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Wallerian Degeneration, Wld^S, and Nmnat

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Abstract

Traditionally, researchers have believed that axons are highly dependent on their cell bodies for long-term survival. However, recent studies point to the existence of axon-autonomous mechanism(s) that regulate rapid axon degeneration after axotomy. Here, we review the cellular and molecular events that underlie this process, termed Wallerian degeneration. We describe the biphasic nature of axon degeneration after axotomy and our current understanding of how Wld^S—an extraordinary protein formed by fusing a Ube4b sequence to Nmnat1—acts to protect severed axons. Interestingly, the neuroprotective effects of Wld^S span all species tested, which suggests that there is an ancient, Wld^S-sensitive axon destruction program. Recent studies with Wld^S also reveal that Wallerian degeneration is genetically related to several dying back axonopathies, thus arguing that Wallerian degeneration can serve as a useful model to understand, and potentially treat, axon degeneration in diverse traumatic or disease contexts.

Keywords

axon; dying back disorder; axonal transport; Wallerian-like degeneration

INTRODUCTION

Axons are huge cellular structures. If a Volkswagen Beetle sprouted a tail proportional to the length of a human motor axon, it would be ~20 miles (or 30 km) long. Maintaining such an enormous cellular outgrowth is a major challenge for the nervous system, and it is accomplished through the combined support of neuronal cell bodies and axon-associated glial cells. Without the delivery of materials from cell bodies by axonal transport (De Vos et al. 2008), axons undergo Wallerian degeneration (Coleman 2005); and without glial support in vivo, axons also degenerate (Nave & Trapp 2008). Consequently, axon and synapse loss is increasingly recognized as a major contributor to neurodegenerative disease.

Despite the importance of extra-axonal support for long-term survival, axons are now thought to initiate their own degeneration when these systems fail. Moreover, the trigger is

DISCLOSURE STATEMENT

M.C. discloses that the patent for the Nmnat2 modulator is pending.

not a general lack of nutrients. Injured axons appear to self-destruct through a regulatable or active process (Buckmaster et al. 1995, Raff et al. 2002) that is distinct from apoptosis (Burne et al. 1996, Deckwerth & Johnson 1994, Finn et al. 2000). Related mechanisms are triggered in some dying back axonopathies that raise the prospect of intervention (Ferri et al. 2003, Mi et al. 2005, Samsam et al. 2003). All of this has come to light because of the fortuitous discovery of the *slow Wallerian degeneration* mutant mouse (*Wld*^{S}) (Lunn et al. 1989), in which axon stumps that are distal to an injury survive ten times longer than normal. Over the past decade, advances in this intriguing and sometimes controversial field have begun to shed light on a novel form of neuroprotection (Araki et al. 2004, Avery et al. 2009, Beirowski et al. 2009, Conforti et al. 2009, Mack et al. 2001, Sasaki et al. 2009b, Yahata et al. 2009).

In this review, we highlight the basic cell biology of Wallerian degeneration and our current understanding of the mechanism by which Wld^S delays injury-induced axon degeneration. Then, we review the relationship between Wallerian degeneration and central and peripheral neuropathies as defined by the use of Wld^S as a tool to block Wallerian-like degeneration. Next, we examine new opportunities created by transferring the Wld^S phenotype to other species. Finally, we discuss several outstanding questions, which include the identity of the molecular trigger for Wallerian degeneration, and we discuss future steps in understanding how Wld^S protects axons.

WALLERIAN DEGENERATION

Wallerian degeneration is classically defined as the degeneration of axons distal to an injury, following Augustus Waller's original nerve transection experiments (Waller 1850). Here, we focus primarily on axonal events, which culminate in the granular disintegration of the axonal cytoskeleton and axon fragmentation that leaves characteristic myelin ovoids behind (Figure 1). However, the glial and macrophage clearance of degenerating axons, which we touch on briefly, is also an important part of Wallerian degeneration (Vargas & Barres 2006). Similar processes occur in unmyelinated axons in mammals and invertebrates (Avery et al. 2009, Ayaz et al. 2008, Macdonald et al. 2006) and in the mammalian central nervous system (CNS). CNS axons exhibit focal swellings that are many times wider than a normal axon many hours before axons fragment (Beirowski et al. 2010, Cajal 1928) and the slower clearance of axonal debris may contribute to the poor regenerative environment of the CNS (Vargas & Barres 2006).

SLOW WALLERIAN DEGENERATION

The extended survival of Wld^S axons without their cell bodies has fundamentally changed our view of axon degeneration. Wld^S transected axons in the sciatic nerve survive for over two weeks, compared to approximately 1.5 days in wild-type mice, and conduct evoked action potentials when stimulated (Lunn et al. 1989) (Figure 1). Transected Wld^S axons in the CNS survive for similar extended periods (Perry et al. 1991). Surprisingly, *Wld*^{S} mice are viable and show normal motor function, although they exhibit a secondary delay in axon regeneration (Brown et al. 1994).

Transected *Wld*^{S} axons eventually degenerate in a process that is more atrophic and gradual than the sudden fragmentation that characterizes wild-type axons (Beirowski et al. 2005). This may reflect the gradual depletion of structural proteins from long-term anucleated axons. Thus, rapid Wallerian degeneration in wild-type nerves may be an active or at least regulated process, similar to apoptosis in principle.

Wld^S is a dose-dependent, semidominant phenotype that is inherited through a single locus (Mack et al. 2001, Perry et al. 1990b). It arose by spontaneous mutation at Harlan UK (then Harlan Olac, hence the original name C57BL/6/Ola) and was discovered by chance after it became homozygous (Lunn et al. 1989). The precise genetic background for *Wld*^{S} is uncertain (Lyon et al. 1993; V.H. Perry, personal communication), and there is further genomic divergence from C57BL/6 (A.L. Wilbrey, J.W. Tsao, and M.P. Coleman, manuscript in preparation).

The phenotype is intrinsic to nerves rather than macrophages (Perry et al. 1990a) and to axons rather than glia (Glass et al. 1993). In Schwann cell grafts between Wld^S and C57BL/6, host axons rather than donor Schwann cells determine the rate of degeneration (Glass et al. 1993); and primary neuronal cultures that lack glia show a remarkably similar delay in Wallerian degeneration after neurite transection, although neurites of both genotypes degenerate faster than in vivo (Buckmaster et al. 1995, Glass et al. 1993). Moreover, neuron-specific, but not glial, expression of the Wld^S gene confers the phenotype in *Drosophila* (Hoopfer et al. 2006, Macdonald et al. 2006). Such an axon-specific effect on Wallerian degeneration is quite unique. Other mutations have been reported to influence Wallerian degeneration but seem to act on Schwann cell or macrophage responses rather than on axons (Keilhoff et al. 2002, Levy et al. 2001, Lopez-Vales et al. 2008, Narciso et al. 2009, Ramaglia et al. 2007).

The use of *Wld*^{S} mice as a genetic tool to explore the basis of cellular destruction pathways shows that neurodegenerative mechanisms are highly compartmentalized. Despite its robust effect on axon degeneration, *Wld*^{S} has no effect on apoptotic death of the cell soma, either in NGF-deprived sympathetic neuronal cultures or in axotomized motor neurons (Adalbert et al. 2006, Deckwerth & Johnson 1994), and no phenotypic change in any other cell type has been reported. Conversely, neither Bcl-2 overexpression nor Bax and Bak deletion alters Wallerian degeneration (Burne et al. 1996, Whitmore et al. 2003), and caspase 3 activation is neither detected in nor required for rapid Wallerian degeneration (Finn et al. 2000). Similar experiments established that axons in several disease models also die by nonapoptotic mechanisms. Bcl-2 overexpression and Bax deletion, respectively, rescue cell bodies in *pmn* mice and the DBA/2J glaucoma model but have no effect on axon degeneration (Libby et al. 2005, Sagot et al. 1995). Wld^S rescues axons in both cases (Ferri et al. 2003, Howell et al. 2007).

Synaptic terminals are also protected by *Wld*^{S} but act as another, partially-distinct compartment with respect to the timing of degeneration after injury (Gillingwater et al. 2002). Transected *Wld*^{S} motor axons support evoked neurotransmitter release at intact neuromuscular junctions for approximately five days compared to the usual 12–20 h (Ribchester et al. 1995), and CNS synapses are also protected (Gillingwater et al. 2006a).

However, NMJ denervation occurs far sooner in wild-type and *Wld*^{S} animals than degeneration of the axon trunk. Moreover, neuromuscular synapse preservation is lost in young adult Wld^S mice without any change in *Wld*^{S} expression, whereas Wld^S continues to preserve injured axon trunks (Gillingwater et al. 2002). Thus axonal and synaptic survival are both enhanced by Wld^S, but either the rate or the nature of the pathways involved differs.

New ENU mutant mice with enhanced synapse protection (Wong et al. 2009) and targeting of Nmnat1 to nerve terminals (E. Babetto, B. Beirowski, L. Janeckova, R. Brown, D. Thomson, R.R. Ribchester, M.P. Coleman, manuscript submitted) should shed more light on events at synapses. Interestingly, developmental synapse elimination is also unaltered in Wld^S mice (Parson et al. 1997), one of several findings that now distinguish developmental axon and synapse loss from injury-induced loss (Bishop et al. 2004, Hoopfer et al. 2006).

WALLERIAN-LIKE DEGENERATION

One central question is whether Wallerian degeneration is relevant to neurodegenerative disease. This occurred immediately to Waller, who stated: “It is particularly with reference to nervous diseases that it will be most desirable to extend these researches” (Waller 1850, p. 423). Decades later, dying-back-type axon degeneration in some peripheral nerve disorders was termed Wallerian-like degeneration based on morphological similarities (Cavanagh 1979, Griffin et al. 1996).

However, axon transection is rare in clinical neuroscience. Spinal injury and traumatic brain injury usually contuse and stretch axons respectively, which result in secondary axon interruption hours or days later. Peripheral nerves may be cut during surgery or wounding; but nerve damage by chronic pressure or metabolic, toxic, or inherited disorders is far more common. Axon transection has been observed directly in animal and cellular models of multiple sclerosis (Neumann et al. 2002; M. Kerschensteiner, personal communication), but whether this is the main mechanism in patients remains unclear. Axon endbulbs (Ferguson et al. 1997, Trapp et al. 1998) could alternatively begin as en passant swellings that precipitate distal axon degeneration (Coleman 2005).

Although axon transection is rare, the disruption of axonal transport is extremely common and also isolates distal axons from cell bodies. As a tool to block Wallerian degeneration, *Wld*^{S} mice made it possible to test the hypothesis that similar mechanisms are triggered in noninjury disorders. Dying back follows a Wallerian-like mechanism in some motor neuron disease and peripheral neuropathy models and in nitric oxide damage (Alvarez et al. 2008, Ferri et al. 2003, Samsam et al. 2003, Wang et al. 2002). CNS studies extended this mechanism to models of Parkinson’s disease, glaucoma, multiple sclerosis, and gracile axonal dystrophy (Beirowski et al. 2008, Hasbani & O’Malley 2006, Howell et al. 2007, Kaneko et al. 2006, Mi et al. 2005, Sajadi et al. 2004); and in primary culture, the neurotoxin vincristine and protein synthesis blockade also trigger Wallerian-like degeneration (Gilley & Coleman 2010, Wang et al. 2000). Thus, similar to using Bcl-2 to define apoptotic cell death, Wld^S sensitivity now provides a genetic definition for Wallerian-like degeneration.

Wld^S does not substantially block pathology in some models of amyotrophic lateral sclerosis or spinal muscular atrophy (Fischer et al. 2005, Kariya et al. 2009, Rose et al. 2008, Velde et al. 2004), whereas axonal swellings precede fragmentation by months in mouse models of familial Alzheimer's disease (Adalbert et al. 2009, Spires et al. 2005). Thus, Wallerian-like degeneration may not be the only outcome when transport is impaired. This may reflect a loss of different transport cargoes in different disorders or the reversion of synapse degeneration to wild type in older animals (Gillingwater et al. 2002). New transgenic mice with stronger synapse protection could help distinguish these possibilities (Beirowski et al. 2009).

THE WLD^S GENE AND PROTEIN

The Wld^S gene was mapped to mouse chromosome 4 (Lyon et al. 1993), in which an unusual genomic rearrangement brings two endogenous genes together. Their mRNAs splice to encode an in-frame fusion protein that is absent in wild-type mice (Figure 2) (Coleman et al. 1998, Conforti et al. 2000). The expression of this protein in transgenic mice replicates the Wld^S phenotype that identifies it as the Wld^S protein (Mack et al. 2001) (Figure 2), and the murine cDNA delays axon degeneration in rat, fly, and cell culture models (Adalbert et al. 2005, Araki et al. 2004, Hoopfer et al. 2006, Macdonald et al. 2006, Wang et al. 2001).

The C-terminal 285 amino acids comprise the complete protein sequence of nicotinamide mononucleotide adenylyltransferase 1 (Nmnat1), a key protein of the NAD^+ salvage pathway in mammals. Nmnat1 normally resides predominantly or exclusively in nuclei (Magni et al. 2004), where Wld^S is also abundant (Mack et al. 2001), and new roles for NAD^+ are emerging (Pollak et al. 2007). Other Nmnat isoforms synthesize NAD^+ in mitochondria and the Golgi apparatus (Berger et al. 2005, Raffaelli et al. 2002). The Wld^S protein synthesizes NAD^+ from nicotinamide mononucleotide and ATP but does not alter basal NAD^+ levels (Araki et al. 2004, Mack et al. 2001), probably owing to rapid NAD^+ catabolism (Pollak et al. 2007). The N-terminal 70 amino acids (N70) of Wld^S are derived from the N-terminus of Ube4b (or Ufd2a), an E4-type ubiquitin ligase that can add multiubiquitin chains to substrates of the ubiquitin fusion degradation (Ufd) pathway (Hatakeyama et al. 2001, Koegl et al. 1999). Only 6% of the Ube4b sequence is incorporated into Wld^S, which excludes the catalytic U box. Thus, Wld^S probably lacks ligase activity, but there is shared protein binding activity (Laser et al. 2006, Morreale et al. 2009). Finally, between the N70 and Nmnat1 sequences lies a unique 18 amino acid sequence generated by a read-through of Nmnat1 5′ UTR, the epitope of the specific Wld18 antibody (Samsam et al. 2003).

GAIN OF FUNCTION

Gain of function appears to be the most likely mechanism for Wld^S function. The genomic rearrangement retains the endogenous Nmnat1 and Ube4b genes, and the corresponding proteins are expressed at normal levels (Conforti et al. 2007b, Gillingwater et al. 2002). Thus, there is no obvious loss of function of either protein. Regarding a dominant negative mechanism, Nmnat activity is increased in Wld^S tissue (Mack et al. 2001), and deleting one allele of Nmnat1 does not alter Wallerian degeneration (L. Conforti, N. Smyth, and M.P.

Coleman, manuscript in preparation), whereas haploin-sufficiency for Ube4b causes axon pathology rather than axon protection (Kaneko-Oshikawa et al. 2005). For a gain of function mechanism, the key question remains whether this is an entirely novel function or whether Wld^S strengthens or mimics the function of an endogenous protein. Recent data support the latter model, indicating that Wld^S substitutes for Nmnat2, an essential axonal protein that is rapidly lost after axon injury (Gilley & Coleman 2010).

DEFINING PROTECTIVE DOMAINS IN THE MOLECULE AND THE CELL

Which domains of Wld^S are essential for the protection of severed axons? This question has been a central focus of the field for the past five years, but attempts to answer it have raised several controversies. Studies in primary culture have generally produced less consistent results regarding whether, where, and how Wld^S and its constituent domains protect severed axons (Araki et al. 2004, Conforti et al. 2007b, Wang et al. 2005) than studies in vivo. Structure-function analyses in mice and *Drosophila* (Avery et al. 2009; Beirowski et al. 2009; Conforti et al. 2007b, 2009; Sasaki et al. 2009a; Yahata et al. 2009) are now converging on a model in which the combinatorial activity of two key domains of Wld^S acts somewhere outside the nucleus to confer maximal axon protection (Figure 2).

Theoretically, Wld^S could promote axon protection through N70, Wld18, Nmnat1, or some combination of these domains. In vitro data argued that Nmnat1 could protect severed neurites in primary culture, although to a significantly lower degree than Wld^S (Araki et al. 2004, Sasaki et al. 2009a, Wang et al. 2005). However, overexpressed Nmnat1 is not sufficient to protect severed axons in transgenic mice (Conforti et al. 2007b), whether they are driven by the same β *actin* promoter used to identify Wld^S previously (Mack et al. 2001) or they express up to threefold higher levels using the *Prp* promoter (Yahata et al. 2009).

Can Nmnat1 protect axons in vivo in any context? In *Drosophila*, the expression of mouse Nmnat1 in ORNs using the Gal4/UAS binary expression system resulted in the strong protection of severed axons (Macdonald et al. 2006), but, importantly, the protection it afforded was consistently weaker and lasted for a shorter period when compared to Wld^S (Avery et al. 2009). Thus, Nmnat1 is likely an important part of Wld^S neuroprotective function, but why is there a discrepancy in Nmnat1-mediated protection between flies and mice? The most plausible explanation is that Gal4/UAS expresses extremely high levels of Nmnat1 in *Drosophila* ORNs. The Gal4 driver line used (*OR22a-Gal4*) is quite strong, which results in high levels of Gal4, and the expression of Nmnat1 (i.e., *UAS-Nmnat1*) is further amplified because Gal4 is an efficient transcriptional activator of *UAS*-regulated target genes. Perhaps experiments aimed at dramatically increasing the levels of Nmnat1 in mouse axons might ultimately provide some level of axon protection in the mouse model. Alternatively, these discrepancies may reflect differences in axon length, diameter, or other characteristics among *Drosophila* and mice that affect the initiation and execution of Wallerian degeneration.

Although wild-type Nmnat1 is not sufficient for the robust protection of severed axons in mice, its activity is clearly an essential part of the protective action of Wld^S. Three groups recently generated animals that express Wld^S variants in which the enzymatic activity of

Nmnat1 was disrupted by mutation (Conforti et al. 2009, Yahata et al. 2009). In each case, the neuroprotective effects of Wld^S were severely reduced (Avery et al. 2009) or abolished (Conforti et al. 2009, Yahata et al. 2009), this time consistent with primary culture results (Araki et al. 2004, Jia et al. 2007). These results are important because they implicate Nmnat1 enzymatic activity in Wld^S-dependent axon protection and because they begin to address a second possible role, that of a chaperone, for Nmnat1 in axon protection. An interesting recent study in *Drosophila* identified mutants in the sole fly Nmnat gene (*dnmnat*), whose neurons appear to develop normally, which extend axons to the appropriate targets but then exhibit age- and activity-dependent degeneration (Zhai et al. 2006). These data raised the intriguing possibility that basic axon integrity in the mature nervous system might be regulated by constitutive dNmnat-dependent suppression of neuronal degeneration.

Surprisingly, in rescue experiments, dNmnat enzymatic activity was found to be dispensable for the rescue of *dnmnat* mutant neurodegenerative phenotypes (Zhai et al. 2006). A subsequent study proposed a novel chaperone-like role for dNmnat and mammalian Nmnat3 (e.g., in the refolding of denatured proteins), independent of NAD^+ biosynthetic activity; and the authors in turn proposed that this novel Nmnat chaperone activity might explain some aspects of the Wld^S-dependent protection of severed axons (Zhai et al. 2008). Such a chaperone-like role, which could stabilize axonal proteins, might fit nicely with the neuroprotective effects observed in Wld^S mice, which essentially express a modified Nmnat1.

However, as mentioned above, the mutation of either the ATP binding site of Nmnat1 (Avery et al. 2009) or the NMN^+ binding site (Conforti et al. 2009, Yahata et al. 2009), which would be expected to leave chaperone function intact, potently blocked the ability of Wld^S to protect severed axons. Is Nmnat-dependent chaperone activity important for Wld^S-mediated axon protection? One possibility is that dNmnat and Nmnat3 chaperone-like functions have nothing to do with Wld^S-mediated axon protection and are more specific to the neurodegenerative phenotypes observed in *dnmnat* mutants. Alternatively, Wld^S may have two essential functions: to generate NAD^+ or another biosynthetic product that would require enzymatic activity and to act as a chaperone. If both activities are essential, then the disruption of either should suppress the axon protective effects of Wld^S, as found in transgenic mice and flies. We can conclude that Nmnat-dependent chaperone activity cannot be the sole role for the Nmnat1 domain of Wld^S.

The weaker axon protective effect of Nmnat1 in flies and mice suggests that other portions of Wld^S are essential for Wld^S-like levels of axon protection, through affecting either its localization or activity. A protein interaction site within the N-terminal 16 amino acids (N16), which is derived from Ube4b, coimmunoprecipitates valosin containing protein (VCP/p97) from mouse brain homogenates (Laser et al. 2006). VCP is a AAA-ATPase with key roles in the UPS and membrane fusion (Wang et al. 2004) and is the only abundant, direct binding partner precipitated by this region.

Subsequent work has shown that N16 is necessary and sufficient to explain the differences in protective effects between Wld^S and Nmnat1 in vivo. The deletion of N16 from Wld^S completely suppresses the axon protection afforded by Wld^S in mice (Conforti et al. 2009)

and greatly weakens the protection of axons in *Drosophila* to a level found by expressing Nmnat1 alone (Figure 2) (Avery et al. 2009). Moreover, fusing N16 directly to Nmnat1 results in levels of axon protection that are indistinguishable from Wld^S (Avery et al. 2009). Does N16 exert its effects on axon preservation through VCP? Two in vivo experiments support this notion. First, replacing N16 in Wld^S with a well-characterized VCP-binding motif from ataxin 3 (Morreale et al. 2009), which shares only five amino acids with N16, restores Wld^S-like axon protection in mice (Conforti et al. 2009). Second, RNAi knockdown of fly VCP is sufficient to suppress axon protection by Wld^S to levels indistinguishable from those afforded by Nmnat1 alone. Together, these data argue strongly that N16, likely working through VCP, and an enzymatically active Nmnat1, are the critical domains and essential activities for Wld^S-like levels of axon protection.

A second major question regarding Wld^S function relates to its site of action. Is it functioning in the nucleus or elsewhere in the cell? Clarifying this point is critical for understanding precisely how Wld^S can so potently suppress Wallerian degeneration. The striking nuclear localization of Wld^S has led to several studies of expression patterns in Wld^S versus wild-type neurons (Gillingwater et al. 2006b, Simonin et al. 2007), in the hope of identifying changes in the expression of key genes that modulate axon autodestruction (see below). However, more recent careful analysis of the localization of Wld^S and potential extranuclear sites of action proposes that Wld^S exerts its neuroprotective effects outside the nucleus.

Nmnat1 contains a strong nuclear localization sequence (NLS), which may account for the nuclear localization of Wld^S in vivo. If Wld^S is required in the nucleus, then deleting the NLS from Nmnat1 should weaken its protective effects. In striking contrast, the mutation of the Nmnat1 NLS from Wld^S doubled the latest timepoint when surviving axons could be identified and greatly enhanced synaptic protection especially in older mice (Beirowski et al. 2009). Moreover, although the expression of Nmnat1 alone in mice fails to suppress Wallerian degeneration (Conforti et al. 2007b, Yahata et al. 2009), the generation of mice that harbor a cytoplasmic mutant of Nmnat1 resulted in robust axon protection (Sasaki et al. 2009a), although the relative contributions of cytoplasmic targeting and high expression levels in these mice remain unclear. Thus, excluding Wld^S and Nmnat1 from the nucleus makes them more protective of severed axons. This observation hints at a possible role for N16 in relocalizing Nmnat1 activity outside the nucleus to another cellular location. Indeed, a careful analysis of Wld^S expression in peripheral nerves revealed low but detectable levels of expression (Beirowski et al. 2009, Yahata et al. 2009), consistent with a potential non-nuclear site of action for Wld^S in mice.

OUTSTANDING QUESTIONS ON THE WLD^S AXON PROTECTIVE MECHANISM

What is the Cellular Site of WLD^S Action?

The discussion above establishes a nonnuclear site of action for Wld^S; now, we need to identify the site. One approach is to distinguish between roles in the axon and soma, and another approach is to identify the appropriate organelle(s) or protein complex. This may be

less straightforward than resolving nuclear or cytoplasmic actions. Wld^S localizes to multiple internal membranous organelles (Beirowski et al. 2009, Yahata et al. 2009). Mitochondria have attracted particular interest because injured axons are protected by overexpressed Nmnat3, the mitochondrial isoform (Avery et al. 2009, Yahata et al. 2009). However, overexpressed proteins may have ectopic locations, and the experience with nuclei reminds us that an observable location does not identify the site of action (Beirowski et al. 2009). Thus, other locations still need to be considered.

Other possible sites include the Golgi apparatus and the ER, particularly because these are sites where VCP is abundant. VCP binding redistributes Wld^S within nuclei, which suggests that cytoplasmic Wld^S is likely to migrate to sites where VCP is abundant (Wilbrey et al. 2008), although this also includes mitochondria (Braun et al. 2006). To complicate matters further, mitochondria are rapidly transported in axons (Misgeld et al. 2007), have close interactions with the ER that may influence axon survival (Merkwirth & Langer 2008), and exchange NAD^+ with their surroundings (Todisco et al. 2006). Thus, any NAD^+ produced within mitochondria could act on nearby structures to protect axons or vice versa. Wld^S becomes ineffective when targeted to the internal surface of the plasma membrane, so this may not be the site of action (Avery et al. 2009), but distinguishing between other sites could be far more complex.

Is NAD^+ a Protective WLD^S Product?

An alternative route forward is to identify functions downstream of Wld^S Nmnat activity. In addition to its long established role in bioenergetic metabolism, NAD^+ is also a substrate for protein deacetylation by sirtuins, for synthesis of cyclic ADP ribose and ADP ribose, both regulators of internal calcium stores, and for mono- and poly-ADP ribosylation of proteins (Hassa et al. 2006, Pollak et al. 2007). It also potentiates the response of sodium-activated potassium channels to sodium (Tamsett et al. 2009), although an action of Wld^S at plasma membranes seems unlikely (see above). NAD^+ is also used to synthesize $NADP^+$, whose reduced form has roles in detoxification and oxidative defense (Pollak et al. 2007). Identifying one of these as an important downstream step could help resolve the site of action because rapid NAD^+ catabolism limits its long-range diffusion (Pollak et al. 2007).

However, despite agreement that Wld^S needs Nmnat activity to protect axons (above), it is less clear whether NAD^+ is the protective enzyme product. No increase in NAD^+ is detectable with Wld^S (Araki et al. 2004, Mack et al. 2001) or when Nmnat activity is raised more than 15-fold (Sasaki et al. 2009a). Knockdown or inhibition of Nampt, the rate-limiting enzyme in the NAD^+ salvage pathway, failed to revert Wld^S phenotype in one study (Sasaki et al. 2009b) and reverted it only partially in another (Conforti et al. 2009). Conversely, increasing cellular NAD^+ by blocking NAD^+ catabolyzing enzyme CD38 does not confer a Wld^S phenotype (Sasaki et al. 2009b; A.L. Wilbrey & M.P. Coleman, unpublished observations). One proposal is that Nmnat activity decreases reactive oxygen species in mitochondria because it protects axons from rotenone-induced damage without restoring normal ATP levels (Press & Milbrandt 2008). Another proposal is that Nmnat catalyzes the reverse reaction under stress conditions, which generates an emergency supply of ATP (Yahata et al. 2009), although this appears unlikely to preserve axons for several

weeks. Many of these observations are also consistent with NAD^+ acting at a highly localized site, but clearly, alternative substrates and products of Nmnat isoforms (Hassa et al. 2006, Sorci et al. 2007) need to be considered alongside NAD^+ as candidates for the axon protective mechanism.

THE MOLECULAR TRIGGER FOR WALLERIAN DEGENERATION

To fully understand how Wld^S, or some Nmnats, delay Wallerian degeneration, we must understand the process they delay. Remarkably, we still do not know the molecular pathway for Wallerian degeneration, a question that far predates Wld^S mice (Lubinska 1977). Almost all recent studies have focused on overexpressed or modified proteins, including Wld^S itself, which is absent in wild-type organisms. The results are exciting but do not tell us directly how endogenous proteins behave when a wild-type axon is injured or sick. The “potential to throw light on the normal processes of nerve degeneration” was a major driving force for identifying Wld^S in the first place (Lyon et al. 1993, p. 9717), and realizing this goal remains a major gap in the field.

However, there are some clues from studying how Wld^S alters disease models. Two alternative models for how injury triggers Wallerian degeneration are a prodegeneration signal generated at the lesion site (e.g., calcium influx through the cut end) and the absence of a prosurvival signal derived from the cell body. The disease studies clearly show that Wallerian-like processes can be triggered in the complete absence of physical injury. In contrast, there is a strong correlation with disorders of axonal transport (Beirowski et al. 2008, Ferri et al. 2003, Howell et al. 2007, Wang et al. 2005). Non-lethal impairment of protein synthesis also triggers Wallerian-like degeneration (Gilley & Coleman 2010). These observations support the survival factor model and argue against the need for any signal derived from the injury site.

SURVIVAL AND CATASTROPHE

We have much to learn from the biphasic degeneration of wild-type axons. An initial latent phase lasts 36–44 hours after injury in a mouse sciatic nerve, followed by a sudden and catastrophic fragmentation phase (Beirowski et al. 2005, Kerschensteiner et al. 2005, Lubinska 1977). During the latent phase, nodal gaps widen and motor axons lose their terminals (Conforti et al. 2007a, Miledi & Slater 1970), but axon trunks remain continuous and can conduct evoked action potentials (Lunn et al. 1989, Moldovan et al. 2009). Fly axons, zebrafish axons (S. Martin & A. Sagasti, personal communication), and transected neurites in primary culture also show a latent phase, albeit shorter than in mice. Both between and within species, there is a correlation with axon stump length.

After surviving 1.5 days without a cell body, several centimeters of mouse wild-type axon undergoes catastrophic fragmentation, possibly in one hour (Figure 3) (Beirowski et al. 2005). An equally rapid process, captured in live in vivo imaging, occurs in CNS axons, where there is also more axon swelling (Kerschensteiner et al. 2005). The onset of this fragmentation phase is heterogeneous among axons in the same nerve and depends on

intrinsic properties such as axon diameter and extrinsic properties such as temperature (Gamble & Jha 1958, Lubinska 1977).

The model posited to explain these data is strikingly similar to the survival signal discussed above. Lubinska suggested that distal axons require the constant delivery of a trophic factor, anterogradely transported from cell bodies (Lubinska 1977) but partially redistributed by retrograde transport (Lubinska 1982). Injured axons degenerate when this putative factor falls below a threshold level. This can explain why cold temperatures and proteasome inhibition extend the latent phase because these are likely to increase the trophic factor half-life (Gamble & Jha 1958, Macinnis & Campenot 2005). This also explains reports that fragmentation begins at the proximal end of a transected axon stump and progresses distally (Beirowski et al. 2005, Lubinska 1977) because steady degradation of the putative survival factor in distal axons will produce a net anterograde flux, which depletes the survival factor in regions closer to the injury sooner than in distal axons. However, the directionality remains controversial (Beirowski et al. 2005). The most consistent, and perhaps the most important, point is the sudden switch.

Lubinska's reasons for suggesting a single trophic factor are unclear but probably relate to the simple biphasic kinetics. A sudden switch is best explained by a single event: the depletion of one axonal component. Multiple survival factors with slightly different half-lives and transport kinetics would be expected to produce a more gradual switch.

Any complete model for the molecular mechanism of Wallerian degeneration must explain what is happening during the latent phase and why it ends so suddenly. A complete model for the action of Wld^S should also explain whether and how the longer latent phase in Wld^S axons relates to the shorter latent phase in wild type.

WLD^S AND SURVIVAL SIGNALING

The key to future progress on this question is to fit the Wld^S protective mechanism into the survival signal, or trophic factor model, of wild-type axon degeneration. The ability of axons to survive for 2–3 weeks originally cast doubt on the survival signal model and suggested that short-term axon survival is independent of cell bodies. However, these are mutant axons in which one or more axonal components must have been altered prior to any nerve lesion. Wld^S could not, and indeed does not (Wishart et al. 2007), cause major alterations to the axonal proteome, but if Lubinska's proposal of a single, endogenous trophic factor is correct, only one or a few changes may be sufficient to circumvent its loss. This might be achieved by the following:

- **a.** directly adding to the pool of a wild-type survival factor,
- **b.** delivering an endogenous survival factor in greater quantities to axons,
- **c.** depleting axons of a factor needed to execute degeneration,
- **d.** stabilizing a survival factor in injured axons,
- **e.** promoting local synthesis of an endogenous survival factor in axons, or
- **f.** substituting for an endogenous survival factor.

Model (*a*) is clearly not the case. Wld^S cannot be identical to a wild-type survival factor because it is absent from wild-type cells.

GENE EXPRESSION

Models (*b*) and (*c*) rely on altering the supply of other axonal components. This could be achieved by altering their axonal transport or their expression level. Wld^S has no obvious similarity to any axonal transport motor or regulator, but it is abundant in nuclei (Mack et al. 2001), and several transcripts and proteins are expressed at different levels in Wld^S and C57BL/6 mice (Chitnis et al. 2007; Gillingwater et al. 2006b; Simonin et al. 2007; Wishart et al. 2007, 2008). Moreover, sirtuin-1, a nuclear NAD^+ dependent gene silencing protein, was reported to be required for axon protection by exogenous NAD^+ (Araki et al. 2004).

However, no causative link has been established yet between gene expression or proteomic changes and axon protection by Wld^S, and sirtuins are not required for Wld^S to protect axons (Avery et al. 2009, Wang et al. 2005). Many of these gene expression changes are also not conserved in Wld^S rats, and some changes reflect the genomic divergence of Wld^S and C57BL/6 mice directly (A.L. Wilbrey, J.W. Tsao, M.R. Cookson, and M.P. Coleman, manuscript in preparation). Moreover, the finding that Wld^S has a cytoplasmic site of action (above) argues against the existence of a mechanism that involves changes in gene expression.

UPS IMPAIRMENT

Model (*d*), stabilizing a survival factor in injured axons, most clearly fits with a possible impairment of the ubiquitin proteasome system (UPS) (Coleman & Ribchester 2004, Ehlers 2003). Impairing the UPS prolongs the survival of injured axons (Hoopfer et al. 2006, Macinnis & Campenot 2005, Zhai et al. 2003), but it is less clear whether this is how Wld^S prolongs axon survival. General impairment of the UPS is more likely to make axons sick than protect them. Proteasome inhibition causes neurite degeneration in culture and peripheral neuropathy in humans (Kane et al. 2003, Laser et al. 2003), whereas several mouse axonopathies are caused by UPS impairment (Kaneko-Oshikawa et al. 2005, Saigoh et al. 1999). An efficient UPS appears to be essential for axon health (Coleman & Ribchester 2004); so the robust health of Wld^S mice, rats, flies, and primary cultures argues against any general UPS impairment.

Interaction between Wld^S and VCP has the potential to impair specific UPS functions. In addition to Ube4b, several other ubiquitin ligases and the deubiquitinating enzyme ataxin 3 bind VCP at the same site, so Wld^S could compete for VCP binding with any or all of these proteins (Morreale et al. 2009). However, the VCP binding site is dispensable in the context of modified Nmnat1 or Nmnat3 (Avery et al. 2009, Sasaki et al. 2009a, Yahata et al. 2009); thus, these actions are not needed to protect axons.

LOCAL PROTEIN SYNTHESIS

Model (*e*) is based on the observation that injured axons elevate local protein synthesis on both sides of a lesion (Court et al. 2008, Perlson et al. 2005), so Wld^S might protect axons

by stimulating the local synthesis of a survival factor. The local synthesis of Wld^S in axons has also been proposed (Fainzilber & Twiss 2006). However, the protein synthesis machinery appears at the same time in injured Wld^S and wild-type axons (Court et al. 2008), and the protective capacity of Wld^S is unabated when protein synthesis is suppressed (Gilley & Coleman 2010). Thus, local synthesis is not required for Wld^S to protect axons.

SUBSTITUTING FOR A SURVIVAL FACTOR

Model (*f*) would fit with Wld^S substituting for an endogenous Nmnat in injured axons. This concept seems implicit in the various Nmnat overexpression studies but has not been phrased this way, perhaps because the nucleus was previously seen as a likely site of action. The recent knowledge that Wld^S acts outside nuclei, and is present in axons, refocuses attention on what it may do there, consistent with an earlier report of a local site of action (Wang et al. 2005). A key requirement is that the respective Nmnat should also be present in axons. Interestingly, it now seems that Nmnat2, like some other Golgi proteins (Merianda et al. 2009), is present in axons and that its depletion is necessary for rapid Wallerian degeneration in vitro of injured axons and sufficient for Wallerian-like degeneration of uninjured axons (Gilley & Coleman 2010). This observation supports a model in which Nmnat2 is an endogenous axon survival factor, and Wld^S, a far more stable protein, can substitute for a prolonged period when it is present.

ACTIVE OR PASSIVE

A related discussion is whether Wallerian degeneration is an active process. Active could have several meanings. Unlike apoptosis, Wallerian degeneration does not require de novo protein synthesis (Gilley & Coleman 2010). Proteases are required to execute it because calpains are involved in the later stages (Schlaepfer 1974), but more exciting would be whether the molecular trigger involves a cascade of regulatory proteases. The involvement of caspase 6 in axonal pruning shows that such cascades can regulate axon degeneration (Nikolaev et al. 2009), although no evidence links this to Wallerian degeneration yet. Kinase cascades may also be involved. Deleting dual leucine kinase or applying a partially specific JNK inhibitor modestly delays Wallerian degeneration (Miller et al. 2009), and proteasome inhibition fails to delay Wallerian degeneration if MEK activity is blocked (Macinnis & Campenot 2005). In both cases, it will be interesting to know whether and how this relates to the axon protective effect of Wld^S. Finally, screens for loss-of-function mutations (below) could be informative. Even a single loss-of-function mutation in a neuronal gene that phenocopied Wld^S would demonstrate that Wallerian degeneration is indeed an active process, driven by an underlying and definable genetic program.

THE WLD^S ZOO: FROM MICE TO FLIES AND BEYOND

For 16 years, Wld^S could only be studied in mice. In the past five years, this has changed dramatically with the generation of Wld^S rats and flies (Adalbert et al. 2005, Hoopfer et al. 2006, Macdonald et al. 2006) and recently also zebra fish (S. Martin & A. Sagasti, personal comunication). This replication in diverse species using mouse cDNA raises intriguing evolutionary questions and provides new tools for mechanism and disease studies.

Wld^S rats were generated for those surgical procedures and disease models in which rats have advantages over mice (Adalbert et al. 2005). For example, ventral root avulsion was used to show that Wld^S neuroprotection is compartment-specific in vivo (Adalbert et al. 2006), and laser photocoagulation of the trabecular meshwork was used to show axon protection in an induced glaucoma model (Beirowski et al. 2008). Wld^S rats have also made an unexpected contribution to mechanism studies. Changes that mediate Wld^S action in mice must also be present in rats, which enables us to eliminate CD200 as a candidate despite its elevation in Wld^S mice (Chitnis et al. 2007). Wld^S rats also provide an abundant source of tissue for biochemical and proteomic studies and establish a precedent for a replicating Wld^S phenotype in other mammals.

The serendipitous identification of *Wld^S* mice by Perry and colleagues was a fortunate event that revolutionized how we think about Wallerian degeneration, but the probability of further spontaneous mutants is low. Moreover, beyond the fact that Wld^S can block it, we know almost nothing about the molecular regulation of Wallerian degeneration. Is there a single or many genetic switches that must be thrown to activate axon autodestruction? What initiates and executes these events? And importantly, what is the mechanism by which Wld^S impinges upon these pathways?

One powerful approach to these and other outstanding questions that has been lacking in the field is unbiased forward genetic screening for mutants that modify Wallerian degeneration or axon protection by Wld^S. A recent screen for dominant ENU-induced mutants in mice has produced a new strain that strengthens protection of neuromuscular synapses (Wong et al. 2009), but screening for loss-of-function mutants in mice is labor intensive, expensive, and slow.

Two essential features to define an invertebrate system as tractable (and relevant) for the study of Wallerian degeneration are activation of an axon autodestruction program after axotomy that is morphologically similar to mammalian Wallerian degeneration and genetic regulation of this degenerative event by Wld^S. The recent development of a simple and reproducible approach for assaying axon degeneration in the adult *Drosophila* olfactory system allowed for the first detailed in vivo analysis of Wallerian degeneration and Wld^S function in invertebrate models (Macdonald et al. 2006) (Figure 1). Interestingly, this work revealed that the events that lead to axon destruction indeed appear morphologically similar to Wallerian degeneration in mammals: Severed axons remain intact for a defined latent phase of 6–8 h, subsequently show beading and cytoskeletal breakdown, and finally undergo wholesale fragmentation (Macdonald et al. 2006). Similarly, when PDF$^+$ CNS axons were severed in primary cultures of the adult *Drosophila* brain, these axons also underwent degeneration within a day (Ayaz et al. 2008). Thus, when *Drosophila* axons are severed, the distal fragments degenerate after a latent phase and ultimately disappear from the CNS.

Can these injury-induced degenerative events in invertebrate axons be regulated by Wld^S? Impressively, in the case of *Drosophila* adult ORNs, mouse Wld^S suppresses Wallerian degeneration for >3 weeks after injury (Avery et al. 2009, Macdonald et al. 2006). Moreover, a recent structure-function study of Wld^S, Nmnat1, Nmnat2, and Nmnat3 in *Drosophila* (Avery et al. 2009) led to results strikingly similar to those found in mammalian systems

with similar molecules (Conforti et al. 2009, Yahata et al. 2009) (Figure 2). Together, these observations argue strongly that the cellular and molecular mechanisms that mediate axon autodestruction are ancient features of neuronal cell types and are well conserved in mice and flies.

Laser axotomy, and the use of mutants with fragile axons that spontaneously break in response to worm movement, are emerging as useful techniques to sever axons in vivo in *C. elegans*. Although the focus of this work is exploring mechanisms of regeneration of the proximal stump (Guo et al. 2008; Hammarlund et al. 2007, 2009; Wu et al. 2007; Yanik et al. 2004), we can glean some information regarding degeneration of the distal fragment, which beads, degenerates, and disappears in a manner similar to Wallerian degeneration in mice and flies (Hammarlund et al. 2007, Wu et al. 2007). Whether Wld^S can suppress this degeneration remains to be determined.

Drosophila and *C. elegans* appear poised to contribute in major ways to understanding the basic cellular and molecular mechanisms that drive Wallerian degeneration and to further our understanding of how Wld^S protects severed axons. Foremost, these organisms are highly amenable to rapid forward genetic analysis, which allows for straightforward genetic screens for Wallerian degeneration mutants or modifiers of Wld^S-function. Exploiting this opportunity, along with other tools available in these systems such as genetically-encoded whole-genome RNAi collections, and genetic mosaic approaches to study cell autonomous roles of essential genes should help tremendously in rapidly defining the pathways that promote Wallerian degeneration or Wld^S function.

Finally, although Wld^S has been an extremely useful tool to explore the molecular relationship between Wallerian degeneration and mouse models of neurodegenerative disease, forward genetic screens in invertebrates are expected to lead to the identification of new Wld^S-independent tools that modulate other steps in the Wallerian degeneration pathway. These, in turn, will represent a new battery of genetic reagents with which to reassess this central question and could ultimately lead to the characterization of a core set of axon destruction genes used in diverse degenerative settings.

LESSONS FROM NATURE: DEGENERATION NOT REQUIRED

Do all severed axons have to degenerate? Some invertebrate axons exhibit very slow Wallerian degeneration, even in wild-type organisms (Benbassat & Spira 1994, Nordlander & Singer 1972). This is particularly well studied in crustaceans in which an evoked transmitter release can occur up to one year after axon transection (Parnas et al. 1991). Similarly, in two species of crickets, *T. commodus* and *G. bimaculatus*, severed PDF-positive axons survive for up to 90 days after axotomy, and behaviors subject to their control appear to remain intact (Stengl 1995). A few wild-type vertebrate axons show a similar phenotype, usually in extremely large-diameter axons (Zottoli et al. 1987). Unlike injured Wld^S axons, these axons are typically invaded by hypertrophic adaxonal glia, which are thought to transfer proteins to axons. Similar events may occur in mammalian axons, but this does not appear to contribute to the Wld^S phenotype (Court et al. 2008). Thus, the means of

resisting Wallerian degeneration may be different, although the identities of any proteins supplied by glia would be very interesting for understanding axon survival mechanisms.

All this leads naturally to the question of whether a similar pathway operates in humans. A repeat of the tandem triplication that gave rise to Wld^S in mice seems unlikely. However, because minor changes to Nmnat1 or Nmnat3 can delay axon degeneration, it will be interesting to determine whether polymorphisms in these proteins, or in homologs of other proteins identified in invertebrate screens, alter susceptibility to neurodegenerative disorders.

ROLES FOR GLIA IN AXON SURVIVAL AND WALLERIAN DEGENERATION

The role of glia in Wallerian degeneration is normally thought to be limited to clearance of axonal debris and myelin ovoids (Vargas & Barres 2006), but could glia also play an instructive role in axon degeneration? To date, no clear genetic evidence links glial phagocytic activity to the destruction of target cell. For example, when glial engulfment activity is blocked by either mutations in the *draper* gene, which encodes an engulfment receptor required for clearance of axonal debris (Macdonald et al. 2006), or by suppressing endocytosis with a glial-expressed dominant temperature-sensitive dynamin (Doherty et al. 2009; J. Zeigenfuss and M.R. Freeman, unpublished observations), severed axons fragment on schedule. Likewise, although the glial clearance of fragmented axons and myelin debris is much slower in the CNS than in the PNS, CNS axons themselves fragment over a normal time frame (Vargas & Barres 2006). However, a potential role for Schwann cells has been suggested, based on the observation that Wallerian degeneration appears to be nucleated in the middle of each internode following the widening of nodes of Ranvier (Lubinska 1977). Future studies that directly address the precise sequence of these events and the neuron-glia signaling mechanisms involved in axon/myelin clearance should shed light on these important events.

What is the role of glia in supporting severed Wld^S-expressing axons during the many weeks they linger (without cell body support) in the CNS? It would be amazing if any axons survived on their own during this time without any contribution from glia. More likely, several key cellular components, including high energy metabolites, are passed from glia to surviving axon stumps (Court et al. 2008). Such a requirement might explain why Wld^S and wild-type axons degenerate more quickly in vitro than in vivo (Buckmaster et al. 1995). A major goal for the field should be to define precisely how axons are nourished by surrounding glia, and how these mechanisms impact axon survival or degeneration in Wallerian degeneration and neurodegenerative disease.

CONCLUSIONS

The past decade has seen a revolution in how we think about axon destruction after injury. We now understand that Wallerian degeneration is a highly regulated process, in which a poorly understood latent phase precedes the rapid and catastrophic destruction of the axon. Amazingly, Wallerian degeneration can be suppressed by a single protein, Wld^S, and this effect is robust even in diverse species. Two domains of Wld^S appear critical for its neuroprotective function and likely function at a highly-localized non-nuclear site, but

precisely how and where they exert their effects remains unclear. There are several extremely interesting outstanding questions in the field, including the following:

1. What is the molecular trigger that activates Wallerian degeneration?
2. What other proteins regulate axon survival/destruction? Do these include executors of an active process as well as inhibitors?
3. What are the endogenous regulators of Wallerian degeneration in vivo, and what are their roles in axon degeneration disorders?
4. Where in the cytoplasm/axoplasm does Wld^S act?
5. Is NAD^+ the Nmnat product responsible for axon survival, and if so, what does it do?
6. Why does Wld^S protect axons robustly in some axonopathies but not in others?
7. Does a Wld^S phenotype occur in the human population, and does this influence neurodegeneration?

It seems that Waller was right and that studies of Wallerian degeneration are informative about the molecular bases of axon degeneration in diverse injury and disease contexts. Answering the above questions is the next critical step in revealing the pathway and will advance our understanding of axon biology in fundamental ways.

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Glossary

Axonal transport
the bidirectional active transport of cargoes between axons and cell bodies and along axons

Wallerian degeneration
the degeneration of an axon distal to a site of injury

Wld^S
the slow Wallerian degeneration protein, an aberrant fusion protein that delays degeneration of injured axons by tenfold

Wallerian-like degeneration
the degeneration of axons in the absence of transection or crush injury with Wallerian-like morphology and/or genetic regulation (Wld^S-sensitivity)

Nmnat
nicotinamide mononucleotide adenylyltransferase; an enzyme that catalyzes NAD^+ synthesis in the salvage pathway. There are three isoforms in mammals

Dying back disorder

a neurodegenerative disorder in which axons die before cell bodies and/or in a retrograde pattern that begins with their distal ends

N70

the N-terminal 70 amino acids of Wld^S and Ube4b

Ube4b

ubiquitin ligase E4b, also known as Ufd2a. It carries out multiubiquitination of substrates in the ubiquitin fusion degradation pathway

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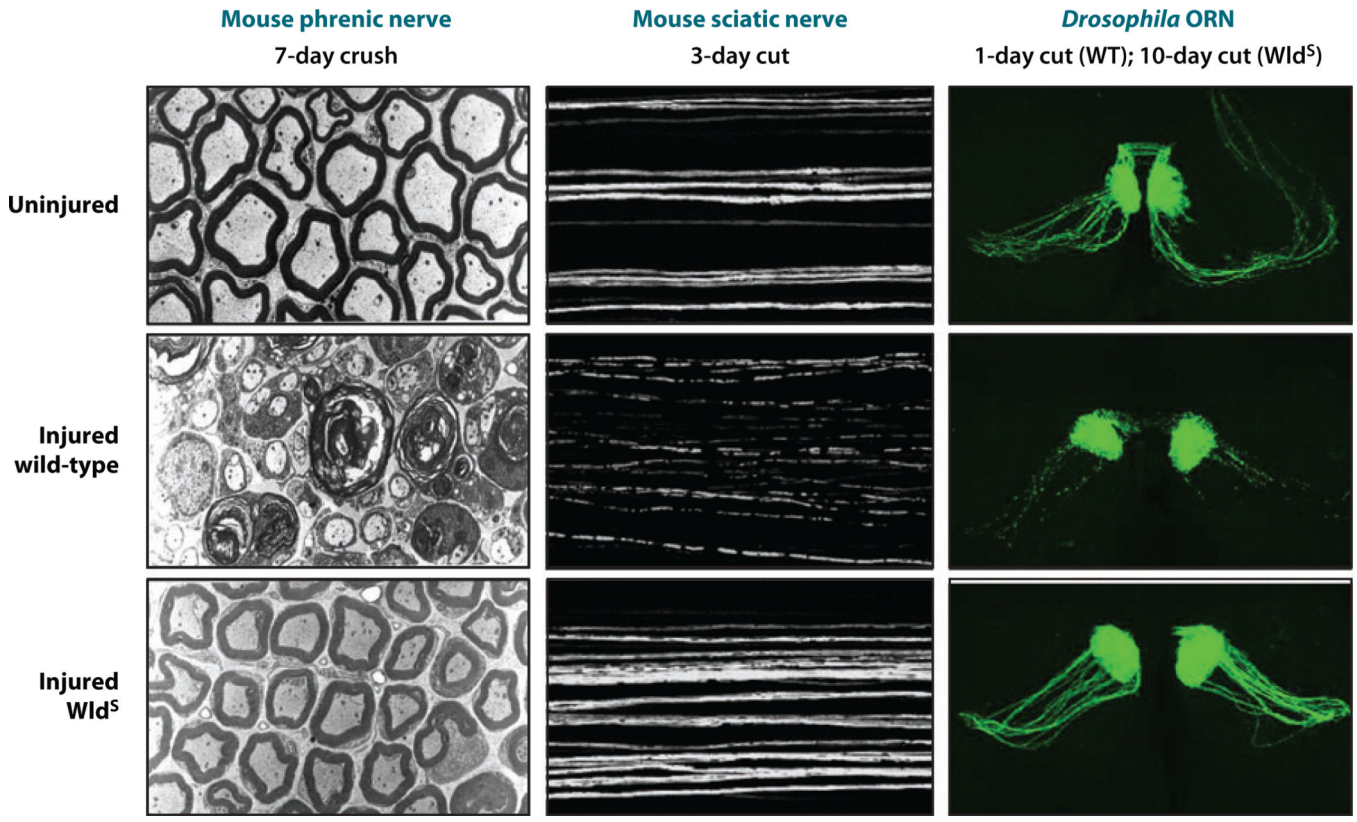

Figure 1.

Wallerian degeneration in wild-type axons and preservation in Wld^S. Injured wild-type axons (middle row) exhibit granular disintegration of the cytoskeleton seen in electron microscopy (left column) and fragmentation visualized by fluorescence microscopy (middle and right columns). Cytoskeletal integrity, unswollen mitochondria, and axon continuity are preserved by the Wld^S gene in each case (bottom row). Note the remarkable consistency of Wallerian degeneration and the neuroprotective Wld^S phenotype between mice and *Drosophila*. ORN, olfactory receptor neuron. Left column from Brown et al. (1994); reprinted with permission from Wiley-Blackwell. Middle column from Conforti et al. (2007b); reprinted by permission from Macmillan Publishers Ltd., Nature Publishing Group.

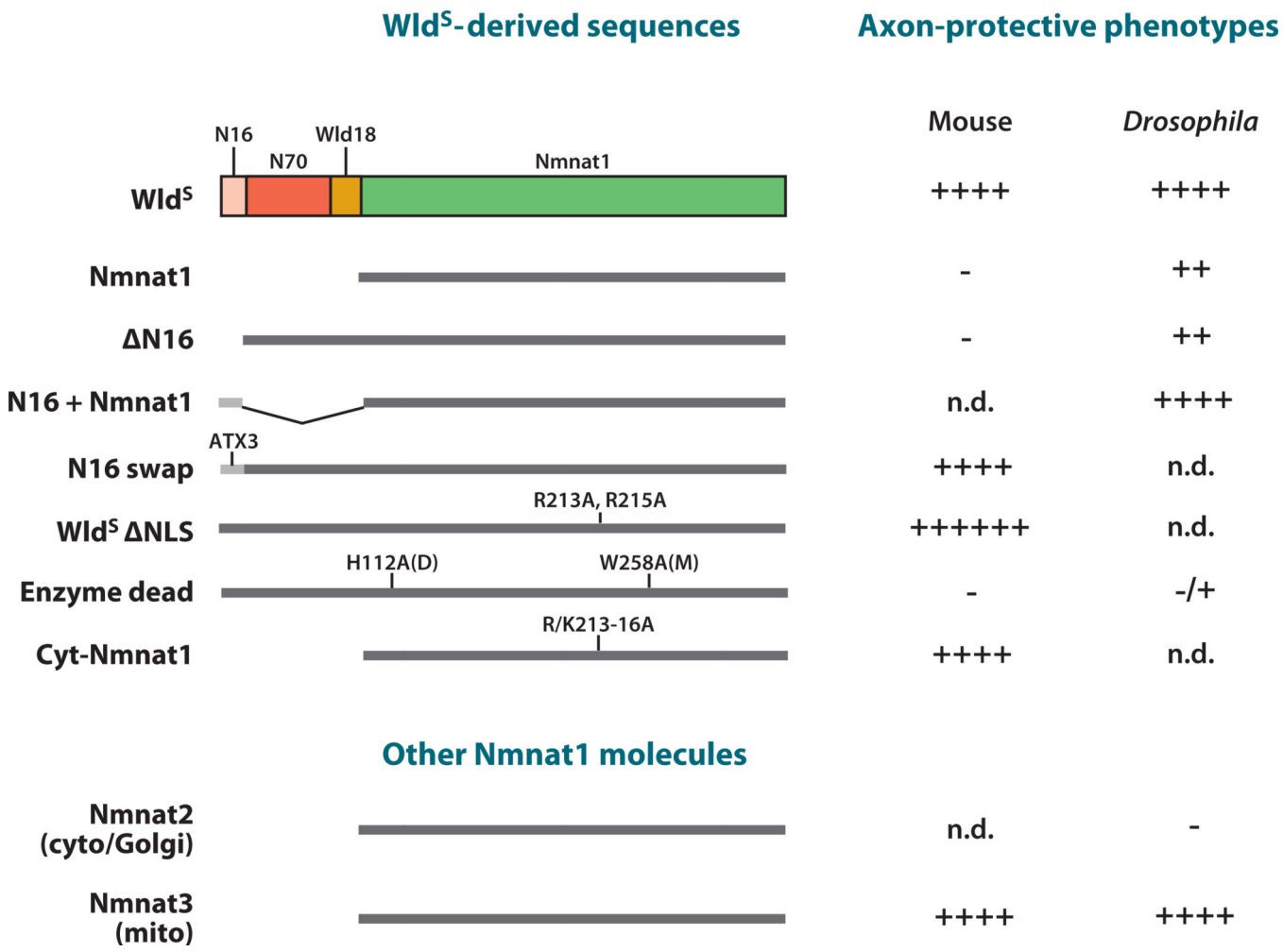

Figure 2.

Axon protection mediated by Wld^S, Wld^S domains, and Nmnat molecules. The in vivo protective effects have been studied extensively in mouse and *Drosophila*. (*Top*) Wld^S-derived sequences represent constructs where specific domains of Wld^S were deleted or mutated. (*Bottom*) Other Nmnat molecules are mouse Nmnat2 and Nmnat3. Protection in either mouse or *Drosophila* is shown to the right. Protection and its relative strength are indicated by (+), a lack of protection is indicated by (−), and those not determined *in vivo* are indicated by n.d. Point mutations or domain swaps are shown above the diagram of each molecule, with point mutation positions that refer to their relative position in Wld^S.

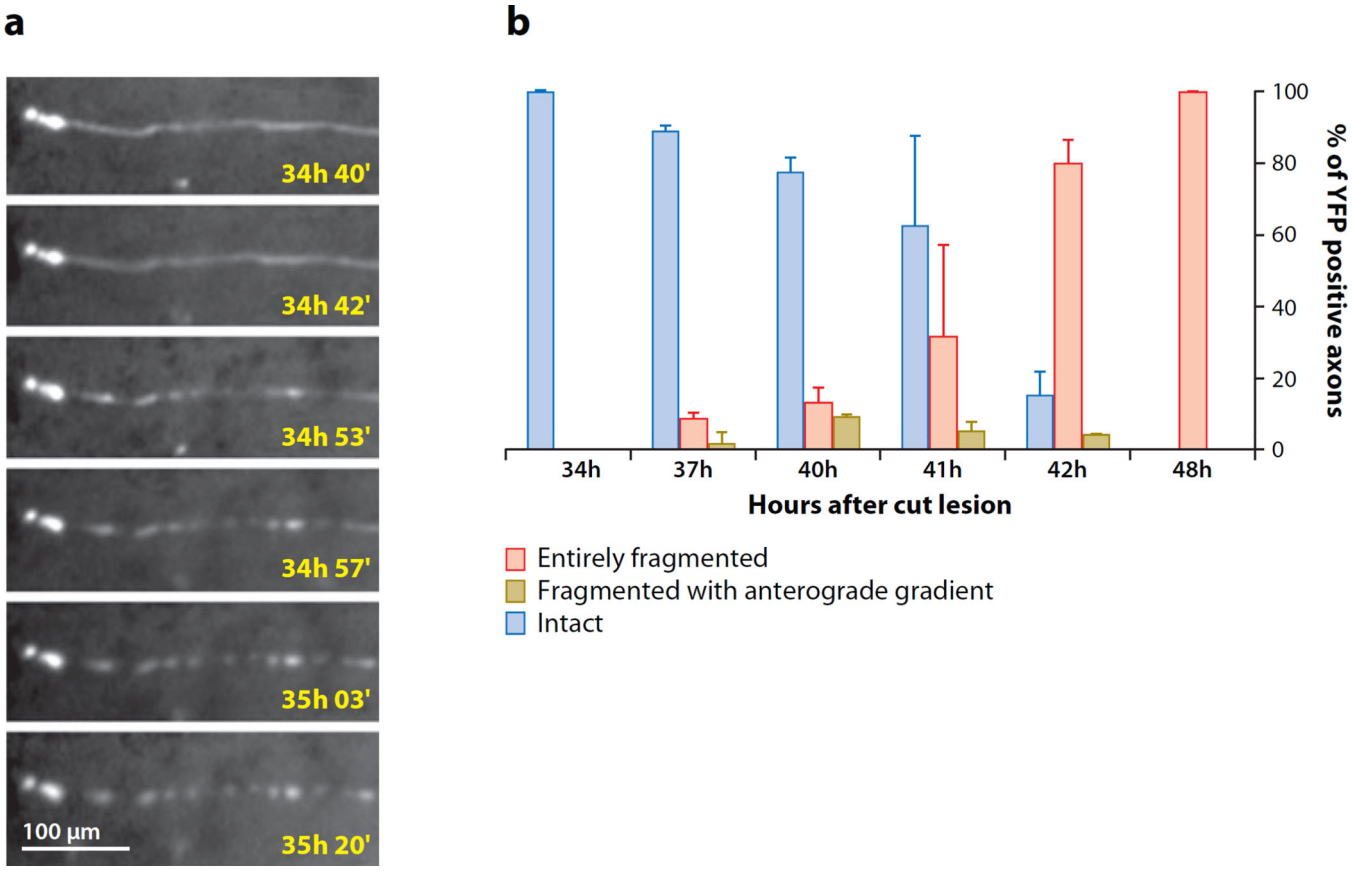

Figure 3.

Wallerian degeneration in wild-type axons is a biphasic process. (*a*) Distal stumps of injured wild-type axons in the dorsal column of mouse spinal cord remain continuous for a latent phase of over 34 h before fragmenting over the course of a few minutes. From Kerschensteiner et al. (2005) reprinted by permission from Macmillan Publishers Ltd., Nature Publishing Group. (*b*) Quantification of fragmented, unfragmented, and partially fragmented wild-type axons in distal sciatic nerve after transection injury shows a similar latent phase followed by fragmentation of all axons in the nerve over the next few hours. The timing of fragmentation is heterogenous within the axon population, but once started, fragmentation is rapid, such that the percentage of partially fragmented axons never exceeds 10% at any one time. From Beirowski et al. (2005) reprinted with permission from BioMed Central.