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Review

NAD$^+$-dependent mechanism of pathological axon degeneration

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ABSTRACT

Pathological axon degeneration is broadly observed in neurodegenerative diseases. This unique process of axonal pathology could directly interfere with the normal functions of neurocircuitries and contribute to the onset of clinical symptoms in patients. It has been increasingly recognized that functional preservation of axonal structures is an indispensable part of therapeutic strategies for treating neurological disorders. In the past decades, the research field has witnessed significant breakthroughs in understanding the stereotyped self-destruction of axons upon neurodegenerative insults, which is distinct from all the known types of programmed cell death. In particular, the novel NAD$^+$-dependent mechanism involving the WLD^S, NMNAT2, and SARM1 proteins has emerged. This review summarizes the landmark discoveries elucidating the molecular pathway of pathological axon degeneration and highlights the evolving concept that neurodegeneration would be intrinsically linked to NAD$^+$ and energy metabolism.

Axons are the unique structure of neurons, forming the building blocks of neurocircuitries. By transducing action potentials, axons bridge neurons with their innervating targets, which is crucial for various neurophysiological functions, e.g., sensing, movement, cognition, and consciousness. Notably, axons can span long distances in the human body, e.g., over 1 m for the sensory or motor axons controlling our hands or feet. As a result, axons are vulnerable to divergent pathological insults. Disruption of axonal integrity, either functionally or structurally, would occur in human neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (also known as Lou Gehrig's disease), multiple sclerosis, Guillain-Barré syndrome, diabetic peripheral neuropathy, and chemotherapy-induced peripheral neuropathy. In the past decades, accumulating research has established that such damage or loss of axons, formally known as pathological axon degeneration, would represent a critical aspect of neurodegeneration and directly contribute to the debilitating symptoms, e.g., pain, ataxia, paralysis, dementia, and even death. Accordingly, functional preservation of axonal structures is essential for effective therapeutic interventions of those neurological disorders (Coleman and Hoke, 2020; DiAntonio, 2019; Neukomm and Freeman, 2014; Wang et al., 2012).

Extensive studies have demonstrated pathological axon degeneration as the stereotyped self-destruction of axons damaged by neurodegenerative insults. Although this process of axonal pathology exhibits certain morphological similarities to programmed cell death, e.g., degradation of cellular proteins, fragmentation of the plasma membrane, and release of proinflammatory molecules, its central mechanism is distinct from all the known types of cell death. In particular, pathological axon degeneration occurring in many disease scenarios would likely not involve the signaling pathways of apoptosis, necroptosis, or pyroptosis. For instance, the genetic blockage of apoptosis would not affect the axonal loss in the mouse models of amyotrophic lateral sclerosis (Gould et al., 2006), prion disease (Chiesa et al., 2005), glaucoma (Libby et al., 2005), or traumatic neural injuries (Simon et al., 2012; Whitmore et al., 2003). In addition, the expression of MLKL protein, the indispensable component of necroptosis, is undetectable in the central nervous system (Wang et al., 2020). Similarly, neuronal populations in the central nervous system have the minor expression of the key pyroptotic protein Gasdermin D (Tsuchiya et al., 2019). On the other hand, it has been increasingly recognized that divergent neurodegenerative cues could converge onto the common mechanism of pathological axon degeneration (Coleman, 2005; Coleman and Hoke, 2020; DiAntonio, 2019; Neukomm and Freeman, 2014; Wang et al., 2012). This review will highlight the

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significant advances in unraveling the molecular pathway governing this unique process of axonal pathology that may lead to the prevention, relief, and cure of human neurodegenerative diseases.

1. Wallerian degeneration and *Wld*^{S} mutation

The research origin of pathological axon degeneration is often attributed to the British physician-scientist Augustus Waller. In the publication in 1850, he firstly reported the phenomenon that traumatic injuries to the peripheral nerves in the frog would cause the destruction of axonal structures in the following weeks (Waller, 1850). Moreover, Augustus Waller prophetically realized the relevance of this axonal pathology to human neurological disorders. In recognition of this landmark discovery, traumatic injury-induced axon degeneration is referred to as Wallerian degeneration. Importantly, traumatic neural injuries in the fruit fly (MacDonald et al., 2006), zebrafish (Feng et al., 2010), chicken (Cavanagh and Webster, 1955), rodents (Mannell, 1952; Olsson and Sjostrand, 1969), and human (Smith, 1975) all induce Wallerian degeneration, suggesting it as a conserved pathological process through evolution. Also, Wallerian degeneration could occur not only in the *in vivo* context but also in cultured neurons (Fig. 1). However, the significance of Wallerian degeneration had been neglected. It is a common belief in axonal biology that neuronal cell bodies are essential for axonal maintenance by providing a variety of cellular components such as RNAs, proteins, membrane-bound vesicles, and mitochondria (De Vos et al., 2008; Maday et al., 2014; Millecamps and Julien, 2013). As a result, a traumatic injury to axons would completely disconnect them from the trophic support of neuronal cell bodies. In line with this view, Wallerian degeneration had been dismissed for over a century as the passive necrosis of trophic-deprived axons without any involvement of particular signaling mechanisms.

1.1. Discovery of the *Wld*^{S} mutant mouse

This long-standing misconception of Wallerian degeneration was challenged in the 1980s by a British research group. They examined the traumatically-injured sciatic nerves in a dozen mouse lines and miraculously identified a mutant strain that could delay Wallerian degeneration for weeks or even longer (Lunn et al., 1989). The damaged axons could transduce action potentials despite being completely cut off from their neuronal cell bodies, indicating both structural and functional protection of axons in this mutant mouse, now known as *Wallerian degeneration slow* (*Wld*^{S}). In addition, the British group proved that this axonal protection is the neuron-intrinsic phenomenon (Perry et al., 1990a). Moreover, studies in the research field showed that the *Wld*^{S} mutant mouse could suppress the axonal pathology in various neurodegenerative conditions besides traumatic injuries, e.g., Parkinson's disease (Cheng and Burke, 2010; Sajadi et al., 2004), multiple sclerosis (Kaneko et al., 2006), progressive motor neuronopathy (Ferri et al., 2003), gracile axonal dystrophy (Mi et al., 2005), Charcot-Marie-Tooth disease (Meyer zu Horste et al., 2011), and chemotherapy-induced peripheral neuropathy (Wang et al., 2002). In addition, the expression of WLD^S mutant protein (see below) in the fruit fly (Avery et al., 2009) or zebrafish (Feng et al., 2010) similarly conferred the axonal protection against traumatic neural injuries, unequivocally suggesting that Wallerian degeneration, or pathological axon degeneration in general, is not a passive necrotic event but rather regulated by the conserved mechanism.

Genetic studies from the 1990s revealed that *Wld*^{S} is an autosomal dominant mutation caused by the triplication of an 85-kb DNA segment in the mouse chromosome 4 (Coleman et al., 1998; Perry et al., 1990b). This triplication mutation generates an in-frame fusion of the N-terminal portion of UBE4b (ubiquitin conjugation factor E4B) and the full-length NMNAT1 (nicotinamide mononucleotide adenylyltransferase 1) (Conforti et al., 2000). Of importance, the UBE4b/NMNAT1 mutant protein is

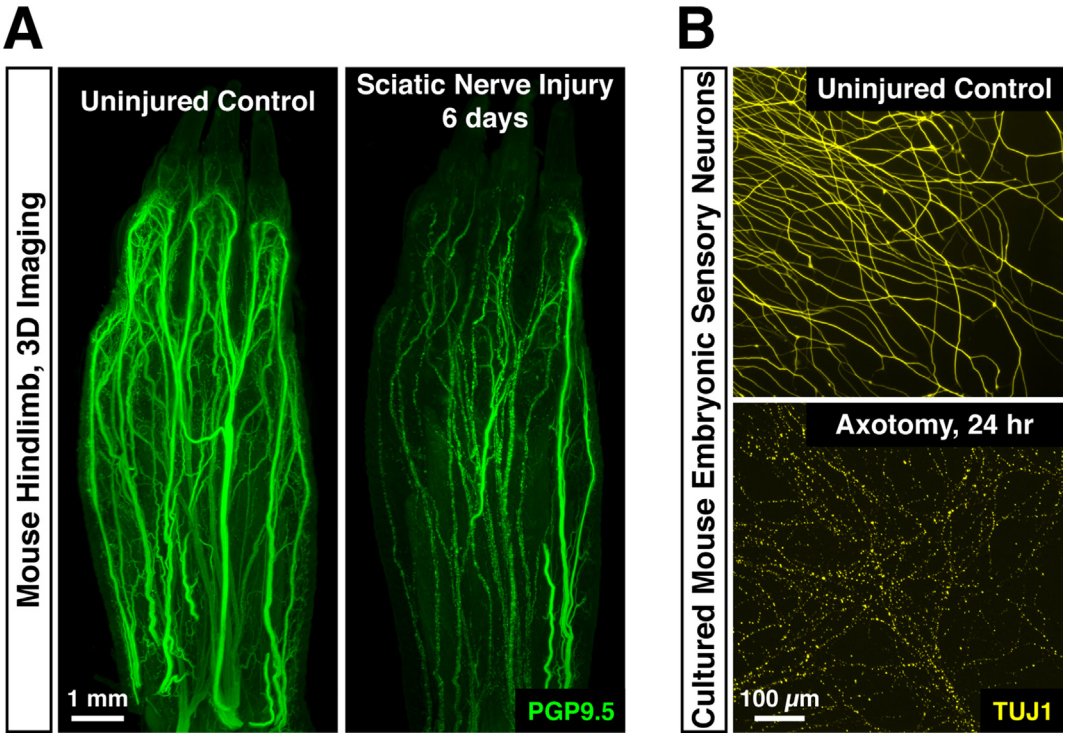

Fig. 1. Pathological degeneration of mouse axons *in vivo* and *in vitro*. (A) The wild-type mouse was subjected to the traumatic injury of the sciatic nerve. The whole-tissue immunolabeling of PGP9.5 (protein gene product 9.5) and lightsheet 3D imaging revealed Wallerian degeneration in the hindlimb. **(B)** Mouse embryonic sensory neurons were cultured, and their axons were then subjected to the traumatic injury (i.e., axotomy). Pathological degeneration of injured axons was visualized by the immunofluorescence labeling of TUJ1 (neuron-specific class III beta-tubulin).

causative for the axonal protective effect observed in the *Wld*^{S} mutant mouse and has been named the WLD^{S} mutant protein (Mack et al., 2001).

1.2. WLD^{S} protein and its relationship to NAD^{+}

NMNAT1 is one of the central enzymes involved in synthesizing NAD^{+} (nicotinamide adenine dinucleotide). There are two major pathways of NAD^{+} synthesis in mammalian cells, i.e., the *de novo* pathway and the salvage pathway (Belenky et al., 2007; Cambronne and Kraus, 2020). In the *de novo* pathway, the amino acid tryptophan is metabolized through multiple steps into NaMN (nicotinic acid mononucleotide). NMNAT proteins convert NaMN into nicotinic acid adenine dinucleotide, which is finally synthesized to NAD^{+} by NAD^{+} synthetase. While the *de novo* pathway could act in most mammalian cells, NAD^{+} production in neurons is primarily catalyzed via the salvage pathway. In the salvage pathway, nicotinamide is converted to NMN (nicotinamide mononucleotide) by NAMPT (nicotinamide phosphoribosyltransferase). NMN is then synthesized into NAD^{+} by NMNAT proteins (Fig. 2).

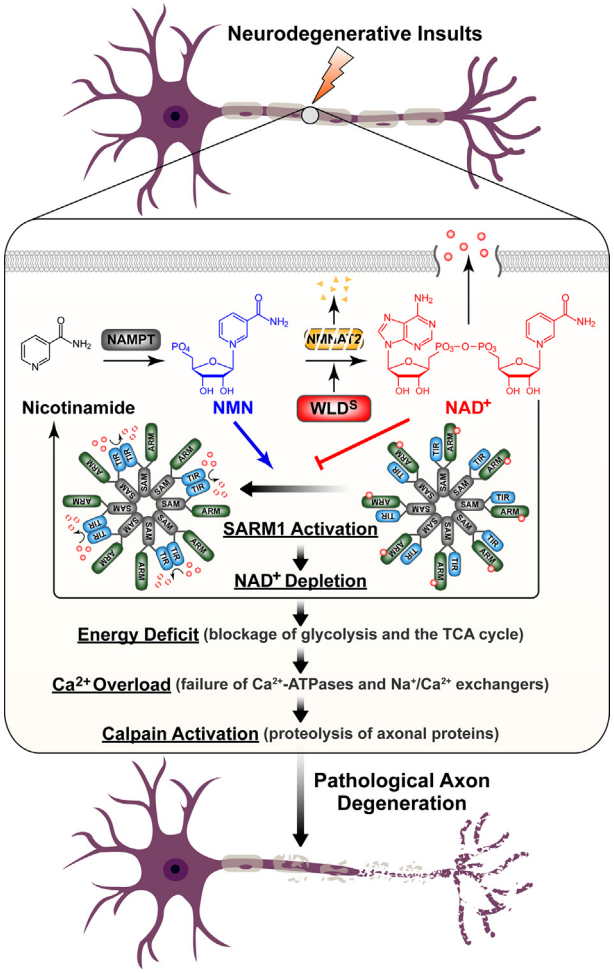

Fig. 2. NAD^{+}-dependent mechanism of pathological axon degeneration. Within damaged axons, the NMNAT2 depletion blocks the NAD^{+} synthesis and results in the NMN buildup. The local membrane breakage of axons may also cause NAD^{+} loss. The WLD^{S} protein prevents such defects of axonal NAD^{+} metabolism, which would otherwise trigger the SARM1 activation. The active SARM1 then degrades NAD^{+} into its metabolic precursor nicotinamide, establishing the positive feedforward of NAD^{+} depletion in axons. This NAD^{+}-dependent mechanism further induces the sequential downstream events of energy deficit, Ca^{2+} overload, and Calpain activation, leading to the execution of pathological axon degeneration. NAMPT, nicotinamide phosphoribosyltransferase; NMN, nicotinamide mononucleotide; NMNAT2, nicotinamide mononucleotide adenylyltransferase 2; WLD^{S}, *Wallerian degeneration slow* mutant protein; SARM1, sterile alpha and Toll/interleukin-1 receptor motif-containing protein 1; TCA cycle, tricarboxylic acid cycle.

Notably, the NAD^{+}-synthesizing activity of the NMNAT1 moiety is indispensable for the axonal protection afforded by the WLD^{S} protein (Araki et al., 2004). Although the endogenous NMNAT1 protein is localized inside the nucleus, the WLD^{S} protein is relocated to the cytosol due to the attachment of the UBE4b portion. Moreover, the axonal protective effect of WLD^{S} protein is entirely independent of neuronal cell bodies (Sasaki and Milbrandt, 2010). In support of the direct relationship between pathological axon degeneration and NAD^{+}, it was observed that the NAD^{+} levels in axons profoundly decreased upon traumatic injuries or other neurodegenerative insults, and the presence of WLD^{S} protein could completely block such loss of axonal NAD^{+} (Wang et al., 2005). These research findings have suggested that this process of axonal pathology is functionally linked to NAD^{+} metabolism.

In addition to NMNAT1, there are two other NMNAT proteins in mammalian cells (Belenky et al., 2007; Cambronne and Kraus, 2020). NMNAT2 is associated with membrane-bound vesicles via its palmitoylation and is responsible for the cytosolic NAD^{+} synthesis. Though it has been debated, NMNAT3 is postulated to reside within mitochondria and catalyze the NAD^{+} production in these organelles. Given that the WLD^{S} protein acts in axons, it became plausible that WLD^{S} would substitute the NAD^{+}-synthesizing activity of an endogenous NMNAT protein. Indeed, studies uncovered that NMNAT2 is primarily synthesized in neuronal cell bodies and then transported into distal axons (Gilley and Coleman, 2010). Interestingly, NMNAT2 has a short half-life of only hours due to the constant degradation by the ubiquitin-proteasome system. As a result, axonal damages would block the NMNAT2 supply from neuronal cell bodies, leading to the consequential depletion of axonal NAD^{+} levels. The small-molecule inhibitors of the proteasome (Gilley and Coleman, 2010) or the genetic deletion of the ubiquitin E3 ligase complex containing PHR1 (Pam/Highwire/RPM-1), SKP1 (S-phase kinase-associated protein 1), and FBXO45 (F-box protein 45) (Babetto et al., 2013; Yamagishi and Tessier-Lavigne, 2016) could stabilize the NMNAT2 protein and prolong the axonal survival following traumatic injuries. In contrast, WLD^{S} appears highly stable in axons, thus replacing the lost NMNAT2 protein in damaged axons to maintain the NAD^{+} synthesis (Gilley and Coleman, 2010). As further evidence supporting the functional relationship between NMNAT2 and WLD^{S}, the genetic deficiency of NMNAT2 would result in severe axonal pathology both in the mouse (Gilley et al., 2013) and the human (Huppke et al., 2019; Lukacs et al., 2019). Such neural defects could be rescued in the *Wld*^{S} mutant mouse (Gilley et al., 2013). This important collection of research has indicated that NAD^{+} metabolism would designate the occurrence of pathological axon degeneration.

2. SARM1 and NAD^{+} metabolism

The significant breakthrough in the past decade has been the identification of SARM1 (sterile alpha and Toll/interleukin-1 receptor motif-containing protein 1) and the revelation of its interaction with axonal NAD^{+} metabolism. In the 2010s, several research groups performed the genetic screens in the cultured mouse neurons or in *Drosophila* for novel signaling components involved in pathological axon degeneration. Impressively, they reached a consensus discovery that the genetic deletion of mouse SARM1 or *Drosophila* dSARM could strongly inhibit the axonal pathology in several models of traumatic injuries (Gerdts et al., 2013; Osterloh et al., 2012). It is worth noting that such genetic deletion of SARM1 or dSARM would not affect the developmental death of neurons or axons in the fruit fly or the mouse (Osterloh et al., 2012), suggesting the mechanistic separation of pathological axon degeneration from the developmental pruning of axons. Similar to that observed before with the WLD^{S} protein, the SARM1 deletion gives the almost universal protection of damaged axons. Studies have shown that pathological axon degeneration under various neurodegenerative conditions, e.g., Parkinson's disease (Peters et al., 2021), amyotrophic lateral sclerosis (White

et al., 2019), multiple sclerosis (Viar et al., 2020), retinopathy (Ozaki et al., 2020), diabetic peripheral neuropathy (Turkiew et al., 2017), and chemotherapy-induced peripheral neuropathy (Geisler et al., 2016; Wang et al., 2019), was mitigated by the SARM1 deletion. In addition, recent works reported that SARM1 also controlled the axonal pathology in some uncommon scenarios, e.g., the destruction of catecholaminergic axons in the colon during inflammation (Sun et al., 2021) or the loss of sympathetic axons in the metabolically-stressed liver (Liu et al., 2021). This commonality of axonal protection afforded by the WLD^S protein and the SARM1 deletion has implicated that they may act through the same molecular pathway.

2.1. *SARM1 is an NAD^+-degrading enzyme*

Surprisingly, studies later found that the SARM1 deletion completely prevented the axonal pathology in the NMNAT2-deficient mice (Gilley et al., 2015). Further, the SARM1 activation induced the robust depletion of axonal NAD^+ levels (Gerdts et al., 2015). These observations have foreseen that the SARM1 protein is directly linked to NAD^+ metabolism. SARM1 belongs to the TIR (Toll/interleukin-1 receptor) domain-containing adaptor family. This protein family includes four other members, i.e., MYD88 (myeloid differentiation primary response protein 88), TRIF (TIR domain-containing adapter protein inducing interferon beta), TIRAP (TIR domain-containing adaptor protein), and TRAM (TRIF-related adapter molecule). These four family members have been extensively documented in immune responses (O'Neill and Bowie, 2007; West et al., 2006). For instance, MYD88 and TRIF are essential for the innate immunity mediated by TLRs (Toll-like receptors). Also, MYD88 transduces the downstream signals of interleukins such as interleukin-1 and interleukin-33. Although SARM1 or its ortholog *tir-1* was reported to regulate innate immune responses in mammals (Carty et al., 2006) or *Caenorhabditis* (Couillault et al., 2004), respectively, the definitive function of SARM1 in mammalian immunity has been debated (Kim et al., 2007; Uccellini et al., 2020).

Studies then observed that the TIR domain of SARM1 exhibits the sequence divergence from other TIR domains identified in mammals. Moreover, recombinant proteins of the TIR domain of SARM1 from the fruit fly, zebrafish, mouse, or human could degrade NAD^+ molecules in biochemical assays (Essuman et al., 2017). This NAD^+-degrading process, referred to as the NADase activity, produces nicotinamide together with ADPR (adenosine diphosphate ribose) or cADPR (cyclic ADPR) (Fig. 2). Accordingly, studies showed that axonal cADPR levels increased in damaged axons, which would be abolished by the SARM1 deletion (Sasaki et al., 2020). In addition, the SARM1 deletion prevented the depletion of axonal NAD^+ levels (Gerdts et al., 2015). Of importance, the NADase activity of mammalian SARM1 proteins depends on a critical glutamate residue Glu642 in the TIR domain. The E642A mutation disabled the NADase activity of SARM1 and its capability to trigger the degeneration of traumatically-injured axons (Essuman et al., 2017). This discovery has revealed that SARM1 controls pathological axon degeneration as an NAD^+-degrading enzyme. Intriguingly, other TIR domain-containing proteins in mammals such as MYD88 and TLR4 do not contain the NADase activity (Essuman et al., 2017). On the other hand, the TIR domains derived from bacteria, archaebacteria, and plants all have a similar ability to degrade NAD^+ (Essuman et al., 2018; Wan et al., 2019), revealing that this unique NADase activity would represent an ancient feature of TIR domains conserved through evolution.

2.2. *SARM1 regulation by NAD^+ and related metabolites*

The SARM1 protein is constantly present within neurons and axons, but its action to mediate the degeneration process only responds to axonal damages. This finding has emphasized that a specific mechanism must exist to maintain SARM1 inactive in the healthy, undamaged condition while ensuring its effective activation upon neurodegenerative insults. In support of this idea, studies recently reported the high-resolution cryo-electron microscopy structures of SARM1 (Bratkowski et al., 2020; Figley et al., 2021; Jiang et al., 2020; Shen et al., 2021; Sporny et al., 2020). It turns out that the protein is organized as an octamer via the interactions between the SAM (sterile alpha motif) domains (Fig. 2). Moreover, the TIR domain could be confined inactive through its interactions with the ARM (Armadillo motif) domain. In particular, the TIR domain forms a hydrophobic interface with the ARM domain, resulting in the physical blockage of the catalytic site of NADase activity. The SARM1 protein missing its N-terminal ARM domain exhibits the NADase hyperactivation (Bratkowski et al., 2020; Jiang et al., 2020) and could trigger the spontaneous degeneration of axons in the absence of any neurodegenerative damage (Gerdts et al., 2013). Similarly, the mutations of several key hydrophobic residues laying within the TIR-ARM interface would significantly enhance the NADase activity of SARM1 and cause the degeneration of undamaged axons (Jiang et al., 2020). Research works implicated that dimerization or oligomerization of the TIR domain would be necessary to induce the SARM1 activation (Gerdts et al., 2015; Yang et al., 2015). However, the structural analyses failed to elucidate the active status of SARM1 till now, likely because of the protein flexibility upon its activation.

With the identification of this SARM1 self-inhibition mechanism, an essential question has emerged as to how SARM1 could sense axonal damages to switch from the inactive to the active status. The first part of the answer to this question has come from the structural analyses of SARM1 (Jiang et al., 2020; Sporny et al., 2020). Strikingly, the ARM domain turns out to contain a previously-unknown binding pocket of NAD^+. The mutations of several positively-charged residues within this pocket would severely disrupt the NAD^+ binding. More importantly, such mutations produced the hyperactive NADase activity of SARM1 that sufficed to initiate axon degeneration without the necessity of neurodegeneration insults. This discovery has suggested that the NAD^+ molecule is a negative allosteric regulator of the SARM1 protein. Further, biochemical assays showed that at NAD^+ concentrations below 150 μM, the NADase activity of SARM1 would be promoted. In contrast, when NAD^+ concentrations increased to over 500 μM, the NADase activity became strongly inhibited. As expected, the SARM1 mutant protein without the ARM domain was insensitive to this inhibitory effect enforced by high NAD^+ levels. Such results intriguingly correlate with the fact that intracellular NAD^+ levels in mammalian cells are maintained at 500–1000 μM in healthy conditions (Yang et al., 2007, 2019). It thus becomes conceivable that the high concentrations of NAD^+ in intact axons are sufficient to keep the SARM1 protein inactive through the NAD^+-enforced inhibition via the ARM domain. On the other hand, such NAD^+-enforced inhibition would be interrupted by the drop of axonal NAD^+ levels due to the NMNAT2 depletion or the local membrane breakage upon neurodegenerative insults. The active SARM1 then degrades more NAD^+ molecules, forming a positive feedforward of NAD^+ depletion within damaged axons. This unique scheme of SARM1 being an NAD^+-degrading enzyme and simultaneously subjected to the allosteric regulation by NAD^+ has designated the precise control of pathological axon degeneration.

In parallel to the NAD^+-enforced inhibition mechanism, recent studies have demonstrated that the mechanistic link of SARM1 to NAD^+ metabolism goes beyond the NAD^+ molecule. As the major pathway of NAD^+ synthesis, nicotinamide is catalyzed to NMN, which is then synthesized into NAD^+ by NMNAT proteins (Fig. 2). However, the NMNAT2 depletion in damaged axons abrogates this last step of converting NMN to NAD^+, leading to a significant accumulation of axonal NMN levels (Di Stefano et al., 2015). Of importance, blocking the NMN production or diverting it to other metabolites could delay pathological axon degeneration (Di Stefano et al., 2015, 2017). These observations have suggested that NMN, as a metabolic precursor of NAD^+, may exert a regulatory role in axonal pathology. Indeed, studies in the field have begun to elucidate that NMN directly stimulates the NADase activity of SARM1 (Bratkowski et al., 2020; Zhao et al., 2019). Moreover, certain neurotoxins such as Vacor (N-3-pyridylmethyl-N-p-nitrophenyl urea)

(Loreto et al., 2021) or 3-acetylpyridine (Wu et al., 2021) could be catalyzed by NAMPT into the NMN mimetics that drastically enhance the NADase activity of SARM1 and induce the rapid occurrence of axon degeneration. Although the structural analyses reported that NMN engages the same NAD^+-binding pocket of the ARM domain (Figley et al., 2021), the biochemical mechanism of the NMN-triggered SARM1 activation has yet to be defined. Nevertheless, this accumulating research has indicated that the imbalanced ratio of NMN to NAD^+ in damaged axons could calibrate the intricate control of SARM1 and pathological axon degeneration.

3. Energy deficit and Ca^{2+} overload

NAD^+ is an essential coenzyme for many metabolic processes, particularly the energy production by glycolysis and the tricarboxylic acid cycle. Therefore, the NAD^+ depletion occurring in damaged axons would lead to the consequential disruption of energy metabolism. Indeed, axonal ATP levels decreased following the NAD^+ depletion after neurodegenerative insults (Wang et al., 2005). Moreover, preservation of axonal NAD^+ by the WLD^S protein (Wang et al., 2005) or the SARM1 deletion (Yang et al., 2015) could prevent such ATP loss, confirming that this energy deficit is the downstream event of the NAD^+-dependent mechanism. Because energy supply is the prerequisite for the maintenance and functions of axons, the energy deficit in damaged axons would thus shut down a variety of cellular events, e.g., axonal transport, protein synthesis, and membrane potential. This systematic failure could block the transduction of neural signals and interfere with corresponding neurocircuitries, functionally disabling axons in neurodegenerative conditions even before their structural destruction.

Ca^{2+} is a central cation involved in numerous neuronal functions, and Ca^{2+} overload is a common phenomenon in different neurodegenerative diseases (Brini et al., 2014; Clapham, 1995; Gleichmann and Mattson, 2011). In the normal condition, intracellular Ca^{2+} levels are kept at submicromolar concentrations. This Ca^{2+} homeostasis is maintained by the constant efflux enabled by Ca^{2+}-ATPases and Na^+/Ca^{2+} exchangers. Notably, Ca^{2+}-ATPases directly demand ATP for their proper function. At the same time, Na^+/Ca^{2+} exchangers rely on the Na^+ gradient across the plasma membrane established by Na^+/K^+-ATPase, which requires the ATP energy input. As a result, the metabolic energy deficit in damaged axons would stop such critical machinery of Ca^{2+} homeostasis, and Ca^{2+} influx through various cation channels then results in the aberrant accumulation of axonal Ca^{2+}. In support of this notion, both the WLD^S protein (Adalbert et al., 2012) and the SARM1 deletion (Ko et al., 2021) could prevent this axonal Ca^{2+} overload. Conversely, the deprivation of energy production in axons would be sufficient to cause the Ca^{2+} accumulation (Shen et al., 2013).

Research back to the 1970s already demonstrated that Ca^{2+} overload is instructive to the structural breakdown of damaged axons (Schlaepfer, 1971, 1974). Chelating extracellular or intracellular Ca^{2+} would be sufficient to preserve the axonal structures but fail to rescue the depletion of ATP levels (Yang et al., 2015), further supporting Ca^{2+} overload as the downstream event of metabolic energy deficit. A specific family of Ca^{2+}-activated proteases, i.e., Calpains, are present in mammalian cells (Goll et al., 2003; Liu et al., 2004). Calpain-1 and Calpain-2 are the two predominant family members expressed in neurons and responsible for the Ca^{2+}-induced axonal destruction. Calpains are typically maintained inactive by the low levels of intracellular Ca^{2+}. In addition, the endogenous specific inhibitor Calpastatin protein suppresses the Calpain activity. However, upon Ca^{2+} overload in damaged axons, Calpain-1 and Calpain-2 are activated by excessive Ca^{2+} levels, overcoming the Calpastatin inhibition. Because they do not have stringent specificity for protein substrates, the active Calpains could rapidly degrade many cellular proteins, e.g., cytoskeletons, channels, and motor proteins. At the same time, the Calpain activation results in fragmentation of the plasma membrane. The WLD^S protein (Bernier et al., 1999) or the SARM1 deletion (Yang et al., 2013) effectively prevents this Calpain-mediated proteolysis in axons. Also, the small-molecule inhibitors of Calpains or the Calpastatin overexpression prolong the structural integrity of damaged axons (Yang et al., 2013), though such manipulations would not affect the upstream energy deficit (Yang et al., 2015). Together, research in the field has established that the NAD^+-dependent mechanism would trigger the sequential events of energy deficit, Ca^{2+} overload, and Calpain activation, leading to the final execution of axons damaged by neurodegenerative insults.

4. Conclusion and future perspectives

Research efforts in the past three decades have revealed the novel molecular pathway that designates the process of pathological axon degeneration. Its signaling components and regulatory mechanism are distinct from all the known types of programmed cell death. In particular, it has become convincingly established that pathological axon degeneration is intrinsically linked to NAD^+ and energy metabolism, with SARM1 being the nexus in such NAD^+-dependent pathway.

With those significant advances, critical questions still await future studies. Answers to those questions would provide more in-depth knowledge of pathological axon degeneration. (1) Although the apoptotic pathway is not involved in most scenarios of pathological axon degeneration, the genetic deletion of BAX, a central apoptotic component inducing the release of Cytochrome *c* from mitochondria, could partially protect the dopaminergic axons in the models of Parkinson's disease (Kim et al., 2011; Vila et al., 2001). Those results suggested the possibility of crosstalk between the NAD^+-dependent mechanism and apoptosis in this particular context of axonal pathology. Notably, SARM1 possesses the N-terminal mitochondria-targeting sequence. How such subcellular localization might preset the SARM1 sensing of mitochondrial permeabilization warrants investigations. (2) The genetic blockage of apoptosis (Gould et al., 2006) or the NAD^+-dependent mechanism (Peters et al., 2018; Vande Velde et al., 2004) both failed to preserve the motor axons in amyotrophic lateral sclerosis induced by the mutant human SOD1 (superoxide dismutase 1). Those findings may reflect the redundancy of different signaling pathways in this specific type of pathological axon degeneration. Alternatively, it might implicate that unknown signals beyond the NAD^+-dependent mechanism exist and control the axonal pathological in amyotrophic lateral sclerosis. (3) The NAD^+-dependent mechanism regulates axonal loss in chemotherapy-induced peripheral neuropathy (Cetinkaya-Fisgin et al., 2020; Geisler et al., 2016; Turkiew et al., 2017; Wang et al., 2019). However, how axonal NAD^+ metabolism would be interrupted by chemotherapeutic drugs remains unknown. It appears plausible that the disruption of NMNAT2 transport from neuronal cell bodies could contribute to pathological axon degeneration caused by the microtubule-targeting drugs vincristine or paclitaxel. On the other hand, the altered metabolism of nucleotides might engage the NAD^+-dependent mechanism in the context of cisplatin-induced axonal damage. (4) The precise role of SARM1 in the immune system has been unsettled (Kim et al., 2007; Uccellini et al., 2020). It remains an important task to unravel this lingering aspect of SARM1, which may be critical to understanding the evolutionary expansion of TIR domain-containing proteins in mammals. In addition, such information would be valuable for evaluating the potential side effects of blocking the SARM1 action for therapeutic purposes.

Finally, the recent revelation of the NAD^+-dependent mechanism in pathological axon degeneration has opened up a new dimension for therapeutic interventions of human neurodegenerative diseases. Strategies focusing on SARM1 or axonal NAD^+ metabolism have garnered attention for their translational potentials. Decades of research endeavors have held up the belief that we could eventually prevent, delay, revert, and even cure neurodegeneration by targeting this unique type of axonal pathology.

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