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Signaling mechanisms regulating Wallerian degeneration

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Summary

Wallerian degeneration (WD) occurs after an axon is cut or crushed and entails the disintegration and clearance of the severed axon distal to the injury site. WD was initially thought to result from the passive wasting away of the distal axonal fragment, presumably because it lacked a nutrient supply from the cell body. The discovery of the *slow Wallerian degeneration* (*Wld*^{S}) mutant mouse, in which distal severed axons survive intact for weeks rather than only 1–2 days, radically changed our thoughts on the autonomy of axon survival. Wld^S taught us that under some conditions the axonal compartment can survive for weeks after axotomy without a cell body. The phenotypic and molecular characterization of *Wld*^{S} and current models for Wld^S molecular function are reviewed herein—the mechanism(s) by which Wld^S spares severed axons remains unresolved. However, recent studies inspired by Wld^S have led to the identification of the first "axon death" signaling molecules whose endogenous activities promote axon destruction during WD.

Introduction

Axons can be enormous structures and constitute the vast majority of the volume of a neuron. Some human sciatic nerve motorneurons are one meter long and attached to a cell body that is only ~50 μm in diameter—meaning the length ratio of cell body to axon is 1:20,000. Maintaining such large and elaborate structures is a major cell biological and bioenergetic challenge for the neuron, but is essential for continued neural circuit function. Axonal injury is quite common in the nervous system, can occur through nerve crush, stretch, or transection, and frequently leads to axonal degeneration. Axon loss is also prominent in neurodegenerative diseases including ALS, Huntington's, and Parkinson's disease. Since axonal and synaptic loss are major contributing factors in neural circuit dysfunction, blockade of axon degeneration by any means is of significant clinical interest.

Cutting an axon (axotomy) leads to the granular disintegration of the axon distal to the injury site [1] — a process termed Wallerian degeneration. For ~150 years it was believed

that Wallerian degeneration occurred because the portion of the axon distal to the injury site lacked a nutrient supply from the soma. This all changed with the serendipitous discovery of the *slow Wallerian degeneration* (*Wld*^{S}) mouse, where distal severed axons survived for weeks after axotomy (rather than ~1.5 days) *in vivo* [2••]. The *Wld*^{S} phenotype was unexpected and remarkable. It demonstrated that—under some conditions—large fragments of severed axons could survive for very long periods of time on their own without a cell body. This observation raised the further intriguing possibility that severed distal axons, rather than waste away, might activate an autonomous "axon death" program akin to apoptotic death [3]. Now 30 years since the discovery of the *Wld*^{s} mutant mouse the Wld^S protective mechanism has proven complex and remains controversial [4]. This review summarizes our current understanding of the mechanism by which Wld^S modulates axon degeneration during Wallerian degeneration, and exciting recent findings that point to the existence of endogenous axon death program(s) required to drive axon death after axotomy.

Dissecting Wld^S neuroprotective function: what is critical, and where?

The *Wld*^{S} strain harbors a tandem triplication that results in the fusion of two genes, *nmnat1* and *ube4b* [5]. The Wld^S protein generated from this locus is composed of 70 amino acids from the N-terminus of the E4 ubiquitin ligase Ube4b (N70), an 18 amino acid linker produced by translation of a short segment of the *nmnat1* 5′UTR (W18), and full length Nmnat1, a component of the NAD$^+$ scavenging pathway (Figure 1) [6••]. Neuronal expression of Wld^S is sufficient to suppress the granular disintegration of both motor and sensory axons, and the axons of multiple types of CNS neurons [4]. Somewhat surprisingly, expression of mouse Wld^S was also shown to robustly suppress Wallerian degeneration in the fruit fly *Drosophila* [7•], and more recently in zebrafish [8], indicating the mechanistic action of Wld^S axon protection is evolutionarily conserved.

The mechanism by which overexpression of Wld^S suppresses axonal degeneration remains incompletely resolved. Studies over the last decade however have clarified the precise domains essential for its axon protective function *in vivo*. Lentivirus-based overexpression of Nmnat1 was first reported to suppress axon degeneration in cultured mammalian dorsal root ganglion (DRG) neurons. It was therefore proposed that Nmnat1, a nuclear NAD$^+$ biosynthetic enzyme, was the key functional component of Wld^S responsible for its axon-sparing activity [9••]; and further that Nmnat1 acted in the nucleus prior to injury, by modulating NAD$^+$-dependent activation of the histone deacetylase enzyme Sirtuin1 [9]. A subsequent study also concluded that Nmnat1 was protective in mouse DRG cultures, but argued that the Wld^S and Nmnat1 acted more locally in axons [10]. NAD$^+$ levels in distal severed axons from wild type animals were found to be maintained for 2–4 hours after axotomy, after which time they dropped precipitously, and this drop in NAD$^+$ along with depletion of ATP immediately preceded axon fragmentation and could be averted by over-expression of Wld^S or Nmnat1. Moreover, degeneration of axon segments could be suppressed by addition of NAD$^+$ or its precursor nicotinamide even 3–5 hours after axotomy. Despite siRNA-based evidence for a role for Sirt1 in Wld^S-mediated axon protection [9], neither NAD$^+$-, Nmnat1-, nor Wld^S-dependent protection of axons was suppressed in DRG cultures from *sirt1* knockout animals [10]. Wld^S-dependent protection of severed *Drosophila* axons was also not affected in by loss of *Sir2*, a fly sirtuin [11].

Together these data argued strongly against a pre-injury requirement for Wld^S in protecting severed axons and the proposed role for Sirt1.

Based on the above *in vitro* studies the field predicted that Nmnat1 over-expression in mice would provide axonal protection similar to Wld^S, but this was not the case. Unexpectedly, mice with levels of Nmnat1 expression and NAD^+ biosynthetic activity comparable to Wld^S showed no axonal protective phenotype after axotomy [12]. Nmnat1 enzymatic activity is certainly crucial as enzymatically dead versions of Wld^S do not suppress Wallerian degeneration strongly in flies [11] or at all in mice [13]. Expression of mouse Nmnat1 in *Drosophila* provided some protection, but this was clearly diminished compared to expression of Wld^S and fully eliminated by blocking enzymatic activity [11]. Therefore the mechanism of Wld^S-dependent axonal preservation is more complex than simply over-expressing Nmnat1. Interestingly, robust axonal protection could be accomplished by including a key portion of the N-terminal Ube4b molecule: the most N-terminal 16 amino acids (N16) that had previously been shown to associate with valosin-containing protein (VCP) [14]. Deletion of N16 from Wld^S suppressed its neuroprotective function in mouse and flies [11,13]; fusing N16 alone to Nmnat1 resulted in Wld^S-like protection in flies [11]; replacing N16 in Wld^S with a VCP-binding motif from Ataxin3 provided Wld^S-like axon protection in mouse [13]; and knocking down VCP in *Drosophila* in the presence of Wld^S reduced its protective capacity to that equivalent to Nmnat1 alone [11]. These observations indicate that both N16 and enzymatically active Nmnat1 are key domains required for maximal protection of axons by Wld^S *in vivo*. Thus, both the N-terminus and Nmnat1 are essential components for full Wld^S-like axon preservation in Wallerian degeneration.

What is N16 doing to promote Wld^S axon-sparing activity? A likely explanation appears to be that N16 drives the relocalization of a portion of the cellular pool of Wld^S out of the nucleus to another site of action. Nmnat1 has a strong nuclear localization sequence (NLS) that is included in the Wld^S fusion protein and results in its primarily nuclear localization [5]. Compelling evidence that extra-nuclear relocalization is a key event in making Nmnat1 neuroprotective came from the observation that deleting the Nmnat1 NLS (to make $Nmnat1^{cyto}$) results in its cytoplasmic localization, and $Nmnat1^{cyto}$ potently suppresses axon degeneration both *in vitro* and *in vivo* [15•]. Deleting the NLS in Nmnat1 from Wld^S in fact makes it an even more potent protective molecule [16], as does the addition of an axonal targeting domain to a cytoplasmically localized Nmnat1 [17]. Wld^S itself has been found at low levels in cytoplasmic fractions and axons [16–18] and in synaptosomal preparations [19]. But perhaps the most convincing data that Wld^S acts in the axon to protect after injury is the observation that lentiviral-based delivery of cytoplasmically targeted Nmnat1 protein directly into axons after axotomy is sufficient to protect them from axonal degeneration [15]. This observation would also seem to eliminate the NAD^+/Sirt1-based nuclear model for Wld^S-mediated axonal protection.

Wld^S should teach us (again) that localization data must be considered quite carefully, especially in situations where the molecule of interest is being over-expressed. Recently the field appears to have unified behind the idea of a non-nuclear, axonal role for Wld^S in axon protection, but it was not easy getting to this point—controversy abounded. A number of technical issues might explain some of the controversy and their careful consideration might

help moving forward. First, in many cases axon protection was compared across vastly different experimental settings, and this is important for phenotypic characterization of protective function. In cultured DRG neurons Nmnat1 and Wld^S provide what appears to be similar levels of protection, promoting axon survival at least 72 hours after axotomy. In striking contrast, wild type Nmnat1 provides no protection by 2–3 days after axotomy *in vivo* [12] while Wld^S protects the majority of axons for at least two weeks in the mouse [5].

A second point to consider is quantification of both temporal and spatial aspects of axon/synapse preservation. Assays of neuroprotection must be taken to endpoints. Direct comparisons of very late time points in Wallerian degeneration in *Drosophila* elucidated important differences in neuroprotective effects of Wld^S versus Nmnat1 and its derivatives [11], as did comparisons of Wld^S and axonally targeted Wld^S [17]. The quality of preservation are also important factors to consider—are axons and synapses preserved equally, and are they functionally intact? If protection were weak or specific to a subdomain of a neurite (e.g. dendrite versus axon) for any manipulation, this would impact its consideration as a therapeutic target in important ways. Though not used as a standard, scoring neuroprotection with single axon resolution should be a key goal for the field. In culture preparations of various ganglia, axonal fibers are not reliably identifiable with single axon resolution using light microscopy, so partial protection of axons from disintegration may appear to be complete, or vice versa.

Finally, we need to consider the potential complexity of genetic pathways driving axon degeneration. Although it is the most common positive empirical readout for Wallerian degeneration, what does it mean when an axon undergoes granular disintegration? Is degeneration always the same, or are there multiple Wallerian degeneration-like programs? There appears to be a remarkable disconnect between molecular programs driving developmental neurite pruning and axon degeneration despite the fact these two processes look very similar morphologically [20,21••]. It is therefore reasonable to suspect that a number of pathways exist that can drive granular disintegration of axons. A systematic use of biomarkers for very specific signaling events (e.g. Ca^{2+} signaling, or reactive oxygen species production) [8,19] or changes in cell biology (e.g. axon trafficking, cytoskeletal breakdown) [22] should go a long way toward determining how distinct genetic pathways might drive the step-wise disassembly of axons in different contexts, and where any genetic or chemical manipulation impinges on these processes.

How does Wld^S act to suppress axon protection?

There is a strong correlation between the time of survival of a detached distal axon fragment and its length —longer detached axon fragments degenerated later than shorter fragments. Based on this observation it was proposed that the soma might provide a trophic factor that was continuously transported down the axon [23,24•]. Once severed, the axon would survive until the pool of trophic factor was depleted and degeneration would ensue (Figure 2). Gilley *et al.* (2010) proposed that this trophic factor was Nmnat2, a cytoplasmic Nmnat molecule that can be found in the axoplasm. Nmnat2 is actively transported down axons, its half-life correlates with the timing of axon degeneration in DRG cultured neuron preparations [24], stabilization of Nmnat2 by mutating is palmitoylation site that tethers it to vesicles stabilizes

Nmnat2 and makes it highly protective [25], and, reciprocally, depletion of Nmnat2 from cultured DRG neurons leads to degeneration that can be suppressed by Wld^S [24]. Wld^S, they speculated, essentially substitutes for the more labile Nmnat2 activity in the axon. Wld^S and Nmant1 are indeed more stable than Nmnat2 *in vitro* [24]. Moreover, while *nmnat2* knockout mice are embryonic lethal and grow axons in the PNS which are significantly shorter than control axons both *in vivo* and DRG cultures [26,27], Wld^S expression is sufficient to rescue both lethality and short axon phenotypes in the *nmnat2* null background [27].

Together these data provide compelling evidence that Nmnat2 is required for maintenance of axonal integrity in cultured neurons and that Wld^S is capable of substituting for Nmnat2 in multiple contexts. But does this fully explain the Wld^S phenotype of axon protection? The Nmnat2 depletion model seems quite logical, but a number of observations and experimental caveats argue that the effects of Wld^S are more complex and not fully explained by Nmnat2 depletion. First, in both mice [18] and *Drosophila* [19] expression of the mitochondrially-localized Nmnat3 molecule is sufficient to recapitulate the strong protective effects of Wld^S, and in synaptosome preparations from the Wld^S mouse brain Wld^S was found exclusively in mitochondria and not the axoplasm [19]. These data are consistent with a mitochondrial role for Wld^S. Two groups have purified mitochondria from *Wld^S* mouse brain and shown they have an enhanced ability to generate ATP and consume O_2 [18,19], and increased capacity to buffer extra-mitochondrial Ca^{2+} and stave off loss of membrane potential and formation of the permeability transition pore (PTP) [19]. Therefore Wld^S animals exhibit significant changes in mitochondrial metabolism and handling of Ca^{2+}. This could be very important for induction of axonal degeneration, a Ca^{2+}-stimulated event (below). Axotomy in *Drosophila* motorneurons leads to a rapid increase in axonal Ca^{2+} in control animals and this is essentially eliminated in Wld^S animals, indicating there is a significant *in vivo* alteration in how severed axons handle Ca^{2+}. Activation of mitochondrial formation of the permeability transition pore (PTP) has long been known to be a key downstream step in the activation of Wallerian degeneration and pathological Ca^{2+} in the axon, its suppression can strongly delay Wallerian degeneration [28,29], and recent work in zebrafish implicates mitochondrial reactive oxygen species (ROS) production as occurring immediately prior to axon degeneration, and ROS production is strongly reduced in Wld^S animals [8]. Finally, it seems mitochondrial function is critical to enable Wld^S to protect severed axons since application of carbonyl cyanide m-chlorophenyl-hydrazone, which uncouples mitochondrial oxidative phosphorylation, eliminates the ability of Wld^S to protect axons [30].

While the above would appear to support an important role for mitochondria in Wallerian degeneration and Wld^S function, the story is not entirely clear. Severed *Drosophila* dendrites also degenerate within a day and this is suppressed by Wld^S, arguing that dendrites use a similar Wallerian-like degenerative program after cut. However, short portions of dendrites (not expressing Wld^S) were able to degenerate in the absence of any mitochondria [31•]. Therefore mitochondria are not strictly required for neurite degeneration, but whether the dendrite degenerative program is the same as in axons, and whether the granular disintegration of the mitochondria-free neurite is molecularly similar to Wallerian degeneration needs further exploration. In addition, depletion of mitochondria from

Drosophila axons did not fully block the ability of Wld^S to protect larval axons after cut [32], although this was only examined over the short term (hours rather than days), and so whether this is true of longer time points remains to be determined.

The mechanism of Wld^S-mediated axon protection it has been difficult to ask key mechanistic questions, often because of experimental limitations. For instance, if the site of action of Wld^S were mitochondria one would like to transplant mitochondria from donor Wld^{S+} animals into control axons, sever axons and see what happens. But this is not practical experimentally for multiple reasons. Because NAD^+ and mitochondria are fundamentally important for axonal and cell survival, determining the precise roles for this molecule and organelle have been challenging—they are needed for cells to simply survive. It is equally important to acknowledge that many experimental manipulations used to dissect Wld^S function have caveats. Molecules of interest such as Nmnats molecules are over-expressed, hence localization studies could be artifactual and dosages might vary significantly. Manipulations used to alter specific molecular properties might not be as specific as we would hope. For instance, mutation of the palmitoylation site in Nmnat2 that tethers it to axon vesicles indeed led to a much longer protein half-life and significantly increased axon protection, which could be used to argue that increasing the stability of Nmnat2 is a key factor to preserve axons. However this manipulation also results in mislocalization of Nmnat2 [25] which could affect neuroprotection—17 amino acids is all that is need to transform Nmnat1 from a non-protective molecule to one that protects as well as Wld^S [13]. As such there have been few really definitive experiments explaining how Wld^S mechanistically blocks Wallerian degeneration. In the future, a greater understanding of the precise cellular and molecular pathways promoting axonal degeneration will be essential to determine precisely where Wld^S impinges on axonal degeneration after axotomy, and which model best explains its neuroprotective function.

Is there an endogenous axon death pathway?

Perhaps the most important idea inspired by the study of Wld^S was the notion that severed axons, rather than simply waste away, might activate an active program of axon auto-destruction [3,33]. Wallerian degeneration in *Drosophila* appears to be genetically distinct from apoptotic cell death and autophagy since elimination of multiple components of these pathways from axons had no effect on Wallerian degeneration [21]. In addition, although caspase activity is required in axons to promote degeneration after trophic factor withdrawal, these same mutants do not suppress Wallerian degeneration [34]. Since Wld^S is a gain-of-function and likely neomorphic mutation, it does not provide evidence of an axon death pathway *per se*. A number of genes have been proposed to suppress Wallerian degeneration when mutated including Dual leucine kinase (Dlk) [35], MORN4 [36•], AKT, GSK3 [37], and IκB kinase (IKK) [38], but these all provided quite weak axon protection: while Wld^S can preserve severed axons for weeks after axotomy, loss of these products only extended survival of severed axons for hours to at most a day.

A major step forward for the field came with the identification of the *Drosophila* sterile α/Armadillo/Toll-Interleukin receptor homology domain (*dsarm*) mutants in a forward genetic screen for mutations that suppress Wallerian degeneration [21••]. This was the first mutant

that approached levels of axon protection found in *Wld*^{S}: *dsarm* loss of function mutations resulted in severed axons remaining morphologically intact for several weeks after axotomy. dSarm thus provided direct evidence that genes in fact exist whose endogenous function is to promote Wallerian degeneration. Elimination of the mouse dSarm ortholog Sarm1 suppressed Wallerian degeneration of the majority of axons in sciatic nerve for at least two weeks, protected multiple types of PNS and CNS neurons both *in vitro* and *in vivo*, suppressed breakdown of the axonal cytoskeleton (e.g. neurofilament) for at least 6 days after axotomy, and extended the maintenance of neuromuscular synapses of severed axons at levels similar to Wld^S [21]. Importantly, *dsarm* null mutations did not suppress apoptotic cell death in *Drosophila*, nor did it block developmental axon pruning [21••], further supporting the notion that Wallerian degeneration, apoptotic cell death, and developmental neurite pruning are driven by genetically distinct molecular programs.

dSarm/Sarm1 encodes a member of the TIR domain-containing family of proteins, best known for acting downstream of Toll receptors in immune functions. Sarm is unique in its structure within the TIR domain family as it contains Armadillo repeats, relatively rigid structural domains that modulates protein-protein interactions, and a number of SAM domains, which mediate protein-protein interactions [39]. TIR domain containing proteins are generally thought to act as kinase scaffolding molecules that couple Toll-like receptors (TLRs) with essential downstream signaling components. However dSarm/Sarm1 might not necessarily require the presence of a TLR to signal. Tir-1, the *C. elegans* ortholog of dSarm/Sarm1 acts in a genetic pathway apparently independent of TLRs [40]. In the context of left-right asymmetry of worm olfactory receptor neurons Tir-1 is activated downstream of the voltage gated Ca^{2+} channel UNC-43 by Ca^{2+}-calmodulin kinase (CaMK), which directly binds to the N-terminal ARM domains in Tir-1. Tir-1 then couples downstream to Apoptosis signaling kinase 1 (Ask1) to execute left-right signaling events [40]. That dSarm/Sarm1 is potentially downstream of a Ca^{2+} signaling pathway is interesting. Entry of extracellular Ca^{2+} through L-type Ca^{2+} channels is essential to activate Wallerian degeneration [41], and a dramatic rise in axoplasmic Ca2+ immediately precedes Wallerian degeneration in zebrafish [42]. An interesting possibility is that dSarm/Sarm1 is activated directly downstream of injury-induced Ca^{2+} elevation, perhaps via CaMK, and in turn activates axonal degeneration.

How dSarm/Sarm1 activates axonal degeneration is not known. As is the case in *C. elegans* with Tir-1, the SAM and TIR domains appear to be essential for prodegenerative signaling in cultured DRG neurons [43]. At least two antibodies have been generated to Sarm1: one results in punctate staining in neurites [21,44], the other a more uniform distribution with some enrichment to mitochondria [43]. Epitope-tagged Sarm1 strongly localizes to mitochondria when over-expresed *in vitro* [45] and mitochondrial localization sequences have been identified [45], but deletion of this motif did not suppress Sarm1-mediated axonal degeneration *in vitro* after axotomy [43]. Thus, while an exciting new molecule that mediates Wallerian degeneration has been identified, where or even when Sarm1 acts (i.e. before or after injury) remains important questions for the future.

A second exciting recent discovery was that the *Drosophila* E3 ubiquitin ligase Highwire (Hiw)(Figure 3B), first identified based on its role in synapse formation at the larval

neuromuscular junction (NMJ), is required for Wallerian degeneration [46••]. *hiw* mutant NMJs exhibit robust overgrowth of the presynaptic motorneuron at the larval NMJ [47]. More recently, in a larval nerve crush model of Wallerian degeneration *hiw* loss of function mutants were shown to suppress axon loss for 2 days longer than wild type, and severed *hiw* axons appear to remain functionally intact by electrophysiological measures for the duration of this time [46]. Loss of Hiw results in very strong protection (up to 2 weeks) in adult *Drosophila*, and so *hiw* mutants also approach levels of protection similar to Wld^S [46]. As it is an E3 ubiquitin ligase, Hiw might be expected to promote the degradation of specific proteins after axotomy to induce axonal degeneration. Based on the rapid degradation of Nmnat2 in mammalian axons, dNmnat (the sole *Drosophila* Nmnat ortholog) was proposed as a Hiw target in the distal axon after axotomy [46]. Consistent with the notion that Hiw negatively regulates dNmnat, *hiw* mutants have elevated dNmnat expression in the nervous system and alterations in the decay of ectopically expressed mouse Nmnat2 [46]. The mouse ortholog of Hiw, called PAM/Highwire/Rpm1 (Phr1), is also essential for Wallerian degeneration. Loss of Phr1 preserves axonal integrity in mouse sciatic nerves for 5–10 days after axotomy *in vivo* [48•], which is robust, but perhaps not quite as strong as the protection observed in *Sarm1*−/− animals [21]. Phr1 elimination increases the levels of Nmnat2, consistent with the notion that Phr1 negatively regulates is degradation [48•]. Direct evidence that Nmnat molecules are ubiquitinated prior to degradation and that this is in a Hiw/Phr1-dependent manner is currently lacking, but are important questions for the future. It is also important to note that while dNmnat and Nmnat2 levels are increased in the absence of Hiw/Phr1 [46••] [48•], the specificity of this increase remains unclear. Could stabilization of Nmnats be an indirect effect of broadly increasing protein stability when the Hiw/Phr1 pathway is depleted? At the moment there is a strong correlation, but whether the axon sparing activity of dNmnat or Nmnat2 explains the Hiw/Phr1 protective effect needs further clarification.

Concluding remarks

The phenotype of Wld^S mutants is fascinating and has inspired intense investigation into the molecular basis of Wallerian degeneration. While excellent progress has been made regarding the essential domains and site of action of Wld^S over the last decade, its mechanism of protection remains unclear. It seems unlikely that it will be simple, or involve only a single mechanism. Wld^S has taught us that axons can be remarkably autonomous structures, and based on its partial suppression of some disease models, that the axon is a viable target for therapeutic intervention in disease [4,49]. For instance, the Wld^S mutation significantly reduced motorneuron loss in the *progressive motorneuronopathy* (*pmn*) model of motorneuron disease [50], axon degeneration in paclitaxel (taxol)-based models of progressive peripheral neuropathy [51], dopaminergic fiber loss in a mouse model of Parkinson's disease [52], and axon degeneration in the DBA/2J glaucoma model [53]. But Wld^S is not a silver bullet and its protective effect does not likely extend to all diseases where axon loss is observed—Wld^S has so far failed to affect disease progression in the mouse SOD-G93A model of ALS [54,55], and likely other models of neurodegenerative that likely went unreported. Whether Sarm1 of Phr1 knockouts might meet with greater success is the realm of disease suppression remains an open and exciting question.

The effect of the *Wld*^{S} mutant on the field of axon biology cannot be overstated. However, unraveling its mechanism has been extremely challenging. An important question to now consider is whether to continue to struggle to understand how Wld^S functions, or to move forward with a deeper analysis of endogenous axon death signaling molecules like dSarm/Sarm1 and Hiw/Phr1? Determining the genetic relationship between these molecules and Wld^S, and identifying additional conserved axon death molecules seems a priority. Perhaps inhibiting one of these with a small molecule will be a more fruitful approach than trying to mimic Wld^S activity in patients?

The current state of field of axon degeneration might be likened to the cell death field after the discovery of *ced-3* and *ced-4* mutants [56]—it is now clear based on genetic evidence that Wallerian degeneration is an active process of auto-destruction driven by an endogenous molecular program. We now need to fully define this genetic program. It is expected that model organisms like *Drosophila* will lead this effort. Ultimately, central goals for the field should include determining whether such axon death genes are activated in neurological disease, designing therapeutics targeted to suppress axon death, and exploring the possibility that blocking axon death will alleviate suffering in patients with neurological conditions involving axon loss.

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Highlights

- The chimeric Wld^S molecule revealed axons can survive for long periods without a cell body
- Wld^S raised the intriguing possibility that axon degeneration is an active process
- Wld^S functional domains are now defined, but its mechanistic action remains unclear
- dSarm/Sarm1 and Hiw/Phr1 are endogenous genes required to drive axon degeneration
- “Axon death” pathways therefore exist, and may be relevant to neurological disease

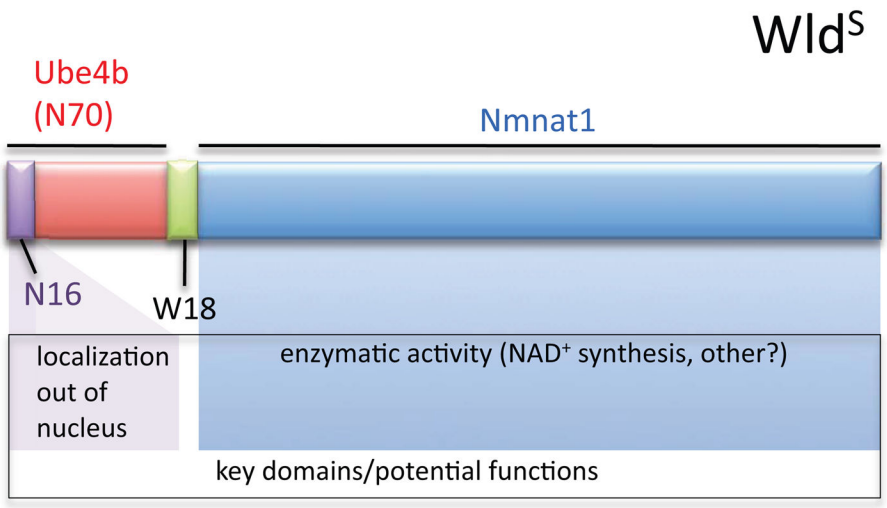

Figure 1. Wld^S protein structure
See text for details.

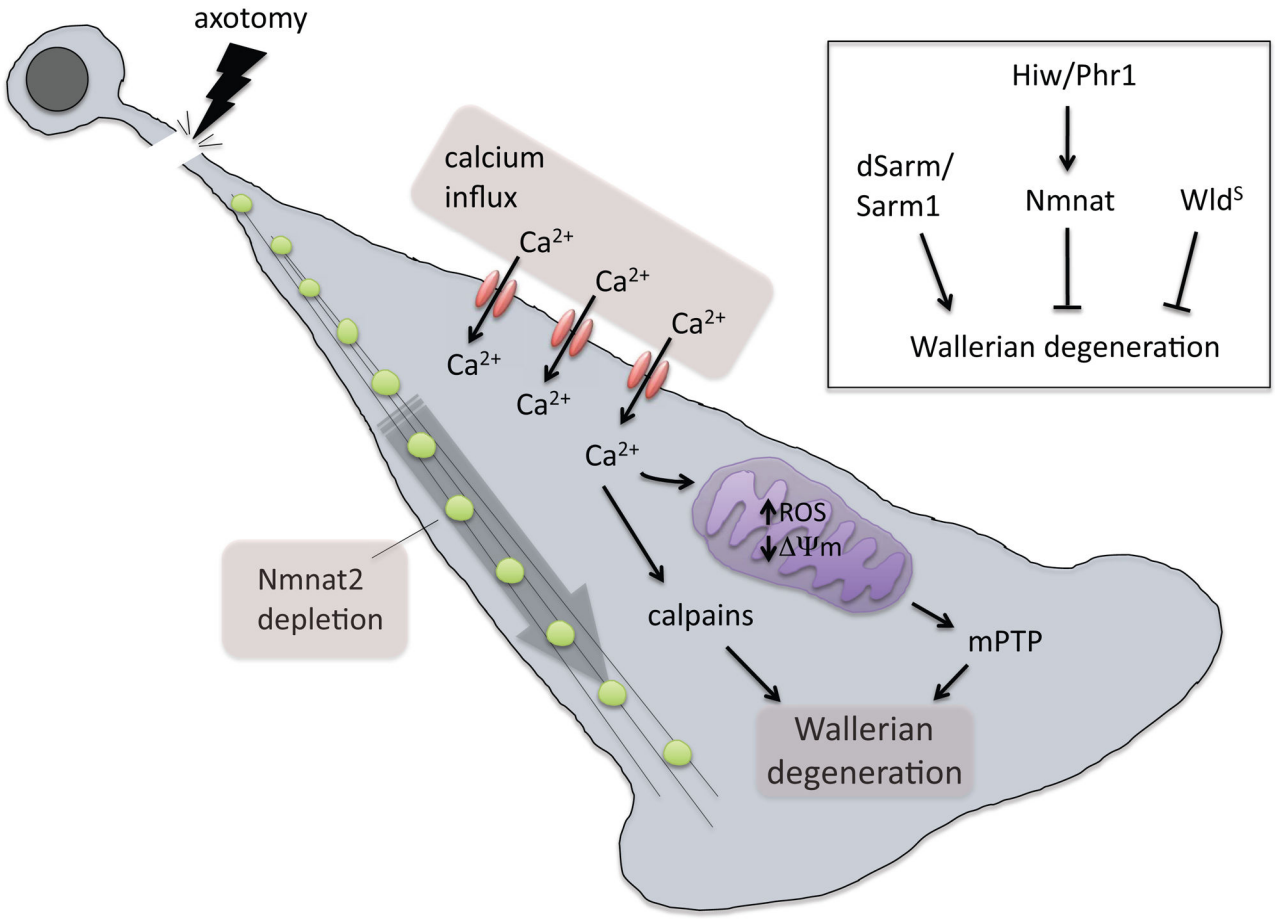

Figure 2. Signaling events initiated by axotomy

Nmnat2 is continuously delivered on vesicles (green) from the soma to the axon. Axotomy terminates delivery and axons survive until Nmnat2 is depleted. Influx of extracellular Ca^{2+} is activated by axotomy, which may be amplified by release from internal stores. Axonal Ca^{2+} activates calpains to drive cytoskeletal degradation, and overwhelms mitochondria, leading to loss of mitochondrial membrane potential and increased ROS production. Eventually mitochondrial undergo PTP formation and granular disintegration of the axon ensues.

Inset box: genetic modulators of Wallerian degeneration, see text for details.