Linking DNA Damage, NAD$^+$/SIRT1, and Aging

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Diseases due to DNA damage repair machinery defects can resemble premature aging. In this issue of *Cell Metabolism*, Scheibye-Knudsen et al. (2014) demonstrate that increasing NAD$^+$ levels may reverse the inactivation of Sirt1 and mitochondrial defects in Cockayne Syndrome B that stem from nuclear NAD$^+$ depletion by the DNA repair protein PARP.

A number of diseases are due to loss of function mutations in DNA repair proteins, such as Cockayne Syndrome or xeroderma pigmentosa, which are characterized by an increase in cancer and metabolic abnormalities. Moreover, aspects of these diseases resemble premature aging, both in humans and mouse models (Hoeijmakers, 2009). Recently, the Bohr lab showed that one of these diseases, xeroderma pigmentosa group A (XPA), resulted in the chronic activation of the DNA repair protein poly-ADP-ribose polymerase (PARP) and a concomitant depletion of NAD$^+$ as PARP consumes it in order to ADP-ribosylate proteins at sites of DNA damage (Fang et al., 2014). This in turn inactivated the NAD$^+$-dependent deacetylase SIRT1 (Imai et al., 2000) and its downstream target PGC-1α, resulting in defective mitochondria with hyperpolarized membranes and increased production of reactive oxygen species. Importantly, many of the metabolic phenotypes of XPA could be rescued by application of PARP inhibitors or NAD$^+$ precursors, such as nicotinamide mononucleotide (NMN) (Ramsey et al., 2008; Yoshino et al., 2011) or nicotinamide riboside (NR) (Cantó et al., 2012), which restored NAD$^+$ levels and SIRT1 activity in cells or animals.

In this issue of *Cell Metabolism* (Scheibye-Knudsen et al., 2014), the authors describe phenotypes that overlap with XPA in a mouse model of Cockayne Syndrome group B (CSB), which also leads to increased DNA damage. In addition to the metabolic studies discussed below, the authors present novel biochemical experiments that suggest that a defect in CSB protein may activate PARP by a novel mechanism yet to be observed in other DNA repair deficiencies. They demonstrate that CSB protein is recruited to sites of DNA damage by poly-ADP-ribosylated proteins generated by PARP and then displaces PARP to allow repair to proceed. Thus, in cells missing CSB, active PARP will persist at damaged sites, thereby exacerbating the depletion of NAD$^+$.

Interestingly, in addition to rescue by NAD$^+$ and PARP inhibitors, the authors now show that a high-fat diet (HFD) can also rescue the mitochondrial defects in CSB tissues and cells resulting from the hyperactivation of PARP (Figure 1). This diet also signals the production of high levels of ketones, such as β-hydroxybuterate. Ketones are made by the liver to bridge a glucose deficit in the brain when dietary carbohydrate is limiting; for example, ketones rise substantially in mammals during fasting. Importantly, the authors demonstrate that β-hydroxybuterate by itself can rescue CSB defects in cells, suggesting that ketone production may be key to the benefits provided by the HFD to *csb*$^{-/-}$ mice. This notion has precedent as ketogenic diets have previously been shown to protect against oxidative stress in mice (Shimazu et al., 2013).

Neurons in *csb*$^{-/-}$ mice are particularly sensitive, exhibiting the characteristic mitochondrial defects and showing extensive damage in the cerebellum and the inner ear. PARP inhibitors or NAD$^+$ precursors rescue these CSB phenotypes, as does the HFD and β-hydroxybuterate. The authors suggest that ketones may function by increasing the low acetyl-CoA (Ac-CoA) levels they observe in *csb*$^{-/-}$ mice, resulting in an increase in the activity of the histone acetyl transferase PCAF, which increases SIRT1 expression and possibly protein stability (Figure 1). To wit, the authors imply that the levels of Ac-CoA are depressed because *csb*$^{-/-}$ mice feature a low NAD$^+$/NADH ratio, which shifts the equilibrium of lactate dehydrogenase toward lactate production, thus shunting pyruvate produced by glycolysis away from mitochondrial metabolism. The HFD fed *csb*$^{-/-}$ mice accordingly display a complete shift to fat catabolism for energy, but this is evidently not sufficient for maintenance of normal Ac-CoA levels. The authors suggest that the provision of ketones raises Ac-CoA levels and activates PCAF, resulting

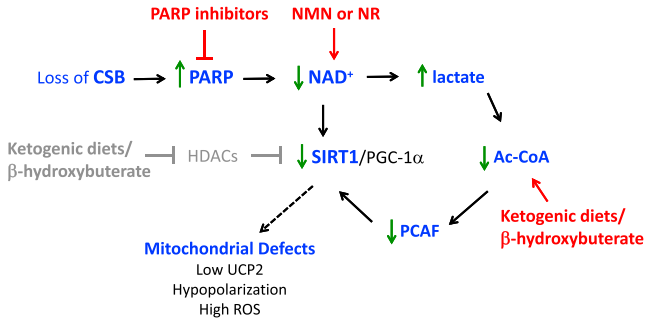

Figure 1. Pathway Leading to Mitochondrial Dysfunction in Cockayne Syndrome B and Possible Therapeutic Interventions
The pathway by which Cockayne Syndrome B results in defective mitochondria is traced in blue. The three interventions that ameliorate this problem, PARP inhibition, NAD$^+$ precursor supplementation, or ketogenic diets, are depicted in red. A second possible pathway emanating from ketogenic diets is shown in gray.

in the increase in SIRT1. However, since β-hydroxybuterate is a well-known inhibitor of class I and II histone deacetylases (HDACs), it is likely that ketones also induce many transcriptional changes via HDAC inhibition, and these may result in SIRT1 activation. The bottom line is that all three interventions for CSB (PARP inhibition, NAD^+ precursor supplementation, and HFD/ketones) require an active SIRT1 to rescue the mitochondrial defects (Figure 1). This outcome may occur, in part, because SIRT1 maintains normal levels of uncoupling protein 2 (UCP2), the lack of which results in tight coupling of electron transport to ATP synthesis, and hyperpolarization of the mitochondrial membrane.

Given the findings of Scheibye-Knudsen et al. (2014), what remains to be done in order to better understand and treat DNA damage repair deficiencies? So far, the rescue of CSB by NAD^+ precursors, PARP inhibitors, or ketones has been demonstrated at the cellular level or in specific tissues like the cerebellum. It will be important to study whether correction of the mitochondrial defects extends to all affected tissues and ameliorates systemic phenotypes. Will supplementation of $csb^{-/-}$ mice with the NAD^+ precursors, PARP inhibitors, or ketones slow aging and extend life span? One might predict a more complex effect of PARP inhibitors, which will not only restore NAD^+ but also exacerbate the DNA repair defect in these animals. These studies may also have translational implications for diseases due to DNA repair defects, since all three treatments that benefit $csb^{-/-}$mice may be feasible in humans.

More generally, a mechanism similar to that represented in Figure 1 has also been demonstrated in normal aging in a variety of organisms (Mouchiroud et al., 2013), suggesting that at least one aspect of aging can be attributed to the metabolic fallout of DNA damage. By this logic, aging induces chronic DNA damage and PARP activation, thereby leading to NAD^+ depletion, SIRT1 inactivation, and mitochondrial dysfunction. Again, youthful NAD^+ levels can be restored in mice by supplementing old animals with the NAD^+ precursors NMN (Ramsey et al., 2008; Yoshino et al., 2011) or NR (Cantó et al., 2012). Supplementation also leads to health benefits in the aged mice, including the restoration of mitochondrial function to youthful levels in skeletal muscle (Gomes et al., 2013). The decline observed in normal aging attributable to chronic DNA damage will, of course, occur more rapidly in mice or humans with XPA or CSB. It remains to be seen whether NAD^+ precursor supplementation improves mitochondrial function and gives rise to overall health benefits in an aging human population. If so, it will be interesting to test whether NAD^+ precursor supplementation synergizes with small molecule activators of SIRT1 to further increase the health span during aging.

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