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Out with the Old, In with the New: Dendrite Degeneration and Regeneration

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Abstract

Dendrites do not form macroscopic structures analogous to axons bundled into nerves. There has therefore been little investigation of how neurons respond to dendrite damage. Although in vertebrate animals, dendrites typically reside under bone in the central nervous system, they are damaged by trauma including stroke, traumatic brain injury, and seizure. Whether neurons can recover from irreversible dendrite damage in these scenarios is not yet known. However, acute removal of dendrites using laser microsurgery in model organisms has been used to probe responses of neurons to dendrite damage. After severing, the detached region of the dendrite undergoes stereotyped degeneration similar to Wallerian degeneration of axons. This degeneration is followed by robust regeneration of the dendrite arbor in *Caenorhabditis elegans* (worms), *Drosophila melanogaster* (flies), and *Danio rerio* (zebrafish). While little is known about the proteins that initiate dendrite regeneration, the core axon injury sensing pathway is not used. Some progress has been made on dissecting key cellular processes required for the outgrowth of new dendrites, but, overall, the molecular drivers of dendrite regeneration remain largely open to discovery. Here, we compare dendrite and axon regeneration and summarize what is known in the new field of dendrite regeneration.

3.1 Historical Perspective of Neuronal Regeneration

For most of human history, nerves were thought not to regenerate after injury (West, 1978). This prevailing view began to crumble when William

Cruikshank presented his findings on vagus nerve severing in dogs to the Royal Society in 1776. Much to his surprise, he found that if he waited for weeks rather than days after the surgery, the animal would completely recover (Cruikshank, 1795). As with many controversial findings, it took some time – 19 years – for this work to actually be published (Ochs, 1977). The same volume of the Philosophical Transactions of the Royal Society of London in which Cruikshank's work was finally published included John Haighton's work on vagus nerve transection (Haighton, 1795). Haighton made similar observations to Cruikshank: that recovery was possible, but only if weeks were allowed. The opening to his article makes it very clear that what was going on during this recovery process was poorly understood. It was debated whether the repair of nerves involved nervous tissue or instead some other bridging material; Haighton argued that it was nerve tissue (Haighton, 1795). Improvements in microscopy allowed the fibrous substance in nerves (axons) to be visualized better, and two camps emerged in the next century: those that thought repair was mediated by reusing the fibers in the part distal to transection, and those that thought new fibers emerged from the proximal part (Ochs, 1977; West, 1978). Note that although most of the body was accepted to be made up of cells by 1850, the intertwined networks of the nervous system meant it was not until the 1890s that it was generally accepted to be composed of individual cells (Shepherd, 1991). However, even before neurons were defined, Augustus Waller's careful microscopy and descriptions of frog and cat nerves after transection in the 1850s laid the foundation for understanding what happens inside nerves after injury. He described internal beading within the distal regions, and fibers extending in from the proximal side (Ochs, 1977). Although acceptance of these observations was not immediate, the basic model that fibers in cut-off pieces of the nerve degenerate and are replaced by growth from the proximal stump forms the framework for our understanding of nervous system regeneration.

Today, when we talk about regeneration in the nervous system, we primarily mean axon regeneration in the periphery. Early anatomists like Cruikshank and Haighton could identify individual nerves, perform surgeries to remove segments of them, and then track whether the animal recovered. Sprouting of fibers from the stump was observed by Waller, Cajal, and others, and this kept the cell body largely out of the picture. This is in contrast to most other tissues, where regeneration refers to rebuilding the tissue through addition of new cells. That most bilaterian animals do not add new neurons (at least in most areas of the nervous system) after early development (Ming and Song, 2011) supports the idea that neuronal regeneration is axon regeneration. However, there may be another conserved type of endogenous

neuronal regeneration that we simply did not consider for historical and technical reasons: dendrite regeneration. Before we move on to considering the evidence that neurons may respond to dendrite injury in a manner similar to axon injury, let us first consider several variations on axon regeneration that are outside the original anatomical framework.

The competing model for the growth of fibers from the stump into the distal damaged nerve was that fibers (axons) in the distal piece contributed to recovery, perhaps by fusing with upstream segments. In fact, this type of repair has been observed in some invertebrates including earthworms, leeches, and crayfish (Neumann et al., 2019). This process has been best documented in the nematode *C. elegans*. In this animal, cut-off pieces of some axons can rejoin with the proximal axon to be reconnected to the cell body (Teoh et al., 2018). This type of regeneration involves plasma membrane fusion and can lead to functional recovery (Abay et al., 2017; Basu et al., 2017). However, it seems restricted to animals with few neurons, perhaps because fusion in a nerve with many axons would be difficult to accomplish without potentially disastrous connections between mismatched proximal and distal axons.

The other variation of axon regeneration that was not accessible to early anatomists was regeneration from a site other than the severed stump. In 2008, the Bradke lab used modern visualization methods to demonstrate that, after axons of cultured mouse hippocampal neurons were severed near the soma, the stump was not competent to reinitiate growth. Instead, a new axon emerged by converting a dendrite and growing out from it (Gomis-Ruth et al., 2008). Precedent for this observation came from studies in sea lampreys (Hall and Cohen, 1983; Hall et al., 1989), cats (MacDermid et al., 2002; MacDermid et al., 2004; Rose and Odlozinski, 1998), hamsters (Cho and So, 1992), and rats (Hoang et al., 2005). This ability to convert a dendrite into a regenerating axon after proximal axotomy seems broadly conserved as it also occurs in *Drosophila* (Stone et al., 2010). One interesting aspect of this type of regeneration is that the new axon typically emerges outside the nerve that would normally guide growth of axons regenerating from the stump. However, in slice culture, these axons can make synaptic connections (Gomis-Ruth et al., 2008). *In vivo* in *Drosophila*, the new axons wander, but if they happen to encounter the nerve that originally carried them, they grow in a directed manner along it (Rao and Rolls, 2017). The ability to track single cells after injury has revealed some unexpected variations on axon regeneration. Is it therefore possible that other types of nervous system regeneration remain to be discovered? One might imagine that, for many neuron types, loss of the receptive field via dendrite damage would be functionally

as problematic as a severed axon. In that context, investigating regeneration of dendrites would seem as important as that of the axon.

3.2 Do Dendrites Get Damaged?

For the possibility of dendrite regeneration to be worth exploring, there should be scenarios where dendrites become damaged and regeneration might be useful. In vertebrate animals, dendrites are largely shielded by bone in the brain and spinal cord. One possible exception is the enteric nervous system, although these neurons have not been characterized enough at the cellular level to know whether they have dendrites. However, even under bone, dendrites have been shown to suffer acute injury in several clinically important scenarios. Traumatic brain injury (TBI) can result in dendrite beading (Gao and Chen, 2011; Gao et al., 2011) and loss of branches (Wang et al., 2016). Dendrite beading and degeneration have also been observed in human samples after surgical removal from epilepsy patients (Isokawa and Levesque, 1991) as well as in experimental kainate-induced seizure *in vivo* (Zeng et al., 2007) and in explants (Al-Noori and Swann, 2000). In fact, excessive electrical activity and neurite beading were linked over 100 years ago by Jean DeMoor (1898). Ischemia also causes dendrite beading and loss (Ji et al., 2021; Purpura et al., 1982). One model for dendritic sensitivity to epilepsy and ischemic stroke is that both conditions lead to high extracellular concentration of glutamate, which in turn activates NMDA receptors on dendrites and leads to massive calcium and sodium influx (Greenwood and Connolly, 2007; Verma et al., 2022).

Dendrite beading is used as a key hallmark of dendrite injury in part because it can be detected in fixed tissues by classic methods including Golgi staining. However, it is not as easy to interpret as, say, cutting out a section of nerve to initiate axon regeneration. Using live cell imaging of neurons in slice culture or *in vivo*, it has become possible to track individual dendrites after seizure or ischemia. These studies indicate that some level of dendrite beading is rapidly (minutes to several hours) reversible once normal activity or blood flow is restored (Murphy et al., 2008; Zeng et al., 2007). However, more global damage (Oliva et al., 2002) or repeated spreading depolarization after initial ischemia (Risher et al., 2010) can lead to irreversible beading and dendrite loss. Thus, it is likely that dendrites are acutely and irreversibly damaged in conditions that have a large impact on human health.

Dendrites also accumulate damage in long-term degenerative diseases including Alzheimer's (Anderton et al., 1998). However, whether regeneration plays a role in mitigating the effects of neurodegenerative disease has not

yet even been determined for axons, so will be set aside for now in favor of acute injury scenarios.

3.3 Does Naturally Occurring Dendrite Damage Lead to Regeneration?

In the previous section, we identified TBI, stroke, and seizure as causes of dendrite damage. So, what are the cellular outcomes of this damage? Reversal of beading is one outcome that has been documented, and, at the other extreme, all of these types of trauma lead to cell death. The live imaging techniques that allowed rapid recovery to be observed are much more difficult on the longer timescales required to track the fate of individual neurons that suffer irreversible dendrite damage. The bottom line then is that, as far as we know, individual cells that have undergone dendrite beading without immediate recovery have not been tracked in mammals. One hint that neurons can recover from at least moderate dendrite damage was, however, published recently. Dendrite complexity was examined at different times after ischemic stroke in mice. Notably, 6–24 h after ischemia, dendrites of pyramidal neurons in the stroke penumbra lost length and complexity (Ji et al., 2021). In 4 days after ischemia, these changes were no longer detectable (Ji et al., 2021). Whether this partial loss and recovery of dendrite complexity is regeneration in the sense we mean for axons, or whether it is a larger scale version of normal dendrite plasticity, remains to be determined. But it does suggest that dendrite regrowth could be an important contributor to nervous system resilience in vertebrates.

If it is not definitively known whether dendrite regeneration happens in clinically relevant conditions, should we pursue it? First, because a phenomenon is technically difficult to observe does not mean it does not happen. One reason to think it may be important is the ability of dendrites to regenerate after controlled injury in invertebrate, and now vertebrate (Stone et al., 2022), model systems. Another reason is the existence of an active program of dendrite degeneration similar to Wallerian axon degeneration. That dendrites can be injured in the same manner as axons and also possess a similar method of injury-induced degeneration suggests that regenerative responses may also exist for both.

3.4 Dendrites have an active program of degeneration

Axon regeneration is preceded by Wallerian degeneration, an active process that clears away pieces of the axon no longer connected to the cell body.

It proceeds in a stereotyped stepwise manner. The basic framework for a molecular understanding of Wallerian degeneration has been pieced together relatively recently (Coleman and Hoke, 2020; Ding and Hammarlund, 2019; Figley and DiAntonio, 2020), even though the process itself was described in some detail in the 19th century. The central player in Wallerian degeneration is Sarm, which was linked to axon degeneration in 2012 (Osterloh et al., 2012). Sarm is an NAD hydrolase that becomes activated after axon injury to rapidly deplete NAD in the cut-off region (Essuman et al., 2017; Gerdts et al., 2015). Either NAD depletion or products of NAD hydrolysis, or both, lead to axon self-destruction (Coleman and Hoke, 2020; Ding and Hammarlund, 2019; Figley and DiAntonio, 2020). Nmnat, an enzyme in both the NAD synthesis and salvage pathways, acts as an axon survival factor by counteracting Sarm (Coleman and Hoke, 2020; Ding and Hammarlund, 2019; Figley and DiAntonio, 2020).

Does a degeneration program also exist for injured dendrites? Dendrite degeneration after injury morphologically resembles axon degeneration, in that there are discrete steps including beading before clearance (Tao and Rolls, 2011). Like axon degeneration, injury-induced dendrite degeneration is blocked by expression of Wlds (Tao and Rolls, 2011), an activated form of Nmnat, hinting that Sarm activation may also drive dendrite degeneration. Indeed, Sarm is required for injury-induced dendrite degeneration and degeneration induced by loss of Nmnat (Ji et al., 2022). There may be some differences between active axon and dendrite degeneration, for example, phosphatidylserine exposure is an important downstream mediator of Sarm activity in dendrites (Ji et al., 2022), but has not been placed downstream of Sarm in axon degeneration. In addition, the microtubule severing protein fidgetin plays a role in dendrite degeneration but not axon degeneration (Tao et al., 2016). However, it seems that dendrites have an active program of injury-induced degeneration, and the core machinery likely overlaps with that of axon degeneration.

3.5 Dendrites Can Regenerate After Controlled Injury

While it remains to be determined whether dendrites can regenerate after tissue-level damage induced by stroke, TBI or other trauma, after controlled dendrite removal, neurons can regrow a new arbor (Figure 3.1). This process has been best described in *Drosophila* dendritic arborization neurons. Although these are peripheral sensory neurons that innervate the body wall, they are polarized in many of the same ways that were initially described in mammalian central neurons (Rolls, 2011; Rolls and Jegla, 2015). For

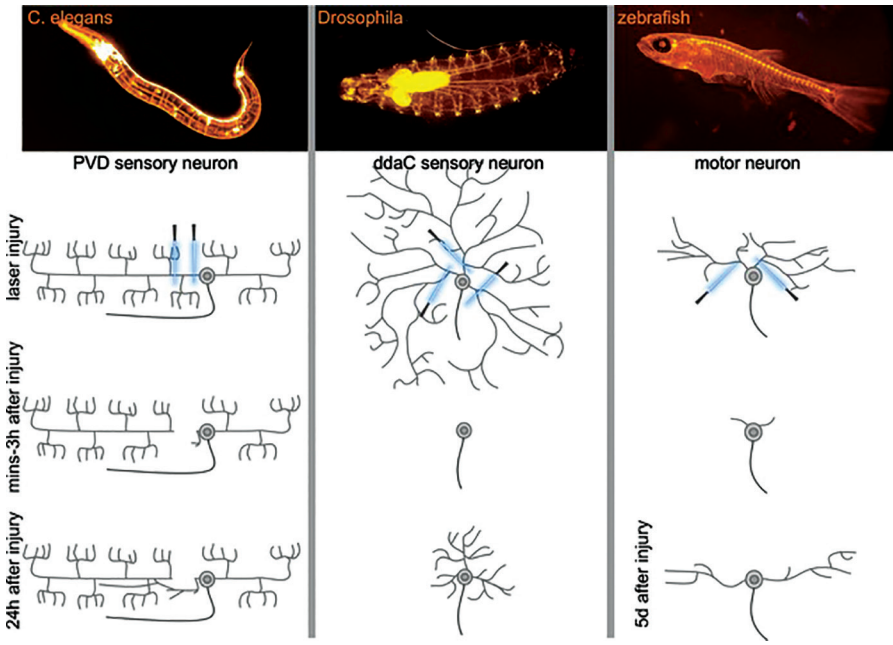

Figure 3.1 Model systems for studying dendrite regeneration. Laser microsurgery (indicated with light saber) has been used to sever dendrites and initiate regeneration in *C. elegans* (left), *Drosophila* larvae (middle), and zebrafish larvae (right). Sensory neurons in *C. elegans* initiate dendrite outgrowth followed by fusion. After complete removal of *Drosophila* sensory dendrites, a new arbor is grown. Similarly, severing dendrites of motor neurons in the zebrafish spinal cord is followed by outgrowth of dendrites until they reach approximately the same length. Times diagrammed for injury responses in each organism are shown at the left of the *C. elegans* column; the same times are shown for all organisms except the last time point for zebrafish (five days).

example, axons and dendrites have different arrangements of microtubules (Stone et al., 2008), dendrites contain Golgi outposts (Ye et al., 2007) and ribosomes (Hill et al., 2012), and the proximal axon houses a diffusion barrier organized by giant ankyrins (Jegla et al., 2016). Perhaps the most important broadly shared feature of these cells in the context of injury is their reliance upon dual leucine zipper kinase (DLK) to initiate axon regeneration. DLK was first identified as essential for axon regeneration in *C. elegans* (Hammarlund et al., 2009; Yan et al., 2009). It was then shown to be critical for axon injury signaling in *Drosophila* motor neurons (Xiong et al., 2010) and for response to peripheral axon injury in rodents (Shin et al., 2012). DLK is absolutely required to initiate axon regeneration in dendritic arborization neurons (Stone et al., 2014) and without DLK and its sister kinase

LZK, motor axon regeneration in zebrafish is eliminated (Adula et al., 2022). Because the *Drosophila* dendritic arborization neurons rely on the conserved core injury signaling machinery and have dendrites near the surface of the animal, they are an excellent model in which to study the response to dendrite injury.

The most complex class of dendritic arborization neurons, class IV, which includes the ddaC neuron, has been used as a model in which to investigate dendrite patterning, pruning, microtubule polarity, and regeneration in *Drosophila* larvae (Corty et al., 2009; Furusawa and Emoto, 2021; Singhania and Grueber, 2014). These neurons are nociceptors that are responsible for detecting oviposition by parasitoid wasps and initiating escape behavior (Hwang et al., 2007). They grow large arbors and display both stereotyped homotypic and heterotypic repulsion – that is, each neuron has a specific receptive field and avoids its own and the dendrites of neighboring neurons (Corty et al., 2009) to most efficiently provide the body with nociceptive capability. Laser surgery was first used to study the effect of dendrite removal in these cells in 2003 (Sugimura et al., 2003). Single dendrite branches were removed in young larvae and the newly empty area was filled in by growth from the stump or neighboring dendrites (Sugimura et al., 2003). This recovery was interpreted as a response to removal of growth inhibition rather than activation of an injury signal and subsequent regeneration. In support of this argument, removal of one branch from the simpler ddaE neuron, which does not exhibit the same type of tiling behavior as ddaC, did not trigger growth to fill the missing area (Sugimura et al., 2003). In 2014, laser microsurgery was used to remove the entire dendrite arbors from both ddaC and ddaE neurons and both cell types were able to regrow dendrite arbors (Stone et al., 2014 and Figure 3.1). For the ddaE cell, the injury was performed after final dendrite shape had been attained and these cells grew a new dendrite arbor of similar complexity, but quite different shape, to the initial arbor (Stone et al., 2014). ddaC neurons continue to add branches throughout larval life; so these cells were still undergoing shape change at the time of injury. They grew to recover their normal territory within four days (Stone et al., 2014 and Figure 3.2). Regeneration of ddaE and ddaC dendrites was corroborated by another group (Thompson-Peer et al., 2016) that also analyzed another type of sensory neuron, the class III ddaA cell, and found it too could regenerate dendrite branches after injury (Thompson-Peer et al., 2016). In these studies, injury initiated a change in cellular behavior, leading to the interpretation that dendrite regeneration is triggered by an injury signal analogous to axon regeneration. However, it is possible that the signal to initiate regeneration is removal of growth inhibition as proposed in

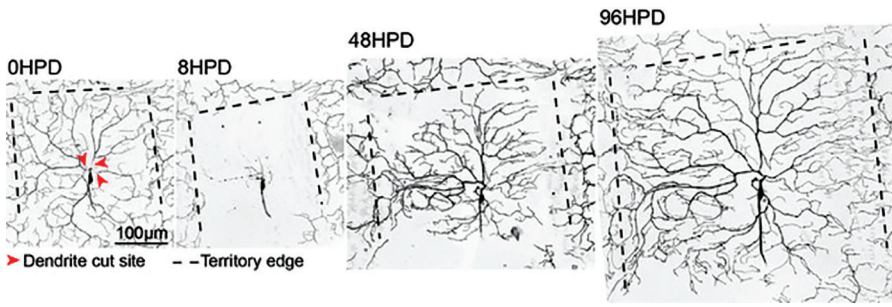

Figure 3.2 Dendrites of *Drosophila* ddaC sensory neurons regenerate to cover their receptive field after laser severing. *Drosophila* ddaC neurons have complex dendrite arbors that cover specific, tiled regions of the larval epidermis. When those dendrites are individually severed with a laser (first panel on left), they start to degenerate within a couple of hours, and most of the debris is cleared by 8 hours post dendrotomy (HPD) (second panel). By 4 HPD, regeneration has already been initiated and new small, branchy dendrites are emerging from the cell body. At 48 HPD the dendrites are long enough to reach the edges of the larval body segment (third panel). This regeneration of dendrites continues over a period of days until the dendrites fully cover the voided area at 96 HPD (fourth panel). Images to show the full dendrite arbor are assembled from multiple image tiles.

2003. In either case, these studies clearly demonstrated that, *in vivo*, mature, fully functional peripheral neurons can regrow dendrites in response to their removal.

Responses to dendrite injury have also been studied in another invertebrate, *C. elegans*. Most *C. elegans* neurons have simple, unbranched dendrites, but the PVD cell is a dramatic exception. This peripheral neuron, which, like *Drosophila* dendritic arborization neurons, innervates the body wall and responds to mechanical stimuli, has two large dendrite branches that elaborate a stereotyped branching pattern along the length of the organism (Tsalik et al., 2003). There is one neuron on each side of the animal, with quaternary branches reaching toward the dorsal and ventral midlines. Laser microsurgery of dendrites triggers dendrite outgrowth and subsequent fusion to repair the arbor (Oren-Suissa et al., 2017 and Figure 3.1). Axon repair by fusion also occurs in *C. elegans* as discussed in the first section. Thus, dendrite injury seems to initiate a regenerative response that is overtly similar to axon regeneration in *C. elegans*. Another injury paradigm has also suggested that *C. elegans* neurons sense and respond to dendrite injury. One key difference in this study was that the cell that was used, the ASJ sensory neuron, has a dendrite that terminates in a cilium rather than a branched dendrite arbor like PVD. Laser injury to the dendrite at the same time as the axon altered the

axon regeneration program so that it no longer required DLK (Chung et al., 2016). Growth of dendrites was not tracked in this study, but may not be possible for ciliated dendrites as the cilium is likely irreplaceable.

In mice, neurons can survive laser-mediated dendrite severing, but cells have only been tracked for 3 h after injury; so regeneration capacity was not determined (Zhao et al., 2017). A recent study in zebrafish has, however, demonstrated that neurons can regrow dendrites after laser surgery in the spinal cord (Stone et al., 2022). Motor neuron dendrites were severed in young fish, and after one or both major branches were removed, regrowth was initiated. By about five days after injury, their former length was restored (Figure 3.1). Thus, the phenomenon of dendrite arbor regrowth after controlled injury has been observed in invertebrates and vertebrates. Therefore, animals may broadly share the capacity to regenerate dendrites just as they share a program of axon regeneration.

Whether regenerated dendrites can restore function to a circuit remained an open question until recently. In *Drosophila*, two earlier studies suggested that functional recovery occurs. Class III dendritic arborization neurons including ddaA normally respond to gentle touch, and action potential bursts were seen in extracellular field recordings when a probe was applied to the cuticle both near and far from the soma (Thompson-Peer et al., 2016). Recently balded (all dendrites removed) neurons showed no response, and neurons with regenerated dendrites had an attenuated but reasonable response to stimulus (Thompson-Peer et al., 2016). A similar paradigm was employed in class IV v'ada neurons in adult *Drosophila*. These neurons are chemically sensitive and display bursts of action potentials upon administration of an acid stimulus. Again, recently balded neurons did not respond to the stimulus, but after seven days, regenerated and uninjured dendrites were almost identical in their ability to transduce an acid stimulus to an action potential burst (DeVault et al., 2018). Note that these neurons that regenerated in the adult had smaller, more compact, dendrite arbors than uninjured counterparts, but their electrical response was identical (DeVault et al., 2018). In 2023, laser surgery was used to remove dendrites or axons of almost all Class IV neurons that innervate the dorsal surface of the animal. This large-scale axon or dendrite removal allowed standard functional assays using a heated probe to target the denervated region. Removal of dendrites strongly reduced the response to noxious heat, but, surprisingly, responsiveness was almost completely restored 24 hours after injury when new dendrite arbors were still small (Hertzler, Bernard, & Rolls, 2023). Functional recovery did not occur in a genetic background in which regenerative growth was blocked, confirming that it is dendrite regeneration that restores neuronal function to

sensory dendrite arbors (Hertzler et al., 2023). It will be interesting to determine whether post-synaptic dendrites can be similarly restored.

3.6 The Molecular Program of Dendrite Regeneration is Distinct from that of Axon Regeneration

The basic process of dendrite regrowth after injury is visually similar to axon regeneration. After injury, cells that had previously attained a stable shape reinitiate massive outgrowth, either from the injured stump or another site. Dendrite regeneration therefore likely involves similar steps to axon regeneration including injury sensing, transcriptional reprogramming to a growth state, growth initiation and extension, and, finally, connecting with a target. It therefore seems possible that some of the machinery that controls axon regeneration could be used for dendrite regeneration. In particular, the injury signaling machinery is a good candidate for shared use. In the case of controlled laser axotomy, exactly the same type of injury can elicit axon and dendrite regeneration. As described above, DLK is at the core of axon injury sensing; so is it also involved in sensing dendrite injury? This question has been addressed in both *Drosophila* dendritic arborization neurons and the *C. elegans* PVD neuron. In *Drosophila*, DLK, as well as downstream kinase JNK, and transcription factor fos, are dispensable for dendrite regeneration (Stone et al., 2014). Importantly, axon injury assays were performed in the same cell type and genetic backgrounds, and DLK, JNK, and fos were required for axon regeneration (Stone et al., 2014). A transcriptional reporter for injury signaling initiated by DLK was robustly activated by axon injury, but not by dendrite injury, indicating that not only is the DLK kinase cascade not required for dendrite regeneration, it is not even activated by dendrite injury (Stone et al., 2014). Similarly, the *C. elegans* PVD neuron requires DLK signaling to initiate axon regeneration, but does not use it for dendrite regeneration (Brar et al., 2022). In addition, while elevation of cAMP by reducing levels of a phosphodiesterase improves axon regeneration in *C. elegans* (Ghosh-Roy et al., 2010), there is no effect on dendrite regeneration (Brar et al., 2022). While axon and dendrite regeneration share some broad requirements for growth machinery (see below), these pathways also do not completely converge at later steps. For example, spastin and atlastin are important for the growth phase of axon regeneration but not dendrite regeneration (Rao et al., 2016; Stone et al., 2012).

If different programs are activated by axon and dendrite injury, it is possible that these programs could interfere with one another. However, this does not seem to be the case. In the example of combined axon and dendrite injury

in the ASJ neuron, injury to the dendrite actually enhances axon outgrowth (Chung et al., 2016). In *Drosophila* dendritic arborization neurons, removal of axons and dendrites together initiates regrowth of both types of processes simultaneously (Shorey et al., 2020).

3.7 Molecular Requirements for Dendrite Regeneration

If much of the core axon regeneration machinery is not used during dendrite regeneration, what do we know about the molecular requirements for dendrite regeneration? Dendrite regeneration seems likely to involve steps similar to axon injury. First, a physiological alteration like voltage change or buildup of a specific protein could signal injury (like activation of DLK in axon regeneration (Xiong et al., 2010)). Second, this signal could be transduced into a pathway that activates transcription factors and leads to pro-regenerative gene expression (similar to regeneration-associated gene (RAG) expression after axon injury (Mahar and Cavalli, 2018)). Finally, dendrite outgrowth would begin and involve microtubule activity and membrane addition (the importance of microtubule dynamics in dendrite regeneration already has experimental support (Feng et al., 2019)). Players in the early steps responsible for injury signal and transcriptional reprogramming have not yet been identified (Figure 3.4). This lack of information is striking in contrast to the breadth of knowledge in the field of axon regeneration. Hundreds to thousands of regeneration-associated genes (RAGs) have been shown to change levels after axon injury (Ma and Willis, 2015). There has been only one set of genes described as upregulated after dendrite injury in *Drosophila* (Hertzler et al., 2020). These genes encode kinetochore proteins, which connect microtubules to chromosomes during mitosis. In neurons, they suppress microtubule nucleation in dendrites, and absence of this suppression has a minor inhibitory effect on dendrite regeneration (Hertzler et al., 2020). However, upstream transcriptional regulators were not identified.

So far, our knowledge of proteins that influence dendrite regeneration is mainly limited to those that facilitate growth. These proteins fall broadly into those that seem more important for dendrite regeneration than other types of neurite outgrowth, and proteins broadly required for cell growth that have a shared role in axon and dendrite regeneration or dendrite development. A theme across both categories is the importance of interactions with surrounding cells.

Cytoskeletal regulators:
Cytoskeletal regulators are the main class of protein known to have a specific role in promoting dendrite regeneration. In *C. elegans*, the RAC GTPase

CED-10 and upstream guanine nucleotide exchange factor (GEF) Tiam-1 were recently demonstrated to be required for PVD dendrite, but not axon, regeneration (Brar et al., 2022). As previously mentioned, *C. elegans* dendrites initiate growth and reconnect dendrite stumps with their severed ends after injury, and CED-10 knockdown severely impairs both processes (Brar et al., 2022). Interestingly, both epidermal- and neuron-specific expression of the wild type gene in a null background rescues the phenotype, but in different ways. Re-expression in neurons rescued both reconnection and ectopic branching defects, while re-expression in epidermal cells only rescued the reconnection defect. This suggests that CED-10 has a cell-autonomous role in neurons for promoting regrowth and branching but also a non-autonomous role in neighboring epidermal cells to promote fusion of severed neurites. The RhoGEF TIAM-1 was found to be the upstream activator of CED-10 in this context; knockdown produced the same phenotypes and expression of a constitutively active version of CED-10 in the TIAM-1 null background rescued both branching and fusion phenotypes (Brar et al., 2022). It is not entirely clear how to extrapolate data on fusion of severed neuronal processes from worms to flies or vertebrates; however, it does illustrate well the ideas that epidermal-derived factors are important for regrowth of dendrites and that specific types of cytoskeletal regulation promote dendrite regeneration.

Cytoskeletal regulators that are specifically important for dendrite regeneration have also been identified in *Drosophila*. One of the most critical drivers of neuronal morphology, and regeneration, is the microtubule cytoskeleton. These long polymers of α-β tubulin heterodimers give neurons their shape and an ability to transport proteins to support the long axonal and dendritic processes. Microtubules have intrinsic polarity, and the plus end, where the β-tubulin subunit is exposed, is highly dynamic and undergoes rapid bouts of growth and shrinkage. Axonal microtubule polarity is typically plus-end-out (microtubule plus end oriented away from the soma) and dendrites are characterized by at least partial minus-end-out polarity (microtubule plus end toward the soma) (Baas and Lin, 2011; Rolls and Jegla, 2015). Because the arrangement of microtubules differs in axons and dendrites, it makes sense that microtubule regulators might be differentially important for the two types of regeneration. Uninjured *Drosophila* dendrites are almost exclusively populated by minus-end-out microtubules (Stone et al., 2008). During the first day of dendrite regeneration, growing dendrites have mixed, rather than minus-end-out, polarity, presumably for plus-end-out microtubules to promote growth. Minus-end-out polarity is restored after the second day of regeneration (Stone et al., 2014). Patronin, a microtubule minus end

binding protein, helps microtubule minus ends grow into dendrites, and is critical for restoring minus-end-out polarity during regeneration (Feng et al., 2019). When Patronin is knocked down, not only is microtubule polarity disrupted, but outgrowth of new dendrites is strongly reduced (Feng et al., 2019). Patronin is also required for establishment of microtubule polarity in development, but developmental dendrite outgrowth is not obviously disrupted and relatively normal dendrite shape is obtained in uninjured Patronin knockdown neurons (Feng et al., 2019; Thyagarajan et al., 2022).

Nucleation of new microtubules has also been shown to be critical for dendrite regeneration in *Drosophila* peripheral neurons. The receptor tyrosine kinase (RTK) Ror was identified in a candidate screen to identify RTKs involved in dendrite regeneration (Nye et al., 2020). Rather than being involved in injury signaling, Ror seems to function constitutively to position microtubule nucleation sites in dendrites (Nye et al., 2020). Ror can itself act as a Wnt receptor (Green et al., 2014; Green et al., 2008; Ripp et al., 2018; Stricker et al., 2017) and acts in concert with three other Wnt receptors (frizzled, frizzled2, and arrow), as well as scaffolding proteins Dishevelled and Axin, to recruit nucleation sites to dendrite branch points (Nye et al., 2020; Weiner et al., 2020). Loss of Ror causes specific reductions in dendrite regeneration, but not dendrite outgrowth or axon regeneration (Nye et al., 2020). However, nucleation sites are reduced in uninjured as well as injured dendrites in Ror knockdown neurons (Nye et al., 2020) indicating that this pathway functions prior to injury. Adding to the evidence that regulation of microtubule nucleation is important for dendrite regeneration is the aforementioned paper showing a novel role for kinetochore proteins in neurons (Hertzler et al., 2020). Reduction of kinetochore proteins caused an increase in microtubule dynamics in dendrites, but not axons, and a deficit in dendrite, but not axon, regeneration (Hertzler et al., 2020). The ability to rescue both microtubule and regeneration phenotypes with partial reduction of γ-tubulin, the core microtubule nucleation protein, indicated that kinetochore proteins normally function to limit dendritic microtubule nucleation (Hertzler et al., 2020). Like Wnt signaling proteins, kinetochore protein function was not limited to injured neurons; microtubule phenotypes were observed in uninjured neurons although shape was normal (Hertzler et al., 2020). Dendrite regeneration may be more sensitive to partial depletion of specific proteins than developmental growth in general because more rapid outgrowth is required. During development, dendrites initially grow out in small animals and then expand as the animal grows. During regeneration, the entire area of a now much bigger animal needs to be recovered.

Together, these studies show that dendrite regeneration is particularly sensitive to alterations in microtubule minus end regulation. Developmental dendrite outgrowth seems more resilient to minus end disruption, perhaps because parallel pathways can compensate in this context. Axon regeneration also involves very rapid neurite outgrowth and is unaffected by loss of Ror or kinetochore proteins. In this case, the lack of minus-end-out microtubules in axons may be responsible for their resilience. While control of microtubule minus ends seems unlikely to fit the broad theme of regulation of regeneration by interactions with surrounding cells, it may actually not be an exception. The involvement of Ror and three other Wnt receptors suggests that microtubule nucleation, and thus dendrite regeneration, may require Wnt ligands to be secreted from surrounding cells.

Receptors for environmental cues:
Clear support for the idea that close interactions between dendrites and their environment facilitate regeneration derives from studies on the role of extra-cellular matrix (ECM) receptors. *Drosophila* dendritic arborization neurons elaborate dendrites on the basal surface of epithelial cells and require proper attachment with the ECM for patterning. Neuronal integrins interact with ECM secreted from epithelial cells during dendrite development in the larva (Han et al., 2012; Kim et al., 2012). A subset of dendritic arborization neurons undergoes dendrite pruning in the pupal stage and regrowth into the adult body wall (Shimono et al., 2009). This adult outgrowth is reduced when integrins are knocked down, as is injury-induced regeneration in the adult (DeVault et al., 2018). Moreover, dendrites preferentially regenerated into collagen-rich areas of the ECM in adult flies, and knockdown of matrix metalloproteinase 2, which remodels the ECM after eclosion (Yasunaga et al., 2010), preserved an ECM permissive to dendrite regrowth (DeVault et al., 2018). Consistent with a key role for dendrite-ECM interactions during dendrite development and regeneration, the Ret RTK works with integrins to promote ECM interactions during dendrite development of large class IV dendritic arborization neurons in the Drosophila larva (Soba et al., 2015). It is also important for regeneration of these same neurons after dendrite injury (Nye et al., 2020 and Figure 3.3). The output of these dendrite–ECM interactions has been suggested to be Rac1 control of actin localization (Soba et al., 2015). In *C. elegans* PVD neurons, Rac1 seems more important for dendrite regeneration than outgrowth (see above; Brar et al., 2022), while in class IV Drosophila neurons integrins/Ret acting through Rac1 are important for normal growth and regeneration.

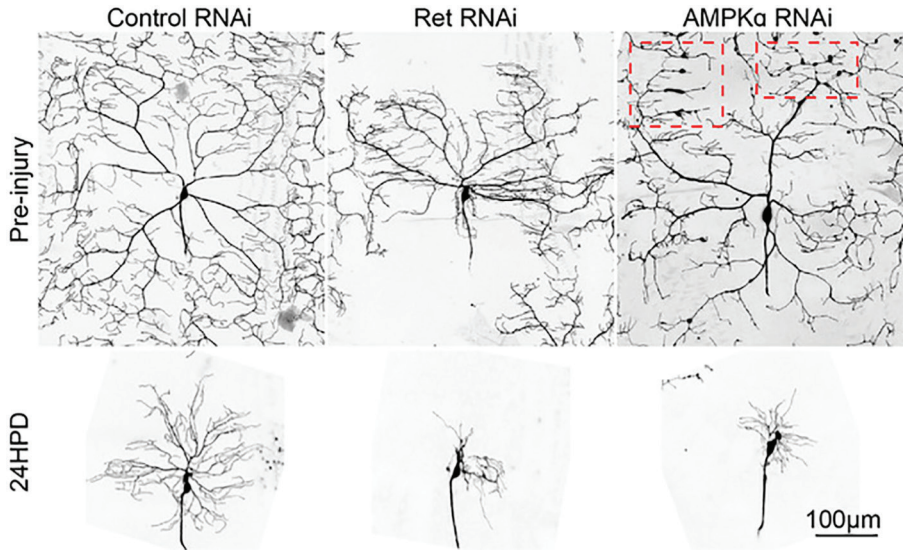

Figure 3.3 Knockdowns that cause dendrite morphology phenotypes usually correspond to defects in dendrite regeneration. While central regulators of dendrite injury signaling and regeneration have not been discovered, there are many proteins that play a role in dendrite morphogenesis and maintenance that also have a role in regeneration. With a control RNAi, dendrites regenerate robustly as early as 24 HPD (left). When Ret is knocked down, there are large gaps in the usually precisely tiled dendrite arbors due to lack of cell adhesion (middle, top). Regeneration after laser severing is also impaired when Ret is knocked down (middle, bottom). AMPKα is responsible for promoting oxidative phosphorylation in neurons, usually reliant on glycolysis, when carbohydrate energy is sparse. This is important for dendrite maintenance during late larval and pupal life of drosophila: when AMPKα is knocked down, dendrites bead and degenerate (right, top, with red dashed regions showing beading). That dendrite regeneration after laser injury is impaired in this knockdown as well suggests that the energy intensive process of regenerating dendrites relies on optimized glycolytic and oxidative phosphorylation energy production.

Cell growth and metabolism regulators:

So far, we have considered regulators of dendrite regeneration that seem to act through the cytoskeleton, either microtubules or actin. The other major class of proteins implicated in dendrite regeneration control cellular growth through membrane delivery, protein synthesis and metabolism. Both axon and dendrite regeneration require rapid neurite outgrowth, and so perhaps unsurprisingly the exocyst complex, which is important for post-Golgi vesicle fusion with the plasma membrane, is strongly required for both (Swope et al., 2022). However, another membrane pathway, concentration of the endoplasmic reticulum near growing neurite tips, is exclusively used for

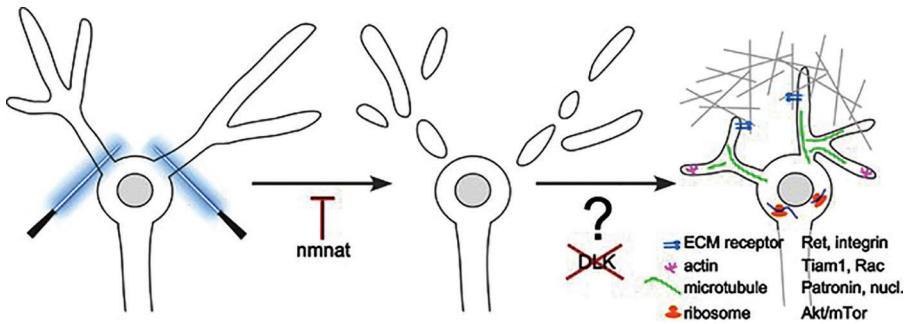

Figure 3.4 Overview of pathways that regulate dendrite degeneration and regeneration. After dendrite removal (left), severed regions undergo beading and then are cleared. Beading and clearance are delayed by expression of the NAD synthesis enzyme nmnat. It is not known what factors initiate regenerative dendrite outgrowth. However, the core DLK-mediated axon injury signaling pathway is not involved. During the growth phase of regeneration, interactions with the ECM through integrins and Ret are important. Akt signaling, likely acting through mTor-mediated increases in translation, is also important for growth. A requirement for Tiam1 and RAC in regeneration in *C. elegans* implicates the actin cytoskeleton, and in *Drosophila* microtubule nucleation (nucl.) and minus end growth mediated by Patronin seems particularly important for dendrite regeneration compared to other types of growth.

axon outgrowth (Rao et al., 2016); so it is not a given that seemingly general growth pathways will be equally used to regrow axons and dendrites.

Akt is a central player downstream of growth factor signaling and upstream of mTor to control cell survival, growth, and metabolism (Manning and Cantley, 2007). PTEN antagonizes Akt signaling (Worby and Dixon, 2014) and its knockout enhances axon regeneration in the mammalian central nervous system (CNS) (Park et al., 2010; Park et al., 2008). Extra Akt or reduced PTEN enhances axon and dendrite regeneration in *Drosophila* (Song et al., 2012) and the pathway also promotes developmental dendrite growth (Parrish et al., 2009). Although upstream regulators of Akt in dendritic arborization neurons have not been identified, the pathway is regulated by signals from epithelial cells. Expression of a microRNA, bantam, in epithelial cells dampens Akt activity in neurons (Parrish et al., 2009). AMPK is another broad regulator of cellular metabolism and growth (Inoki et al., 2012). Its reduction in *Drosophila* class IV dendritic arborization neurons leads to dendrite blebbing (Marzano et al., 2021; Swick et al., 2013) and gaps in coverage (Figure 3.3). Dendrite regeneration is reduced similarly to Akt knockdown in AMPK RNAi neurons (Figure 3.3). While it makes intuitive sense that proteins required generally for cell growth and metabolism would be important for dendrite regeneration (Figure 3.4), regeneration of PVD dendrites in

C. elegans is not affected in *Akt* mutants (Brar et al., 2022). Perhaps compensatory pathways make some cellular processes, cell types, or animals more resilient to reduction of broadly used growth pathways.

Beyond sensory dendrites:
All studies on molecular requirements for dendrite regeneration have so far been performed in peripheral neurons that have sensory, rather than post-synaptic, dendrites. How applicable are these findings likely to be to central neurons, whose dendrites contain synapses and are surrounded by glia and other neurons? This question has not been addressed, as it is much more difficult to access and manipulate dendrites in the CNS, but it seems likely that at least early steps of regeneration would be shared between sensory and post-synaptic dendrites. Injury would still need to be sensed, and outgrowth of new dendrites initiated. Forming synapses would be a late-stage, necessary addition intrinsic to central neurons that may involve neuron-to-neuron interaction that sensory dendrites do not require. Interactions with ECM, cytoskeletal organization, and regulation of cell growth pathways are all likely to be similarly important for regeneration of all types of dendrites.

Research has uncovered a plethora of proteins required for axon injury signaling, and many that promote both dendrite and axon regeneration. However, finding proteins required specifically for a dendrite injury response has been elusive, and so it remains unclear how similar axon and dendrite regenerations really are. More work is needed in this field to compare how axons and dendrites signal damage and respond in morphologically distinct ways.

3.8 Where We Stand with Dendrite Injury Responses

The study of axon regeneration is hundreds of years old. While there is also a long history that suggests that dendrites can be damaged in physiologically important scenarios, the discovery that neurons possess the capacity to regenerate dendrites de novo after acute cellular injury is very recent and relies on controlled delivery of dendrite damage. The laser injury paradigm used to perform dendrite microsurgery is less than 20 years old (Galbraith and Terasaki, 2003; Sugimura et al., 2003). Important work has since differentiated dendrite from axon regeneration (Brar et al., 2022; Nye et al., 2020; Rao et al., 2016; Stone et al., 2014; Stone et al., 2012); however, the major pathways and proteins that govern regeneration of dendrites have yet to be discovered (Figure 3.4).

Most work on dendrite regeneration has been in the optically friendly *Drosophila* and *C. elegans* model systems. A recently published paper

demonstrates the first evidence of de novo dendrite regeneration in a vertebrate model system (Stone et al., 2022). Much of what is currently known about dendrite regeneration falls into two categories: factors that are required for a permissive extracellular environment, and factors that enhance cell growth (Figure 3.4). An extracellular space that does not inhibit growth is critical for regeneration of both axons (Yiu and He, 2006) and dendrites (DeVault et al., 2018). In both *Drosophila* and *C. elegans*, sensory neurons embedded in skin cells require interaction with the epithelial cells and/or ECM for both normal growth and regeneration (Brar et al., 2022; DeVault et al., 2018; Jiang et al., 2019; Liu et al., 2016; Oren-Suissa et al., 2017; Poe et al., 2017; Soba et al., 2015; Zou et al., 2016). On the intracellular side, for instance, Akt activation promotes dendrite regeneration (Song et al., 2012). Microtubule-related proteins have also been shown to have dendrite outgrowth and regeneration phenotypes when knocked down: if new microtubules cannot nucleate or grow from the minus end, dendrite outgrowth is significantly restricted (Feng et al., 2019; Hertzler et al., 2020; Nye et al., 2020).

However, much remains to be discovered about the actual signals and physiological changes that kick off dendrite regeneration. That dendrites of multiple neuron types in both vertebrates and invertebrates show regeneration and re-elaboration implies that a mechanism exists for sensing injury or incomplete function of the dendritic compartment, but it remains unknown how neurons sense this damage and which pathways are activated as a result. While transcriptional changes are almost certainly required, these transcription factors and genes involved have yet to be identified.

The biggest question to be answered is whether there exists a conserved dendrite regeneration pathway that is analogous to the DLK-mediated axon regeneration pathway. Many different neuron types have stereotyped dendrite arbors and extracellular interactions that may require transcription of different genes for their proper regeneration. However, as microscope technology and genetic tools continue to evolve in multiple model organisms, we can start to gain a clearer view (pun intended) of how neurons sense and respond to injury.

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