



has been identified as a potential target for developing drugs to treat neurodegenerative diseases such as AD, PD, and multiple sclerosis (6, 7).

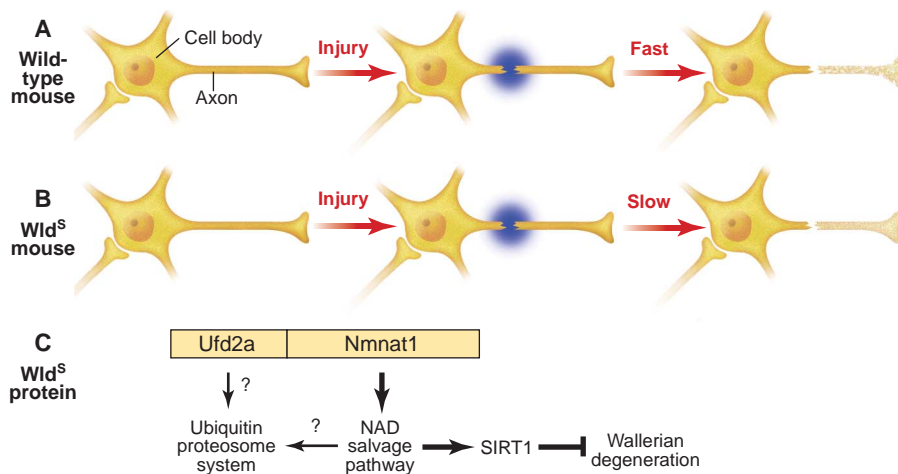
Araki *et al.* (1) developed an *in vitro* model of Wallerian degeneration comprising cultures of primary dorsal root ganglion neurons derived from wild-type mice. The neurons overexpressed either the *Wld<sup>s</sup>* fusion protein or one of the fusion protein fragments. Surprisingly, the authors found that overexpression of the *Ufd2a* protein fragment alone did not delay degeneration of axons injured by removal of the neuronal cell body (transec-

tion) or treatment with the neurotoxin vincristine. In contrast, overexpression of *Nmnat1* or the addition of NAD to the neuronal cultures before injury delayed axonal degeneration in response to mechanical or chemical damage.

fusion cultures after injury only when SIRT1 expression was reduced. The same effect was observed when SIRT1 activity was blocked with a small-molecule inhibitor; a SIRT1 activator, on the other hand, boosted neuronal survival following injury. These data suggest that protection against Wallerian degeneration is the result of increased expression of *Nmnat1*, a rise in nuclear NAD levels, and a consequent increase in SIRT1 activity. This conclusion does not negate the involvement of the proteasome in Wallerian degeneration, but it does indicate that the protective effect of the *Wld<sup>s</sup>*

In intact neurons of *C57BL/Wld<sup>s</sup>* mice, the *Wld<sup>s</sup>* fusion protein is expressed almost exclusively in the nucleus (4). In fibroblasts (9)—and, presumably, in neurons—SIRT1 also is expressed in the nucleus. SIRT1 and other NAD-dependent deacetylases alter gene expression by targeting histone proteins as well as key nuclear transcription factors such as p53 (9, 10), forkhead (11, 12), and NF- $\kappa$ B (13). In addition, Sirtuins also deacetylate cytoplasmic proteins, including  $\alpha$ -tubulin. The protective effect of the *Wld<sup>s</sup>* fusion protein appears to be exerted in the nucleus, because addition of NAD after removal of cell bodies in the neuronal cultures is no longer protective. This suggests that an alternative program of gene expression is initiated by elevated NAD levels in the nucleus, leading to the production of protective factors that actively block Wallerian degeneration. The therapeutic implication of this finding is that it may be possible to design neuroprotective drugs that boost SIRT1 activity and prevent further neurodegeneration in diseases like AD and PD.

The Araki *et al.* study (1) addresses the long-standing question of how the *Wld<sup>s</sup>* fusion protein prevents Wallerian degeneration. As with most groundbreaking studies, new questions emerge. For example, what is the direct result of increased *Nmnat1* expression? Overexpression of *Nmnat1* leads to increased activity of this enzyme but does not change total NAD levels or the ratio of NAD to NADH, raising the possibility that increased *Nmnat1* activity may result in a decrease in nicotinamide or other inhibitory molecules. It is possible that the relevant target of SIRT1's neuroprotective activity may be a transcription factor that responds to changes in the cell's metabolic state by switching on expression of genes that encode neuroprotective proteins. Identifying the targets of SIRT1 that mediate the neuroprotective effect may broaden the options for therapeutic intervention in AD, PD, and other neurodegenerative diseases.



**Energizing neuroprotection.** (A) In wild-type mice, axons of injured neurons rapidly degenerate (Wallerian degeneration) in a process that may be relevant to the neurodegeneration seen in diseases like AD and PD. (B) In mice with the *Wld<sup>s</sup>* dominant mutation (a tandem triplication of a region on mouse chromosome 4), injured neurons show a delay in Wallerian degeneration due to activity of the *Wld<sup>s</sup>* fusion protein. (C) The fusion protein consists of the amino terminus of *Ufd2a* (an E4 ubiquitin-conjugating enzyme) and the entire sequence of *Nmnat1* (an enzyme in the NAD salvage pathway). Neuroprotection in the *Wld<sup>s</sup>* mouse may result from increased synthesis of NAD, leading to a concomitant increase in the activity of the NAD-dependent deacetylase, SIRT1, which may activate a transcription factor that induces expression of genes involved in neuroprotection (7).

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It is well established that increased expression of NAD salvage pathway genes in yeast, including the yeast homologs of *Nmnat1* (*NMA1* and *NMA2*), lengthens life-span and boosts resistance to stress, an effect that depends on the NAD-dependent deacetylase Sir2 (8). Based on this observation, Araki *et al.* tested whether the protective effect of increased *Nmnat1* expression required NAD-dependent deacetylase activity. Expression of small interfering RNAs that target each of the seven Sir2 mammalian homologs (SIRT1 through SIRT7) decreased survival of the dorsal root gan-

glion cultures after injury only when SIRT1 activity was reduced. The same effect was observed when SIRT1 activity was blocked with a small-molecule inhibitor; a SIRT1 activator, on the other hand, boosted neuronal survival following injury. These data suggest that protection against Wallerian degeneration is the result of increased expression of *Nmnat1*, a rise in nuclear NAD levels, and a consequent increase in SIRT1 activity. This conclusion does not negate the involvement of the proteasome in Wallerian degeneration, but it does indicate that the protective effect of the *Wld<sup>s</sup>*

The enzymes SIRT1 through SIRT7 belong to a unique enzyme class that requires a boost in NAD levels to maintain activity, because they consume this cofactor during deacetylation of target proteins. Another enzyme that depletes cellular NAD levels is PARP. In the presence of NAD, inhibition of PARP has little effect on Wallerian degeneration; however, in the absence of exogenous NAD, inhibition of PARP increases the survival of dorsal root ganglion cultures after injury (1). This suggests that neuronal survival requires the maintenance of adequate NAD levels, but that a boost in NAD levels beyond this point confers no additional benefit.

## References

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