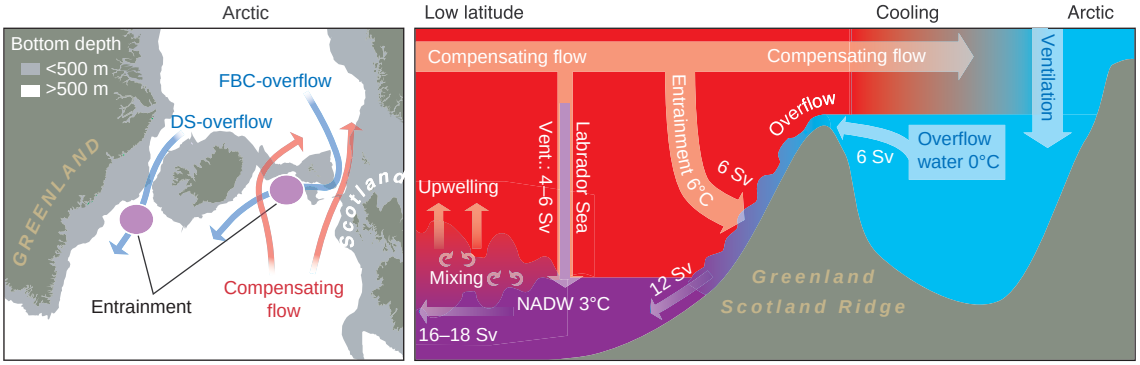
spired a public debate focused on a potential cooling of northern Europe, which has the compensating flow just off the coast. Note that this part of the North Atlantic THC is especially dependent on ventilation north of the Greenland-Scotland Ridge, overflow, and entrainment (*3*).



North Atlantic flow. The exchange of water across the Greenland-Scotland Ridge is a fundamental component of the North Atlantic THC. Arrows on the map indicate the main overflow (blue) and compensating inflow (red) branches. On the schematic section to the right, temperatures in °C and volume transports in Sv (1 Sv = 10^6 m^3/s) are approximate values. DS, Denmark Strait; FBC, Faroe Bank Channel.

The concept of a weakened THC is supported by some numerical climate models (*8*), but not by all. Increased salinity of the compensating flow may balance the salinity decrease from the increased freshwater supply and maintain ventilation (*9*). Climate models, so far, do not provide a unique answer describing the future development of the THC, but what is the present observational evidence?

It is argued that early evidence for changes should primarily be sought in the ventilation and overflow rates. Indeed, some such changes have been reported. Since around 1960, large parts of the open sea areas north of the Greenland-Scotland Ridge have freshened (*10*), and so have the overflows (*11*). At the same time, low-latitude Atlantic waters became more saline in the upper layer (*12*), and this is also reflected in the compensating flow. Long-term observations in both of the main branches of compensating flow across the Greenland-Scotland Ridge have shown increasing salinity since the mid-1970s, with a record high in 2003.

Even more convincing evidence for a reduction of the North Atlantic THC has been gained from monitoring both the overflows and the compensating northward flow by direct current measurements (*13*). For the Denmark Strait overflow, no persistent long-term trends in volume transport have been reported (*2*, *14*), but the Faroe Bank Channel overflow was found to have decreased by about 20% from 1950 to 2000 (*15*).

We find evidence of freshening of the Nordic Seas and a reduction of the strength of the overflow, both of which will tend to weaken the North Atlantic THC. On the other hand, the compensating northward flow is getting more saline, which may maintain ventilation and counterbalance the THC decrease. So the jury is still out. This emphasizes the need for more refined climate models and long-term observational systems that are capable of identifying potential changes in our climate system.

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NEUROSCIENCE

NAD to the Rescue

Antonio Bedalov and Julian A. Simon

The cofactor nicotinamide adenine dinucleotide (NAD)—once consigned to the oblivion of metabolic pathway wall charts—has recently attained celebrity status as the link between metabolic activity, cellular resistance to stress or injury, and longevity. NAD influences many cell fate decisions—for example, NAD-dependent enzymes such as poly (ADP-ribose) polymerase (PARP) are important for the DNA damage response, and NAD-dependent protein deacetylases (Sirtuins) are involved in transcriptional regulation, the stress response, and cellular differentiation. On page 1010 of this issue, Araki and colleagues (*1*) extend the influence of NAD with their demonstration that an increase in NAD biosynthesis or enhanced activity of the NAD-dependent deacetylase SIRT1 protects mouse neurons from mechanical or chemical injury (*2*).

Axonal degeneration (termed Wallerian degeneration) often precedes the death of neuronal cell bodies in neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's (PD). Mice carrying the spontaneous dominant *Wld*^{s} mutation show delayed axonal degeneration following neuronal injury. The *Wld*^{s} mutation on mouse chromosome 4 is a rare tandem triplication of an 85-kb DNA fragment that harbors a translocation. The translocation encodes a fusion protein comprising the amino-terminal 70 amino acids of Ufd2a (ubiquitin fusion degradation protein 2a), an E4 ubiquitin ligase, and the entire coding region of Nmnat1 (nicotinamide mononucleotide adenylyltranferase 1), an NAD biosynthetic enzyme. Although the C57BL/*Wld*^{s} mouse was described 15 years ago (*3*) and expression of the Wld^s fusion protein is known to delay Wallerian degeneration (*4*), the mechanism of neuroprotection has remained elusive. Given that proteasome inhibitors block Wallerian degeneration both in vitro and in vivo (*5*), the Ufd2a protein fragment (a component of the ubiquitin proteasome system) has been the prime candidate for mediator of neuroprotection in the *Wld*^{s} mouse. Indeed, ubiquitin-mediated protein degradation by the proteasome

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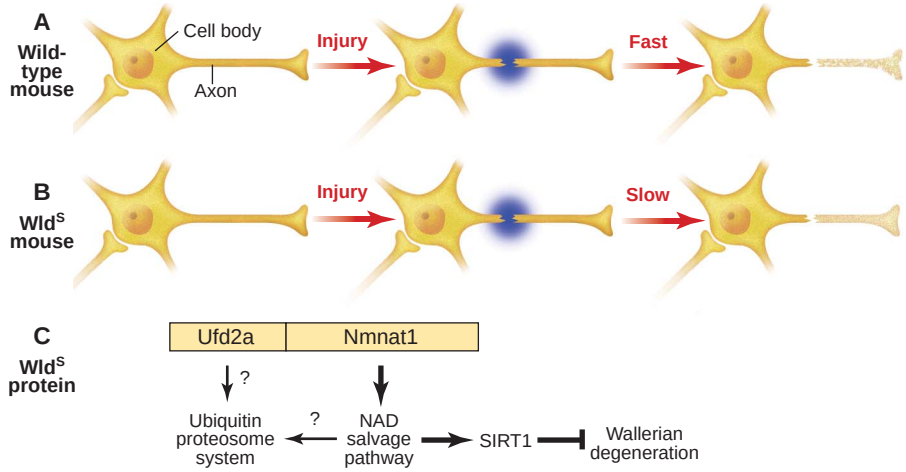
has been identified as a potential target for developing drugs to treat neurodegenerative diseases such as AD, PD, and multiple sclerosis (*6, 7*).

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^S 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 (transection) 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.



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^S* 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^S 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^S* 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 (*1*).

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 ganglion 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^S fusion protein is independent of Ufd2a activity. Indeed, the new findings throw open the possibility that changes in NAD levels may indirectly regulate the ubiquitin-proteasome system.

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.

In intact neurons of C57BL/*Wld^S* mice, the Wld^S 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-κB (*13*). In addition, Sirtuins also deacetylate cytoplasmic proteins, including α-tubulin. The protective effect of the Wld^S 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^S 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.

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ERRATUM

Post date 29 April 2005

Perspectives: "NAD to the rescue" by A. Bedalov and J. A. Simon (13 Aug. 2004, p. 954). In reference 7, the first author's name was mispelled. It should be A. Sajadi.