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Axon degeneration: context defines distinct pathways

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Axon degeneration is an essential part of development, plasticity, and injury response and has been primarily studied in mammalian models in three contexts: 1) Axotomy-induced Wallerian degeneration, 2) Apoptosis-induced axon degeneration (axon apoptosis), and 3) Axon pruning. These three contexts dictate engagement of distinct pathways for axon degeneration. Recent advances have identified the importance of SARM1, NMNATs, NAD+ depletion, and MAPK signaling in axotomy-induced Wallerian degeneration. Interestingly, apoptosis-induced axon degeneration and axon pruning have many shared mechanisms both in signaling (*e.g.* DLK, JNKs, GSK3α/β) and execution (*e.g.* Puma, Bax, caspase-9, caspase-3). However, the specific mechanisms by which caspases are activated during apoptosis *versus* pruning appear distinct, with apoptosis requiring Apaf-1 but not caspase-6 while pruning requires caspase-6 but not Apaf-1.

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Introduction

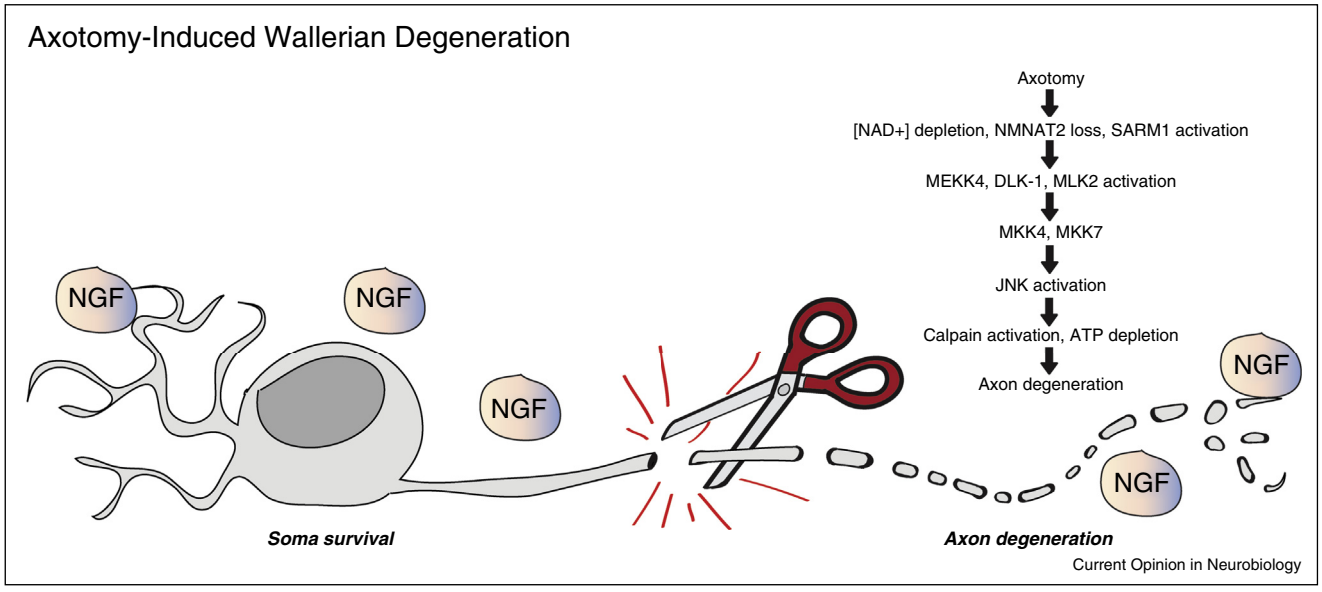
Neuronal development and target innervation by axons and dendrites are essential processes required for the establishment of the nervous system. Simultaneously however, the selective destruction of neurons or only their projections is also important for the refinement of the wired brain [1]. While the mechanisms regulating the survival and elimination of neurons *via* apoptosis are well studied, the pathways governing the selective degeneration of the axons are less understood. Axon degeneration has been observed to occur in many contexts (*e.g.* pruning, axotomy, apoptosis, dying back) and models (*e.g. D. melanogaster*, *C. elegans*, mice), which has been discussed in several recent reviews [2,3,4,5,6].

In the mammalian model, mechanistic details of axon degeneration has been predominantly studied *in vitro* in three contexts: 1) **Axotomy** (also known as Wallerian degeneration), where the severing of axons results in the degeneration of axons distal to the cut site; 2) **Apoptosis-induced Axon Degeneration**, which we define here as '**Axon Apoptosis**', where the entire neuron is exposed to apoptotic stimuli (*e.g.* global deprivation of trophic factors) resulting in the degeneration of both axons and soma; and 3) **Pruning-induced Axon Degeneration**, which we refer to here as '**Axon Pruning**', where a subset of axons are selectively exposed to a pruning stimuli (*e.g.* axon-only or 'local' deprivation of trophic factors) which results in the selective degeneration of only the axons exposed to the stimulus. Axon degeneration within these three contexts appears to engage overlapping but also distinct pathways. While axon degeneration is ultimately the convergent outcome of these pathways, the molecular mechanisms engaged are defined by the context of degeneration (just as cell death can be executed by distinct mechanisms, dependent on context). In this article, we primarily focus on the recent progress in mammalian models and define the shared and distinct mechanisms of axon degeneration that occur in these three contexts. These *in vitro* studies have been instrumental in providing significant insight into the distinct mechanisms of axon degeneration, which are now poised for future *in vivo* investigations.

Axotomy (Wallerian degeneration)

Wallerian degeneration is a unique and structured form of axon degeneration that occurs when a neuronal axon is crushed or severed (axotomized) from its cell body (illustrated in Figure 1). Wallerian degeneration has been well examined in the Wld^s mouse, where axotomy-induced axon degeneration is strikingly inhibited [7]. Wld^s mice express a unique gain-of-function gene product responsible for this protection, which was identified as the fusion of the N-terminal fragment of ubiquitination factor E4B (UBE4b) with NMNAT1 (nicotinamide mononucleotide adenyltransferase 1) [8]. These findings implicated the role of NAD+ (nicotinamide adenine dinucleotide) metabolism in Wallerian degeneration, as NMNATs are key enzymes in the NAD+ salvage pathway [9]. NAM (Nicotinamide), which is a byproduct of NAD+ metabolism, is recycled into NMN (nicotinamide mononucleotide). NAD+ can then be regenerated from NMN by the NMNAT enzymes to maintain steady-state pools of intracellular NAD+. The rapid depletion of NAD+ has been identified to be a central event in axons undergoing Wallerian degeneration and addition of exogenous NAD+ (albeit at high concentrations) is sufficient to inhibit this

Figure 1



Axotomy activates the Wallerian degeneration pathway for the selective elimination and degeneration of severed axons. This mechanism of axon degeneration occurs in response to the physical severing of axons from their cell bodies. Wallerian degeneration occurs independently of NGF signaling and the presence of NGF is illustrated in this context only for contrast with the axon apoptosis and axon pruning pathways.

degeneration [9,10,11]. However, recent evidence suggest that rather than NAD+ depletion, it could be the accumulation of NMN instead that triggers axon degeneration [12].

Recently, SARM1 (Sterile Alpha and TIR Motif 1) was identified as the first loss-of-function mutation to provide protection against Wallerian degeneration [13•,14]. SARM1-deficiency robustly protects axons following axotomy *in vivo* and *in vitro* [13•,14] and maintains NAD+ levels *in vitro* [15••]. Conversely, direct activation of SARM1 rapidly depletes intracellular NAD+ and is sufficient to induce axon degeneration [14,15••]. A key event that occurs after axotomy is the depletion of NMNAT2, which is sufficient to trigger axon degeneration [16]. SARM1 appears to be the effector of NMNAT2-depletion induced axon degeneration, as the degeneration trigged by NMNAT2 depletion can be fully rescued by the co-deletion of SARM1 [17]. The exact role of SARM1 in degeneration is not fully understood, but these data and a recent review suggest that NMNAT2 depletion causes the reduction of NAD+ levels which leads to subsequent SARM1 activation and axon degeneration [4,15••,17]. Downstream of SARM1, a MAP Kinase signaling pathway is activated to propagate the degenerative signal [18••,19]. This cascade includes DLK (Dual Leucine Zipper Kinase), MEKK4, and MLK2 which leads to the activation of c-Jun-N-terminal kinases (JNKs) *via* MKK4 and MKK7 [18••]. Exactly how these signaling pathways induce axon degeneration is not fully understood, but this involves additional mediators of Wallerian degeneration which include the ubiquitin-proteasome system [20], the ubiquitin ligase Phr1/Highwire [21,22], and SCG10 [23,24]. Furthermore, Ca^{2+} signaling and calpain activation are also important for mediating late events in axon degeneration [25,26,27].

Axon apoptosis (apoptosis-induced axon degeneration)

Neuronal apoptosis, which results in the degeneration of both the soma and axons, occurs extensively during development and in response to various neuronal insults [28,29]. This pathway has been well studied in the developing peripheral nervous system where sympathetic and sensory neurons, which are produced in excess, are acutely dependent on the limiting amounts of target-derived nerve growth factor (NGF). During target innervation, only those neurons that obtain robust NGF signaling survive whereas those deprived of NGF undergo apoptosis, a phenomenon that serves to match the number of innervating neurons with the size of target [30,31]. While neurons can undergo developmental or pathological apoptosis in multiple contexts, the model of NGF deprivation-induced apoptosis is a useful and well-recognized model for studying the molecular mechanisms of neuronal apoptosis. This phenomenon can also be recapitulated in cell culture where dissociated sympathetic or sensory neurons, or ganglia explants, are maintained in the presence of NGF. It should be noted that in these models both the soma and axons are exposed to direct NGF signaling, while *in vivo* NGF signaling primarily occurs at the axon terminals and the signal is retrogradely

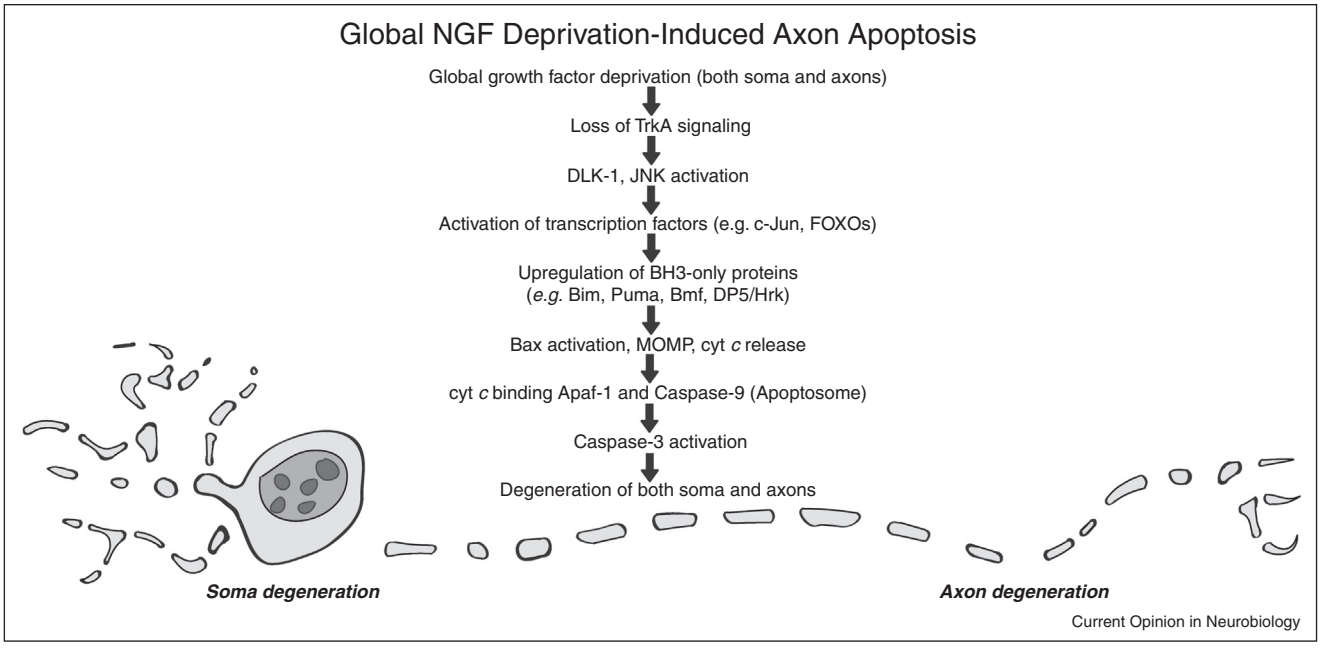
transported to the cell body. Importantly, elimination of NGF from the culture media, to deprive the entire neuron of NGF (often referred to as 'global deprivation'), results in the apoptotic degeneration of both the soma and axons (Illustrated in Figure 2) [30,32]. Since axon degeneration in this context is a consequence of the activation of the global apoptotic program, we refer to this apoptosis-induced axon degeneration here as 'axon apoptosis'.

Global deprivation of NGF initiates a well-defined apoptotic pathway that we briefly outline here. NGF deprivation induces the dephosphorylation of its receptor, Tropomysin receptor kinase A (TrkA), which leads to the activation of a DLK-mediated apoptotic signal. DLK signaling activates downstream mediators (including JNKs, MAPKs, and GSKα/β) and leads to the transcriptional upregulation of proapoptotic BH3-only family proteins (including Bim, Puma, Bmf, Hrk/DP5) by transcription factors such as c-Jun, NF-Y, and FOXOs [30,33••]. Recently, Puma was shown to be a key mediator of axon apoptosis. Not only is Puma induced in the cell body after global NGF deprivation, but Puma deficiency markedly reduces axon apoptosis [33••]. A major function of the BH3-only proteins such as Puma is to activate Bax, a pro-apoptotic member of the Bcl-2 family, which is required for neuronal apoptosis [30]. Some BH3-only proteins directly interact with Bax to induce its conformation activation, while others inhibit anti-apoptotic proteins of the Bcl-2 protein family (Bcl-2, Bcl-w, Bcl-XL, Mcl-1) [34]. In particular, Bcl-w and Bcl-XL are known to be localized to axons where they are important for maintaining axonal survival [33••,35].

Once Bax is activated, it translocates to mitochondria and inserts into the outer mitochondrial membrane, leading to the release of cytochrome *c* (cyt *c*) into the cytoplasm [36]. Released cyt *c* can then bind to Apaf-1 (Apoptotic protease activating factor 1) to induce a conformational change in Apaf-1 that results in the recruitment of and activation of procaspase-9 (Casp9). Activated Casp9 functions to activate Caspase-3 (Casp3), and subsequently calpains, which together execute the degeneration of both soma and axons by targeting many cellular proteins for proteolysis [26]. Interestingly, while there are numerous mammalian caspases with diverse cellular functions, only Casp9 and Casp3 appear to be strictly required for global NGF-deprivation induced sympathetic neuronal apoptosis [37]. Lastly, X-linked Inhibitor of Apoptosis Protein (XIAP) inhibits caspase activity in both soma and axons, but is degraded during global NGF deprivation [38,39•]. Consistent with this, XIAP-deficient neurons exhibit enhanced axon degradation when globally deprived of NGF [39•].

This apoptotic pathway activated upon global deprivation of NGF has been recognized to be important for the degeneration of both the cell bodies and axons. Indeed, deletion or depletion of the key effectors of this apoptotic

Figure 2



Global NGF deprivation activates the apoptotic pathway to degenerate both soma and axons, in the model of sympathetic neurons. We refer to the axon degeneration that occurs during apoptosis as 'axon apoptosis' for clarity and to distinguish this axon degeneration from that observed during axon pruning. Here, major components of the apoptotic pathway engaged during axon apoptosis are highlighted for clarity.

program (*e.g.* DLK, JNKs, c-Jun, FOXO3a, Puma, Bax, Apaf-1, Casp9, Casp3) blocks the death of neurons in response to global NGF deprivation [30,33••,37,40•,41]. Overall, these data suggest that in the context of global apoptotic stimulation, axonal degeneration is a consequence of the apoptotic program activated in the cell body.

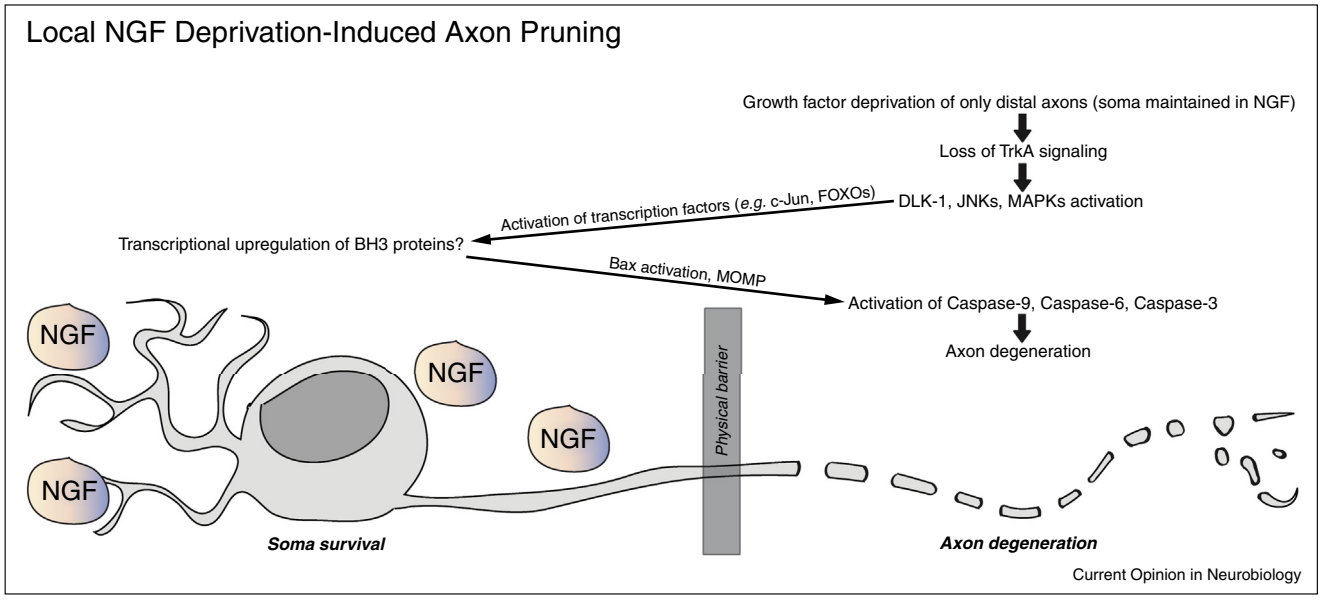
Axon pruning (pruning-induced axon degeneration)

In addition to neuronal apoptosis, the nervous system undergoes substantial refinement by the pruning of axons and dendritic connections, which are selectively dismantled and rewired. Pruning is critical not only for establishing specific and appropriate neuronal circuitry during development but also for neuronal plasticity in the adult nervous system [1,42]. Precise spatial and temporal control of the degenerative machinery is important for pruning to ensure that only the targeted region of the axons are degraded. This spatial regulation is in contrast to apoptosis, where the entire neuron undergoes degeneration.

Similarly to axon apoptosis, mechanistic insights into the axon pruning pathway have primarily come from studies in peripheral neurons (sensory and sympathetic) subjected to NGF deprivation. An important distinction however is that while axonal apoptosis is triggered by 'global' deprivation of NGF (where neurons are completely deprived of NGF), axon pruning is observed in response to the selective deprivation of NGF from the axon while maintaining the neuronal cell body in NGF (illustrated in Figure 3). To study this pathway *in vitro*, neurons are cultured in compartmentalized chambers (*e.g.* Campenot [43] or microfluidic chambers [44]) where the cell bodies and the axons are maintained in different compartments, separated by a physical barrier, to keep them spatially and fluidically isolated. Selective deprivation of NGF from only the axon compartment (known as 'local' NGF deprivation), with cell bodies maintained in NGF, triggers the selective degeneration (pruning) of only the axons in the NGF-deprived axon compartment [43]. Although axon apoptosis and axon pruning are both induced by NGF deprivation, the distinction between global and local NGF deprivation is important as it defines the context, and thus, the specific pathway activated.

While multiple factors are known to mediate axon pruning *in vivo* [1], the molecular pathway of axon pruning has been best studied *in vitro* in the context of local (axon-only) NGF deprivation. Amyloid Precursor Protein (APP) and Death Receptor 6 (DR6) have been shown to regulate pruning *in vivo* [45•,46]. However, loss of either APP or DR6 was not protective against apoptosis (global NGF deprivation) *in vitro* [46], suggesting that APP and DR6 may play a role in axon pruning but are dispensable for apoptosis. During local NGF deprivation-mediated pruning, the loss of TrkA activity leads to the activation of DLK to induce a retrograde signal to the soma [33••]. This signal is important, as DLK-deficient neurons are protected from axon pruning [33••,40•]. DLK activation

Figure 3



Pruning is the selective degeneration of only the axon exposed to the pruning stimulus, leaving the cell body intact. Pruning can be studied in compartmentalized (illustrated by the gray barrier) cultures where NGF can be selectively deprived from the axons, while cell bodies are maintained in NGF. The axon pruning pathway shares many of the same machinery as the axon apoptosis pathway, but several significant differences exist which are discussed in the text and are highlighted in Table 1.

leads to the local phosphorylation of JNKs to propagate the pruning signal [40•,47•]. Interestingly, inhibition of JNK (and also p38 MAPK) in only the axons, and not the cell bodies, is sufficient to protect against axon pruning, confirming a function of JNK in axons during pruning [47•]. One of the functions of JNK in axons could be to mediate the transmission of the retrograde signal to soma. Interestingly, GSK3 (both α and β) could be a potential JNK-regulated mediator of this retrograde signal as inhibition of GSK3 activity in the soma, but not the axons, protects against axon pruning [47•]. While GSK3 could be activated locally in axons, its kinase activity may be required only in the soma where its substrates important for pruning could be spatially localized. c-Jun has been identified as another soma-dependent transcriptional regulator that is activated during pruning [40•,48]. Whether c-Jun is required for axon pruning is undetermined however, as previous results examined the requirement of c-Jun in the context of axon apoptosis (global NGF deprivation) [33••,40•].

Recently, the BH3-only protein Puma was identified as an essential mediator for axon pruning where its deficiency protects axons against local NGF deprivation [33••]. As Bax is also required for axon pruning [45•,49••,50•], the role of Puma is likely to directly activate Bax. Consistent with Bax activation, cyt *c* is released from mitochondria in axons undergoing pruning [49••], but whether cyt *c* is required for pruning is not known. This is particularly relevant as cyt *c* is known to activate Casp9 *via* Apaf-1 on the apoptosome. However, Apaf-1 is not required for axon pruning [49••]. While Apaf-1-deficient neurons are protected from undergoing apoptosis after global NGF deprivation [33••,37], they still undergo pruning after local NGF deprivation [49••]. Interestingly, despite not requiring Apaf-1, pruning requires both Casp9 [49••] and Casp3 [49••,51]. Thus, Casp9 appears to be activated *via* an Apaf-1-independent mechanism during pruning. This is particularly relevant in post-developmental mature neurons, which are known to shut down the apoptotic pathway by turning off Apaf-1 expression [52], yet maintain plasticity and remain competent to undergo pruning [49••]. This finding not only supports but highlights the Apaf-1-independent nature of the pruning pathway and illustrates how neurons are able to exert precise and differential control over the apoptosis and pruning pathways. Additionally, pruning is dependent on Casp6, a caspase which is not essential for apoptosis [45•,49••,51]. However, exactly how caspases are activated during pruning, or even their precise order of activation, remains unknown. As pruning allows for the selective degeneration only the targeted axon, without degeneration outside of that target region, it is likely that caspase activation during pruning is spatially restricted [53]. One such mediator that restricts caspase activity during pruning is XIAP (dIAP in Drosophila) [49••,54], which could serve to spatially constrain degeneration to the desired region. Indeed, XIAP-deficient neurons are unable to spatially restrict caspase activity and inappropriately accumulate active Casp3 in their cell bodies during pruning [49••].

Mechanistic overlap in the three distinct pathways of axon degeneration

Despite the fact that the three pathways of axon degeneration that we highlight in this review are engaged in different contexts, there are intriguing overlaps in both the signaling as well as the execution of these pathways. We highlight here some of these similarities and differences, although in many instances the function of individual proteins has not been examined in all three contexts of axon degeneration (Table 1). While it is too early to tell whether the shared proteins have exactly the same functional role in all contexts of axon degeneration, future research will help clarify these roles.

An obvious difference between these axon degeneration pathways is that during axotomy-induced Wallerian degeneration, the cell body is not involved as it is physically separated from the axon undergoing degeneration. In contrast, the cell body is required, and in fact orchestrates,

Table 1

Shared and distinct components of the axon degeneration pathways:

	Axotomy	Apoptosis	Pruning
Wld^S	+++ [57]	Axons ++ Soma – [55]	?
NMNAT overexpression	+++ [58]	Axons ++ Soma – [56]	?
Sarm1 –/–	+++ [13•,14]	+/– [13•,14]	?
MLK-depletion (DLK, MLK, MEKK4)	+++ [18••,19,59]	+++ [40•,59,60]	+++ [40•]
Inhibition or loss of JNKs	+++ [19,33••]	+++ [19,40•]	+++ [47•]
Inhibition of Calpains	+ [26,61]	+ [26]	?
NGF/TrkA stimulation	– [33••]	+++ [33••]	+++ [33••]
Bax –/–	– [62]	+++ [63]	+++ [45,49••]
Apaf-1 –/–	?	+++ [33••,37]	– [49••]
Caspase 9 –/–	?	+++ [37]	+++ [49••]
Caspase 6 –/–	?	– [49••]	+++ [49••,51]
Caspase 3 –/–	– [51]	+++ [37]	+++ [45,49••]

Summary of the functional importance of select key proteins in mediating axon degeneration in the contexts of axotomy, apoptosis, and pruning. The degree of protection reported is represented as: +++ excellent or complete protection, ++ significant protection, + some protection, – no protection,? not yet known. The relevant publications (not an exhaustive list) are also indicated.

the degeneration programs during both apoptosis and pruning. Despite this fundamental contextual difference, key proteins essential for Wallerian degeneration (*e.g.* Wld^{s}, NMNATs, SARM1) appear to be involved in axon apoptosis as well. Demonstrating this overlap, neurons isolated from Wld^{s} mice degenerate their cell bodies but surprisingly not their axons upon global NGF deprivation [55]. Similarly, overexpression of cytosolic NMNAT1 also provides protection against axon apoptosis during global NGF deprivation [56]. Interestingly, this block appears to be in parallel to or after the point of caspase activation [56] but exactly how this protection is mediated remains to be determined. The exact importance of SARM1 for axon apoptosis is unclear as one study found SARM1 deficiency to protect axons [14] while another failed to see protection [13•] after global NGF deprivation. The ability of these proteins to protect axons but not the cell bodies in the context of apoptosis suggests that there could be mechanistically distinct pathways mediating the degeneration of axons *versus* cell bodies during neuronal apoptosis. Interestingly, all three contexts of axon degeneration mediate degeneration by engaging many of the same signaling pathways and proteins (*e.g.* various MAPKs and JNKs). While during apoptosis and pruning these signaling proteins are required for the transcriptional activation of the axon degeneration [33••,40•,47•], their function in Wallerian degeneration is downstream of SARM1 and independent of transcription as the degenerating axons are separate from the cell body [18••].

Significant mechanistic similarities exist between axon apoptosis and axon pruning. Both pathways are initiated by a common signal (NGF deprivation) and in both contexts (global or local NGF deprivation), the initiating signals appear to originate from axons [47•,48]. Also, most of the upstream signals up to the point of Bax activation appear to identical in both contexts. This includes DLK, JNKs, p38 MAPK, GSK3, the BH3-only protein Puma, and Bax itself [33••,40•,45•,47•,49••,51]. However, downstream of Bax, these two pathways diverge. Axon apoptosis strictly requires the Apaf-1 and Casp9-dependent apoptosome pathway to activate Casp3 [33••,37]. In this context, Casp6 is cleaved but its deficiency does not block axon apoptosis [49••]. In contrast, axon pruning requires Casp6 as well as Casp9 and Casp3 [49••,51], but importantly not Apaf-1[49••]. This is surprising as there is very limited precedence of a Casp9-dependent but Apaf-1-independent pathway for activating Casp3. Thus, many exciting details of how these two distinct pathways use similar signaling machinery yet diverge after the point of Bax activation remain to be discovered. Undoubtedly, future research will help uncover the mechanisms by which neurons exert both spatial and temporal control of these axon degeneration pathways.

Conflict of interest statement

Nothing declared.

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