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Commentary

SARM: From immune regulator to cell executioner

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ABSTRACT

SARM is the fifth and most conserved member of the Toll/Il-1 Receptor (TIR) adaptor family. However, unlike the other TIR adaptors, MyD88, Mal, TRIF and TRAM, SARM does not participate in transducing signals downstream of TLRs. By contrast SARM inhibits TLR signalling by interacting with the adaptors TRIF and MyD88. In addition, SARM also has positive roles in innate immunity by activating specific transcriptional programs following immune challenge. SARM has a pivotal role in activating different forms of cell death following cellular stress and viral infection. Many of these functions of mammalian SARM are also reflected in SARM orthologues in lower organisms such as *C. elegans* and *Drosophila*. SARM expression is particularly enriched in neurons of the CNS and SARM has a critical role in neuronal death and in axon degeneration. Recent fascinating molecular insights have been revealed as to the molecular mechanism of SARM mediated axon degeneration. SARM has been shown to deplete NAD+ by possessing intrinsic NADase activity in the TIR domain of the protein. This activity can be activated experimentally by forced dimerization of the TIR domain. It is thought that this activity of SARM is normally switched off by the axo-protective activities of NMNAT2 which maintain low levels of the NAD+ precursor NMN. Therefore, there is now great excitement in the field of SARM research as targeting this enzymatic activity of SARM may lead to the development of new therapies for neurodegenerative diseases such as multiple sclerosis and motor neuron disease.

1. Introduction

Sterile alpha and HEAT/Armadillo motif (SARM), a member of the Toll/Il-1 Receptor (TIR) domain protein family, is highly conserved in worm, fly and mammals [1]. The presence of a TIR domain predicts a role for SARM in innate immunity, however SARM is very different from the other mammalian cytosolic TIR proteins MyD88, Mal, TRIF and TRAM, as it is not required for signalling downstream of the Toll-Like Receptors (TLRs) [2]. By contrast mammalian SARM was initially described in 2006 as an inhibitor of TLR signalling [3]. In the intervening years other roles have been assigned to SARM, and these include the activation of gene expression following immune challenge [4,5], indicating that SARM has both positive and negative contributions to innate immunity. Another major role for SARM is in mediating cell death. In fact, throughout evolution immune defence and cell death are reoccurring themes of SARM biology, in all species examined ranging from *C. elegans*, *Drosophila* to mammals. In mice, SARM is enriched in the CNS, particularly in neurons and SARM has a critical function in neuronal death and in eliminating damaged nerve axons [6]. The most intriguing recent advance regarding SARM is the discovery that SARM is an enzyme that degrades NAD+ and this activity is required for SARM to execute axonal destruction of neurons [7]. Since SARM is the only TIR domain protein that displays this activity, this suggests that at some point in early evolution the functions of the other TIR proteins diverged. In this review we will examine the evolutionary origins of SARM and discuss the physiological roles of this ancient protein which encompass development, immune regulation and cell death.

2. The evolutionary origins of SARM

SARM was initially discovered in 2001 as a protein containing a SAM domain which had an orthologue in *C. elegans*, *Drosophila* and mammals [8]. It was soon discovered that SARM contained a TIR domain (Fig. 1) thus predicting an important biological connection to

Abbreviations: ALS, amyotrophic lateral sclerosis; ARM, armadillo; CNS, central nervous system; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAVS, mitochondrial antiviral-signalling protein; MND, motor neurone disease; NAD+, nicotinamide adenine dinucleotide; NMN, nicotinamide mononucleotide; PBMCs, peripheral blood mononuclear cells; PN, peripheral neuropathy; PNS, peripheral nervous system; PRR, pattern recognition receptor; RLR, rig-like receptors; ROS, reactive oxygen species; SAM, sterile alpha motif; SARM, sterile alpha and HEAT/Armadillo motif; TIR, Toll/Il-1 Receptor; TLR, Toll-Like Receptors; TRAF, TNF receptor-associated factor; VSV, vesicular stomatitis virus; WD, Wallerian degeneration; Wld^S, Wallerian degeneration slow; WNV, West Nile Virus

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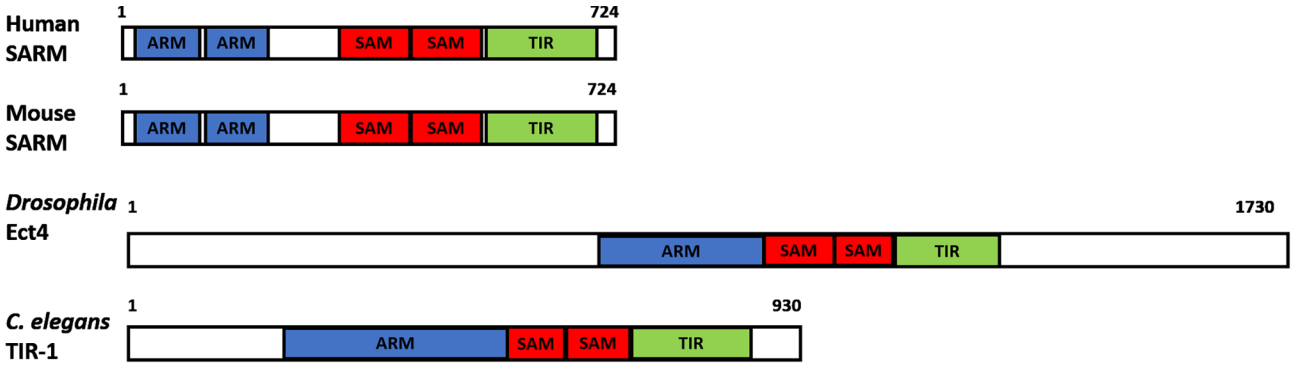

Fig. 1. Schematic comparison of human and mouse SARM domains [25] with that of orthologous *Drosophila* Ect4 and *C. elegans* TIR-1 [15].

TLRs and innate immunity [1]. Indeed SARM is the only TIR protein with a clear orthologue in *C. elegans* and is therefore the most conserved member of the TIR family [2]. Structurally, the SARM protein also contains ARM repeats and SAM motifs, both of which are proposed to be important in mediating protein-protein interactions. The presence of SARM throughout evolution has led to studies investigating its evolutionary origin. The observation that SARM clusters more with bacterial TIR proteins than animal TIR proteins in phylogenetic trees, suggests a bacterial origin for animal SARM [9]. It has been suggested that this may have arisen from a horizontal gene transfer event between bacteria and the ancestor of ecdysozoa and deuterostomes [10]. Interestingly, the bacterial TIR protein TirS attenuates the macrophage response to infection [11] and also possesses NADase activity [7], indicating that the immune regulatory and NADase enzymatic function of mammalian SARM may have its origins in bacteria.

2.1. *C. elegans SARM functions in immunity and development*

The *C. elegans* SARM orthologue, TIR-1, was shown to have both an immune defence and a developmental role. In response to fungal and bacterial infection, TIR-1 was required for expression of antimicrobial peptides, which occurred independently of the sole Toll receptor in *C. elegans*, Tol-1 [12]. In response to bacterial pathogen challenge, *C. elegans* survival was reduced when *tir-1* expression was suppressed [13]. That study also provided evidence that TIR-1 activated the worm p38 MAP kinase cassette NSY-1–SEK-1-PMK-1 [13], through which the antimicrobial role for TIR-1 is mediated. The link between TIR-1 and the p38 pathway was further supported by studies that resistance of *C. elegans* to both bacteria and heavy metals requires expression of the ABC transporter *pgp-5* which was shown to be TIR-1 and p38 MAP kinase-dependent [14].

A developmental role for TIR-1 was also described where the asymmetry associated with odour receptor expression on olfactory neurons was governed by TIR-1 [15]. In this context TIR-1 is activated downstream of calcium and the *C. elegans* orthologue of CamKII, resulting in TIR-1 dependent p38 and JNK kinase activation. Interestingly it was shown that splice variants of *tir-1* that contained both the SAM motifs and TIR domain was capable of fulfilling the functions of the full length protein, indicating that the N terminus of TIR-1 has inhibitory properties [15]. Subsequently it was found that microtubules and a kinesin motor protein were necessary to locate TIR-1 at the correct postsynaptic site to regulate neuronal asymmetry [16]. A further developmental role for TIR-1 was demonstrated where TIR-1 participates in non-apoptotic developmental death of linker cells [17]. In response to anoxic stress, TIR-1 activates the p38 MAP kinase pathway, leading to anoxic cell death [18]. Tissue specific functions were also described for TIR-1 in *C. elegans*, where in the intestine and epidermis, TIR-1 activation of the p38 MAP kinase pathway is triggered upon encountering pathogenic microbes. In contrast encounters with pathogenic microbes or nutrient rich microbes alters behaviour due to TIR-1 dependent activation of the MAP kinase pathway in sensory neurons leading to serotonin production [19]. Higher order functions have also been described for *tir-1* in *C. elegans*, where forgetting of olfactory adaptation and salt chemotaxis learning is activated by TIR-1 dependent JNK kinase activation [20]. Therefore in *C. elegans* immune defence, development and cell death all depend on the ability of TIR-1 to activate MAP kinase pathways.

2.2. *Drosophila SARM regulates fly immunity and cell death*

The SARM orthologue in *Drosophila,* Ect4, like *C. elegans* TIR-1, was similarly reported to regulate both immune defence and development. In *Drosophila* antimicrobial peptide production downstream of the IMD pathway is limited by constitutive signalling via Tollo (Toll8) which is mediated by Ect4 [21]. Interestingly this inhibitory effect of Ect4 occurs in the respiratory epithelium but not in the fat body or gut [21], suggesting tissue specific regulation of immune signalling by Ect4. Expression of *Ect4* was shown to be upregulated by virus infection, and survival following viral infection was shown to be dependent on *Ect4* [22]. However, the mechanism of this antiviral role for *Ect4* has not been elucidated but has been suggested to involve restoration of homeostasis by down regulating Toll signalling after an immune response [22]. Further work is required to understand the functional relationship between Ect4 and the 9 Toll receptors expressed in *Drosophila* and to determine if Ect4 can activate cell death after viral infection, similar to its mammalian role [23]. In developing tissues, cells that are unfit, known as loser cells, are removed. In *Drosophila* this occurs via a cell death pathway that requires the Toll receptors and the NF-κB orthologue Relish (Rel). Here, *Drosophila* Toll receptors activate Rel through a signalling axis that involves Ect4 [24]. Therefore, *Drosophila* Ect4 has a key role in immune responses and development-associated cell death.

3. Mammalian SARM

Both human and mouse SARM consists of 724 amino acids. At the N terminal region there are 2 armadillo (ARM) motifs followed by 2 SAM motifs fused to a C terminal TIR domain [2,25] (Fig. 1). This structure of SARM is unique since no other mammalian protein consists of a SAM motif adjacent to a TIR domain. The domain architecture of SARM, similar to its amino acid sequence, is conserved throughout evolution, from *C. elegans* to mammals [15]. Interestingly, some lower eukaryotes such as *Clonorchis sinensis*, the Chinese liver fluke and human pathogen, encode a protein with a centrally located TIR domain followed by a distant SAM motif located at its C terminal end.

3.1. *Cellular location of SARM*

SARM expression studies are often difficult given that commercially available SARM antibodies are unreliable, thus hampering analysis of

the endogenous protein. Nonetheless, studies examining the subcellular location of SARM have found it to be located predominantly in the mitochondria [26]. Similarly to its sequence conservation and domain architecture this feature of SARM is also conserved, such that SARM has been found to be located at the mitochondria in lower organisms such as amphioxus [27] shrimp [28] as well as in mice [29]. There is however some confusion about which domains of SARM mediate this mitochondrial localisation. A study by Ding and co-workers showed that the N terminal 27 amino acids of SARM were necessary for mitochondrial localisation [30]. However a follow-up study by the same group showed that the SAM and TIR domains alone of SARM were capable of eliciting mitochondrial depolarisation [25], suggesting that the SAM motifs or TIR domain of SARM must also have mitochondrial localisation capabilities. Work by Kim et al [29] suggested that the N terminal ARM domains of SARM were needed for SARM mitochondria localisation, but either or both of the SAM and TIR domains were needed to cluster mitochondria. Subsequent studies reported that the N terminal 106 amino acids of SARM were required for mitochondrial localisation [31]. Recent data shows that SARM exists both as a cytosolic and a mitochondrial pool [26]. In neurons, the site of greatest SARM abundance, SARM was shown to be mostly present in the axon and not to be localised with mitochondria [32]. Together these studies show that while the N terminus of SARM can locate the protein to the mitochondria, the SAM motifs and TIR domain also impact the mitochondria, and depending on the cellular context, SARM is also present in the cytoplasm.

3.2. Regulation of SARM expression

Although SARM is expressed mainly in neurons, few studies have examined how SARM expression is regulated in these cells. We previously showed that SARM protein levels are enhanced with LPS treatment in human PBMCs [3]. However unpublished work in our lab has shown that PRR stimulation, including by LPS, suppresses SARM mRNA in human PBMCs. This indicates that SARM mRNA and protein are differentially regulated. Further investigation is warranted to fully understand the cellular mechanisms that govern SARM expression both at the protein and mRNA level. Despite the fact that SARM is predominantly expressed in neurons of mice, a recent study of SARM in high fat diets showed that the SARM protein is expressed in murine astrocytes which is increased with high fructose treatments [33]. In addition, SARM is also expressed in the liver which is increased when the mice are fed high fat diets. This indicates that SARM expression is more widely distributed than previously thought. It has been reported that SARM protein levels are enhanced by mitochondrial depolarisation, artificially induced by the H^+ protonophore CCCP in the human neuronal cell line SH-SY5Y [31], indicating that cell stress may increase SARM protein expression. In studies using rats, celasterol, a pentacyclic triterpene, increased SARM protein levels in the skin [34], while intracerebroventricular treatment of the angiotensin II receptor antagonist telmisartan together with LPS, strongly induced SARM protein levels in the brain [35]. Kainic acid causes enhanced expression of SARM in retinal ganglion cells of the eye [36], as does infection with Bunyavirus in neurons [23]. Together these data indicate that SARM is regulated at both the mRNA and the protein level following infection and agents that induce cellular stress.

3.3. SARM regulates TLR signalling

The first report demonstrating that SARM regulates TLR signalling was in 2006, where we reported that mammalian SARM negatively regulated TRIF dependent TLR signalling [3]. Other reports followed showing similar negative regulatory properties of SARM, where SARM inhibited both TRIF and MyD88 dependent TLR signalling [37]. Indeed a number of studies showed that SARM from lower organisms, such as the horseshoe crab [38], amphioxus [27], shrimp [28], to mammals such as pigs [39] were all capable of inhibiting the TLR pathways, clearly demonstrating functional conservation. This negative regulation by SARM is likely due to homotypic interactions between the TIR domain of SARM and the other TIR adaptors, TRIF and MyD88. This proposition is supported by data showing that SARM interacts with both MyD88 and TRIF TIR domains via the BB loop of the SARM TIR domain and revealed a critical role for a glycine residue at position 601 in SARM [40]. Work carried out to date on mice lacking SARM has found no discernible increase in TLR signalling [29,41]. This is perplexing given the sequence conservation of SARM, especially between mouse and human SARM but also compared to lower organisms. Whether there are indeed mouse-human differences regarding the role of SARM or whether SARM regulates TLR signalling in specific cells in different species is unclear. There is one context where mouse SARM has been shown to regulate TLR signalling, namely during infection of murine macrophages with *Burkholderia pseudomallei*. *Burkholderia pseudomallei* is the causative agent of melioidosis, and during macrophage infection SARM expression increased leading to inhibition of TRIF and thus reduced IFNβ production [42]. This work was developed further when it was reported that SARM, following *Burkholderia pseudomallei* infection, was required for the expression of SIRPα, another negative regulator of TLR signalling [43]. The net effect of increased SARM was the inhibition of iNOS upregulation which allowed the bacteria to multiply in the mouse macrophage. These observations show that SARM is a negative regulator of innate immune signalling in the context of live bacterial infection, via inhibition of TLR signalling.

Apart from the specific case of a negative role for SARM in TLR regulation in *Burkholderia pseudomallei* infection, there are reports suggesting a positive role for SARM in mouse TLR signalling in specific contexts, in both neurons and in the liver. In terms of neurons, SARM, and not MyD88, was shown to mediate TLR7 and TLR9-induced apoptosis [44], although it is possible that this was due to a more general role for SARM in killing neurons (see later) rather than a TLR signalling role *per se*. A second case for a possible positive role for SARM in mouse TLR function came from a study showing that SARM contributes to non-alcohol fatty acid liver disease (NAFALD). SARM is expressed in the liver and in mouse models of NAFALD, deletion of SARM was shown to reduce inflammation, oxidative stress and prevent lipid accumulation [33]. Interestingly these effects of SARM deletion appeared to be mediated via a reduction of TLR4/7/9 responses, whereby SARM was shown to drive TLR and proinflammatory cytokine expression in response to high fat diets both in the liver and hypothalamus [33]. Furthermore, deletion of SARM protected the mice against the development of insulin resistance and promoted the expression of NRF2, a master regulator and cytoprotective factor against oxidative stress [33].

3.4. Transcriptional induction of cytokines and chemokines by SARM

The initial evidence of a role for SARM in driving gene expression was the observation that SARM was required for TNF expression in the brain stem of mice following challenge with West Nile Virus [41]. This response was shown to contribute to protective immunity, since mice lacking SARM were more susceptible to infection with West Nile Virus (WNV) compared with WT mice. Further evidence in support of such a role was found as SARM was shown to be required for pro-inflammatory cytokine expression in response to VSV infection in the brain. Here expression of IL-6, TNF, MIP1- α, MCP-1, RANTES, IFNα, IFNβ all required SARM [5]. However, in contrast to the situation for WNV, the SARM dependent transcriptional response to VSV was detrimental, where mice lacking SARM showed increased survival in response to the virus. Together this work shows that SARM contributes to the transcriptional immune response to viral infection in the CNS, albeit with different outcomes. Our studies showed that SARM was required for *Ccl5* gene induction, but not for *Tnf*, *Ccl2* or *Cxcl10*, following stimulation by multiple PRRs in mouse macrophages [4]. This work revealed

that SARM was required to recruit RNA pol II to the *Ccl5* promoter. Why SARM is specifically required for *Ccl5* induction in macrophages is currently unclear. Therefore, further investigation involving full transcriptome analysis will be required to determine what subset of genes require SARM for expression in macrophages. Recently it was shown that neurons respond to traumatic injury by chemokine production. This response required SARM-dependent JNK pathway activation, resulting in the expression of *Ccl2*, *Ccl7*, *Ccl12* and *Csf1* [45]. This further emphasises a positive role for SARM in inflammatory gene expression in the CNS. Interestingly while SARM is required for JNK kinase to induce a transcriptional response following neuronal injury, no such requirement of SARM is needed to induce the JNK pathway following PRR activation in macrophages [4]. Therefore, it would be interesting to examine if cellular stress in macrophages induces a SARM dependent JNK kinase response.

3.5. Role of SARM in mitophagy

Mitophagy is an important cellular process to remove damaged mitochondria from cells and since damaged mitochondria can drive innate immunity, their timely removal prevents inappropriate inflammation. This process involves the recruitment of parkin by PINK1 to depolarised mitochondria to execute mitophagy. SARM participates in this cellular process by recruiting the ubiquitin E3 ligase TRAF6 to form lysine 63-linked ubiquitin chains on PINK1, resulting in PINK1 stabilisation and recruitment of Parkin to damaged mitochondria [31]. This highlights that SARM has cell intrinsic roles that contributes to homeostasis.

3.6. SARM triggers cell death in the periphery

SARM is now best known for its role in mediating neuronal cell death (see next section). However, there are also studies demonstrating a role for SARM in peripheral cell death. SARM activates apoptosis in CD8+ T cells after immune challenge in order to aid in T cell clearance [25]. Mechanistically SARM reduced ERK MAP kinase phosphorylation, suppressed anti-apoptotic BCL-xL expression and upregulated the production of reactive oxygen species. In addition, SARM triggered mitochondrial depolarisation, mediated by the SAM and TIR domains, resulting in the activation of the intrinsic apoptotic pathway. In NK/T cell lymphoma patients SARM expression was reduced compared with healthy controls [25], indicating that SARM expression might become a useful biomarker to predict cancer outcomes. Further mechanistic insights have recently been provided on the role of SARM in non-neuronal cell death outside the CNS, since SARM was shown to bind to NLRX1 when overexpressed in HEK293T cells and be required for NLRX1-mediated apoptosis in mouse fibroblasts [26]. It has been reported in hepatocellular carcinoma that SARM, along with Noxa, are targets for SAG (sensitive to apoptosis gene)-dependent ubiquitination. This resulted in proteosomal degradation of both SARM and Noxa, presumably representing an apoptosis evasion strategy by the tumour [46]. In addition macrophage survival in response to PAMP challenge can be regulated by modulating SARM protein levels via the SAG dependent ubiquitination system [47]. Another study showed that the proapoptotic functions of SARM were increased by the binding of UTX2 (ubiquitously expressed transcript) leading to increased mitochondrial depolarisation and caspase 8 activation. This activity is regulated through the binding of SARM to UTX 1 resulting in the maintenance of healthy mitochondria [48]. Thus, the examples described here indicate that SARM regulates cell death in many cell types apart from neurons.

Despite the contributions of SARM to developmental cell death in lower organisms, to date there is no evidence that SARM functions in developmental cell death in mammals, since mice lacking SARM appear to be born without any abnormalities [29,41]. Rather SARM has a critical role in cell death of T cells and neurons, indicating that SARM participates in the death of terminally differentiated cells in mammals [6,25]. This may indicate that SARM has evolved from having a role in developmental cell death in *Drosophila*, to regulating death of differentiated cells in higher organisms [24].

4. SARM regulates multiple types of neuronal cell death

Comprehensive expression analysis has shown that in mice, SARM is expressed mainly in the CNS, particularly in neurons [29]. Early studies in *Sarm*$^{-/-}$ mice showed that SARM mediated death of hippocampal neurons following oxygen and glucose deprivation [29]. The exact mechanism of cell death was not defined, but SARM was shown to localise to mitochondria and to recruit JNK kinase 3 to the mitochondrial compartment [29]. These early studies linking neuronal SARM to cell death and MAP kinase activation after pathological insults built a foundation for what has now become an exciting era for SARM research whereby SARM has been shown to control an ancient molecular program to dismantle damaged neuronal axons (this section) and has also revealed a conserved function of SARM as an NADase (Section 5).

4.1. SARM participates in Wallerian degeneration

Axon degeneration is a key pathological feature in a range of neurodegenerative diseases including Parkinson's disease, Huntington's disease, Alzheimer's disease, motor neuron disease (MND) and multiple sclerosis [49]. Wallerian degeneration (WD) is a specific form of axon degeneration whereby after axotomy (i.e. transection of a neuron that separates the cell body from its axon) the axon distal to the cell body disintegrates. For decades this destruction was regarded as a passive process, thought to be due to a reduction in nutrients from the cell body [50]. However, this belief was challenged by the discovery of the WD slow (*Wld*^{S}) mice [51,52]. Expression of *Wld*^{S}, that occurred due to a spontaneous mutation, resulted in a tenfold delay in axon degeneration [49]. These observation raised the possibility that WD involved a molecular program as is the case for apoptosis [53,54]. The *Wld*^{S} mice express a chimeric mutant fusion protein that is formed between full length Nmnat1 and the N terminal 70 amino acids of the ubiquitination factor Ube4b [55]. Considerable confusion surrounded the protective activity of *Wld*^{S} until many years later it was shown that this protein possessed nicotinamide mononucleotide adenylyltransferase (NMNAT) activity, that resulted in NAD+ biosynthesis [49]. The WLD^{S} protein prevents axonal degradation as it translocates the NAD+ enzymatic activity of NMNAT1 to the axon to replace the activity of NMNAT2, an enzyme which is rapidly degraded upon axotomy [49,56–59]. These data supported the notion that NAD+ is protective in WD, as was the NAD+ salvage pathway, and also helped to explain why NAD+ depletion results in WD [49].

However, *Wld*^{S} is an artificial neomorph possibly with activities that may not reflect normal physiological regulation of the WD process. Moreover, WD is a property that is unique to nerve axons and it is not blocked by known inhibitors of apoptosis [49]. Therefore, attempts were made to identify players in the pathway to WD. To do so a forward genetic screen in *Drosophila* was performed and flies defective in WD were isolated. It was found that in mutant flies that showed delayed degeneration, a single gene was affected, SARM (Ect4). Remarkably neurons from *Drosophila* containing Ect4 mutants showed protection from axotomy for 30 and in some instances up to 50 days. The protective effects of SARM deficient mutants in *Drosophila* to axotomy were also observed in mice lacking SARM. Cultures of superior cervical ganglia from mice lacking SARM showed similar protection to axotomy as the *Wld*^{S} mice. In a sciatic nerve injury model SARM-sufficient nerves degenerated within 3 days after damage, while nerves from the SARM KO were intact up to 14 days post injury. These striking observations highlight that the role of SARM in WD is conserved from insects to mammals. Interestingly the study also showed that monocyte and macrophage recruitment to lesioned nerves were also decreased in the SARM KO. [32]. This last observation is an interesting one since nerve

injury induces SARM dependent expression of chemokines which are likely to recruit macrophages to the site of damage [45].

Further, an independent group performed an siRNA screen of cultured mouse DRG neurons and identified SARM as a mediator of axon degeneration [60]. The SAM and TIR domain of SARM was shown to be required for the axonal degenerative capacity of SARM, which occurred even in the absence of injury. A SARM mutant lacking the TIR domain was capable of SARM binding and displayed strong dominant negative activity, suggesting that SARM SAM domains oligomerise, which thus may be important for the ability of SARM to kill neurons [60]. In addition, the authors discovered that the ability of SARM to mediate axon degeneration did not require the mitochondrial localisation of SARM [60].

4.2. Contribution of SARM to traumatic brain injury pathology

There are strong similarities between WD and axonal degeneration after traumatic brain injury (TBI). However, the role of WD in TBI is poorly understood. Interestingly the WLD^S protein does not protect against axon degeneration in a traumatic brain injury model [49,61]. In an attempt to test the contribution of SARM-dependent Wallerian degeneration in TBI, SARM KO mice were examined in a TBI model and found to be protected [61]. Specifically, absence of SARM protected mice from brain trauma induced axonal loss and as a consequence displayed less of an axon injury marker, phosphorylated neurofilament subunit H, in the blood. In response to this injury SARM KO mice were protected from neurological defects, neurometabolic dysfunction and from a reduction in cerebral blood flow [61]. Furthermore, the behavioural deficit associated with traumatic brain injury was also reduced in the SARM KO mice. Since loss of SARM is protective in TBI whereas *Wld*^{s} expression is not, this may indicate that there are different contributions of SARM to WD and TBI. For example, SARM-dependent immune activation might contribute to pathology in TBI. This is consistent with the observation that neuronal injury induces SARM dependent recruitment of macrophages [32], likely due to SARM dependent chemokine expression that occurs upon such trauma [45].

4.3. SARM mediates peripheral neuropathy

Peripheral neuropathy (PN) is another case of neuronal degeneration that is reminiscent of WD. PN is an important clinical consideration and major side effect in patients receiving chemotherapy during cancer treatment and in individuals suffering from obesity associated metabolic disorder. Studies by two independent research groups reported that genetic deletion of SARM protected mice from chemotherapy induced PN [62,63] and PN due to high fat diet associated metabolic disorder [63]. Deletion of SARM resulted in a preservation of intraepidermal nerve fibres, myelinated axons in distal sensory nerves and nerve conductance following vincristine treatment [62]. Furthermore, SARM loss significantly inhibited the development of a hyperalgesic response associate with vincristine treatment [62]. Since the destruction of axons following SARM expression is rescued by expression of NMNAT1, NAMPT or by the addition of the NAD+ precursor nicotinamide riboside (vitamin B) [64], suggests that nicotinamide riboside might be therapeutically useful in this condition [62]. The protective role for NAD+ in PN is further supported by the observation that *Wld*^{s} protects against taxol induced PN in mouse models [65]. Therefore, SARM mediates axonal destruction in response to a range of insults including axotomy, chemotherapy induced neurotoxicity and metabolic syndrome associated neuronal damage.

4.4. The association of SARM with amyotrophic lateral sclerosis (ALS)

The destruction of axons that occurs in WD share morphological features common to ALS also known as motor neuron disease (MND) [66]. The superoxide dismutase 1 (SOD1) is a gene that is strongly associated with ALS and SOD1 mutant mice, such as the G93A are commonly used as a model of this disease [67]. SOD1 mutant mice crossed with the *Wld*^{s} mice partially rescued the disease phenotype, suggesting that the Wallerian degeneration pathway may contribute to ALS [66]. Also, a genome wide study of individuals suffering with sporadic ALS identified a SNP in 17q11.2, the genomic location of human SARM [66]. These observations raised the intriguing possibility that SARM may participate in human ALS. This contention was further supported by the observation that *tir-1* contributed to ALS using a *C. elegans* model of the disease [68]. However a recent study of the $SOD1^{G93A}$ mouse model of ALS showed that loss of SARM does not slow disease progression [69]. This finding suggests that SARM acts redundantly, and that other molecular players contribute to the demise of motor neurons in this condition. Examples of other proteins that have been reported to have axon degenerating abilities comparable to that of SARM, include the ubiquitin E3 ligase Phr1 (also known as Highwire), and Axundead [69]. Further work will be needed to ascertain their relative contributions to ALS. It is possible that the role of SARM in WD is more reflected in a situation of traumatic brain injury than in the pathological condition of ALS [69]. Indeed there is such a precedent in *Drosophila*, where an ALS causing mutant of TDP-43 expressed in *Drosophila* was not rescued by loss of *Ect4* [70], despite the known role for *Ect4* in WD [32]. Interestingly the genes required for loss of motor neurons in the TDP-43 model are distinct from the genes required for WD [70]. Therefore, there is contradictory evidence regarding the role of SARM in different models of ALS. In the *C. elegans* model which uses a mutant of TDP-43 a role of *tir-1* in ALS was described [68], whereas in the *Drosophila* model of ALS again using a mutant of TDP-43, no role of *Ect4* in ALS could be found [70]. One possibility that explains the observed difference in the requirement for SARM might be due to the different TDP-43 mutants or species used. In *C. elegans* the TDP-43 A315T mutant was used [68], whereas in *Drosophila* the TDP-43 Q331K mutant was used [70]. Therefore, further investigation is required to determine if SARM contributes to ALS, however the observation linking the SARM locus to sporadic ALS is tantalising and may indicate that the role of SARM in WD and ALS are somehow linked.

4.5. Role of SARM in pathological conditions of the eye

A number of studies have shown that SARM mediates degeneration in retinal ganglion cells of the eye following trauma or excitotoxic stress. Excitotoxicity induced by the treatment of kainic acid induced the upregulation of SARM protein levels and was shown to cause Wallerian-like degeneration of the cells and axons of the retinal ganglion, and this was reduced by siRNA knockdown of SARM [36]. Using an optic nerve crush model of injury, loss of SARM was shown to protect distal axons of the retinal ganglia [71]. Crucially the preserved axons were electrophysiologically active [71], highlighting that therapeutic means to inhibit SARM will not just physically preserve the axons, but this intervention will also preserve function. By contrast SARM was not involved in degeneration of the soma of the retinal ganglion cells, which was dependent on the JNK kinase pathway and BAX [71]. The authors also report that both SARM and *Wld*^{s} act on the same degenerative pathway, since expression of *Wld*^{s} on the SARM null background did not provide any better protection than either knocking out SARM or expression of *Wld*^{s} alone [71]. The role of SARM in the eye is supported by an earlier study showing that SARM is expressed in retinal photoreceptor and bipolar cells, forming an association with the Na^+/K^+ ATPase [72]. The significance of this pattern of expression was not addressed, but together with other studies shows that there is widespread expression of SARM anatomically throughout the eye. These studies do raise the important question, what is the physiological function of SARM in the eye? Currently more is known about the pathological role of SARM than the physiological role of SARM in the eye. For example, it may be that SARM has a positive role in dendritic arborisation or branching of the retinal ganglion cells, similar to its

neuronal role in the CNS [73]. It would be interesting to determine if mice lacking SARM experience visual impairment as they age.

4.6. SARMOPTOSIS in response to mitochondrial poisons

A fascinating study of sensory neurons showed that their death induced by rotenone or CCCP was mediated by cytosolic SARM and was not blocked by known inhibitors of apoptosis, necroptosis, or parthanatos [74]. The study showed that these mitochondrial poisons induced ROS accumulation, and deletion of SARM protected these neurons from oxidative stress. SARM was shown to be activated downstream of cytosolic calcium influx, and apart from SARM deletion the only intervention capable of rescuing these poisoned neurons was calcium chelation. The authors termed this unique form of cell death sarmoptosis [74]. Interestingly the survival of SARM deficient neurons treated with mitochondrial poisons was dependent on a metabolic switch to glycolysis and treatment of the poisoned SARM KO neurons with 2-deoxyglucose resulted in their death [74]. The authors suggested that SARM might be activated directly or indirectly either by calcium influx or by ROS accumulation, cellular events that may somehow impact on the N terminal inhibitory region of SARM, in order to activate the protein.

4.7. Role of SARM in mediating death in response to viral infection

As well as mediating neuronal death in response to physical injury and cellular poisons, SARM can also activate neuronal death following infection with neurotropic viruses. In response to neuronal infection with Bunyavirus, SARM triggers apoptosis [23]. Specifically, Bunyavirus infection increased SARM protein levels, and caused SARM recruitment to the mitochondria, resulting in apoptosis of neurons, all of which required the RIG-I-like receptor adapter MAVS. Infection with this virus resulted in SARM binding to ATP synthase at the mitochondria, but in contrast to the study by Kim and colleagues [29], JNK3 was not recruited to the mitochondria by SARM [23]. Compared with MAVS, SARM itself is not directly antiviral, as Bunyavirus titres were unaltered in mice lacking SARM. However, in the context of this infection SARM mediates detrimental immunity in the mammalian host, as mice lacking SARM show enhanced survival after Bunyavirus infection [23]. This study highlights that in response to specific viral infection, outcomes might be improved by methods to inhibit the death promoting abilities of SARM.

4.8. Indicators of physiological roles for SARM in neurons

The previous sections clearly demonstrate a role for SARM in mediating neuronal death during pathogenic scenarios. This begs the question as to what other roles, if any, SARM has under more physiological conditions in neurons. One study indicated a role for SARM in regulating neuronal morphogenesis. A key feature of neuronal development in the CNS is the process of dendritic arborisation, where new dendritic outgrowths are established. This process is controlled by a transmembrane heparan sulphate proteoglycan known as syndecan-2 that is localised to synapses. Signals downstream of syndecan-2 where shown to be transduced by SARM to activate the downstream JNK pathway to drive dendritic arborisation [73]. This work revealed that SARM was required for initiation and elongation of dendrites, axonal outgrowth and polarity of neurons. Part of the mechanism whereby SARM contributes to this process is via the stabilisation of microtubules [73].

Other indications of physiological roles for neuronal SARM come from studies analysing behaviour and cognition in transgenic SARM knockdown mice. These mice displayed reduced associative memory, cognitive inflexibility and impaired social interactions [75]. Interestingly even though TIR-1 in *C. elegans* has a role in odour receptor expression, no deficit in olfaction was seen in these SARM knockdown mice [75]. The authors proposed that these behavioural alterations might be due to defective neuronal morphogenesis [73], and dysregulated cytokine expression in the CNS [76]. The observation was also made that these SARM knockdown mice exhibit an autistic phenotype and thus defects in SARM may be associated with autism [75]. Interestingly the human SARM gene is within the autism susceptibility locus 6 (Auts6) [75]. It has also been reported that SARM expression is decreased in patients with autism [75] and SARM mRNA has been predicted to be recognised by the Fragile X mental retardation protein [77]. Work by the same group gained electrophysiological insights to explain why SARM knockdown mice exhibited this altered behaviour. In hippocampal neurons, SARM knockdown inhibited glutamate receptor (mGlut) dependent long term depression but increased N-methyl-D-aspartate receptor (NMDAR)-dependent long term potentiation [78]. In an elegant experiment, intraperitoneal administration of the Glut5 agonist CDPPB enhanced mGlut5 transmission and corrected the behavioural deficit in the SARM knockdown mice [78]. While this work indicates that SARM has an important contribution to behaviour and cognition, this work would need to be independently verified using the SARM KO mice. Fascinating work lies ahead to determine if indeed SARM alterations do occur in autism and associated neurological disorders.

5. The SARM protein is an NADase

How the SARM protein is 'activated' or mobilised to mediate the various functions described above has been an intense area of research, and recently there has a been a major breakthrough in understanding SARM protein function with reports from the Milbrandt group that the protein has NADase activity (see below). Prior to this, it was appreciated that removal of the N terminus leaves the protein functionally intact, a feature that is conserved in worms and mammals. The C terminal SAM and TIR domains of SARM are capable of regulating neuronal asymmetry in *C elegans*, TLR signalling and axonal degradation in mammals in the absence of the N terminal domain [3,15,60]. These observations have led to the hypothesis that the N terminus of SARM is somehow inhibitory (Fig. 2). While the activating mechanism of SARM is unclear, it may involve either the unfolding of the N terminus of SARM away from the SAM motifs and TIR domain or perhaps cleavage to remove the N terminus, although to date no data has been presented showing cleavage of SARM. One study showed that the N terminus was capable of binding the TIR domain of SARM, thus providing a potential mechanism for SARM autoinhibition [79] (Fig. 2). Indeed, TRIF and other death promoting TIR proteins expressed in plants are also subject to similar N terminal dependent auto-inhibition [80,81], suggesting a common auto-regulatory mechanism of TIR proteins. It has also been suggested that given the propensity of SAM motifs to multimerise, activation of SARM may depend on dimerisation or oligomerisation of the protein [60] (Fig. 2). In fact, other proteins pivotal in innate immunity and indeed cell death rely on oligomerisation or co-operative assembly for activation, notable examples being MyD88 forming the Myddosome and NLRP3 forming the NLRP3 inflammasome [82]. Other cellular alterations may also be required for the activation of SARM which may lead to the inactivation of the N terminus and/or oligomerisation of the protein. For example, in *C. elegans* TIR-1 acts downstream of calcium and Cam kinase II to specify neuronal asymmetry [15]. In mammals SARM dependent neuronal degeneration can be driven by an increase in intracellular calcium [32] and SARM dependent death of sensory neurons requires calcium [74]. These observations strongly suggest that SARM activation depends upon intracellular calcium mobilisation [61]. This infers that a rise in intracellular calcium may lead to unfolding of the N terminus, followed by SARM oligomerisation that is mediated by the SAM motifs, resulting in full activation of the protein (Fig. 2). It is unclear as to which of these events are required to activate SARM in its different roles (Fig. 2). For example, in participating in gene induction events, SARM activation may simply require the unfolding of the N terminus of SARM to allow

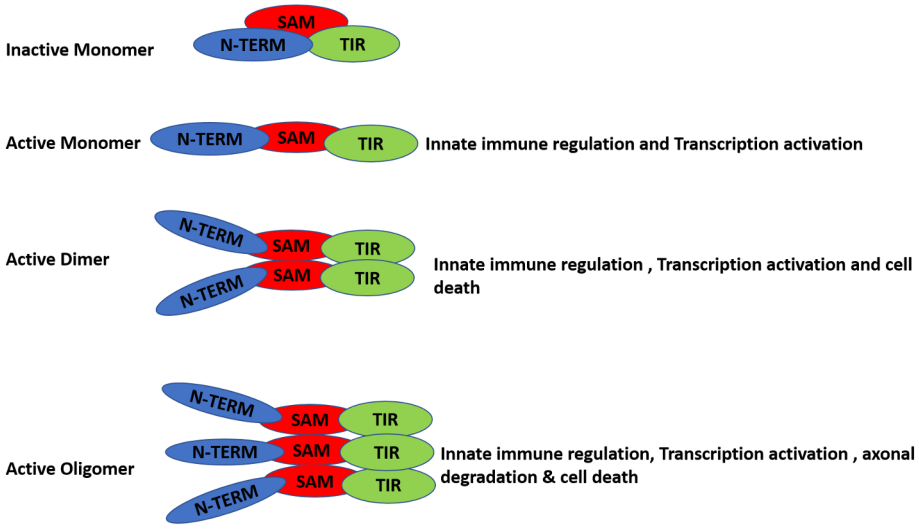

Fig. 2. Possible mechanisms whereby SARM is activated resulting in immune regulation, transcription responses and cell death. SARM remains inactive due to N terminus binding to the TIR domain. Monomeric unfolded SARM may regulate innate immune signalling and activate a specific transcriptional program. Dimerisation and oligomerisation of SARM may further lead to cell death which may also activate a transcriptional response.

monomeric SARM to be active. Whereas for SARM to drive cell death, particularly in neurons, calcium mobilisation, N terminus unfolding and oligomerisation may all be required (Fig. 2).

5.1. Discovery of the NADase activity of SARM

A series of studies by the Milbrandt group in particular addressing the molecular basis as to how SARM activates WD led to the discovery that SARM is an NADase. Prior to this discovery, it was shown that activation of SARM, which could be triggered experimentally by the dimerization of the TIR domain, resulted in NAD+ depletion, ATP loss and induction of mitochondrial depolarisation which all occur prior to axonal destruction [64,79]. These SARM activities were conserved since the TIR domain of *C. elegans* TIR-1 elicited the same effects [79]. A SARM specific (SS) loop was identified in the TIR domain that was essential for this NAD+ depletion. It remained completely unknown how dimerization of the TIR domain led to depletion of NAD+. Two possibilities existed, either that SARM activated a downstream NADase enzyme or alternatively that SARM itself acted as a NADase enzyme. Surprisingly, it was then revealed that the latter was the case, since recombinant SARM TIR domain expressed in vitro possessed intrinsic NADase activity, and cleaved NAD+ to ADP-ribose (ADPR), cyclic ADPR, and nicotinamide [7]. Interestingly, nicotinamide feeds back to inhibit this enzymatic activity of SARM. The study showed that SARM is unique among the TIR adaptors as the TIR domains of either TLR4 or MyD88 lacked this activity. Once again, this activity of SARM is conserved as expression of *Drosophila*, zebrafish, and mouse SARM TIR all cleaved NAD+ [7]. A key glutamate at position 642 in the TIR domain was shown to be critically required for this enzymatic activity. In a vincristine induced injury model it was shown that the NAD+ depletion activities of SARM were associated with its pro-degenerative function, both of which were abolished upon expression of the TIR E642A mutant of SARM [7].

5.2. Regulation of SARM NADase activity in WD

Clearly the activities of the SARM NADase must be tightly regulated and means to support NAD+ biosynthesis would seem to be vital to prevent aberrant axonal degradation. The protective effects of *Wld*^{s} in damaged neurons is due to the substitution of NMNAT2 activity for that of NMNAT1 [49]. However, the exact mechanism that explains the preservation of damaged axons following NMNAT expression was unknown. NMNAT enzymes utilise nicotinamide mononucleotide (NMN) and ATP to synthesis NAD+. Two different mechanisms were proposed to lead to axonal destruction, NAD+ depletion or alternatively NMN accumulation [83]. Surprisingly this study revealed that neither NAD+ maintenance or NMN consumption accounted for the protective effects of NMNAT expression. Rather the protective effects of NMNAT1 were due to the inhibition of the NAD+ depletion activity of SARM [83]. Interestingly expression of E Coli NMN deamidase, which was also shown to protect damaged axons [84], also blocked SARM dependent depletion of NAD+ [83]. That study presented biochemical evidence that NMNAT enzymes directly block the NAD+ depletion activity of SARM [83]. The study also showed that means to support NAD+ biosynthesis, such as NAMPT expression, which synthesises the NAD+ precursor NMN, or treatment with nicotinamide riboside along with expression of nicotinamide riboside kinase to synthesise NMN, showed much weaker protection of damaged axons than NMNAT expression [83]. The exact mechanism whereby NMNAT enzymes inhibit SARM are unknown. The authors cast doubt on a direct interaction between SARM and NMNAT, however they speculate that since enzymatically dead mutants of NMNAT or NMN deamidase can no longer protect damaged axons, metabolites from these enzymes may well inhibit the NADase activity of SARM [83].

The above study does explain why NMAT2 KO is embryonically lethal, as it unleashes SARM activation to commit catastrophic axonal destruction [85]. In that study it was shown that loss of NMNAT2 restricts outgrowth of developing axons, which is rescued by deletion of SARM, resulting in perinatal survival and the resulting healthy mice surviving into adulthood [85]. Given that both pharmacological and genetic inhibition of NMN accumulation prevents degeneration in the absence of NMNAT2, indicates that NMN accumulation may well be sufficient to activate SARM [85]. Therefore there is genetic [85] and biochemical evidence [83] to show that NMNAT activity inhibits SARM [6].

Other studies also support the notion that axon destruction might be triggered by NMN accumulation. It was found that inhibition of the NMN biosynthetic enzyme NAMPT or expression of *E. coli* NMN deamidase protected damaged neurons, which was lost upon addition of exogenous NMM [84]. In addition, expression of the axon protective WLD^{S} protein prevented this injury induced rise in NMN. The study suggests that the protective effect of WLD^{S} and NMNAT is due to the prevention of NMN accumulation upon damage to neurons. Of note the protection of axons provided by the use of NAMPT inhibitors occurred even though NAD+ levels dropped dramatically [84]. These observations can be accommodated into a model where reduced axonal NMNAT2 activity results in increased NMN leading to SARM dependent axonal destruction via its NADase enzymatic activity [6].

Further possible insights as to how NMN accumulation might activate the SARM dependent axonal destruction program was provided in

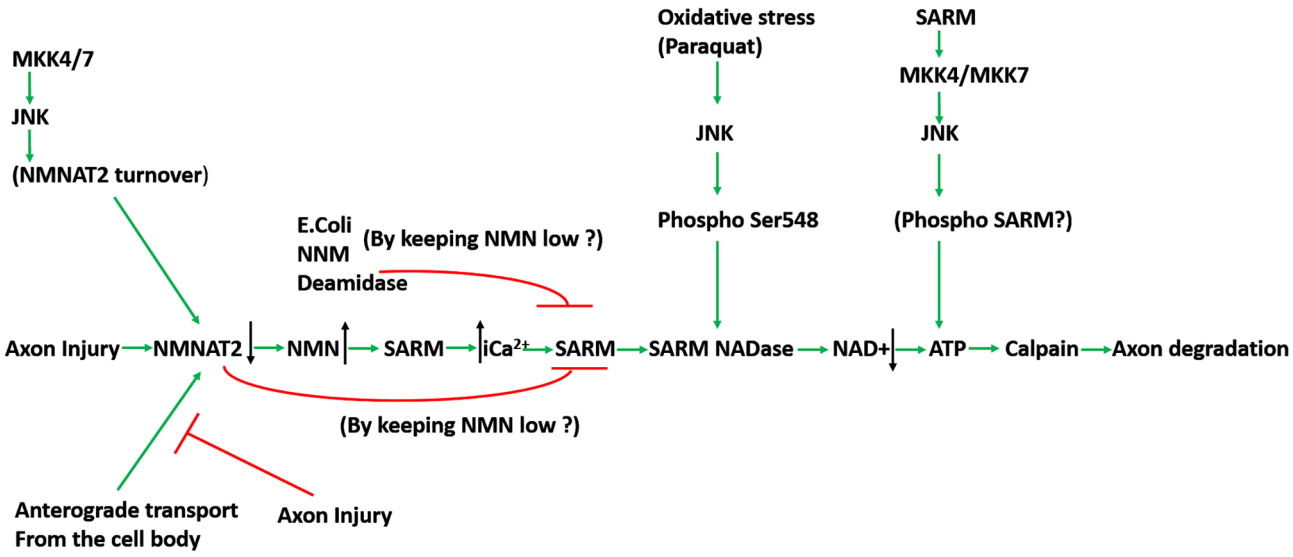

Fig. 3. Overview of the mechanism whereby the SARM NADase is activated leading to axon destruction or Wallerian degeneration. The axoprotective NMNAT2 is delivered to the nerve axon from the cell body via anterograde transport which is interrupted by axotomy. In addition JNK pathway activation results in the turnover of NMNAT2. A decrease in NMNAT2 activity results in NMN accumulation which somehow results in the activation of the SARM NADase activity. SARM has been reported to activate an increase in intracellular calcium and SARM is also activated by increased intracellular calcium. Oxidative stress activates JNK dependent phosphorylation of SARM at serine 548 triggering its NADase activity. SARM has been shown to be upstream of the JNK pathway which leads to localised ATP deficits. SARM dependent NADase activity depletes NAD+ levels resulting in ATP loss, calpain activation and axon destruction.

a study where NMN was shown to result in calcium influx which occurs just prior to axonal degradation [86]. Interestingly SARM is required for NMN to execute a rise in intra axonal calcium, which originated extracellularly [86]. The study found no requirement of mitochondrial depolarisation for accumulated NMN to trigger axonal degeneration [86]. This is the first report indicating that SARM might be upstream of calcium influx but also raises the question as to how can cytosolic SARM activate calcium influx from the extracellular compartment?

In addition to SARM activating axonal degeneration via depleting levels of NAD+, it has been shown that SARM is upstream of the MAPKKK member MLK2 to activate the JNK kinase pathway, which leads to a localised ATP deficit, calpain activation, and axon degeneration [87] (Fig. 3). Interestingly another member of the MAPKKK family DLK, the first protein found to participate in Wallerian Degeneration [88], mediates axon destruction, again due to activation of the JNK pathway [87].

The axonal protective cytoNmnat1 of WLD^S also prevented injury induced activation of the MAPK pathway [87], raising the possibility that in addition to NMNAT inhibiting SARM NADase, NMNAT of WLD^S may be also protective as it blocks injury-induced SARM dependent activation of the MAP kinase pathway. Therefore, it is possible that SARM activates two pathways to axonal destruction. One, through the direct NADase enzymatic activity of SARM and secondly via an upstream role for SARM to deplete ATP via activation of the JNK kinase pathway (Fig. 3). However, it should also be mentioned that another study failed to show an upstream role for SARM in activating the MAP kinase pathway upon damage to neurons [89]. There it was shown that the JNK kinase pathway activated the turnover of NMNAT2 resulting in the activation of SARM dependent axonal degradation [89] (Fig. 3). In order to directly compare the protective effects of SARM knockout with expression of Wld^s in WD, Nmnat2 KO mice, destined to succumb to death due to truncated axons, were crossed with SARM KO or with the Wld^s mice. Interestingly whereas the Wld^s mice developed hind limb paralysis at 3 months, the mice lacking SARM survived into old age with no overt pathologies. The authors concluded that therapies directed at SARM may be more efficacious that therapies related to Wld^s in neurodegenerative conditions [90].

5.3. SARM phosphorylation may regulate NADase activity

Apart from the hypothesis that N terminal cleavage or disruption can activate SARM, and the fact that enforced dimerization of the TIR domain also does so, little is known about how the SARM NADase activity is regulated in intact cells. However recently it was found that oxidative stress induced the phosphorylation SARM on serine 548 by JNK kinase to promote the NADase activity of SARM [91]. The study is noteworthy since to our knowledge it is the first paper showing a post translational modification of SARM. The study also shows that the NADase activity of SARM is only employed following oxidative stress and not under resting state [91]. This is consistent with the notion that under normal conditions SARM NADase activity is minimal, since NAD + levels are normal in undamaged axons lacking SARM [83]. It is possible that JNK kinase phosphorylation of SARM at serine 548 causes the N terminal portion of the protein to disassociate from the TIR domain resulting in its enzymatic activation [79]. It is already established that axotomy drives the JNK pathways, which can be activated by SARM [87]. It will be interesting to establish if axotomy and /or NMN accumulation drives JNK dependent phosphorylation of SARM on Ser 548. Thus, the JNK kinase pathway may activate WD by both the turnover of axo-protective NMNAT2 and the induction of the axo-destructive capabilities of the SARM NADase (Fig. 3).

6. Conclusion and future directions

Since SARM has been conserved from *C. elegans* to humans, this points to an indispensable role for this protein throughout 600 million years of animal evolution. However, it is still unclear as to what the roles of SARM are that have made it so indispensable. This is a compelling question to address given the very high degree of amino acid sequence conservation of SARM and since mice lacking SARM appear completely normal. Thus, whether SARM has been maintained throughout evolution due to its role in limiting immune activation, activating gene expression following immune challenge, NAD+ regulation or as an inducer of cell death is entirely unclear. Indeed, it is also possible that there are other as yet to be discovered roles for SARM that account for its conservation. On this point it is interesting to note that in SARM knockdown mice, behaviour and cognition were impaired

[75,78]. If roles in cognition and behaviour explain the conservation of SARM the question then would be, which if any of the described molecular functions for SARM, such as NADase activity, gene regulation or indeed cell death, contribute to its role in cognition and behaviour? We recall work done in *C. elegans* where forgetting behaviour required TIR-1 dependent activation of the JNK kinase pathway [20]. Furthermore, we do know that SARM drives the JNK pathway in the CNS to promote neuronal morphogenesis in mice [73]. Therefore, it would be fascinating to determine if SARM drives JNK activation in the CNS of mammals to control behaviour and cognition.

There are other possibilities to explain the conservation of SARM. Regarding WD and therein SARM, it has been proposed that WD which arose in a common ancestor of flies and mammals provided a survival advantage, but one associated with immunity and not injury [49]. Most invertebrates only have an innate immune system. Many neurotropic viruses spread using the axonal transport system and WD may have been a convenient method of limiting the spread of viruses [49]. Later in evolution WD became an injury response, useful in the peripheral nervous system (PNS) to remove damaged axons [49]. In the context of SARM such an idea is attractive, since SARM does mediate neuronal death in response to bunyavirus and WNV [23,41]. SARM activates apoptosis in response to infection with Bunyavirus, however what mode of death SARM activates in response to infection with WNV is unknown, but worthy of further exploration. It is possible therefore that SARM evolved as an indispensable component of the WD machinery as a mode of immune defence. To support this theory further work would need to be performed to determine if *Ect4* has a role in immune defence against neurotropic viruses in *Drosophila*. For now, however, the mystery concerning why SARM is so strongly conserved remains unsolved.

Apart from understanding why SARM is so conserved, another outstanding issue relates to which of the multiple functions of SARM described in this review depend on its NADase activity? Since other TIR domain proteins such as Myd88 and TLR4 do not appear to have NADase activity, and given that SARM regulates some TLR pathways, it is likely that at least some of SARM's activities are mediated by protein interactions via its TIR domain rather than by NADase activity. Thus, both NADase-dependent and -independent TIR functions may be mediated by SARM in mammals and indeed lower organisms too. Further work using NADase dead mutants, which would not be expected to affect the overall TIR fold structure, to try to rescue phenotypes in SARM KO animals and cells should bring clarity to this issue.

Since SARM has a prominent role in cell death of terminally differentiated cells such as neurons and T cells, one might predict a key function for SARM in blocking tumour progression, and indeed a small number of studies hint at such a role. Thus, future studies should also examine tumour models in SARM KO mice.

Given the role of SARM in driving axonal destruction following axotomy, traumatic brain injury and its role in peripheral neuropathy, there is now great interest in the pharmacological inhibition of the SARM NADase activity. And since the NADase activity is a response to injury and not a physiological function, this makes it an ideal drug candidate. The discovery of such inhibitors could have a great impact in the treatment of a range of neurodegenerative diseases.

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