Boosting NAD to Spare Hearing

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Ex vivo experiments have strangely shown that inhibition or stimulation of NAD metabolism can be neuroprotective. In this issue of *Cell Metabolism*, Brown et al. (2014) demonstrate that cochlear NAD is diminished by deafening noise but protected by nicotinamide riboside or *WldS* mutation. Hearing protection by nicotinamide riboside depends on Sirt3.

The Wallerian degeneration slow (*WldS*) mouse has been trying to tell us something about neurodegeneration for over 20 years, but it has been difficult for us to hear an unequivocal message. Wallerian degeneration is the active process by which axons degenerate distal to injuries caused by cutting or crushing nerve fibers. Wallerian-related degeneration is found in multiple diseases and conditions, including chemotherapy-induced nerve damage, at least in nerve cell culture. Because the *WldS* mutation allows mice and nerves analyzed ex vivo to resist these insults, we'd like to know how it does this and how we can exploit this information to oppose neurodegeneration (Coleman and Freeman, 2010). New data indicate that nicotinamide riboside (NR) protects mice from noise-induced hearing loss (Brown et al., 2014). This discovery is mechanistically insightful and translationally promising.

WldS acts as a dominant trait consisting of triplication of a fusion between the N terminus of polyubiquitylation factor Ube4b and the full-length of NAD biosynthetic enzyme NMN adenylyltransferase 1 (Nmnat1) (Coleman and Freeman, 2010). When *WldS* was cloned, there was an impression that NAD biosynthesis is ubiquitous and unregulated, suggesting the possibility that *WldS* functions as a dominant-negative Ube4b. However, neuronal overexpression of Nmnat1 protects cultured neurons from vincristine-induced axonapathy (Araki et al., 2004). Moreover, addition of any of three NAD precursor vitamins protects cultured neurons against axonopathy. Two of three vitamins required concomitant introduction of a biosynthetic gene, while NR, a recently discovered NAD precursor vitamin (Bieganowski and Brenner, 2004), protected because the NR kinase 2 gene is transcriptionally upregulated by nerve damage (Sasaki et al., 2006).

These results emphasized the potential for dysregulation and modulation of NAD in pathophysiology because cellular damage can induce activities such as poly (ADP-ribose) polymerase (PARP), which degrades nucleocytoplasmic NAD (Belenky et al., 2007). Indeed, the length and specialized structures inherent in sensory and peripheral neurons may render them particularly sensitive to NAD-dependent bioenergetics. If this is the case, it might follow that PARP activation—secondary to reactive oxygen species (ROS) produced in response to drugs or elevated glucose metabolism—is a central player in chemotherapy-induced and diabetic peripheral neuropathies. Because NAD salvage from NR is inducible by damage (Sasaki et al., 2006), NR has strong potential as a neuroprotective agent.

The *WldS* story became more complicated with conflicting claims about the role of Nmnat isozymes in neuroprotection. Nmnat1 is reported to be mostly nuclear, while Nmnat2 is associated with the cytosolic face of Golgi membranes, and Nmnat3 is reported to be inside the mitochondrial matrix (Coleman and Freeman, 2010). Nmnat1 loss-of-function mutations cause retinal neurodegeneration, and Nmnat2 knockdown in cultured neurons promotes neurite degeneration, while Nmnat3 overexpression has *WldS*-like activity. However, in some systems, overexpression of Nmnat active site mutants also provided *WldS*-like activity (Coleman and Freeman, 2010). In such systems, the potential for NAD-dependent protection was discounted and *WldS* was interpreted as a protein that protects against nerve damage by relief of proteotoxicity (Zhai et al., 2008). The site of action of *WldS* is also controversial. Some studies conclude that mitochondrial localization is required for neuroprotection, while other studies claim to exclude mitochondria as sites of *WldS*-dependent neuroprotection.

Pharmacological experiments did not settle these issues. In one system, FK866, a specific inhibitor of NAD biosynthetic enzyme nicotinamide phosporibosyltransferase, induces atrophy in cultured neurons (Wang and He, 2009). In another, FK866 protects transected axons from degeneration, leading to the conclusion that *WldS* uses its active site not to make NAD but to rid neurons of NMN, a putative cell death signal that would arise when the unstable Nmnat2 protein falls below a critical concentration in the distal axon (Di Stefano et al., 2014). In the absence of an in vivo test, current models are starkly different. Does NAD protect from Wallerian degeneration because axonal and/or mitochondrial NAD are under attack by nerve damage (Araki et al., 2004; Sasaki et al., 2006; Wang and He, 2009), or does NMN signal damaged nerves to die back because the axon has determined that Nmnat2 transport has ceased (Di Stefano et al., 2014)? The former model predicts that NR would be neuroprotective in vivo while the latter model predicts that NR-dependent formation of NMN would accelerate nerve loss. Models that reject NAD biosynthesis (Zhai et al., 2008) would be emboldened by a finding that NR is inert in vivo.

The results were remarkably clean. In mice, loud noises sufficient to produce temporary loss of hearing drove down cochlear NAD. The *WldS* mutation or NR prevented loss of NAD and maintained good hearing. Moreover, NR-dependent protection depended on the mitochondrial NAD-dependent protein lysine

deacetylase, Sirt3 (Brown et al., 2014). All of this is wonderful news for the future of NR and NAD-boosting neurotherapeutic approaches. Because Sirt3 was required for NR function, it stands to reason that NR increased mitochondrial NAD synthesis. However, these results do not rule out *WldS* functions in the axonal cytosol nor exclude a role for axonal NMN as a degenerative signal.

The way to synthesize the concepts of NMN as neurotoxic and NAD as neuroprotective is to appreciate that cytosolic NAD is required to generate ATP for anterograde vesicular transport. Indeed, increasing evidence indicates that glycolytic enzymes and Nmnat2 are vesicle-associated, which allows such organelles to traverse the length of neurons with "on-board" ATP production (Zala et al., 2013). Because Nmnat2 is unstable, depletion of the ability to convert NMN to NAD could limit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and damage neuronal energetics because the preparatory phase of glycolysis (upstream of GAPDH) costs two ATP per input glucose. Without an ATP payoff and the resulting production of cytosolic GTP, the ability to translate and transport new Nmnat2 and other proteins would be greatly compromised, and NMN accumulation could be read by such damaged neurons as a signal to die back. In the presence of *WldS* or NR, I predict that cytosolic and mitochondrial NAD formation are improved, with axonal NAD preserving transport of GAPDH, Nmnat2, and other molecules along lengthy microtubules. So long as anterograde transport is functioning, there should not be accumulation of NMN or a degenerative signal produced by NMN.

The most recent result (Brown et al., 2014) also demands a role for mitochondrial NAD, which is thought to be produced from mitochondrial transport of cytosolic NMN. Why Sirt3 would need to deacetylate mitochondrial proteins to prevent neurodegeneration is not known, though mitochondrial proteins are frequently inactivated by acetylation. Future experiments are expected to clarify whether loud noise induces PARP in a way that makes GAPDH require increased axonal synthesis of NAD, whether noise alters mitochondrial metabolism in a manner that drives acetyl modifications onto protein targets, to what degree ROS damage is attenuated by provision of NR, and whether NR can be used to prevent or treat additional neurodegenerative diseases and conditions.

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Purloined Mechanisms of Bacterial Immunity Can Cure Muscular Dystrophy

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Myriad strategies have been explored to compensate for the lack of dystrophin or to skip mutations that cause the lethal disease Duchenne muscular dystrophy (DMD). A new study shows that gene editing strategies used by bacteria can be applied in zygotes of a mouse model of DMD to correct the genetic defect that causes muscular dystrophy (Long et al., 2014).

Duchenne muscular dystrophy (DMD) is a lethal, X-linked recessive disease affecting approximately 1 of 3,500 born males. Upon discovery that DMD is a monogenic disease caused by mutations of the dystrophin gene, hopes were high that targeting the wild-type gene to dystrophic muscle would provide a cure. In intervening decades, an impressive array of