *xbp-1*. Conversely, the RNA cyclase RtcA has been shown to repress regeneration in *Drosophila* and mammalian neurons (Song *et al.* 2015). In *Drosophila*, this effect was interpreted as involving canonical RtcA functions in *xbp-1* splicing. *xbp-1* itself is required for efficient axon regeneration in *C. elegans* and in *Drosophila* (Nix *et al.* 2014; Song *et al.* 2015). RtcA and RtcB catalyze opposing reactions, so it is less evident why loss of function in either gene should have the same regeneration-enhancing effect. As *C. elegans* lacks a clear ortholog of RtcA, the biochemical function of RtcB may have diverged. These studies reveal an unexpected role for RNA ligation in axon regeneration.

Developmental timing and aging: microRNAs and insulin signaling

Motor axon regrowth capacity is robust throughout larval development, as is the regeneration of PLM and ALM axons (Wu *et al.* 2007). In contrast, the regenerative capacity of

AVM declines after the L3 stage (Gabel *et al.* 2008). This decline has been traced to expression of the heterochronic pathway miRNA *let-7* in older neurons (Zou *et al.* 2013). Loss of function in *let-7* or in the miRNA processing enzyme *alg-1* prevents developmental decline in AVM regrowth; regrowth eventually declines in adults. *let-7* represses *lin-41*, whose expression in young neurons correlates with regrowth capacity. The enhanced regrowth of *let-7* animals requires the *DLK-1* pathway, suggesting downregulation of *DLK-1* pathway expression might correlate with the developmental decline in regrowth capacity.

Distinct from developmental decline, regrowth of motor and touch axons progressively slows in adult life, although even very old animals display some regenerative responses (A. D. Chisholm and Yishi Jin, unpublished data). Importantly, this age-dependent decline in axon regrowth capacity is separable from organismal aging, as animals that display increased longevity do not always exhibit extended regenerative capacity (Byrne *et al.* 2014). Reduced function of the insulin/IGF receptor *DAF-2* prolongs lifespan and extends regenerative capacity, but appears to do so by distinct mechanisms, in that the *DAF-2* target *DAF-16/FOXO* acts independently in neurons and non-neuronal cells to promote regeneration and longevity.

In mammals, the PTEN phosphatase is a potent intrinsic inhibitor of axon regrowth, acting as a negative regulator of the TOR signaling pathway (He and Jin 2016). In *C. elegans*, loss of function in PTEN ortholog *DAF-18* enhances axon regeneration in older adults, independently of *DAF-16*, and dependent on TOR signaling (Byrne *et al.* 2014). Interestingly, *DAF-18* also promotes developmental outgrowth of specific neurons, independent of TOR signaling, and via PI3K (Christensen *et al.* 2011). The enhanced regeneration of *daf-2* and *daf-18* mutants requires the *DLK-1* pathway, and *DAF-16/FOXO* itself may regulate *dlk-1* transcription. Transcriptional profiling of neurons has also identified the forkhead factor *fkh-9* as a target of *DAF-16* required for *daf-2*-dependent adult axon regeneration (Kaletsky *et al.* 2016). *C. elegans* should be a tractable model to explore how age affects axon regenerative capacity.

Summary and Future Directions in *C. elegans* Axon Regeneration

C. elegans has a robust capacity to regenerate injured axons, without new neurogenesis. The regrown axons can restore circuit function. The selective advantage of this regenerative capability in the natural environment remains to be determined. However, as heat shock can enhance regeneration, the ability of axons to regrow may play a role in surviving thermal or other stresses. In contrast to the highly stereotyped development of axons in *C. elegans*, regenerative axon regrowth is variable in extent and direction. In part, this may reflect experimental variability, and, in part, it may reflect the greater sensitivity of regeneration to minor variations in genetic background, epigenetic influences, or environmental

conditions. Several major axon outgrowth/guidance pathways function in regeneration, but, in general, axon regeneration does not appear to be a simple recapitulation of developmental axon growth or guidance processes. To date, most studies have addressed morphological regeneration. An important goal in the future will be to explore more deeply how synapses are regenerated, and to what extent behavioral function is restored.

C. elegans has become a tractable model for gene discovery of conserved regeneration factors, as exemplified by the *DLK-1* pathway. Other potentially conserved pathways may include PTEN/TOR, *HIF-1*, RNA ligation, and MT regulation. Despite vast differences in neuronal scale and complexity, some intrinsic mechanisms of axon regeneration appear to have been highly conserved. While vertebrate-specific extrinsic inhibitory factors such as myelin are absent, the *C. elegans* extracellular matrix may have regeneration-promoting and inhibiting roles. Thus *C. elegans* may provide insights into intrinsic and extrinsic regulators of axon regrowth, both positive and negative. Studies to date have exploited laser axotomy and axon fragility to generate axon breakages, yet have typically been restricted to testing selected sets of candidate genes. The advent of fully automated laser axotomy may one day allow the power of unbiased forward genetic screens to be applied to axon regeneration.

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Literature Cited

- Ackley, B. D., J. R. Crew, H. Elamaa, T. Pihlajaniemi, C. J. Kuo *et al.*, 2001 The NC1/endostatin domain of *Caenorhabditis elegans* type XVIII collagen affects cell migration and axon guidance. *J. Cell Biol.* 152: 1219–1232.
- Ackley, B. D., S. H. Kang, J. R. Crew, C. Suh, Y. Jin *et al.*, 2003 The basement membrane components nidogen and type XVIII collagen regulate organization of neuromuscular junctions in *Caenorhabditis elegans*. *J. Neurosci.* 23: 3577–3587.
- Adler, C. E., R. D. Fetter, and C. I. Bargmann, 2006 UNC-6/Netrin induces neuronal asymmetry and defines the site of axon formation. *Nat. Neurosci.* 9: 511–518.
- Alam, T., H. Maruyama, C. Li, S. I. Pastuhov, P. Nix *et al.*, 2016 Axotomy-induced HIF-serotonin signalling axis promotes axon regeneration in *C. elegans*. *Nat. Commun.* 7: 10388.
- Alexander, M., K. K. Chan, A. B. Byrne, G. Selmán, T. Lee *et al.*, 2009 An UNC-40 pathway directs postsynaptic membrane extension in *Caenorhabditis elegans*. *Development* 136: 911–922.

- Alexander, M., G. Selman, A. Seetharaman, K. K. Chan, S. A. D'Souza *et al.*, 2010 MADD-2, a homolog of the Opitz syndrome protein MID1, regulates guidance to the midline through UNC-40 in *Caenorhabditis elegans*. *Dev. Cell* 18: 961–972.
- Altun, Z. F., and D. H. Hall, 2011 Nervous system, general description, in *WormAtlas*, edited for the web by Herndon, L. A. In *WormAtlas*. DOI:10.3908/wormatlas.1.18. Last revision: June 19, 2013.
- Andrusiak, M. G., and Y. Jin, 2016 Context specificity of stress-activated mitogen-activated protein (MAP) kinase signaling: the story as told by *Caenorhabditis elegans*. *J. Biol. Chem.* 291: 7796–7804.
- Asakura, T., K. Ogura, and Y. Goshima, 2007 UNC-6 expression by the vulval precursor cells of *Caenorhabditis elegans* is required for the complex axon guidance of the HSN neurons. *Dev. Biol.* 304: 800–810.
- Asan, A., S. A. Raiders, and J. R. Priess, 2016 Morphogenesis of the *C. elegans* intestine involves axon guidance genes. *PLoS Genet.* 12: e1005950.
- Aurelio, O., D. H. Hall, and O. Hobert, 2002 Immunoglobulin-domain proteins required for maintenance of ventral nerve cord organization. *Science* 295: 686–690.
- Bader, B. L., N. Smyth, S. Nedbal, N. Miosge, A. Baranowsky *et al.*, 2005 Compound genetic ablation of nidogen 1 and 2 causes basement membrane defects and perinatal lethality in mice. *Mol. Cell. Biol.* 25: 6846–6856.
- Baird, S. E., D. H. Fitch, I. A. Kassem, and S. W. Emmons, 1991 Pattern formation in the nematode epidermis: determination of the arrangement of peripheral sense organs in the *C. elegans* male tail. *Development* 113: 515–526.
- Baum, P. D., and G. Garriga, 1997 Neuronal migrations and axon fasciculation are disrupted in *ina-1* integrin mutants. *Neuron* 19: 51–62.
- Bénard, C. Y., C. Blanchette, J. Recio, and O. Hobert, 2012 The secreted immunoglobulin domain proteins ZIG-5 and ZIG-8 cooperate with L1CAM/SAX-7 to maintain nervous system integrity. *PLoS Genet.* 8: e1002819.
- Bernadskaya, Y. Y., A. Wallace, J. Nguyen, W. A. Mohler, and M. C. Soto, 2012 UNC-40/DCC, SAX-3/Robo, and VAB-1/Eph polarize F-actin during embryonic morphogenesis by regulating the WAVE/SCAR actin nucleation complex. *PLoS Genet.* 8: e1002863.
- Bhat, J. M., and H. Hutter, 2016 Pioneer axon navigation is controlled by AEX-3, a guanine nucleotide exchange factor for RAB-3 in *Caenorhabditis elegans*. *Genetics* 203: 1235–1247.
- Bhat, J. M., J. Pan, and H. Hutter, 2015 PLR-1, a putative E3 ubiquitin ligase, controls cell polarity and axonal extensions in *C. elegans*. *Dev. Biol.* 398: 44–56.
- Blanchette, C. R., P. N. Perrat, A. Thackeray, and C. Y. Bénard, 2015 Glypican is a modulator of netrin-mediated axon guidance. *PLoS Biol.* 13: e1002183.
- Blelloch, R., S. S. Anna-Arriola, D. Gao, Y. Li, J. Hodgkin *et al.*, 1999 The *gon-1* gene is required for gonadal morphogenesis in *Caenorhabditis elegans*. *Dev. Biol.* 216: 382–393.
- Boulin, T., R. Pocock, and O. Hobert, 2006 A novel Eph receptor-interacting IgSF protein provides *C. elegans* motoneurons with midline guidepost function. *Curr. Biol.* 16: 1871–1883.
- Bounoutas, A., J. Kratz, L. Emtage, C. Ma, K. C. Nguyen *et al.*, 2011 Microtubule depolymerization in *Caenorhabditis elegans* touch receptor neurons reduces gene expression through a p38 MAPK pathway. *Proc. Natl. Acad. Sci. USA* 108: 3982–3987.
- Bradke, F., J. W. Fawcett, and M. E. Spira, 2012 Assembly of a new growth cone after axotomy: the precursor to axon regeneration. *Nat. Rev. Neurosci.* 13: 183–193.
- Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. *Genetics* 77: 71–94.
- Broadbent, I. D., and J. Pettitt, 2002 The *C. elegans hmr-1* gene can encode a neuronal classic cadherin involved in the regulation of axon fasciculation. *Curr. Biol.* 12: 59–63.
- Bülow, H. E., and O. Hobert, 2004 Differential sulfations and epimerization define heparan sulfate specificity in nervous system development. *Neuron* 41: 723–736.
- Bülow, H. E., K. L. Berry, L. H. Topper, E. Peles, and O. Hobert, 2002 Heparan sulfate proteoglycan-dependent induction of axon branching and axon misrouting by the Kallmann syndrome gene *kal-1*. *Proc. Natl. Acad. Sci. USA* 99: 6346–6351.
- Bülow, H. E., N. Tjoe, R. A. Townley, D. Didiano, T. H. van Kuppevelt *et al.*, 2008 Extracellular sugar modifications provide instructive and cell-specific information for axon-guidance choices. *Curr. Biol.* 18: 1978–1985.
- Byrne, A. B., T. Walradt, K. E. Gardner, A. Hubbert, V. Reinke *et al.*, 2014 Insulin/IGF1 signaling inhibits age-dependent axon regeneration. *Neuron* 81: 561–573.
- Cang, J., and D. A. Feldheim, 2013 Developmental mechanisms of topographic map formation and alignment. *Annu. Rev. Neurosci.* 36: 51–77.
- Chan, K. K., A. Seetharaman, R. Bagg, G. Selman, Y. Zhang *et al.*, 2014 EVA-1 functions as an UNC-40 co-receptor to enhance attraction to the MADD-4 guidance cue in *Caenorhabditis elegans*. *PLoS Genet.* 10: e1004521.
- Chan, S. S., H. Zheng, M. W. Su, R. Wilk, M. T. Killeen *et al.*, 1996 UNC-40, a *C. elegans* homolog of DCC (Deleted in Colorectal Cancer), is required in motile cells responding to UNC-6 netrin cues. *Cell* 87: 187–195.
- Chang, C., C. E. Adler, M. Krause, S. G. Clark, F. B. Gertler *et al.*, 2006 MIG-10/Lamellipodin and AGE-1/PI3K promote axon guidance and outgrowth in response to slit and netrin. *Curr. Biol.* 16: 854–862.
- Chedotal, A., G. Kerjan, and C. Moreau-Fauvarque, 2005 The brain within the tumor: new roles for axon guidance molecules in cancers. *Cell Death Differ.* 12: 1044–1056.
- Chen, L., Z. Wang, A. Ghosh-Roy, T. Hubert, D. Yan *et al.*, 2011 Axon regeneration pathways identified by systematic genetic screening in *C. elegans*. *Neuron* 71: 1043–1057.
- Chen, L., M. Chuang, T. Koorman, M. Boxem, Y. Jin *et al.*, 2015 Axon injury triggers EFA-6 mediated destabilization of axonal microtubules via TACC and doublecortin like kinase. *eLife* 4: e08695.
- Chin-Sang, I. D., S. E. George, M. Ding, S. L. Moseley, A. S. Lynch *et al.*, 1999 The ephrin VAB-2/EFN-1 functions in neuronal signaling to regulate epidermal morphogenesis in *C. elegans*. *Cell* 99: 781–790.
- Chin-Sang, I. D., S. L. Moseley, M. Ding, R. J. Harrington, S. E. George *et al.*, 2002 The divergent *C. elegans* ephrin EFN-4 functions in embryonic morphogenesis in a pathway independent of the VAB-1 Eph receptor. *Development* 129: 5499–5510.
- Chisholm, A. D., 2013 Cytoskeletal dynamics in *Caenorhabditis elegans* axon regeneration. *Annu. Rev. Cell Dev. Biol.* 29: 271–297.
- Chisholm, A., and M. Tessier-Lavigne, 1999 Conservation and divergence of axon guidance mechanisms. *Curr. Opin. Neurobiol.* 9: 603–615.
- Cho, Y., J. E. Shin, E. E. Ewan, Y. M. Oh, W. Pita-Thomas *et al.*, 2015 Activating injury-responsive genes with hypoxia enhances axon regeneration through neuronal HIF-1 α . *Neuron* 88: 720–734.
- Christensen, R., L. de la Torre-Ubieta, A. Bonni, and D. A. Colon-Ramos, 2011 A conserved PTEN/FOXO pathway regulates neuronal morphology during *C. elegans* development. *Development* 138: 5257–5267.
- Christensen, R. P., A. Bokinsky, A. Santella, Y. Wu, J. Marquina-Solis *et al.*, 2015 Untwisting the *Caenorhabditis elegans* embryo. *eLife* 4: .10.7554/eLife.10070

- Chuang, M., A. Goncharov, S. Wang, K. Oegema, Y. Jin *et al.*, 2014 The microtubule minus end binding protein Patronin/PTRN-1 is required for axon regeneration in *C. elegans*. *Cell Reports* 9: 874–883.
- Chung, S. H., D. A. Clark, C. V. Gabel, E. Mazur, and A. D. Samuel, 2006 The role of the AFD neuron in *C. elegans* thermotaxis analyzed using femtosecond laser ablation. *BMC Neurosci.* 7: 30.
- Chung, S. H., M. R. Awal, J. Shay, M. M. McLoed, E. Mazur *et al.*, 2016 Novel DLK-independent neuronal regeneration in *Caenorhabditis elegans* shares links with activity-dependent ectopic outgrowth. *Proc. Natl. Acad. Sci. USA* 113: E2852–E2860.
- Clark, S. G., and C. Chiu, 2003 *C. elegans* ZAG-1, a Zn-finger-homeodomain protein, regulates axonal development and neuronal differentiation. *Development* 130: 3781–3794.
- Colavita, A., and J. G. Culotti, 1998 Suppressors of ectopic UNC-5 growth cone steering identify eight genes involved in axon guidance in *Caenorhabditis elegans*. *Dev. Biol.* 194: 72–85.
- Colavita, A., S. Krishna, H. Zheng, R. W. Padgett, and J. G. Culotti, 1998 Pioneer axon guidance by UNC-129, a *C. elegans* TGF-beta. *Science* 281: 706–709.
- Colon-Ramos, D. A., M. A. Margeta, and K. Shen, 2007 Glia promote local synaptogenesis through UNC-6 (netrin) signaling in *C. elegans*. *Science* 318: 103–106.
- Dai, Y., H. Taru, S. L. Deken, B. Grill, B. Ackley *et al.*, 2006 SYD-2 Liprin-alpha organizes presynaptic active zone formation through ELKS. *Nat. Neurosci.* 9: 1479–1487.
- Demarco, R. S., and E. A. Lundquist, 2010 RACK-1 acts with Rac GTPase signaling and UNC-115/abLIM in *Caenorhabditis elegans* axon pathfinding and cell migration. *PLoS Genet.* 6: e1001215.
- Demarco, R. S., E. C. Struckhoff, and E. A. Lundquist, 2012 The Rac GTP exchange factor TIAM-1 acts with CDC-42 and the guidance receptor UNC-40/DCC in neuronal protrusion and axon guidance. *PLoS Genet.* 8: e1002665.
- Dent, E. W., S. L. Gupton, and F. B. Gertler, 2011 The growth cone cytoskeleton in axon outgrowth and guidance. *Cold Spring Harb. Perspect. Biol.* 3: pii: a001800.
- Díaz-Balzac, C. A., M. I. Lázaro-Peña, E. Tecle, N. Gomez, and H. E. Bülow, 2014 Complex cooperative functions of heparan sulfate proteoglycans shape nervous system development in *Caenorhabditis elegans*. *G3 (Bethesda)* 4: 1859–1870.
- Díaz-Balzac, C. A., M. I. Lázaro-Peña, G. A. Ramos-Ortiz, and H. E. Bülow, 2015 The adhesion molecule KAL-1/anosmin-1 regulates neurite branching through a SAX-7/L1CAM-EGL-15/FGFR receptor complex. *Cell Reports* 11: 1377–1384.
- Dickson, B. J., and Y. Zou, 2010 Navigating intermediate targets: the nervous system midline. *Cold Spring Harb. Perspect. Biol.* 2: a002055.
- Dong, B., M. Moseley-Alldredge, A. A. Schwieterman, C. J. Donelson, J. L. McMurtry *et al.*, 2016 EFN-4 functions in LAD-2-mediated axon guidance in *Caenorhabditis elegans*. *Development* 143: 1182–1191.
- Dong, X., K. Shen, and H. E. Bülow, 2015 Intrinsic and extrinsic mechanisms of dendritic morphogenesis. *Annu. Rev. Physiol.* 77: 271–300.
- Durbin, R., 1987 Studies on the development and organisation of the nervous system of *Caenorhabditis elegans*. Ph.D. Thesis, University of Cambridge.
- Edwards, D. R., M. M. Handsley, and C. J. Pennington, 2008 The ADAM metalloproteinases. *Mol. Aspects Med.* 29: 258–289.
- Edwards, S. L., L. M. Morrison, R. M. Yorks, C. M. Hoover, S. Boominathan *et al.*, 2015a UNC-16 (JIP3) acts through synapse-assembly proteins to inhibit the active transport of cell soma organelles to *Caenorhabditis elegans* motor neuron axons. *Genetics* 201: 117–141.
- Edwards, S. L., R. M. Yorks, L. M. Morrison, C. M. Hoover, and K. G. Miller, 2015b Synapse-assembly proteins maintain synaptic vesicle cluster stability and regulate synaptic vesicle transport in *Caenorhabditis elegans*. *Genetics* 201: 91–116.
- Edwards, T. J., and M. Hammarlund, 2014 Syndecan promotes axon regeneration by stabilizing growth cone migration. *Cell Reports* 8: 272–283.
- El Bejjani, R., and M. Hammarlund, 2012 Neural regeneration in *Caenorhabditis elegans*. *Annu. Rev. Genet.* 46: 499–513.
- Fleming, T., S. C. Chien, P. J. Vanderzalm, M. Dell, M. K. Gavin *et al.*, 2010 The role of *C. elegans* Ena/VASP homolog UNC-34 in neuronal polarity and motility. *Dev. Biol.* 344: 94–106.
- Forrester, W. C., and G. Garriga, 1997 Genes necessary for *C. elegans* cell and growth cone migrations. *Development* 124: 1831–1843.
- Fujisawa, K., J. L. Wrana, and J. G. Culotti, 2007 The slit receptor EVA-1 coactivates a SAX-3/Robo mediated guidance signal in *C. elegans*. *Science* 317: 1934–1938.
- Gabel, C. V., F. Antoine, C. F. Chuang, A. D. Samuel, and C. Chang, 2008 Distinct cellular and molecular mechanisms mediate initial axon development and adult-stage axon regeneration in *C. elegans*. *Development* 135: 1129–1136.
- Garriga, G., C. Desai, and H. R. Horvitz, 1993 Cell interactions control the direction of outgrowth, branching and fasciculation of the HSN axons of *Caenorhabditis elegans*. *Development* 117: 1071–1087.
- Geoffroy, C. G., and B. Zheng, 2014 Myelin-associated inhibitors in axonal growth after CNS injury. *Curr. Opin. Neurobiol.* 27: 31–38.
- George, S. E., K. Simokat, J. Hardin, and A. D. Chisholm, 1998 The VAB-1 Eph receptor tyrosine kinase functions in neural and epithelial morphogenesis in *C. elegans*. *Cell* 92: 633–643.
- Gettner, S. N., C. Kenyon, and L. F. Reichardt, 1995 Characterization of beta pat-3 heterodimers, a family of essential integrin receptors in *C. elegans*. *J. Cell Biol.* 129: 1127–1141.
- Ghosh-Roy, A., Z. Wu, A. Goncharov, Y. Jin, and A. D. Chisholm, 2010 Calcium and cyclic AMP promote axonal regeneration in *Caenorhabditis elegans* and require DLK-1 kinase. *J. Neurosci.* 30: 3175–3183.
- Ghosh-Roy, A., A. Goncharov, Y. Jin, and A. D. Chisholm, 2012 Kinesin-13 and tubulin posttranslational modifications regulate microtubule growth in axon regeneration. *Dev. Cell* 23: 716–728.
- Giger, R. J., E. R. Hollis, II, and M. H. Tuszynski, 2010 Guidance molecules in axon regeneration. *Cold Spring Harb. Perspect. Biol.* 2: a001867.
- Ginzburg, V. E., P. J. Roy, and J. G. Culotti, 2002 Semaphorin 1a and semaphorin 1b are required for correct epidermal cell positioning and adhesion during morphogenesis in *C. elegans*. *Development* 129: 2065–2078.
- Gitai, Z., T. W. Yu, E. A. Lundquist, M. Tessier-Lavigne, and C. I. Bargmann, 2003 The netrin receptor UNC-40/DCC stimulates axon attraction and outgrowth through enabled and, in parallel, Rac and UNC-115/AbLIM. *Neuron* 37: 53–65.
- Gokce, S. K., S. X. Guo, N. Ghorashian, W. N. Everett, T. Jarrell *et al.*, 2014 A fully automated microfluidic femtosecond laser axotomy platform for nerve regeneration studies in *C. elegans*. *PLoS One* 9: e113917.
- Gotenstein, J. R., R. E. Swale, T. Fukuda, Z. Wu, C. A. Giurumescu *et al.*, 2010 The *C. elegans* peroxidase PNX-2 is essential for embryonic morphogenesis and inhibits adult axon regeneration. *Development* 137: 3603–3613.
- Grill, B., R. K. Murphey, and M. A. Borgen, 2016 The PHR proteins: intracellular signaling hubs in neuronal development and axon degeneration. *Neural Dev.* 11: 8.
- Grossman, E. N., C. A. Giurumescu, and A. D. Chisholm, 2013 Mechanisms of ephrin receptor protein kinase-independent signaling in amphid axon guidance in *Caenorhabditis elegans*. *Genetics* 195: 899–913.

- Guo, S. X., F. Bourgeois, T. Chokshi, N. J. Durr, M. A. Hilliard *et al.*, 2008 Femtosecond laser nanoaxotomy lab-on-a-chip for in vivo nerve regeneration studies. *Nat. Methods* 5: 531–533.
- Gysi, S., C. Rhiner, S. Flibotte, D. G. Moerman, and M. O. Hengartner, 2013 A network of HSPG core proteins and HS modifying enzymes regulates netrin-dependent guidance of D-type motor neurons in *Caenorhabditis elegans*. *PLoS One* 8: e74908.
- Hagedorn, E. J., and D. R. Sherwood, 2011 Cell invasion through basement membrane: the anchor cell breaches the barrier. *Curr. Opin. Cell Biol.* 23: 589–596.
- Hagedorn, E. J., H. Yashiro, J. W. Ziel, S. Ihara, Z. Wang *et al.*, 2009 Integrin acts upstream of netrin signaling to regulate formation of the anchor cell's invasive membrane in *C. elegans*. *Dev. Cell* 17: 187–198.
- Hagedorn, E. J., J. W. Ziel, M. A. Morrissey, L. M. Linden, Z. Wang *et al.*, 2013 The netrin receptor DCC focuses invadopodia-driven basement membrane transmigration in vivo. *J. Cell Biol.* 201: 903–913.
- Hahn, A. C., and S. W. Emmons, 2003 The roles of an ephrin and a semaphorin in patterning cell-cell contacts in *C. elegans* sensory organ development. *Dev. Biol.* 256: 379–388.
- Hall, D. H., and Z. F. Altun, 2008 *C. elegans* Atlas. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Hallam, S. J., A. Goncharov, J. McEwen, R. Baran, and Y. Jin, 2002 SYD-1, a presynaptic protein with PDZ, C2 and rho-GAP-like domains, specifies axon identity in *C. elegans*. *Nat. Neurosci.* 5: 1137–1146.
- Hamelin, M., Y. Zhou, M. W. Su, I. M. Scott, and J. G. Culotti, 1993 Expression of the UNC-5 guidance receptor in the touch neurons of *C. elegans* steers their axons dorsally. *Nature* 364: 327–330.
- Hammarlund, M., and Y. Jin, 2014 Axon regeneration in *C. elegans*. *Curr. Opin. Neurobiol.* 27: 199–207.
- Hammarlund, M., W. S. Davis, and E. M. Jorgensen, 2000 Mutations in beta-spectrin disrupt axon outgrowth and sarcomere structure. *J. Cell Biol.* 149: 931–942.
- Hammarlund, M., E. M. Jorgensen, and M. J. Bastiani, 2007 Axons break in animals lacking beta-spectrin. *J. Cell Biol.* 176: 269–275.
- Hammarlund, M., P. Nix, L. Hauth, E. M. Jorgensen, and M. Bastiani, 2009 Axon regeneration requires a conserved MAP kinase pathway. *Science* 323: 802–806.
- Hao, J. C., T. W. Yu, K. Fujisawa, J. G. Culotti, K. Gengyo-Ando *et al.*, 2001 *C. elegans* slit acts in midline, dorsal-ventral, and anterior-posterior guidance via the SAX-3/robo receptor. *Neuron* 32: 25–38.
- Hao, J. C., C. E. Adler, L. Mebane, F. B. Gertler, C. I. Bargmann *et al.*, 2010 The tripartite motif protein MADD-2 functions with the receptor UNC-40 (DCC) in netrin-mediated axon attraction and branching. *Dev. Cell* 18: 950–960.
- He, Z., and Y. Jin, 2016 Intrinsic control of axon regeneration. *Neuron* 90: 437–451.
- Hedgecock, E. M., J. G. Culotti, J. N. Thomson, and L. A. Perkins, 1985 Axonal guidance mutants of *Caenorhabditis elegans* identified by filling sensory neurons with fluorescein dyes. *Dev. Biol.* 111: 158–170.
- Hedgecock, E. M., J. G. Culotti, D. H. Hall, and B. D. Stern, 1987 Genetics of cell and axon migrations in *Caenorhabditis elegans*. *Development* 100: 365–382.
- Hedgecock, E. M., J. G. Culotti, and D. H. Hall, 1990 The *unc-5*, *unc-6*, and *unc-40* genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in *C. elegans*. *Neuron* 4: 61–85.
- Hekimi, S., and D. Kershaw, 1993 Axonal guidance defects in a *Caenorhabditis elegans* mutant reveal cell-extrinsic determinants of neuronal morphology. *J. Neurosci.* 13: 4254–4271.
- Hill, E., I. D. Broadbent, C. Chothia, and J. Pettitt, 2001 Cadherin superfamily proteins in *Caenorhabditis elegans* and *Drosophila melanogaster*. *J. Mol. Biol.* 305: 1011–1024.
- Hilliard, M. A., and C. I. Bargmann, 2006 Wnt signals and frizzled activity orient anterior-posterior axon outgrowth in *C. elegans*. *Dev. Cell* 10: 379–390.
- Hobert, O., and H. Bülow, 2003 Development and maintenance of neuronal architecture at the ventral midline of *C. elegans*. *Curr. Opin. Neurobiol.* 13: 70–78.
- Honigberg, L., and C. Kenyon, 2000 Establishment of left/right asymmetry in neuroblast migration by UNC-40/DCC, UNC-73/Trio and DPY-19 proteins in *C. elegans*. *Development* 127: 4655–4668.
- Huang, C. C., D. H. Hall, E. M. Hedgecock, G. Kao, V. Karantza *et al.*, 2003 Laminin alpha subunits and their role in *C. elegans* development. *Development* 130: 3343–3358.
- Huang, X., H. J. Cheng, M. Tessier-Lavigne, and Y. Jin, 2002 MAX-1, a novel PH/MyTH4/FERM domain cytoplasmic protein implicated in netrin-mediated axon repulsion. *Neuron* 34: 563–576.
- Huang, X., P. Huang, M. K. Robinson, M. J. Stern, and Y. Jin, 2003 UNC-71, a disintegrin and metalloprotease (ADAM) protein, regulates motor axon guidance and sex myoblast migration in *C. elegans*. *Development* 130: 3147–3161.
- Hubert, T., Z. Wu, A. D. Chisholm, and Y. Jin, 2014 S6 kinase inhibits intrinsic axon regeneration capacity via AMP kinase in *Caenorhabditis elegans*. *J. Neurosci.* 34: 758–763.
- Hudson, M. L., T. Kinnunen, H. N. Cinar, and A. D. Chisholm, 2006 *C. elegans* Kallmann syndrome protein KAL-1 interacts with syndecan and glypican to regulate neuronal cell migrations. *Dev. Biol.* 294: 352–365.
- Hutter, H., 2003 Extracellular cues and pioneers act together to guide axons in the ventral cord of *C. elegans*. *Development* 130: 5307–5318.
- Hutter, H., B. E. Vogel, J. D. Plenefisch, C. R. Norris, R. B. Proenca *et al.*, 2000 Conservation and novelty in the evolution of cell adhesion and extracellular matrix genes. *Science* 287: 989–994.
- Hutter, H., I. Wacker, C. Schmid, and E. M. Hedgecock, 2005 Novel genes controlling ventral cord asymmetry and navigation of pioneer axons in *C. elegans*. *Dev. Biol.* 284: 260–272.
- Ishii, N., W. G. Wadsworth, B. D. Stern, J. G. Culotti, and E. M. Hedgecock, 1992 UNC-6, a laminin-related protein, guides cell and pioneer axon migrations in *C. elegans*. *Neuron* 9: 873–881.
- Jarrell, T. A., Y. Wang, A. E. Bloniarz, C. A. Brittin, M. Xu *et al.*, 2012 The connectome of a decision-making neural network. *Science* 337: 437–444.
- Johnson, R. P., and J. M. Kramer, 2012 *C. elegans* dystroglycan coordinates responsiveness of follower axons to dorsal/ventral and anterior/posterior guidance cues. *Dev. Neurobiol.* 72: 1498–1515.
- Kaletsky, R., V. Lakhina, R. Arey, A. Williams, J. Landis *et al.*, 2016 The *C. elegans* adult neuronal IIS/FOXO transcriptome reveals adult phenotype regulators. *Nature* 529: 92–96.
- Kang, S. H., and J. M. Kramer, 2000 Nidogen is nonessential and not required for normal type IV collagen localization in *Caenorhabditis elegans*. *Mol. Biol. Cell* 11: 3911–3923.
- Kao, G., C. C. Huang, E. M. Hedgecock, D. H. Hall, and W. G. Wadsworth, 2006 The role of the laminin beta subunit in laminin heterotrimer assembly and basement membrane function and development in *C. elegans*. *Dev. Biol.* 290: 211–219.
- Kasthuri, N., K. J. Hayworth, D. R. Berger, R. L. Schalek, J. A. Conchello *et al.*, 2015 Saturated reconstruction of a volume of neocortex. *Cell* 162: 648–661.
- Katidou, M., N. Tavernarakis, and D. Karagogeos, 2013 The contactin RIG-6 mediates neuronal and non-neuronal cell migration in *Caenorhabditis elegans*. *Dev. Biol.* 373: 184–195.

- Kawano, T., H. Zheng, D. C. Merz, Y. Kohara, K. K. Tamai *et al.*, 2009 *C. elegans mig-6* encodes papilin isoforms that affect distinct aspects of DTC migration, and interacts genetically with mig-17 and collagen IV. *Development* 136: 1433–1442.
- Kawano, Y., T. Yoshimura, D. Tsuboi, S. Kawabata, T. Kaneko-Kawano *et al.*, 2005 CRMP-2 is involved in kinesin-1-dependent transport of the Sra-1/WAVE1 complex and axon formation. *Mol. Cell. Biol.* 25: 9920–9935.
- Keino-Masu, K., M. Masu, L. Hinck, E. D. Leonardo, S. S. Chan *et al.*, 1996 Deleted in Colorectal Cancer (DCC) encodes a netrin receptor. *Cell* 87: 175–185.
- Kennedy, T. E., T. Serafini, J. R. de la Torre, and M. Tessier-Lavigne, 1994 Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. *Cell* 78: 425–435.
- Kennerdell, J. R., R. D. Fetter, and C. I. Bargmann, 2009 Wnt-Ror signaling to SIA and SIB neurons directs anterior axon guidance and nerve ring placement in *C. elegans*. *Development* 136: 3801–3810.
- Killeen, M. T., and S. S. Sybingco, 2008 Netrin, Slit and Wnt receptors allow axons to choose the axis of migration. *Dev. Biol.* 323: 143–151.
- Kim, S., and W. G. Wadsworth, 2000 Positioning of longitudinal nerves in *C. elegans* by nidogen. *Science* 288: 150–154.
- Kim, S., X. C. Ren, E. Fox, and W. G. Wadsworth, 1999 SDQR migrations in *Caenorhabditis elegans* are controlled by multiple guidance cues and changing responses to netrin UNC-6. *Development* 126: 3881–3890.
- Kirszenblat, L., D. Pattabiraman, and M. A. Hilliard, 2011 LIN-44/Wnt directs dendrite outgrowth through LIN-17/Frizzled in *C. elegans* Neurons. *PLoS Biol.* 9: e1001157.
- Kirszenblat, L., B. Neumann, S. Coakley, and M. A. Hilliard, 2013 A dominant mutation in *mec-7/beta-tubulin* affects axon development and regeneration in *Caenorhabditis elegans* neurons. *Mol. Biol. Cell* 24: 285–296.
- Knobel, K. M., E. M. Jorgensen, and M. J. Bastiani, 1999 Growth cones stall and collapse during axon outgrowth in *Caenorhabditis elegans*. *Development* 126: 4489–4498.
- Kolodkin, A. L., 1998 Semaphorin-mediated neuronal growth cone guidance. *Prog. Brain Res.* 117: 115–132.
- Kolodkin, A. L., and M. Tessier-Lavigne, 2011 Mechanisms and molecules of neuronal wiring: a primer. *Cold Spring Harb. Perspect. Biol.* 3: pii: a001727.
- Kosmaczewski, S. G., S. M. Han, B. Han, B. Irving Meyer, H. S. Baig *et al.*, 2015 RNA ligation in neurons by RtcB inhibits axon regeneration. *Proc. Natl. Acad. Sci. USA* 112: 8451–8456.
- Kramer, J. M., 2005 Basement membranes (September 1, 2005), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.16.1, <http://www.wormbook.org>.
- Krause, M., J. D. Leslie, M. Stewart, E. M. Lafuente, F. Valderrama *et al.*, 2004 Lamellipodin, an ena/VASP ligand, is implicated in the regulation of lamellipodial dynamics. *Dev. Cell* 7: 571–583.
- Kubota, Y., R. Kuroki, and K. Nishiwaki, 2004 A fibulin-1 homolog interacts with an ADAM protease that controls cell migration in *C. elegans*. *Curr. Biol.* 14: 2011–2018.
- Kulkarni, G., H. Li, and W. G. Wadsworth, 2008 CLEC-38, a transmembrane protein with C-type lectin-like domains, negatively regulates UNC-40-mediated axon outgrowth and promotes pre-synaptic development in *Caenorhabditis elegans*. *J. Neurosci.* 28: 4541–4550.
- Kulkarni, G., Z. Xu, A. M. Mohamed, H. Li, X. Tang *et al.*, 2013 Experimental evidence for UNC-6 (netrin) axon guidance by stochastic fluctuations of intracellular UNC-40 (DCC) outgrowth activity. *Biol. Open* 2: 1300–1312.
- Kurup, N., D. Yan, A. Goncharov, and Y. Jin, 2015 Dynamic microtubules drive circuit rewiring in the absence of neurite remodeling. *Curr. Biol.* 25: 1594–1605.
- Lai, T., and G. Garriga, 2004 The conserved kinase UNC-51 acts with VAB-8 and UNC-14 to regulate axon outgrowth in *C. elegans*. *Development* 131: 5991–6000.
- Lee, M., E. J. Cram, B. Shen, and J. E. Schwarzbauer, 2001 Roles for beta(pat-3) integrins in development and function of *Caenorhabditis elegans* muscles and gonads. *J. Biol. Chem.* 276: 36404–36410.
- Lemke, G., and M. Reber, 2005 Retinotectal mapping: new insights from molecular genetics. *Annu. Rev. Cell Dev. Biol.* 21: 551–580.
- Leonardo, E. D., L. Hinck, M. Masu, K. Keino-Masu, S. L. Ackerman *et al.*, 1997 Vertebrate homologues of *C. elegans* UNC-5 are candidate netrin receptors. *Nature* 386: 833–838.
- Leung-Hageteijn, C., A. M. Spence, B. D. Stern, Y. Zhou, M. W. Su *et al.*, 1992 UNC-5, a transmembrane protein with immunoglobulin and thrombospondin type 1 domains, guides cell and pioneer axon migrations in *C. elegans*. *Cell* 71: 289–299.
- Levy-Strumpf, N., 2016 Orchestrating A/P and D/V guidance - A Wnt/netrin tale. *Worm* 5: e1146857.
- Levy-Strumpf, N., and J. G. Culotti, 2007 VAB-8, UNC-73 and MIG-2 regulate axon polarity and cell migration functions of UNC-40 in *C. elegans*. *Nat. Neurosci.* 10: 161–168.
- Levy-Strumpf, N., and J. G. Culotti, 2014 Netrins and Wnts function redundantly to regulate antero-posterior and dorso-ventral guidance in *C. elegans*. *PLoS Genet.* 10: e1004381.
- Levy-Strumpf, N., M. Krizus, H. Zheng, L. Brown, and J. G. Culotti, 2015 The Wnt frizzled receptor MOM-5 regulates the UNC-5 netrin receptor through small GTPase-dependent signaling to determine the polarity of migrating Cells. *PLoS Genet.* 11: e1005446.
- Li, C., N. Hisamoto, P. Nix, S. Kanao, T. Mizuno *et al.*, 2012 The growth factor SVH-1 regulates axon regeneration in *C. elegans* via the JNK MAPK cascade. *Nat. Neurosci.* 15: 551–557.
- Li, C., N. Hisamoto, and K. Matsumoto, 2015 Axon regeneration is regulated by Ets-C/EBP transcription complexes generated by activation of the cAMP/Ca2+ signaling pathways. *PLoS Genet.* 11: e1005603.
- Li, H., G. Kulkarni, and W. G. Wadsworth, 2008 RPM-1, a *Caenorhabditis elegans* protein that functions in presynaptic differentiation, negatively regulates axon outgrowth by controlling SAX-3/robo and UNC-5/UNC5 activity. *J. Neurosci.* 28: 3595–3603.
- Loria, P. M., A. Duke, J. B. Rand, and O. Hobert, 2003 Two neuronal, nuclear-localized RNA binding proteins involved in synaptic transmission. *Curr. Biol.* 13: 1317–1323.
- Lucanic, M., M. Kiley, N. Ashcroft, N. Etoile, and H. J. Cheng, 2006 The *Caenorhabditis elegans* P21-activated kinases are differentially required for UNC-6/netrin-mediated commissural motor axon guidance. *Development* 133: 4549–4559.
- Lundquist, E. A., R. K. Herman, J. E. Shaw, and C. I. Bargmann, 1998 UNC-115, a conserved protein with predicted LIM and actin-binding domains, mediates axon guidance in *C. elegans*. *Neuron* 21: 385–392.
- Lundquist, E. A., P. W. Reddien, E. Hartwig, H. R. Horvitz, and C. I. Bargmann, 2001 Three *C. elegans* Rac proteins and several alternative Rac regulators control axon guidance, cell migration and apoptotic cell phagocytosis. *Development* 128: 4475–4488.
- MacNeil, L. T., W. R. Hardy, T. Pawson, J. L. Wrana, and J. G. Culotti, 2009 UNC-129 regulates the balance between UNC-40 dependent and independent UNC-5 signaling pathways. *Nat. Neurosci.* 12: 150–155.
- Maniar, T. A., M. Kaplan, G. J. Wang, K. Shen, L. Wei *et al.*, 2012 UNC-33 (CRMP) and ankyrin organize microtubules and localize kinesin to polarize axon-dendrite sorting. *Nat. Neurosci.* 15: 48–56.
- Marcette, J. D., J. J. Chen, and M. L. Nonet, 2014 The *Caenorhabditis elegans* microtubule minus-end binding homolog PTRN-1 stabilizes synapses and neurites. *eLife* 3: e01637.

- Maro, G. S., M. P. Klassen, and K. Shen, 2009 A beta-catenin-dependent Wnt pathway mediates anteroposterior axon guidance in *C. elegans* motor neurons. *PLoS One* 4: e4690.
- McIntire, S. L., G. Garriga, J. White, D. Jacobson, and H. R. Horvitz, 1992 Genes necessary for directed axonal elongation or fasciculation in *C. elegans*. *Neuron* 8: 307–322.
- McShea, M. A., K. L. Schmidt, M. L. Dubuke, C. E. Baldiga, M. E. Sullender *et al.*, 2013 Abelson interactor-1 (ABI-1) interacts with MRL adaptor protein MIG-10 and is required in guided cell migrations and process outgrowth in *C. elegans*. *Dev. Biol.* 373: 1–13.
- Mehlen, P., C. Delloye-Bourgeois, and A. Chédotal, 2011 Novel roles for Slits and netrins: axon guidance cues as anticancer targets? *Nat. Rev. Cancer* 11: 188–197.
- Meighan, C. M., and J. E. Schwarzbauer, 2007 Control of *C. elegans* hermaphrodite gonad size and shape by vab-3/Pax6-mediated regulation of integrin receptors. *Genes Dev.* 21: 1615–1620.
- Meng, L., C. H. Chen, and D. Yan, 2016 Regulation of gap junction dynamics by UNC-44/ankyrin and UNC-33/CRMP through VAB-8 in *C. elegans* neurons. *PLoS Genet.* 12: e1005948.
- Merz, D. C., H. Zheng, M. T. Killeen, A. Krizus, and J. G. Culotti, 2001 Multiple signaling mechanisms of the *unc-6*/netrin receptors *unc-5* and *unc-40/dcc* in vivo. *Genetics* 158: 1071–1080.
- Merz, D. C., G. Alves, T. Kawano, H. Zheng, and J. G. Culotti, 2003 UNC-52/perlecan affects gonadal leader cell migrations in *C. elegans* hermaphrodites through alterations in growth factor signaling. *Dev. Biol.* 256: 173–186.
- Mizuno, T., N. Hisamoto, T. Terada, T. Kondo, M. Adachi *et al.*, 2004 The *Caenorhabditis elegans* MAPK phosphatase VHP-1 mediates a novel JNK-like signaling pathway in stress response. *EMBO J.* 23: 2226–2234.
- Moffat, L. L., R. E. Robinson, A. Bakoulis, and S. G. Clark, 2014 The conserved transmembrane RING finger protein PLR-1 downregulates Wnt signaling by reducing Frizzled, Ror and Ryk cell-surface levels in *C. elegans*. *Development* 141: 617–628.
- Mohamed, A. M., and I. D. Chin-Sang, 2006 Characterization of loss-of-function and gain-of-function Eph receptor tyrosine kinase signaling in *C. elegans* axon targeting and cell migration. *Dev. Biol.* 290: 164–176.
- Mohamed, A. M., J. R. Boudreau, F. P. Yu, J. Liu, and I. D. Chin-Sang, 2012 The *Caenorhabditis elegans* Eph receptor activates NCK and N-WASP, and inhibits Ena/VASP to regulate growth cone dynamics during axon guidance. *PLoS Genet.* 8: e1002513.
- Mohler, W. A., G. Shemer, J. J. del Campo, C. Valansi, E. Opoku-Serebuoh *et al.*, 2002 The type I membrane protein EFF-1 is essential for developmental cell fusion. *Dev. Cell* 2: 355–362.
- Morrissey, M. A., R. Jayadev, G. R. Miley, C. A. Blebea, Q. Chi *et al.*, 2016 SPARC promotes cell invasion in vivo by decreasing type IV collagen levels in the basement membrane. *PLoS Genet.* 12: e1005905.
- Murshed, M., N. Smyth, N. Miosge, J. Karolat, T. Krieg *et al.*, 2000 The absence of nidogen 1 does not affect murine basement membrane formation. *Mol. Cell. Biol.* 20: 7007–7012.
- Nakamura, F., K. Kumeta, T. Hida, T. Isono, Y. Nakayama *et al.*, 2014 Amino- and carboxyl-terminal domains of Filamin-A interact with CRMP1 to mediate Sema3A signalling. *Nat. Commun.* 5: 5325.
- Nakata, K., B. Abrams, B. Grill, A. Goncharov, X. Huang *et al.*, 2005 Regulation of a DLK-1 and p38 MAP kinase pathway by the ubiquitin ligase RPM-1 is required for presynaptic development. *Cell* 120: 407–420.
- Nash, B., A. Colavita, H. Zheng, P. J. Roy, and J. G. Culotti, 2000 The forkhead transcription factor UNC-130 is required for the graded spatial expression of the UNC-129 TGF-beta guidance factor in *C. elegans*. *Genes Dev.* 14: 2486–2500.
- Nelson, J. C., and D. A. Colón-Ramos, 2013 Serotonergic neurosecretory synapse targeting is controlled by netrin-releasing guidepost neurons in *Caenorhabditis elegans*. *J. Neurosci.* 33: 1366–1376.
- Neumann, S., and C. J. Woolf, 1999 Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury. *Neuron* 23: 83–91.
- Neumann, B., K. C. Nguyen, D. H. Hall, A. Ben-Yakar, and M. A. Hilliard, 2011 Axonal regeneration proceeds through specific axonal fusion in transected *C. elegans* neurons. *Dev. Dyn.* 240: 1365–1372.
- Neumann, B., S. Coakley, R. Giordano-Santini, C. Linton, E. S. Lee *et al.*, 2015 EFF-1-mediated regenerative axonal fusion requires components of the apoptotic pathway. *Nature* 517: 219–222.
- Nichols, A. L., E. Meelkop, C. Linton, R. Giordano-Santini, R. K. Sullivan *et al.*, 2016 The apoptotic engulfment machinery regulates axonal degeneration in *C. elegans* neurons. *Cell Reports* 14: 1673–1683.
- Nishiwaki, K., N. Hisamoto, and K. Matsumoto, 2000 A metalloprotease disintegrin that controls cell migration in *Caenorhabditis elegans*. *Science* 288: 2205–2208.
- Nix, P., N. Hisamoto, K. Matsumoto, and M. Bastiani, 2011 Axon regeneration requires coordinate activation of p38 and JNK MAPK pathways. *Proc. Natl. Acad. Sci. USA* 108: 10738–10743.
- Nix, P., M. Hammarlund, L. Hauth, M. Lachnit, E. M. Jorgensen *et al.*, 2014 Axon regeneration genes identified by RNAi screening in *C. elegans*. *J. Neurosci.* 34: 629–645.
- Norris, A. D., and E. A. Lundquist, 2011 UNC-6/netrin and its receptors UNC-5 and UNC-40/DCC modulate growth cone protrusion in vivo in *C. elegans*. *Development* 138: 4433–4442.
- Norris, A. D., J. O. Dyer, and E. A. Lundquist, 2009 The Arp2/3 complex, UNC-115/abLIM, and UNC-34/enabled regulate axon guidance and growth cone filopodia formation in *Caenorhabditis elegans*. *Neural Dev.* 4: 38.
- Norris, A. D., L. Sundararajan, D. E. Morgan, Z. J. Roberts, and E. A. Lundquist, 2014 The UNC-6/netrin receptors UNC-40/DCC and UNC-5 inhibit growth cone filopodial protrusion via UNC-73/Trio, Rac-like GTPases and UNC-33/CRMP. *Development* 141: 4395–4405.
- O'Rourke, S. M., S. N. Christensen, and B. Bowerman, 2010 *Caenorhabditis elegans* EFA-6 limits microtubule growth at the cell cortex. *Nat. Cell Biol.* 12: 1235–1241.
- Onishi, K., E. Hollis, and Y. Zou, 2014 Axon guidance and injury-lessons from Wnts and Wnt signaling. *Curr. Opin. Neurobiol.* 27: 232–240.
- Opperman, K. J., and B. Grill, 2014 RPM-1 is localized to distinct subcellular compartments and regulates axon length in GABAergic motor neurons. *Neural Dev.* 9: 10.
- Oren-Suissa, M., D. H. Hall, M. Treinin, G. Shemer, and B. Podbilewicz, 2010 The fusogen EFF-1 controls sculpting of mechanosensory dendrites. *Science* 328: 1285–1288.
- Otsuka, A. J., R. Franco, B. Yang, K. H. Shim, L. Z. Tang *et al.*, 1995 An ankyrin-related gene (*unc-44*) is necessary for proper axonal guidance in *Caenorhabditis elegans*. *J. Cell Biol.* 129: 1081–1092.
- Pan, C. L., J. E. Howell, S. G. Clark, M. Hilliard, S. Cordes *et al.*, 2006 Multiple Wnts and frizzled receptors regulate anteriorly directed cell and growth cone migrations in *Caenorhabditis elegans*. *Dev. Cell* 10: 367–377.
- Park, J., P. L. Knezevich, W. Wung, S. N. O'Hanlon, A. Goyal *et al.*, 2011 A conserved juxtacrine signal regulates synaptic partner recognition in *Caenorhabditis elegans*. *Neural Dev.* 6: 28.
- Pastuhov, S. I., K. Fujiki, P. Nix, S. Kanao, M. Bastiani *et al.*, 2012 Endocannabinoid-Galpha signalling inhibits axon regeneration in *Caenorhabditis elegans* by antagonizing Gqalpha-PKC-JNK signalling. *Nat. Commun.* 3: 1136.

- Pastuhov, S. I., N. Hisamoto, and K. Matsumoto, 2015 MAP kinase cascades regulating axon regeneration in *C. elegans*. *Proc. Jpn. Acad., Ser. B, Phys. Biol. Sci.* 91: 63–75.
- Pastuhov, S. I., K. Matsumoto, and N. Hisamoto, 2016 Endocannabinoid signaling regulates regenerative axon navigation in *Caenorhabditis elegans* via the GPCRs NPR-19 and NPR-32. *Genes Cells* 21: 696–705.
- Patel, M. R., E. K. Lehrman, V. Y. Poon, J. G. Crump, M. Zhen *et al.*, 2006 Hierarchical assembly of presynaptic components in defined *C. elegans* synapses. *Nat. Neurosci.* 9: 1488–1498.
- Peckol, E. L., J. A. Zallen, J. C. Yarrow, and C. I. Bargmann, 1999 Sensory activity affects sensory axon development in *C. elegans*. *Development* 126: 1891–1902.
- Perkins, L. A., E. M. Hedgecock, J. N. Thomson, and J. G. Culotti, 1986 Mutant sensory cilia in the nematode *Caenorhabditis elegans*. *Dev. Biol.* 117: 456–487.
- Pilon, M., 2014 Developmental genetics of the *Caenorhabditis elegans* pharynx. *Wiley Interdiscip. Rev. Dev. Biol.* 3: 263–280.
- Pinan-Lucarre, B., C. V. Gabel, C. P. Reina, S. E. Hulme, S. S. Shevkopyas *et al.*, 2012 The core apoptotic executioner proteins CED-3 and CED-4 promote initiation of neuronal regeneration in *Caenorhabditis elegans*. *PLoS Biol.* 10: e1001331.
- Pocock, R., and O. Hobert, 2008 Oxygen levels affect axon guidance and neuronal migration in *Caenorhabditis elegans*. *Nat. Neurosci.* 11: 894–900.
- Poinat, P., A. De Arcangelis, S. Sookhareea, X. Zhu, E. M. Hedgecock *et al.*, 2002 A conserved interaction between beta1 integrin/PAT-3 and Nck-interacting kinase/MIG-15 that mediates commissural axon navigation in *C. elegans*. *Curr. Biol.* 12: 622–631.
- Pollard, T. D., and G. G. Borisy, 2003 Cellular motility driven by assembly and disassembly of actin filaments. *Cell* 112: 453–465.
- Pollard, T. D., and J. A. Cooper, 2009 Actin, a central player in cell shape and movement. *Science* 326: 1208–1212.
- Polleux, F., and W. Snider, 2010 Initiating and growing an axon. *Cold Spring Harb. Perspect. Biol.* 2: a001925.
- Poon, V. Y., M. P. Klassen, and K. Shen, 2008 UNC-6/netrin and its receptor UNC-5 locally exclude presynaptic components from dendrites. *Nature* 455: 669–673.
- Poulain, F. E., and H. J. Yost, 2015 Heparan sulfate proteoglycans: a sugar code for vertebrate development? *Development* 142: 3456–3467.
- Prasad, B. C., and S. G. Clark, 2006 Wnt signaling establishes anteroposterior neuronal polarity and requires retromer in *C. elegans*. *Development* 133: 1757–1766.
- Prasad, B. C., B. Ye, R. Zackhary, K. Schrader, G. Seydoux *et al.*, 1998 *unc-3*, a gene required for axonal guidance in *Caenorhabditis elegans*, encodes a member of the O/E family of transcription factors. *Development* 125: 1561–1568.
- Quinn, C. C., and W. G. Wadsworth, 2008 Axon guidance: asymmetric signaling orients polarized outgrowth. *Trends Cell Biol.* 18: 597–603.
- Quinn, C. C., D. S. Pfeil, E. Chen, E. L. Stovall, M. V. Harden *et al.*, 2006 UNC-6/netrin and SLT-1/slit guidance cues orient axon outgrowth mediated by MIG-10/RIAM/lamellipodin. *Curr. Biol.* 16: 845–853.
- Quinn, C. C., D. S. Pfeil, and W. G. Wadsworth, 2008 CED-10/Rac1 mediates axon guidance by regulating the asymmetric distribution of MIG-10/lamellipodin. *Curr. Biol.* 18: 808–813.
- Ren, X. C., S. Kim, E. Fox, E. M. Hedgecock, and W. G. Wadsworth, 1999 Role of netrin UNC-6 in patterning the longitudinal nerves of *Caenorhabditis elegans*. *J. Neurobiol.* 39: 107–118.
- Rhiner, C., S. Gysi, E. Frohli, M. O. Hengartner, and A. Hajnal, 2005 Syndecan regulates cell migration and axon guidance in *C. elegans*. *Development* 132: 4621–4633.
- Richardson, C. E., K. A. Spilker, J. G. Cueva, J. Perrino, M. B. Goodman *et al.*, 2014 PTRN-1, a microtubule minus end-binding CAMSAP homolog, promotes microtubule function in *Caenorhabditis elegans* neurons. *eLife* 3: e01498.
- Rogalski, T. M., B. D. Williams, G. P. Mullen, and D. G. Moerman, 1993 Products of the *unc-52* gene in *Caenorhabditis elegans* are homologous to the core protein of the mammalian basement membrane heparan sulfate proteoglycan. *Genes Dev.* 7: 1471–1484.
- Rohde, C. B., and M. F. Yanik, 2011 Subcellular in vivo time-lapse imaging and optical manipulation of *Caenorhabditis elegans* in standard multiwell plates. *Nat. Commun.* 2: 271.
- Roy, P. J., H. Zheng, C. E. Warren, and J. G. Culotti, 2000 mab-20 encodes Semaphorin-2a and is required to prevent ectopic cell contacts during epidermal morphogenesis in *Caenorhabditis elegans*. *Development* 127: 755–767.
- Salzberg, Y., C. A. Díaz-Balzac, N. J. Ramirez-Suarez, M. Attreed, E. Tecle *et al.*, 2013 Skin-derived cues control arborization of sensory dendrites in *Caenorhabditis elegans*. *Cell* 155: 308–320.
- Samara, C., C. B. Rohde, C. L. Gilleland, S. Norton, S. J. Haggarty *et al.*, 2010 Large-scale in vivo femtosecond laser neurosurgery screen reveals small-molecule enhancer of regeneration. *Proc. Natl. Acad. Sci. USA* 107: 18342–18347.
- Sasakura, H., H. Inada, A. Kuhara, E. Fusaoka, D. Takemoto *et al.*, 2005 Maintenance of neuronal positions in organized ganglia by SAX-7, a *Caenorhabditis elegans* homologue of L1. *EMBO J.* 24: 1477–1488.
- Sawa, H., and H. C. Korswagen, 2013 Wnt signaling in *C. elegans* (December 9, 2013), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.7.2, <http://www.wormbook.org>.
- Schaefer, A. M., G. D. Hadwiger, and M. L. Nonet, 2000 *rpm-1*, a conserved neuronal gene that regulates targeting and synaptogenesis in *C. elegans*. *Neuron* 26: 345–356.
- Schmidt, K. L., N. Marcus-Gueret, A. Adeleye, J. Webber, D. Baillie *et al.*, 2009 The cell migration molecule UNC-53/NAV2 is linked to the ARP2/3 complex by ABI-1. *Development* 136: 563–574.
- Schmitz, C., P. Kinge, and H. Hutter, 2007 Axon guidance genes identified in a large-scale RNAi screen using the RNAi-hypersensitive *Caenorhabditis elegans* strain *nre-1(hd20) lin-15b(hd126)*. *Proc. Natl. Acad. Sci. USA* 104: 834–839.
- Schwarz, V., J. Pan, S. Voltmer-Irsch, and H. Hutter, 2009 IgCAMs redundantly control axon navigation in *Caenorhabditis elegans*. *Neural Dev.* 4: 13.
- Schwieterman, A. A., A. N. Steves, V. Yee, C. J. Donelson, M. R. Bentley *et al.*, 2016 The *Caenorhabditis elegans* ephrin EFN-4 functions non-cell autonomously with heparan sulfate proteoglycans to promote axon outgrowth and branching. *Genetics* 202: 639–660.
- Schymeinsky, J., S. Nedbal, N. Miosge, E. Pöschl, C. Rao *et al.*, 2002 Gene structure and functional analysis of the mouse nidogen-2 gene: nidogen-2 is not essential for basement membrane formation in mice. *Mol. Cell. Biol.* 22: 6820–6830.
- Seetharaman, A., G. Selman, R. Puckrin, L. Barbier, E. Wong *et al.*, 2011 MADD-4 is a secreted cue required for midline-oriented guidance in *Caenorhabditis elegans*. *Dev. Cell* 21: 669–680.
- Serafini, T., T. E. Kennedy, M. J. Galko, C. Mirzayan, T. M. Jessell *et al.*, 1994 The netrins define a family of axon outgrowth-promoting proteins homologous to *C. elegans* UNC-6. *Cell* 78: 409–424.
- Shaham, S., 2015 Glial development and function in the nervous system of *Caenorhabditis elegans*. *Cold Spring Harb. Perspect. Biol.* 7: a020578.
- Shakir, M. A., J. S. Gill, and E. A. Lundquist, 2006 Interactions of UNC-34 Enabled with Rac GTPases and the NIK kinase MIG-15 in *Caenorhabditis elegans* axon pathfinding and neuronal migration. *Genetics* 172: 893–913.

- Shakir, M. A., K. Jiang, E. C. Struckhoff, R. S. Demarco, F. B. Patel *et al.*, 2008 The Arp2/3 activators WAVE and WASP have distinct genetic interactions with Rac GTPases in *Caenorhabditis elegans* axon guidance. *Genetics* 179: 1957–1971.
- Shaye, D. D., and I. Greenwald, 2011 OrthoList: a compendium of *C. elegans* genes with human orthologs. *PLoS One* 6: e20085.
- Sherwood, D. R., and P. W. Sternberg, 2003 Anchor cell invasion into the vulval epithelium in *C. elegans*. *Dev. Cell* 5: 21–31.
- Siddiqui, S. S., and J. G. Culotti, 1991 Examination of neurons in wild type and mutants of *Caenorhabditis elegans* using antibodies to horseradish peroxidase. *J. Neurogenet.* 7: 193–211.
- Siddiqui, S. S., E. Aamodt, F. Rastinejad, and J. Culotti, 1989 Anti-tubulin monoclonal antibodies that bind to specific neurons in *Caenorhabditis elegans*. *J. Neurosci.* 9: 2963–2972.
- Song, S., B. Zhang, H. Sun, X. Li, Y. Xiang *et al.*, 2010 A Wnt-Frizzled pathway regulates neurite outgrowth in *Caenorhabditis elegans*. *PLoS Genet.* 6: e1001056.
- Song, Y., D. Sretavan, E. A. Salegio, J. Berg, X. Huang *et al.*, 2015 Regulation of axon regeneration by the RNA repair and splicing pathway. *Nat. Neurosci.* 18: 817–825.
- Soto, M. C., H. Qadota, K. Kasuya, M. Inoue, D. Tsuboi *et al.*, 2002 The GEX-2 and GEX-3 proteins are required for tissue morphogenesis and cell migrations in *C. elegans*. *Genes Dev.* 16: 620–632.
- Stavoe, A. K., and D. A. Colón-Ramos, 2012 Netrin instructs synaptic vesicle clustering through Rac GTPase, MIG-10, and the actin cytoskeleton. *J. Cell Biol.* 197: 75–88.
- Stavoe, A. K., J. C. Nelson, L. A. Martínez-Velázquez, M. Klein, A. D. Samuel *et al.*, 2012 Synaptic vesicle clustering requires a distinct MIG-10/Lamellipodin isoform and ABI-1 downstream from Netrin. *Genes Dev.* 26: 2206–2221.
- Steimel, A., L. Wong, E. H. Najjarro, B. D. Ackley, G. Garriga *et al.*, 2010 The Flamingo ortholog FMI-1 controls pioneer-dependent navigation of follower axons in *C. elegans*. *Development* 137: 3663–3673.
- Stringham, E. G., and K. L. Schmidt, 2009 Navigating the cell: UNC-53 and the navigators, a family of cytoskeletal regulators with multiple roles in cell migration, outgrowth and trafficking. *Cell Adhes. Migr.* 3: 342–346.
- Struckhoff, E. C., and E. A. Lundquist, 2003 The actin-binding protein UNC-115 is an effector of Rac signaling during axon pathfinding in *C. elegans*. *Development* 130: 693–704.
- Sun, L., J. Shay, M. McLoed, K. Roodhouse, S. H. Chung *et al.*, 2014 Neuronal regeneration in *C. elegans* requires subcellular calcium release by ryanodine receptor channels and can be enhanced by optogenetic stimulation. *J. Neurosci.* 34: 15947–15956.
- Takenawa, T., and S. Suetsugu, 2007 The WASP-WAVE protein network: connecting the membrane to the cytoskeleton. *Nat. Rev. Mol. Cell Biol.* 8: 37–48.
- Tamai, K. K., and K. Nishiwaki, 2007 bHLH transcription factors regulate organ morphogenesis via activation of an ADAMTS protease in *C. elegans*. *Dev. Biol.* 308: 562–571.
- Tang, N. H., and A. D. Chisholm, 2016 Regulation of microtubule dynamics in axon regeneration: insights from *C. elegans*. *F1000Res* 5. pii: F1000.
- Tang, X., and W. G. Wadsworth, 2014 SAX-3 (Robo) and UNC-40 (DCC) regulate a directional bias for axon guidance in response to multiple extracellular cues. *PLoS One* 9: e110031.
- Tank, E. M., K. E. Rodgers, and C. Kenyon, 2011 Spontaneous age-related neurite branching in *Caenorhabditis elegans*. *J. Neurosci.* 31: 9279–9288.
- Tedeschi, A., 2011 Tuning the orchestra: transcriptional pathways controlling axon regeneration. *Front. Mol. Neurosci.* 4: 60.
- Tessier-Lavigne, M., and C. S. Goodman, 1996 The molecular biology of axon guidance. *Science* 274: 1123–1133.
- Teulière, J., C. Gally, G. Garriga, M. Labouesse, and E. Georges-Labouesse, 2011 MIG-15 and ERM-1 promote growth cone directional migration in parallel to UNC-116 and WVE-1. *Development* 138: 4475–4485.
- Tian, D., M. Diao, Y. Jiang, L. Sun, Y. Zhang *et al.*, 2015 Anillin regulates neuronal migration and neurite growth by linking RhoG to the actin cytoskeleton. *Curr. Biol.* 25: 1135–1145.
- Torpe, N., and R. Pocock, 2014 Regulation of axonal midline guidance by prolyl 4-hydroxylation in *Caenorhabditis elegans*. *J. Neurosci.* 34: 16348–16357.
- Toth, M. L., I. Melentijevic, L. Shah, A. Bhatia, K. Lu *et al.*, 2012 Neurite sprouting and synapse deterioration in the aging *Caenorhabditis elegans* nervous system. *J. Neurosci.* 32: 8778–8790.
- Tu, H., B. Pinan-Lucarré, T. Ji, M. Jospin, and J. L. Bessereau, 2015 *C. elegans* punctin clusters GABA(A) receptors via neuroligin binding and UNC-40/DCC recruitment. *Neuron* 86: 1407–1419.
- Tucker, M., and M. Han, 2008 Muscle cell migrations of *C. elegans* are mediated by the alpha-integrin INA-1, Eph receptor VAB-1, and a novel peptidase homologue MNP-1. *Dev. Biol.* 318: 215–223.
- Tulgren, E. D., S. M. Turgeon, K. J. Opperman, and B. Grill, 2014 The nesprin family member ANC-1 regulates synapse formation and axon termination by functioning in a pathway with RPM-1 and β -Catenin. *PLoS Genet.* 10: e1004481.
- Unsoeld, T., J. O. Park, and H. Hutter, 2013 Discoidin domain receptors guide axons along longitudinal tracts in *C. elegans*. *Dev. Biol.* 374: 142–152.
- Viveiros, R., H. Hutter, and D. G. Moerman, 2011 Membrane extensions are associated with proper anterior migration of muscle cells during *Caenorhabditis elegans* embryogenesis. *Dev. Biol.* 358: 189–200.
- Wacker, I., V. Schwarz, E. M. Hedgecock, and H. Hutter, 2003 *zag-1*, a Zn-finger homeodomain transcription factor controlling neuronal differentiation and axon outgrowth in *C. elegans*. *Development* 130: 3795–3805.
- Wadsworth, W. G., 2002 Moving around in a worm: netrin UNC-6 and circumferential axon guidance in *C. elegans*. *Trends Neurosci.* 25: 423–429.
- Wadsworth, W. G., and E. M. Hedgecock, 1996 Hierarchical guidance cues in the developing nervous system of *C. elegans*. *Bio-Essays* 18: 355–362.
- Wadsworth, W. G., H. Bhatt, and E. M. Hedgecock, 1996 Neuroglia and pioneer neurons express UNC-6 to provide global and local netrin cues for guiding migrations in *C. elegans*. *Neuron* 16: 35–46.
- Wang, F., 2009 The signaling mechanisms underlying cell polarity and chemotaxis. *Cold Spring Harb. Perspect. Biol.* 1: a002980.
- Wang, X., P. J. Roy, S. J. Holland, L. W. Zhang, J. G. Culotti *et al.*, 1999 Multiple ephrins control cell organization in *C. elegans* using kinase-dependent and -independent functions of the VAB-1 Eph receptor. *Mol. Cell* 4: 903–913.
- Wang, X., J. Kweon, S. Larson, and L. Chen, 2005 A role for the *C. elegans* L1CAM homologue *lad-1/sax-7* in maintaining tissue attachment. *Dev. Biol.* 284: 273–291.
- Wang, X., W. Zhang, T. Cheever, V. Schwarz, K. Opperman *et al.*, 2008 The *C. elegans* L1CAM homologue LAD-2 functions as a coreceptor in MAB-20/Sema2 mediated axon guidance. *J. Cell Biol.* 180: 233–246.
- Wang, Z., Y. Hou, X. Guo, M. van der Voet, M. Boxem *et al.*, 2013 The EBAX-type Cullin-RING E3 ligase and Hsp90 guard the protein quality of the SAX-3/Robo receptor in developing neurons. *Neuron* 79: 903–916.
- Wang, Z., L. M. Linden, K. M. Naegeli, J. W. Ziel, Q. Chi *et al.*, 2014 UNC-6 (netrin) stabilizes oscillatory clustering of the UNC-40 (DCC) receptor to orient polarity. *J. Cell Biol.* 206: 619–633.
- Ward, S., N. Thomson, J. G. White, and S. Brenner, 1975 Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *J. Comp. Neurol.* 160: 313–337.

- Ware, R. W., D. Clark, K. Crossland, and R. L. Russell, 1975 The nerve ring of the nematode *Caenorhabditis elegans*: sensory input and motor output. *J. Comp. Neurol.* 162: 71–110.
- Watari-Goshima, N., K. Ogura, F. W. Wolf, Y. Goshima, and G. Garriga, 2007 *C. elegans* VAB-8 and UNC-73 regulate the SAX-3 receptor to direct cell and growth-cone migrations. *Nat. Neurosci.* 10: 169–176.
- Watkins, T. A., B. Wang, S. Huntwork-Rodriguez, J. Yang, Z. Jiang *et al.*, 2013 DLK initiates a transcriptional program that couples apoptotic and regenerative responses to axonal injury. *Proc. Natl. Acad. Sci. USA* 110: 4039–4044.
- White, J. G., 1985 Neuronal connectivity in *Caenorhabditis elegans*. *Trends Neurosci.* 8: 277–283.
- White, J., E. Southgate, J. Thompson, and S. Brenner, 1986 The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 314: 1–340.
- Wightman, B., R. Baran, and G. Garriga, 1997 Genes that guide growth cones along the *C. elegans* ventral nerve cord. *Development* 124: 2571–2580.
- Williams, B. D., and R. H. Waterston, 1994 Genes critical for muscle development and function in *Caenorhabditis elegans* identified through lethal mutations. *J. Cell Biol.* 124: 475–490.
- Williams, W., P. Nix, and M. Bastiani, 2011 Constructing a low-budget laser axotomy system to study axon regeneration in *C. elegans*. *J. Vis. Exp.* 57: 3331.
- Wu, Z., A. Ghosh-Roy, M. F. Yanik, J. Z. Zhang, Y. Jin *et al.*, 2007 *Caenorhabditis elegans* neuronal regeneration is influenced by life stage, ephrin signaling, and synaptic branching. *Proc. Natl. Acad. Sci. USA* 104: 15132–15137.
- Xu, Y., H. Taru, Y. Jin, and C. C. Quinn, 2015 SYD-1C, UNC-40 (DCC) and SAX-3 (Robo) function interdependently to promote axon guidance by regulating the MIG-2 GTPase. *PLoS Genet.* 11: e1005185.
- Xu, Z., H. Li, and W. G. Wadsworth, 2009 The roles of multiple UNC-40 (DCC) receptor-mediated signals in determining neuronal asymmetry induced by the UNC-6 (netrin) ligand. *Genetics* 183: 941–949.
- Yam, P. T., and F. Charron, 2013 Signaling mechanisms of non-conventional axon guidance cues: the Shh, BMP and Wnt morphogens. *Curr. Opin. Neurobiol.* 23: 965–973.
- Yan, D., and Y. Jin, 2012 Regulation of DLK-1 kinase activity by calcium-mediated dissociation from an inhibitory isoform. *Neuron* 76: 534–548.
- Yan, D., Z. Wu, A. D. Chisholm, and Y. Jin, 2009 The DLK-1 kinase promotes mRNA stability and local translation in *C. elegans* synapses and axon regeneration. *Cell* 138: 1005–1018.
- Yang, Y., W. S. Lee, X. Tang, and W. G. Wadsworth, 2014 Extracellular matrix regulates UNC-6 (Netrin) axon guidance by controlling the direction of intracellular UNC-40 (DCC) outgrowth activity. *PLoS One* 9: e97258.
- Yanik, M. F., H. Cinar, H. N. Cinar, A. D. Chisholm, Y. Jin *et al.*, 2004 Neurosurgery: functional regeneration after laser axotomy. *Nature* 432: 822.
- Yanik, M. F., H. Cinar, H. N. Cinar, A. Gibby, A. D. Chisholm *et al.*, 2006 Nerve regeneration in *Caenorhabditis elegans* after femtosecond laser axotomy. *IEEE J. Sel. Top. Quantum Electron.* 12: 1283–1290.
- Yaniv, S. P., N. Issman-Zecharya, M. Oren-Suissa, B. Podbilewicz, and O. Schuldiner, 2012 Axon regrowth during development and regeneration following injury share molecular mechanisms. *Curr. Biol.* 22: 1774–1782.
- Yee, C. S., S. S. Sybingco, V. Serdetchania, G. Kholkina, M. Bueno de Mesquita *et al.*, 2011 ENU-3 is a novel motor axon outgrowth and guidance protein in *C. elegans*. *Dev. Biol.* 352: 243–253.
- Yoshimura, S., J. I. Murray, Y. Lu, R. H. Waterston, and S. Shaham, 2008 *mls-2* and *vab-3* Control glia development, *hlh-17/Olig* expression and glia-dependent neurite extension in *C. elegans*. *Development* 135: 2263–2275.
- Yu, T. W., J. C. Hao, W. Lim, M. Tessier-Lavigne, and C. I. Bargmann, 2002 Shared receptors in axon guidance: SAX-3/robo signals via UNC-34/enabled and a netrin-independent UNC-40/DCC function. *Nat. Neurosci.* 5: 1147–1154.
- Zallen, J. A., S. A. Kirch, and C. I. Bargmann, 1999 Genes required for axon pathfinding and extension in the *C. elegans* nerve ring. *Development* 126: 3679–3692.
- Zhen, M., X. Huang, B. Bamber, and Y. Jin, 2000 Regulation of presynaptic terminal organization by *C. elegans* RPM-1, a putative guanine nucleotide exchanger with a RING-H2 finger domain. *Neuron* 26: 331–343.
- Zhou, S., K. Opperman, X. Wang, and L. Chen, 2008 *unc-44* Ankyrin and *stn-2* gamma-syntrophin regulate *sax-7* L1CAM function in maintaining neuronal positioning in *Caenorhabditis elegans*. *Genetics* 180: 1429–1443.
- Ziel, J. W., E. J. Hagedorn, A. Audhya, and D. R. Sherwood, 2009 UNC-6 (netrin) orients the invasive membrane of the anchor cell in *C. elegans*. *Nat. Cell Biol.* 11: 183–189.
- Zou, Y., H. Chiu, A. Zinovyeva, V. Ambros, C. F. Chuang *et al.*, 2013 Developmental decline in neuronal regeneration by the progressive change of two intrinsic timers. *Science* 340: 372–376.

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