Protein Cell 2010, 1(3): 237–245
DOI 10.1007/s13238-010-0021-2

Protein & Cell

REVIEW

Wld^S, Nmnats and axon degeneration—progress in the past two decades

Yan Feng[1,2], Tingting Yan[1,2], Zhigang He[3], Qiwei Zhai[1,2] ✉

[1] Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Shanghai 200031, China
[2] Graduate School of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai 200031, China
[3] F. M. Kirby Neurobiology Center, Children's Hospital, and Department of Neurology, Harvard Medical School, Boston, MA 02115, USA
✉ Correspondence: qwzhai@sibs.ac.cn
Received December 9, 2009 Accepted December 21, 2009

ABSTRACT

A chimeric protein called Wallerian degeneration slow (Wld^S) was first discovered in a spontaneous mutant strain of mice that exhibited delayed Wallerian degeneration. This provides a useful tool in elucidating the mechanisms of axon degeneration. Over-expression of Wld^S attenuates the axon degeneration that is associated with several neurodegenerative disease models, suggesting a new logic for developing a potential protective strategy. At molecular level, although Wld^S is a fusion protein, the nicotinamide mononucleotide adenylyl transferase 1 (Nmnat1) is required and sufficient for the protective effects of Wld^S, indicating a critical role of NAD biosynthesis and perhaps energy metabolism in axon degeneration. These findings challenge the proposed model in which axon degeneration is operated by an active programmed process and thus may have important implication in understanding the mechanisms of neurodegeneration. In this review, we will summarize these recent findings and discuss their relevance to the mechanisms of axon degeneration.

KEYWORDS axon degeneration, Wallerian degeneration, Wld^S, NAD, neurodegenerative diseases

Wld^S AS A MODEL TO STUDY THE MECHANISMS OF AXON DEGENERATION

Axon degeneration is a common pathological event that is associated with different types of pathological conditions such as acute trauma, toxic insults and chronic neurodegenerative diseases (Raff et al., 2002; Coleman, 2005; Hilliard, 2008). The degeneration of an axon separates the neuronal soma from its synaptic targets, which disrupts neuronal function. Thus, understanding the underlying mechanisms of axon degeneration may facilitate the development of novel protective strategies.

A simple model in studying the mechanisms of axon degeneration is the Wallerian degeneration, a self-destructive process observed at the distal portion of a transected axon (Waller, 1850). In vertebrates, the distal portion of a severed axon remains viable and conducts action potentials *in vivo* for up to a few days, after which they undergo a rapid structural destruction where the axolemma and axonal cytoskeleton are dismantled (Raff et al., 2002; Coleman, 2005; Luo and O'Leary, 2005). Morphologically, such a degenerative process is characterized by a beading appearance followed by granular disintegration of the axons. In addition, initial studies suggested that the axons undergoing Wallerian degeneration do not seem to possess detectable activation of the caspase 3 protease (Finn et al., 2000), suggesting that Wallerian degeneration and apoptosis may represent two distinct self-destruction programs. However, this concept has been challenged by recent observations that suggest the involvement of other types of caspases in axon degeneration (Nikolaev et al., 2009).

An important landmark is the discovery of a spontaneous mutant mouse strain, C57BL/*Wld*^{S}, whose axons survived for as long as weeks after transection (Lunn et al., 1989; Glass et al., 1993). Genetic analysis suggests that the slow Wallerian degeneration phenotype can be attributed to a dominant mutation that acts intrinsically in neurons (Perry et al., 1990; Deckwerth and Johnson, 1994; Ferri et al., 2003). By *in vivo* imaging methods, Kerschensteiner et al. also reported that after axotomy, the proximal ends of severed

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2010

axons undergo a rapid retrograde (dying-back) degeneration, which could be efficiently protected in Wld^S mice (Kerschensteiner et al., 2005). *In vivo* protective effect of the Wld^S protein has also been demonstrated in different species. For example, it was also found to protect physically injured axons in transgenic rats (Adalbert et al., 2005), as well as surgically severed Drosophila olfactory receptor neurons (ORN) (Hoopfer et al., 2006; MacDonald et al., 2006). In addition, the Wld^S is able to protect axon degeneration in other disease models (reviewed by Coleman et al., 2005, also see below). These studies suggest that these different types of axon degeneration may share similar mechanisms that might be relevant to Wld^S.

AXON DEGENERATION, Wld^S AND NEURODEGENERATIVE DISEASE

It has become clear that many types of neurodegenerative diseases involve major synapse and axon losses before the appearance of symptoms, and even before the loss of neurons (Raff et al., 2002; Bjartmar et al., 2003; Saxena and Caroni, 2007). A variety of neurological disorders, including amyotrophic lateral sclerosis (ALS), spinal muscular atrophy, spinocerebellar disorders, peripheral neuropathies, nutritional neurological disorders, various intoxications and AIDS, are characterized by initial degeneration of the distal regions of long axons (Luo and O'Leary, 2005). Preventing the axon degeneration but not the apoptosis of the motoneuron cell body was able to rescue the symptoms and extends life span in *pmn* mice, a mouse model of motoneuron disease (Sagot et al., 1995; Sagot et al., 1998; Ferri et al., 2003). Clinical symptoms in the SOD1 G93A model of ALS result specifically from damage to the distal motor axon and not from activation of the death pathway (Gould et al., 2006). Therefore, it is intriguing to prevent the loss of axon and dendrites for therapeutic treatment of these neurodegenerative diseases.

Does Wld^S protect these different forms of axon degeneration? *In vitro*, Wld^S protein can be neuroprotective against neuropathy that is induced by vincristine or Taxol (Wang et al., 2001a, b, 2002). In disease models, Wld^S mice show resistance to the 6-hydroxydopamine-induced dopamine fiber loss in Parkinson disease (Sajadi et al., 2004) or the MPTP-induced nigrostriatal axon degeneration (Hasbani and O'Malley, 2006). In addition, the protective roles of Wld^S have been observed in mouse models of different neurodegenerative diseases, including progressive motor neuropathy (*pmn* mice) (Ferri et al., 2003), multiple sclerosis (experimental autoimmune encephalomyelitis) (Kaneko et al., 2006), myelin-related axonapathy (myelin protein zero null mutants) (Samsam et al., 2003), gracile axonal dystrophy (gad mutant mice) (Mi et al., 2005) and glaucoma (Beirowski et al., 2008). However, Wld^S fails to protect axon degeneration in other degenerative disease models, such as the SOD1 transgenic mouse model of ALS (Vande Velde et al., 2004; Fischer et al., 2005), the proteolipid protein (Plp) null animal model of hereditary spastic paraplegia, an spinal muscular atrophy model (Kariya et al., 2009) and prion infected models (Gultner et al., 2009). On the other hand, Wld^S also cannot prevent developmentally regulated axonal pruning in both Drosophila and mice (Hoopfer et al., 2006), suggesting th existence of Wld^S-independent pathways (Hilliard, 2008).

MOLECULAR MECHANISMS OF Wld^S-MEDIATED PROTECTION

Genetic studies showed that the slow Wallerian degeneration phenotype in the Wld^S mice is due to the over-expression of an fusion protein Wld^S that consists of the amino-terminal 70 amino acids of UFD2, which is an evolutionally conserved protein involved in protein poly-ubiquitination (Koegl et al., 1999), and the nicotinamide mononucleotide adenylyltransferase (Nmnat1) (Conforti et al., 2000). Initially, there was some controversy regarding whether individual fragments of the Wld^S gene (UFD2 or Nmnat1), or the entire chimeric gene, are responsible for the phenotypes (Araki et al., 2004; Wang et al., 2005; Zhai et al., 2006; Conforti et al., 2007). In cultured neurons, we and others found that while inhibiting the ubiquitin proteosome system activity can slow down Wallerian degeneration (Zhai et al., 2003; MacInnis and Campenot, 2005), over-expressing Nmnat1 alone could mimic the protective effects of Wld^S (Araki et al., 2004; Wang et al., 2005; Sasaki et al., 2006). Such protective effects could be mimicked by exogenously provided NAD or its biosynthetic precursors such as nicotinamide or nicotinamide riboside (Araki et al., 2004; Wang et al., 2005; Sasaki et al., 2006), suggesting that Wld^S/Nmnat1 may act, at least partially, through NAD biosynthesis. Similarly, independent studies also showed that Nmnat1 could protect axon degeneration in Drosophila-based *in vivo* models (MacDonald et al., 2006; Zhai et al., 2006).

However, a study reported that over-expression of Nmnat1 in transgenic mice model failed to protect Wallerian degeneration of sciatic nerves *in vivo* (Conforti et al., 2007). The exact mechanisms for these different results are unclear. It is possible that the differences in both the expression levels and protein localizations of the over-expressed Nmnat1 vs. Wld^S contribute to these discrepancies due to the nature of these gain-of-function studies. For example, while endogenous Nmnat1 is a nuclear protein (Emanuelli et al., 2003), some of the over-expressed Wld^S/Nmnat1 protein can be detected in the axons of cultured neurons (Wang et al., 2005; Zhai et al., 2006). Perhaps the Nmnat1 expressed in transgenic mice generated by Conforti (Conforti et al., 2007) did not reach a high level and still primarily localized in the nucleus; therefore, insufficient amounts of Nmnat1 were available in the axons for the protective effects. In addition, although many reports suggest that Wld^S is localized to the

 © Higher Education Press and Springer-Verlag Berlin Heidelberg 2010

nucleus of neurons in the Wld^S mice (Coleman, 2005), all of these studies relied on an antibody prepared against an 18-amino acid peptide in Wld^S (between UFD2 and Nmnat1) (Conforti et al., 2000; Fang et al., 2005). UFD2 itself localizes to both the nucleus and axons (Fang et al., 2005), and thus the fusion protein Wld^S might be brought to axons by the UFD2 fragment. In support of this hypothesis, recent genetic studies demonstrated that a mutant form of Wld^S with mutations at its nuclear localization signal (thus leading to increased cytoplasmic localization of the protein) has dramatically increased protective effects on Wallerian degeneration (Beirowski et al., 2009), which substantiates our finding that non-nuclear Wld^S determines its neuroprotective efficacy for axons (Wang et al., 2005). Furthermore, more recent studies revealed the existence of the Wld^S protein in axon in Wld^S mice *in vivo*, likely in the mitochondria (Yahata et al., 2009). It appears that such cytoplasmically localized Wld^S shows protective effects on axon degeneration. Various mutants of Wld^S in axon degeneration are summarized in Table 1.

Recent structure-function studies suggested that N70 and/or W18 portion of Wld^S is likely to contribute for axonal protection (Watanabe et al., 2007). Wld^S directly binds valosin-containing protein (VCP)/TER94 and is partially relocated it in the nucleus through N70 (Laser et al., 2006; Avery et al., 2009). N70 binds VCP through a VCP-binding motif in its N-terminal 16 amino acids (Laser et al., 2006). Knocking down TER94 by RNAi reduced the axon-protective effects of Wld^S to levels indistinguishable from Nmnat1 and Wld$^{S\text{-}\Delta N16}$. Removing the N-terminal 16 amino acids in N70 portion of Wld^S (Wld$^{S\text{-}\Delta N16}$) decreased axon protection in transgenic mice and also in Drosophila (Avery et al., 2009;

Table 1 Mutants of Wld^S in axon degeneration

gene	species	localization	models	type of injury	effectiveness	literature
Wld^S (W258A)	mice	NT[a]	DRG	transection	N[b]	(Araki et al., 2004)
	mice	as Wld^S mice, nucleus, mitochondria, other fractions as well	transgenic mice	transection	N	(Yahata et al., 2009)
	mice	nucleus	transgenic mice	transection	N	(Conforti et al., 2009)
	mice	nucleus	SCG from transgenic mice	vincristine	N	(Conforti et al., 2009)
Wld^S (F28A)	mice	nucleus	SCG	transection	N	(Jia et al., 2007)
Wld$^{S\text{-}dead}$ (H24A)	mice	nucleus	transgenic Drosophila	transection	W, dramatically reduced	(Avery et al., 2009)
ΔNLS-Wld^S	mice	cytoplasm	transgenic mice	transection	Y, 14 days after injury, stronger than wild-type Wld^S	(Beirowski et al., 2009)
ΔN16-Wld^S	mice	without amino acids 2–16, cytoplasm	transgenic mice	transection	N	(Conforti et al., 2009)
	mice	cytoplasm	SCG from transgenic mice	vincristine	N	(Conforti et al., 2009)
Wld$^{S\text{-}\Delta N16}$	mice	Wld^S with a deletion of the first 16 amino acids, nucleus	transgenic Drosophila	transection	W, significantly reduced	(Avery et al., 2009)
ATX3-Wld^S	mice	replaced the Wld^S VCP-binding domain with an ataxin-3-derived VCP-binding sequence, nucleus	transgenic mice	transection	Y, similar to Wld^S	(Conforti et al., 2009)
	mice	nucleus	SCG from transgenic mice	vincristine	Y, similar to Wld^S	(Conforti et al., 2009)

[a] NT, Not tested; [b] Y, Yes, have protective function; W, Weak protection; N, No protective function.

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2010

Conforti et al., 2009). Replacing the Wld^S VCP-binding domain with an alternative ataxin-3-derived VCP-binding sequence restores its protective function (Conforti et al., 2009). N16-Nmnat1, a fusion of N16 to full-length Nmnat1, robustly increased the ability of Nmnat1 to protect severed Drosophila ORN axons from degeneration *in vivo* (Avery et al., 2009).

How N16-VCP/TRE94 interaction modulates axon degeneration is still an open question. A possible mechanism is related to the ubiquitin-proteasome system (UPS). It has been reported that the UPS is intrinsically required for both developmental and injury-induced axon degeneration (Zhai et al., 2003; Hoopfer et al., 2006). In cultured neurons, inhibiting the activity of the UPS can slow down Wallerian degeneration (Zhai et al., 2003). It was reported that VCP binds Npl4 and Ufd2 to target proteins for proteasomal degradation, or bind Ufd1 and Plap to release refolded proteins into the cytoplasm (Halawani and Latterich, 2006). Knocking down the Drosophila orthologue of above genes failed to suppress the ability of Wld^S to protect degenerated axons (Avery et al., 2009). Another possible model is that N16-VCP interaction allows the Nmnat1 protein to relocate to cytoplasmic compartment.

Nmnats AND AXON DEGENERATION

The requirement of Nmnat1 for the protective effect of Wld^S prompts further analysis of this NAD synthetic enzyme. The Nmnat enzymes catalyze the last step in three major NAD biosynthesis pathways in both prokaryotes and eukaryotes: the de novo, salvage, and Preiss-Handler-independent pathway (Sasaki et al., 2006, 2009b). Three Nmnat isoforms have been identified in mammals and they differ in subcellular localization and tissue distribution (Emanuelli et al., 2001; Zhang et al., 2003; Yalowitz et al., 2004). While endogenous Nmnat1 is localized exclusively to nucleus (Schweiger et al., 2001; Zhang et al., 2003), Nmnat2 is mainly expressed in Golgi complex and Nmnat3 is in mitochondria (Berger et al., 2005). In terms of tissue distribution, Nmnat1 appears to be almost ubiquitously expressed while Nmnat2 and Nmnat3 exhibit rather specific tissue distributions. For example, Nmnat2 is predominantly expressed in brain and weakly expressed in heart and skeletal muscle (Emanuelli et al., 2001; Raffaelli et al., 2002; Berger et al., 2005). Specifically, Nmnat2 is highly expressed in cerebral cortex and detectable in midbrain, cerebellum, olfactory bulb, striatum and hippocampus (Yan et al., 2009). On the other hand, mitochondrial Nmnat3 is strongly expressed in kidney, lung and spleen, where Nmnat2 expression is low (Lau et al., 2009).

The protective effects of different Nmnat isoforms on axon degeneration was intentively analyzed (Table 2). Over-expression of Nmnat1 delayed axon degeneration after axotomy in primary dorsal root ganglia (DRG) cultures (Araki et al., 2004; Wang et al., 2005). Nmnat1 also prevented axon degeneration caused by exposure to vincristine in cultured DRG (Araki et al., 2004; Press and Milbrandt, 2008) and differentiated Neuro2A cells (Watanabe et al., 2007). Axon damage induced by rotenone, a mitochondria complex I inhibitor, was inhibited by Nmnat1 as well (Press and Milbrandt, 2008). It is important to note that although the endogenous Nmnat1 is a nuclear protein, over-expressed Nmnat1 is detected in the cytoplasmic compartments and axons. Because NAD or nicotinamide, a NAD synthetic precursor, could protect axon degeneration in the axonal preparation without cell bodies (Wang et al., 2005), it is likely that such axonal and perhaps mitochondrial localized Nmnat1 functions in protecting axon degeneration.

Our recent research revealed that Nmnat2 mRNA level was remarkably decreased in hippocampus of aged APPswe/PS1dE9 transgenic mice (Yan et al., 2009), a model for Alzheimer's disease (Morrissette et al., 2009). Over-expression of human Nmnat2 was reported to protect the axons from degeneration in transected cultured SCGs (Yan et al., 2009), while over-expression of mouse Nmnat2 failed to suppress axon degeneration in transected Drosophila ORN (Avery et al., 2009).

Similar to Nmnat1, Nmnat3 was also reported to protect axon against mitochondrial dysfunction induced by a variety of insults like injury or chemicals in culture DRG (Sasaki et al., 2006; Press and Milbrandt, 2008) (Table 2). Nmnat3 had increased protection compared to Nmnat1 in DRG (Sasaki et al., 2006) and transgenic Drosophila (Avery et al., 2009). Interestingly, Nmnat3 transgenic mice showed robust protection as Wld^S (Yahata et al., 2009), again supporting the protective effects of non-nuclear Nmnat and Wld^S proteins.

Furthermore, Nmnat-mediated axonal protection is evolutionarily conserved. Nmnat enzymes with diverse sequences and structures from various species, including Drosophila, yeast, and archaebacterium (Lau et al., 2009), all mediate robust axonal protection in cultured mouse DRG (Sasaki et al., 2009b).

NAD SYNTHESIS PATHWAY AND AXON DEGENERATION

Despite a lot of efforts, how increased Nmnat activity influences axon degeneration is not entirely known. The total content of NAD was not significantly altered in Wld^S transgenic mice (Mack et al., 2001) and the steady state NAD levels did not change in the PrP-cytNmnat1 transgenic mice as well (Sasaki et al., 2009a). *In vitro* cultured DRG study showed that neither Wld^S nor Nmnat1 significantly increased neuronal NAD levels in intact neurons (Araki et al., 2004; Wang et al., 2005; Sasaki et al., 2006). However, by measuring NAD levels in degenerating axons, Wang et al. found a rapid NAD depletion in degenerating axons and this occurs prior to the development of degeneration signs, suggesting that NAD depletion is an early event in the process of axon degeneration (Wang et al., 2005). Importantly, both

 © Higher Education Press and Springer-Verlag Berlin Heidelberg 2010

Table 2 Nmnats and axon degeneration

gene	species	localization	models	type of injury	effectiveness	citation
Nmnat1	mice	nucleus	DRG	transection	Y[a], comparable to Wld^S	(Araki et al., 2004)
	mice	nucleus	DRG	vincristine	Y, up to 9 days	(Araki et al., 2004)
	mice	NT[b]	DRG	transection	Y, up to 5 days	(Wang et al., 2001b)
	mice	nucleus	DRG	transection	Y, > 72 h	(Sasaki et al., 2006; Sasaki et al., 2009a)
	mice	NT	Neuro2A cell	vincristine	Y, but weaker than Wld^S	(Watanabe et al., 2007)
	mice	nucleus	transgenic mice	transection	N	(Conforti et al., 2007)
	mice	nucleus	transgenic primary DRG	transection	N	(Conforti et al., 2007)
	mice	nucleus	DRG	vincristine	W	(Conforti et al., 2007)
	mice	nucleus	transgenic mice	transection	N	(Yahata et al., 2009)
	mice	NT	DRG	rotenone	Y, > 72 h	(Press and Milbrandt, 2008)
	mice	NT	DRG	transection	Y	(MacDonald et al., 2006)
	mice	nucleus	transgenic Drosophila	transection	Y, less effective than Wld^S	(Avery et al., 2009)
Nmnat3	mice	mitochondria	DRG	axotomy	Y, > 72 h	(Sasaki et al., 2006)
	mice	NT	DRG	rotenone, vincristine, complex i inhibitors, h_2o_2	Y	(Press and Milbrandt, 2008)
	mice	mitochondria	transgenic mice	transection	Y, similar to Wld^S	(Yahata et al., 2009)
	mice	cytoplasm	transgenic Drosophila	transection	Y, similar to Wld^S	(Avery et al., 2009)
Nmnat2	mice	cytoplasm	transgenic Drosophila	transection	N	(Avery et al., 2009)
Nmnat2	human	Golgi apparatus	SCG	transection	Y	(Yan et al., 2009)
Nmnat1(W170A)	mice	NT	DRG	transection	N	(Araki et al., 2004)
	mice	NT	DRG	transection	N	(Sasaki et al., 2009b)
Nmnat1(H24A)	mice	nucleus	transgenic Drosophila	transection	N	(Avery et al., 2009)
	mice	NT	DRG	transection	N	(Sasaki et al., 2009b)
Nmnat2(H24D)	human	Golgi apparatus	SCG	transection	N	(Yan et al., 2009)
N16-Nmnat1	mice	a fusion of N16 to full-length Nmnat1, nucleus	transgenic Drosophila	transection	Y, increased protection relative to Nmnat1	(Avery et al., 2009)

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2010

(*Continued*)

gene	species	localization	models	type of injury	effectiveness	citation
cytNmnat1	mice	cytoplasm	DRG	axotomy	Y, similar as Nmnat1	(Sasaki et al., 2006)
	mice	cytoplasm	DRG from $Cd38^{-/-}Parp1^{-/-}$ mice	transection	Y	(Sasaki et al., 2009b)
	mice	cytoplasm, neuronal projections	transgenic mice	axotomy	Y, 7 days after injury	(Sasaki et al., 2009a)
	mice	cytoplasm, high in axon	DRG	axotomy, vincristine	Y, more efficiently than Nmnat1	(Sasaki et al., 2009a)
nuc-Nmnat3	mice	nucleus	DRG	axotomy	Y, similar as Nmnat1	(Sasaki et al., 2006)
dNmnat[c]	Drosophila	cytoplasm	transgenic Drosophila	transection	Y, weaker than Wld^S	(MacDonald et al., 2006)
	Drosophila	NT	DRG	transection	Y	(Sasaki et al., 2009b)
yNmnat[d]	yeast	NT	DRG	transection	Y	(Sasaki et al., 2009b)
mjNmnat[e]	archaebacterium	NT	DRG	transection	Y	(Sasaki et al., 2009b)

[a] Y, Yes, have protective function, W, Weak protection, N, No protection; [b] NT, Not tested; [c] Drosophila melanogaster Nmnat; [d] *Saccharomyces cerevisiae* Nmnat; [e] archaebacterium *Methanocaldococcus jannaschii* Nmnat.

Nmnat1 and Wld^S prevent the decrease of NAD levels in transcected axonal segments *in vitro* (Wang et al., 2005). However, a recent study showed that elevated neuronal NAD levels in cultured DRG neurons from $Cd38^{-/-}Parp1^{-/-}$ mice do not provide axonal protection (Sasaki et al., 2009b). It is suggested that the Wld^S or Nmnats protect the axon loss by maintaining the homeostasis of NAD level. As it is reported that NAD levels in mitochondria remain at physiological levels following genotoxic stress when nuclear and cytoplasmic pools of NAD are depleted (Yang et al., 2007), it is also possible that they changed the NAD levels in specific organelles, which are not reflected by the whole cell NAD levels.

Another line of evidence that supports the role of NAD in protecting axon degeneration is from the observations that over-expression of other NAD-synthesizing enzymes, in addition to Nnmats, also showed protective effects. These enzymes include nicotiamide phosphoribosyltransferase, nicotinamide phosphoribosyltransferase (Nampt) and nicotinic acid phosphoribosyl transferase (Sasaki et al., 2006). In addition, exogenous application the substrate or intermediates of the NAD synthesis pathway, including nicotinamide, NAD, nicotinic acid mononucleotide and nicotinamide mononucleotide all promoted axonal protection (Araki et al., 2004; Wang et al., 2005; Kaneko et al., 2006; Sasaki et al., 2006). Exogenously pyruvate, a critical intermediate product of glycolysis, could partially protect axon degeneration (Wang et al., 2005). Together with the observed localization of Wld^S in mitochondria (Yahata et al., 2009), it is likely that NAD protects axon degeneration via impacting energy metabolism in the axon. Although it remains to be determined whether maintaining energy balance is the sole or major protective mechanism of Wld^S, these findings highly suggested a passive wasting-away mechanism involved in axon degeneration, which challenges the proposed active program process of axon degeneration (Raff et al., 2002).

However, the axonal protection provided by these chemical treatments is less potent than that provided by Nmnat over-expression. In addition, inhibition of Nampt that catalyzes the rate-limiting step for NAD biosynthesis from nicotinamide, could strongly reduced NAD levels in Wld^S SCG cultures, but only incompletely reversed the Wld^S phenotype. Inhibition of Nampt did not affect the protective activity of Nmnat1 as well (Sasaki et al., 2009b). These data suggest the potential NAD-independent functions of Nmnat1. In this aspect, other downstream metabolites should be considered. Alternatively, NAD synthesis activity may function at specific loci such as mitochondria, Golgi apparatus or endoplasmic reticulum, and could not be measured in the whole cell lysates.

In addition to the critical role in energy metabolism, NAD is also a substrate for the Sirtuin family of histone deacetylases. Enhancing the dosage of Nampt could increase total NAD levels and regulate the transcriptional repressive activity of SIRT1 in mouse NIH3T3 fibroblasts (Revollo et al., 2004). It was shown that SIRT1 protein are effector of the axonal protection mediated by increased Nmnat activity (Araki et al., 2004), but this mechanism is not supported by subsequent studies. Genetic deletion of SIRT1 in mice and Drosophila does not affect the protective effects of Wld^S on axon degeneration (Wang et al., 2005; Avery et al., 2009). However, there

 © Higher Education Press and Springer-Verlag Berlin Heidelberg 2010

are several cytoplasmic SIRT members, such as SIRT2, SIRT3 and SIRT4 (Haigis and Guarente, 2006), whose roles in NAD-dependent protection remain to be determined.

OTHER MECHANISMS

Recent studies in Drosophila showed that Nmnat functions as a chaperone in protecting neuronal degeneration, which is independent of its NAD synthesis activity (Zhai et al., 2008). Another study in mice showed that chaperone activity in the mutant protein Wld^S (W258A) was similar to that reported for enzyme-dead Nmnat, but the Wld^S (W258A) transgenic mice show no axon protection (Conforti et al., 2009). Thus, the chaperon activity was unlikely to be sufficient in the axonal protection of Nmnat or Wld^S (Conforti et al., 2009).

Other researches focus on the downstream effectors of Wld^S. Pituitary tumour-transforming gene-1 (Pttg1) could be down-regulated by Wld^S, but Pttg1$^{-/-}$ mice did not showed neuroprotective phenotype (Gillingwater et al., 2006). A recent study suggested that Wld^S could regulate cell cycle status, which possibly involve Pttg1/Ube1, NAD and VCP pathways (Wishart et al., 2008). In addition, previous observations showed decreased macrophage/microglial activation in transected nerves of Wld^S mice as well as in the experimental autoimmune encephalomyelitis model mediated by elevated expression of CD200 (Chitnis et al., 2007). But how these pathways influence Wld^S/Nmnat-related axonal protective function remains to be further investigated.

SUMMARY AND PERSPECTIVES

As discussed above, great progress has been achieved for understanding axon degeneration during the past two decades. However, many challenges remain. Neither the exactly cell organelles nor the downstream pathway that Wld^S or Nmnat works on has been elucidated. Furthermore, in addition to Wld^S, the protective function of Nmnats in neurodegenerative diseases, and the correlation of Nmnats and neurodegenerative diseases have not been fully characterized. There are still a lot of puzzles to be solved.

ACKNOWLEDGEMENTS

This study was supported by grants from National Natural Science Foundation of China (30570558 and 30825009), National Basic Research Program of China (973 Program, 2009CB918403 and 2007CB914501), Q. Zhai is a scholar of the Hundred Talents Program from Chinese Academy of Sciences, and a scholar of the Shanghai Rising-Star Program from Science and Technology Commission of Shanghai Municipality (08QH1402600).

ABBREVIATIONS

ALS, amyotrophic lateral sclerosis; DRG, dorsal root ganglia; NAD, nicotinamide adenine dinucleotide; Nampt, nicotinamide phosphoribosyltransferase; Nmnat, nicotinamide mononucleotide adenylyl transferase; ORN, olfactory receptor neuron; SCG, superior cervical ganglia; VCP, valosin containing protein; UPS, ubiquitin-proteasome system

REFERENCES

Adalbert, R., Gillingwater, T.H., Haley, J.E., Bridge, K., Beirowski, B., Berek, L., Wagner, D., Grumme, D., Thomson, D., Celik, A., *et al*. (2005). A rat model of slow Wallerian degeneration (WldS) with improved preservation of neuromuscular synapses. Eur J Neurosci 21, 271–277.

Araki, T., Sasaki, Y., and Milbrandt, J. (2004). Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. Science 305, 1010–1013.

Avery, M.A., Sheehan, A.E., Kerr, K.S., Wang, J., and Freeman, M.R. (2009). Wld S requires Nmnat1 enzymatic activity and N16–VCP interactions to suppress Wallerian degeneration. J Cell Biol 184, 501–513.

Beirowski, B., Babetto, E., Coleman, M.P., and Martin, K.R. (2008). The WldS gene delays axonal but not somatic degeneration in a rat glaucoma model. Eur J Neurosci 28, 1166–1179.

Beirowski, B., Babetto, E., Gilley, J., Mazzola, F., Conforti, L., Janeckova, L., Magni, G., Ribchester, R.R., and Coleman, M.P. (2009). Non-nuclear Wld(S) determines its neuroprotective efficacy for axons and synapses in vivo. J Neurosci 29, 653–668.

Berger, F., Lau, C., Dahlmann, M., and Ziegler, M. (2005). Subcellular compartmentation and differential catalytic properties of the three human nicotinamide mononucleotide adenylyltransferase isoforms. J Biol Chem 280, 36334–36341.

Bjartmar, C., Wujek, J.R., and Trapp, B.D. (2003). Axonal loss in the pathology of MS: consequences for understanding the progressive phase of the disease. J Neurol Sci 206, 165–171.

Chitnis, T., Imitola, J., Wang, Y., Elyaman, W., Chawla, P., Sharuk, M., Raddassi, K., Bronson, R.T., and Khoury, S.J. (2007). Elevated neuronal expression of CD200 protects Wlds mice from inflammation-mediated neurodegeneration. Am J Pathol 170, 1695–1712.

Coleman, M. (2005). Axon degeneration mechanisms: commonality amid diversity. Nat Rev Neurosci 6, 889–898.

Conforti, L., Fang, G., Beirowski, B., Wang, M.S., Sorci, L., Asress, S., Adalbert, R., Silva, A., Bridge, K., Huang, X.P., *et al*. (2007). NAD(+) and axon degeneration revisited: Nmnat1 cannot substitute for Wld(S) to delay Wallerian degeneration. Cell Death Differ 14, 116–127.

Conforti, L., Tarlton, A., Mack, T.G., Mi, W., Buckmaster, E.A., Wagner, D., Perry, V.H., and Coleman, M.P. (2000). A Ufd2/D4Cole1e chimeric protein and over-expression of Rbp7 in the slow Wallerian degeneration (WldS) mouse. Proc Natl Acad Sci U S A 97, 11377–11382.

Conforti, L., Wilbrey, A., Morreale, G., Janeckova, L., Beirowski, B., Adalbert, R., Mazzola, F., Di Stefano, M., Hartley, R., Babetto, E., *et al*. (2009). Wld S protein requires Nmnat activity and a short N-terminal sequence to protect axons in mice. J Cell Biol 184, 491–500.

Deckwerth, T.L., and Johnson, E.M., Jr. (1994). Neurites can remain viable after destruction of the neuronal soma by programmed cell death (apoptosis). Dev Biol 165, 63–72.

Emanuelli, M., Amici, A., Carnevali, F., Pierella, F., Raffaelli, N., and Magni, G. (2003). Identification and characterization of a second NMN adenylyltransferase gene in *Saccharomyces cerevisiae*.

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2010

Protein Expr Purif 27, 357–364.

Emanuelli, M., Carnevali, F., Saccucci, F., Pierella, F., Amici, A., Raffaelli, N., and Magni, G. (2001). Molecular cloning, chromosomal localization, tissue mRNA levels, bacterial expression, and enzymatic properties of human NMN adenylyltransferase. J Biol Chem 276, 406–412.

Fang, C., Bernardes-Silva, M., Coleman, M.P., and Perry, V.H. (2005). The cellular distribution of the Wld s chimeric protein and its constituent proteins in the CNS. Neuroscience 135, 1107–1118.

Ferri, A., Sanes, J.R., Coleman, M.P., Cunningham, J.M., and Kato, A.C. (2003). Inhibiting axon degeneration and synapse loss attenuates apoptosis and disease progression in a mouse model of motoneuron disease. Curr Biol 13, 669–673.

Finn, J.T., Weil, M., Archer, F., Siman, R., Srinivasan, A., and Raff, M. C. (2000). Evidence that Wallerian degeneration and localized axon degeneration induced by local neurotrophin deprivation do not involve caspases. J Neurosci 20, 1333–1341.

Fischer, L.R., Culver, D.G., Davis, A.A., Tennant, P., Wang, M., Coleman, M., Asress, S., Adalbert, R., Alexander, G.M., and Glass, J.D. (2005). The WldS gene modestly prolongs survival in the SOD1G93A fALS mouse. Neurobiol Dis 19, 293–300.

Gillingwater, T.H., Wishart, T.M., Chen, P.E., Haley, J.E., Robertson, K., Macdonald, S.H., Middleton, S., Wawrowski, K., Shipston, M.J., Melmed, S., *et al*. (2006). The neuroprotective WldS gene regulates expression of PTTG1 and erythroid differentiation regulator 1-like gene in mice and human cells. Hum Mol Genet 15, 625–635.

Glass, J.D., Brushart, T.M., George, E.B., and Griffin, J.W. (1993). Prolonged survival of transected nerve fibres in C57BL/Ola mice is an intrinsic characteristic of the axon. J Neurocytol 22, 311–321.

Gould, T.W., Buss, R.R., Vinsant, S., Prevette, D., Sun, W., Knudson, C.M., Milligan, C.E., and Oppenheim, R.W. (2006). Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. J Neurosci 26, 8774–8786.

Gultner, S., Laue, M., Riemer, C., Heise, I., and Baier, M. (2009). Prion disease development in slow Wallerian degeneration (Wld(S)) mice. Neurosci Lett 456, 93–98.

Haigis, M.C., and Guarente, L.P. (2006). Mammalian sirtuins–emerging roles in physiology, aging, and calorie restriction. Genes Dev 20, 2913–2921.

Halawani, D., and Latterich, M. (2006). p97: The cell's molecular purgatory? Mol Cell 22, 713–717.

Hasbani, D.M., and O'Malley, K.L. (2006). Wld(S) mice are protected against the Parkinsonian mimetic MPTP. Exp Neurol 202, 93–99.

Hilliard, M.A. (2009). Axonal degeneration and regeneration: a mechanistic tug-of-war. J Neurochem 108, 23–32.

Hoopfer, E.D., McLaughlin, T., Watts, R.J., Schuldiner, O., O'Leary, D. D., and Luo, L. (2006). Wlds protection distinguishes axon degeneration following injury from naturally occurring developmental pruning. Neuron 50, 883–895.

Jia, H., Yan, T., Feng, Y., Zeng, C., Shi, X., and Zhai, Q. (2007). Identification of a critical site in Wld(s): Essential for Nmnat enzyme activity and axon-protective function. Neurosci Lett 413, 46–51.

Kaneko, S., Wang, J., Kaneko, M., Yiu, G., Hurrell, J.M., Chitnis, T., Khoury, S.J., and He, Z. (2006). Protecting axonal degeneration by increasing nicotinamide adenine dinucleotide levels in experimental autoimmune encephalomyelitis models. J Neurosci 26, 9794–9804.

Kariya, S., Mauricio, R., Dai, Y., and Monani, U.R. (2009). The neuroprotective factor Wld(s) fails to mitigate distal axonal and neuromuscular junction (NMJ) defects in mouse models of spinal muscular atrophy. Neurosci Lett 449, 246–251.

Kerschensteiner, M., Schwab, M.E., Lichtman, J.W., and Misgeld, T. (2005). In vivo imaging of axonal degeneration and regeneration in the injured spinal cord. Nat Med 11, 572–577.

Koegl, M., Hoppe, T., Schlenker, S., Ulrich, H.D., Mayer, T.U., and Jentsch, S. (1999). A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. Cell 96, 635–644.

Laser, H., Conforti, L., Morreale, G., Mack, T.G., Heyer, M., Haley, J. E., Wishart, T.M., Beirowski, B., Walker, S.A., Haase, G., *et al*. (2006). The slow Wallerian degeneration protein, WldS, binds directly to VCP/p97 and partially redistributes it within the nucleus. Mol Biol Cell 17, 1075–1084.

Lau, C., Niere, M., and Ziegler, M. (2009). The NMN/NaMN adenylyltransferase (NMNAT) protein family. Front Biosci 14, 410–431.

Lunn, E.R., Perry, V.H., Brown, M.C., Rosen, H., and Gordon, S. (1989). Absence of Wallerian degeneration does not hinder regeneration in peripheral nerve. Eur J Neurosci 1, 27–33.

Luo, L., and O'Leary, D.D. (2005). Axon retraction and degeneration in development and disease. Annu Rev Neurosci 28, 127–156.

MacDonald, J.M., Beach, M.G., Porpiglia, E., Sheehan, A.E., Watts, R.J., and Freeman, M.R. (2006). The Drosophila cell corpse engulfment receptor Draper mediates glial clearance of severed axons. Neuron 50, 869–881.

MacInnis, B.L., and Campenot, R.B. (2005). Regulation of Wallerian degeneration and nerve growth factor withdrawal-induced pruning of axons of sympathetic neurons by the proteasome and the MEK/Erk pathway. Mol Cell Neurosci 28, 430–439.

Mack, T.G., Reiner, M., Beirowski, B., Mi, W., Emanuelli, M., Wagner, D., Thomson, D., Gillingwater, T., Court, F., Conforti, L., *et al*. (2001). Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. Nat Neurosci 4, 1199–1206.

Mi, W., Beirowski, B., Gillingwater, T.H., Adalbert, R., Wagner, D., Grumme, D., Osaka, H., Conforti, L., Arnhold, S., Addicks, K., *et al*. (2005). The slow Wallerian degeneration gene, WldS, inhibits axonal spheroid pathology in gracile axonal dystrophy mice. Brain 128, 405–416.

Morrissette, D.A., Parachikova, A., Green, K.N., and LaFerla, F.M. (2009). Relevance of transgenic mouse models to human Alzheimer disease. J Biol Chem 284, 6033–6037.

Perry, V.H., Brown, M.C., Lunn, E.R., Tree, P., and Gordon, S. (1990). Evidence that very slow Wallerian degeneration in C57BL/Ola mice is an intrinsic property of the peripheral nerve. Eur J Neurosci 2, 802–808.

Press, C., and Milbrandt, J. (2008). Nmnat delays axonal degeneration caused by mitochondrial and oxidative stress. J Neurosci 28, 4861–4871.

Raff, M.C., Whitmore, A.V., and Finn, J.T. (2002). Axonal self-destruction and neurodegeneration. Science 296, 868–871.

Raffaelli, N., Sorci, L., Amici, A., Emanuelli, M., Mazzola, F., and Magni, G. (2002). Identification of a novel human nicotinamide mononucleotide adenylyltransferase. Biochem Biophys Res Commun 297, 835–840.

Revollo, J.R., Grimm, A.A., and Imai, S. (2004). The NAD biosynthesis

 © Higher Education Press and Springer-Verlag Berlin Heidelberg 2010

pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells. J Biol Chem 279, 50754–50763.

Sagot, Y., Dubois-Dauphin, M., Tan, S.A., de Bilbao, F., Aebischer, P., Martinou, J.C., and Kato, A.C. (1995). Bcl-2 over-expression prevents motoneuron cell body loss but not axonal degeneration in a mouse model of a neurodegenerative disease. J Neurosci 15, 7727–7733.

Sagot, Y., Rosse, T., Vejsada, R., Perrelet, D., and Kato, A.C. (1998). Differential effects of neurotrophic factors on motoneuron retrograde labeling in a murine model of motoneuron disease. J Neurosci 18, 1132–1141.

Sajadi, A., Schneider, B.L., and Aebischer, P. (2004). Wld^S-mediated protection of dopaminergic fibers in an animal model of Parkinson disease. Curr Biol 14, 326–330.

Samsam, M., Mi, W., Wessig, C., Zielasek, J., Toyka, K.V., Coleman, M.P., and Martini, R. (2003). The Wld^S mutation delays robust loss of motor and sensory axons in a genetic model for myelin-related axonopathy. J Neurosci 23, 2833–2839.

Sasaki, Y., Araki, T., and Milbrandt, J. (2006). Stimulation of nicotinamide adenine dinucleotide biosynthetic pathways delays axonal degeneration after axotomy. J Neurosci 26, 8484–8491.

Sasaki, Y., Vohra, B.P., Baloh, R.H., and Milbrandt, J. (2009a). Transgenic mice expressing the Nmnat1 protein manifest robust delay in axonal degeneration in vivo. J Neurosci 29, 6526–6534.

Sasaki, Y., Vohra, B.P., Lund, F.E., and Milbrandt, J. (2009b). Nicotinamide mononucleotide adenylyl transferase-mediated axonal protection requires enzymatic activity but not increased levels of neuronal nicotinamide adenine dinucleotide. J Neurosci 29, 5525–5535.

Saxena, S., and Caroni, P. (2007). Mechanisms of axon degeneration: from development to disease. Prog Neurobiol 83, 174–191.

Schweiger, M., Hennig, K., Lerner, F., Niere, M., Hirsch-Kauffmann, M., Specht, T., Weise, C., Oei, S.L., and Ziegler, M. (2001). Characterization of recombinant human nicotinamide mononucleotide adenylyl transferase (NMNAT), a nuclear enzyme essential for NAD synthesis. FEBS Lett 492, 95–100.

Vande Velde, C., Garcia, M.L., Yin, X., Trapp, B.D., and Cleveland, D. W. (2004). The neuroprotective factor Wlds does not attenuate mutant SOD1-mediated motor neuron disease. Neuromolecular Med 5, 193–203.

Waller, A.V. (1850). Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations on the alterations produced thereby in the structure of their primitive fibres. Philos Trans RSoc Lond B Biol Sci 140, 423–429.

Wang, J., Zhai, Q., Chen, Y., Lin, E., Gu, W., McBurney, M.W., and He, Z. (2005). A local mechanism mediates NAD-dependent protection of axon degeneration. J Cell Biol 170, 349–355.

Wang, M., Wu, Y., Culver, D.G., and Glass, J.D. (2001a). The gene for slow Wallerian degeneration (Wld(s)) is also protective against vincristine neuropathy. Neurobiol Dis 8, 155–161.

Wang, M.S., Davis, A.A., Culver, D.G., and Glass, J.D. (2002). WldS mice are resistant to paclitaxel (taxol) neuropathy. Ann Neurol 52, 442–447.

Wang, M.S., Fang, G., Culver, D.G., Davis, A.A., Rich, M.M., and Glass, J.D. (2001b). The WldS protein protects against axonal degeneration: a model of gene therapy for peripheral neuropathy. Ann Neurol 50, 773–779.

Watanabe, M., Tsukiyama, T., and Hatakeyama, S. (2007). Protection of vincristine-induced neuropathy by Wld(S) expression and the independence of the activity of Nmnat1. Neurosci Lett 411, 228–232.

Wishart, T.M., Pemberton, H.N., James, S.R., McCabe, C.J., and Gillingwater, T.H. (2008). Modified cell cycle status in a mouse model of altered neuronal vulnerability (slow Wallerian degeneration; Wlds). Genome Biol 9, R101.

Yahata, N., Yuasa, S., and Araki, T. (2009). Nicotinamide mononucleotide adenylyltransferase expression in mitochondrial matrix delays Wallerian degeneration. J Neurosci 29, 6276–6284.

Yalowitz, J.A., Xiao, S., Biju, M.P., Antony, A.C., Cummings, O.W., Deeg, M.A., and Jayaram, H.N. (2004). Characterization of human brain nicotinamide 5'-mononucleotide adenylyltransferase-2 and expression in human pancreas. Biochem J 377, 317–326.

Yan, T., Feng, Y., Zheng, J., Ge, X., Zhang, Y., Wu, D., Zhao, J., and Zhai, Q. (2009). Nmnat2 delays axon degeneration in superior cervical ganglia dependent on its NAD synthesis activity. Neurochem Int. In press.

Yang, H., Yang, T., Baur, J.A., Perez, E., Matsui, T., Carmona, J.J., Lamming, D.W., Souza-Pinto, N.C., Bohr, V.A., Rosenzweig, A., *et al*. (2007). Nutrient-sensitive mitochondrial NAD + levels dictate cell survival. Cell 130, 1095–1107.

Zhai, Q., Wang, J., Kim, A., Liu, Q., Watts, R., Hoopfer, E., Mitchison, T., Luo, L., and He, Z. (2003). Involvement of the ubiquitin-proteasome system in the early stages of wallerian degeneration. Neuron 39, 217–225.

Zhai, R.G., Cao, Y., Hiesinger, P.R., Zhou, Y., Mehta, S.Q., Schulze, K.L., Verstreken, P., and Bellen, H.J. (2006). Drosophila NMNAT maintains neural integrity independent of its NAD synthesis Activity. PLoS Biol 4, e416.

Zhai, R.G., Zhang, F., Hiesinger, P.R., Cao, Y., Haueter, C.M., and Bellen, H.J. (2008). NAD synthase NMNAT acts as a chaperone to protect against neurodegeneration. Nature 452, 887–891.

Zhang, X., Kurnasov, O.V., Karthikeyan, S., Grishin, N.V., Osterman, A.L., and Zhang, H. (2003). Structural characterization of a human cytosolic NMN/NaMN adenylyltransferase and implication in human NAD biosynthesis. J Biol Chem 278, 13503–13511.

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2010