Adult stem cells (SCs) are essential for tissue maintenance and regeneration yet are susceptible to senescence during aging. We demonstrate the importance of the oxidized form of cellular nicotinamide adenine dinucleotide (NAD⁺) and its effect on mitochondrial activity as a pivotal switch to modulate muscle SC (MuSC) senescence. Treatment with the NAD⁺ precursor nicotinamide riboside (NR) induced the mitochondrial unfolded protein response and synthesis of prohibitin proteins, and this rejuvenated MuSCs in aged mice. NR also prevented MuSC senescence in the mdx (C57BL/10ScSn-Dmd<sup>mdx<sub>2</sub>/J) mouse model of muscular dystrophy. We furthermore demonstrate that NR delays senescence of neural SCs and melanocyte SCs and increases muscle life span. Strategies that conserve cellular NAD⁺ may reprogram dysfunctional SCs and improve life span in mammals.

In adults, tissue homeostasis is highly dependent on stem cell (SC) function. Adult SCs are not only essential in continuously proliferating tissues (like the hematopoietic, intestinal, and skin systems) but also in normally quiescent tissues (such as skeletal muscle and the brain) that require regeneration after damage or exposure to disease (1). Aging is accompanied by a decline in adult SC function, termed SC senescence, which leads to the loss of tissue homeostasis and regenerative capacity (2, 3).

Homeostasis and regeneration of skeletal muscle depend on normally quiescent muscle SCs (MuSCs), which are activated upon muscle damage to expand and give rise to differentiated myogenic cells that regenerate damaged muscle fibers (4, 5). These responses are blunted in aged muscle, probably because of the reduced number and function of MuSCs (6–8). In aging, MuSC dysfunction may be caused by extrinsic signals (9, 10), intrinsic cellular senescence signaling pathways (11), or both. One general regulator of cellular senescence, cyclin-dependent kinase inhibitor 2A (CDKN2A, also known as p16<sup>INK4a</sup>), shows increased expression in geriatric MuSCs, which causes permanent cell cycle withdrawal and senescence of MuSCs in very old mice (28 to 32 months of age) (11). However, reductions in MuSC number and function can already be observed before this stage (6, 11), indicating that MuSC senescence may be initiated at an earlier time point. Pre-geriatric mice, approximately 2 years old, can exhibit features of MuSC senescence (8, 12–15). However, the early mechanisms that instigate MuSC senescence are still largely unknown.

One of the hallmarks of organismal aging is the appearance of mitochondrial dysfunction (2, 3). Induced by calorie-dense diets or aging, mitochondrial dysfunction can result from depletion of the oxidized form of nicotinamide adenine dinucleotide (NAD⁺), whereas NAD⁺ repletion, with precursors such as nicotinamide riboside (NR), can reverse this process (16–20). SCs are thought to rely predominantly on glycolysis for energy, a process that would reduce cellular concentrations of NAD⁺ (21). Mitochondrial function is linked to SC maintenance and activation (22–25), yet its role in senescence is unknown.

**Mitochondrial dysfunction is a biomarker of MuSC senescence**

To identify the role of mitochondrial function in SCs, we compared MuSCs from young and aged mice. For the purpose of identifying the principal mechanisms that initiate MuSC senescence, we examined publically available MuSC gene expression data sets from young (~3 months) and aged (~24 months) mice using gene set enrichment analysis (GSEA; Gene Expression Omnibus (GEO) accession numbers GSE47177 (14), GSE47104 (22), and GSE47401 (8)) enrichment scores of data sets from young versus aged mice revealed up-regulation of senescence pathways and down-regulation of cell cycle pathways with age (Fig. 1A, tables S1 to S3, and fig. S1, A and B). This finding is consistent with the idea that irreversible cell cycle arrest is a primary marker of cellular senescence (2, 3). In all three data sets, tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS) pathways were among the most down-regulated pathways in aged MuSCs (Fig. 1A, tables S1 to S3, and fig. S1, A and B). Analysis of gene ontology terms that were significantly (GSEA; family-wise error rate < 0.05) down-regulated in aged MuSCs further demonstrated links to mitochondrial function (fig. S1C). Genes that were commonly down-regulated during aging showed a substantial overlap (113 genes; 11.59%) with mitochondrial genes (26) (Fig. 1B and table S4), in contrast to the minimal overlap (11 genes; 1.92%) among commonly up-regulated genes and mitochondrial genes (fig. S1D and table S4). Among the 113 down-regulated mitochondrial genes in aged MuSCs, 41.6% were related to the TCA cycle and OXPHOS (fig. S1E), which is higher than their percent composition of the whole mitochondrial proteome (~14%) (27, 28). This indicates a dominant decline in expression of mitochondrial respiratory genes in aged MuSCs. The reduction in mitochondrial OXPHOS and TCA cycle genes was consistent for all independent data sets (fig. S1F and G). We isolated primary aged and young MuSCs and confirmed reduced abundance of OXPHOS and TCA cycle transcripts (Fig. 1C), as well as reduced oxidative respiratory rates in both freshly isolated (Fig. 1D) and cultured MuSCs (fig. S1H). MuSC mitochondrial dysfunction in aged mice was further confirmed by the loss of mitochondrial membrane potential (Fig. 1E) and a reduction in cellular adenosine triphosphate (ATP) concentrations (Fig. 1F). Several important markers and regulators of the mitochondrial unfolded protein response (UPR<sup>mit</sup>), a stress-response pathway that mediates adaptations in mitochondrial content and function, were down-regulated in aged MuSCs (fig. S1F and Fig. 1G). Despite the absence of consistent changes in the CDKN2A (fig. S1I and table S4) or mitogen-activated protein kinase 14 (MAPK14, also known as p38) pathways (table S4), previously reported to regulate MuSC senescence, expression of cell cycle genes was decreased and expression of genes encoding the senescent proinflammatory secretome was increased (fig. S1, I to K). The reduction in cell cycle signaling was accompanied by increased expression of genes in the cyclin-dependent kinase inhibitor 1 (CDKN1A; also known as p21<sup>WAF1/CIP1</sup>)-mediated pathway (fig. S1K and table S4), which suggests that early senescence in MuSCs may involve CDKN1A.

**NAD⁺ repletion improves MuSC function in aged mice**

Given the importance of NAD⁺ concentrations in the control of mitochondrial function (16, 29), we examined the potential of NAD⁺ repletion to improve MuSC numbers and muscle function in aged mice. Amounts of NAD⁺ in freshly isolated MuSCs were lower in those isolated from aged mice, and 6 weeks of NR treatment increased NAD⁺ concentration in MuSCs from young and old mice (Fig. 2A). Amounts of NADH (the reduced form of NAD⁺) were relatively stable...
Muscle from aged mice contained fewer MuSCs compared with that of young animals (Fig. 2, B and C, and fig. S2, B and C). However, NR treatment increased MuSC numbers in young and old mice (Fig. 2, B and C, and fig. S2, B and C). The increase in MuSC numbers was confirmed by staining with paired box protein Pax-7 (PAX7), a known MuSC marker (4) (Fig. 2D and fig. S2, D and E). In both young and aged mice, the effect of NR did not appear to result from changes in muscle or body mass, as these measures remained comparable among all groups over the short treatment period (fig. S2, F to I). NR treatment also enhances muscle function in aged animals through an independent mechanism acting directly on the muscle fibers (36), as was apparent from improvements in maximal running times and distances, along with limb grip strength in aged mice (Fig. 2, E to G). Young animals showed no such changes (fig. S2, J to L).

Impairments in muscle regeneration efficiency have been linked to the decline in MuSC function in aged mice (6). We therefore examined the benefits of NR on muscle regeneration with cardiotoxin (CTX)-induced muscle damage (4). NR treatment accelerated muscle regeneration in aged and young mice (Fig. 2H and fig. S2M). NR-induced improvements in regeneration were paralleled by increases in Pax7-stained MuSCs in aged mice (Fig. 2I and fig. S2N) but not in young mice (Fig. S2, O and P). NAD+ repletion also improved the stemness of the aged MuSCs, as demonstrated by a reduction in the ratio of myoblast determination protein 1 (MYOD1) and Pax7–double-positive to Pax7-positive myofibers (Fig. 2J and fig. S2Q), as MYOD1 is a transcriptional factor that activates MuSC differentiation. Seven days after CTX-induced damage, aged mice treated with NR exhibited elevated levels of fibers positive for embryonic myosin heavy chain, a protein expressed in fetal and newly regenerating adult muscle fibers (30) (Fig. 2K). MuSCs were then transplanted from NR-treated or control aged mice into mdx (C57BL/10ScSn-Dmd<sup>mdx<sup>ym</sup>/J) mice (Fig. S2R) (a mouse model of Duchenne muscular dystrophy that gradually loses Musc function in aging due to the strain of continual muscle regeneration). MuSCs isolated from NR-treated donors more effectively replenished the MuSC compartment and stimulated myogenesis of dystrophin-positive myofibers when transplanted into either young or aged mdx recipients (Fig. S2S and Fig. 2L, respectively). Thus, NR treatment can improve both muscle regeneration and MuSC transplantation efficiency.

The inappropriate accumulation of nonmyogenic fibro-adipogenic progenitors (FAPs) and inflammatory cells has been reported to impair MuSC function and muscle regeneration, especially in aged or chronically damaged muscle, as found in mdx mice (31). NR treatment largely attenuated increases in FAP numbers 7 days after CTX-induced damage in aged mice but had no effect on FAPs under basal conditions (Fig. S2, T to V). This finding is consistent with benefits to FAP clearance in later periods of muscle regeneration (31). NR also alleviated macrophage infiltration 7 days after CTX-induced regeneration in aged mice (fig. S2, W and X).

NR prevents MuSC senescence by improving mitochondrial function

To explain the improvements in MuSCs from aged animals after NAD+ repletion, we examined effects on MuSC senescence. Freshly isolated MuSCs from NR-treated young and aged mice were immunostained for phosphorylation of histone 2AX at Ser139 (γH2AX), a marker of DNA damage (2). γH2AX-stained nuclei were more abundant in MuSCs from aged animals, and staining was reduced with NR treatment (Fig. 3, A and B; results for young controls can be found in fig. S3, A and B). The reduction of the nuclear damage response in MuSCs was confirmed by a single-cell gel electrophoresis (comet) assay, a sensitive measure of DNA strand breaks as an indicator of senescence (Fig. 3C), as well as by staining for β-galactosidase (β-Gal), another classical marker of senescence (32). Immunostaining for β-Gal was significantly reduced in aged MuSCs treated with NR (Fig. 3, A and B).

**Fig. 1. Mitochondrial dysfunction in MuSCs during aging.** (A) GSEA demonstrates up- and down-regulated signaling pathways in MuSCs from 2-year-old mice, as compared with 3-month-old mice. This panel shows the results of our analysis of microarray data from the publicly available GEO data set (accession number GSE47177), using Kyoto encyclopedia of genes and genomes (KEGG) enrichment. Signaling pathways were ranked on the basis of normalized enrichment scores (NESs); positive and negative NESs indicate down- or up-regulation, respectively, in aged MuSCs. Specific pathways related to MuSC function are highlighted in red and blue. TGFβ transforming growth factor-β. (B) Area-proportional Venn diagram representing 113 common genes between the significantly down-regulated genes (P < 0.05) in MuSC transcriptomes originating from aged mice [GSE47177 and GSE47401 (J2)] and genes from the human mitochondrial transcriptome (26). (C to G) Young (1 month old) and aged (22 to 24 months old) C57BL/6J mice received a dietary supplement with NR for 6 weeks. (C) Quantitative real-time fluorescence polymerase chain reaction validation of transcriptional changes in mitochondrial genes of freshly sorted MuSCs. (D) Oxygen consumption rate (OCR) in freshly isolated MuSCs after 16 hours of recovery at 37°C. Pmole, picomoles. (E and F) Mitochondrial membrane potential, as measured by a tetramethylrhodamine, methyl ester (TMRM) assay (E) and cellular ATP levels (F) in freshly isolated MuSCs. (G) Relative gene expression for UPR<sup>mt</sup> genes and cell senescence markers in freshly sorted MuSCs. Data are normalized to 36b4 mRNA transcript levels. All statistical significance was calculated by Student’s t test. All data are shown as mean ± SEM (error bars). *P < 0.05; **P < 0.01; ***P < 0.001. In (C), (D), (F), and (G), n = 6 mice per group; in (E), n = 3 mice per group.
proteins and increased the expression of cell encoding CDKN1A and related proinflammatory proteins and increased the expression of cell senescence marker (2) (fig. S3C). A 6-hour NR treatment of late-passage C2C12 myoblasts also reduced the expression of cell senescence and apoptosis markers (32) (Fig. 3D). MuSCs isolated from NR-treated aged mice showed enhanced potential to form myogenic colonies (Fig. 3E and fig. S3D). Thus, NR exerts a protective effect against intrinsic MuSC senescence.

Nicotinamide riboside treatment of MuSCs from aged mice reduced abundance of mRNAs after NR supplementation [two-way analysis of variance (ANOVA) test, \( P < 0.05 \) (Fig. 3H and table S6)]. Protein immunoblotting of freshly positive MuSCs. Insets (20 \( \mu m \) by 20 \( \mu m \)) show a single MuSC. Scale bar, 50 \( \mu m \).

**Fig. 2.** Improved MuSC numbers and muscle function in NR-treated aged mice. Young (1 month old) and aged (22 to 24 months old) C57BL/6J mice received a chow diet (CD) or a CD supplemented with NR for 6 weeks. All results are compared with those of age-matched mice given a control diet. (A) NAD\(^+\) concentrations in freshly isolated MuSCs. (B and C) Percentage of fluorescence-activated cell sorting (FACS)-quantified CD34\(^+\)/integrin \( \alpha7 \)/Lin\(^-\)/Sca-1\(^+\) MuSCs relative to the total Lin\(^-\)/Sca-1\(^+\) cell population (B) or to muscle weight (C). (D) Representative images of PAX7- and laminin-immunostained tibialis anterior (TA) muscle. Arrows point to PAX7-positive SCs. Insets (20 \( \mu m \) by 20 \( \mu m \)) show a single MuSC. Scale bar, 50 \( \mu m \). DAPI, 4′,6-diamidino-2-phenylindole. (E to G) Comparison of maximal running duration (E), running distance (F), and grip strength (G) in NR-treated aged mice. (H) Hematoxylin and eosin (H&E)-stained TA tissue sections from NR-treated aged mice 7 and 14 days after CTX-induced muscle damage. Scale bar, 100 \( \mu m \). (I) Images of PAX7- and laminin-immunostained TA muscle cross sections taken from NR-treated aged mice 7 days after CTX-induced muscle damage. Arrows point to PAX7-positive MuSCs. Insets (20 \( \mu m \) by 20 \( \mu m \)) show a single MuSC. Scale bar, 50 \( \mu m \). (J) Quantification of MYOD1 and PAX7–double positive to PAX7-positive myofibers, performed on sections isolated 7 days after muscle damage in aged mice. (K) Newly regenerated muscle fibers stained by embryonic myosin heavy chain (eMyHC) 7 days after muscle damage in aged mice. Scale bar, 50 \( \mu m \). (L) Dystrophin immunostaining of TA muscle sections in aged (16 months old) mdx mice 4 weeks after receiving transplantations of MuSCs isolated from control or NR-treated aged C56BL/6J donors. Scale bar, 100 \( \mu m \). All statistical significance was calculated by Student’s \( t \) test or two-way ANOVA. All data are shown as mean ± SEM (error bars). *\( P < 0.05 \); **\( P < 0.01 \); ***\( P < 0.001 \). Main effects for treatment or age are denoted with * or † symbols, respectively. † or ‡, \( P < 0.05 \); †† or ‡‡, \( P < 0.01 \); ††† or ‡‡‡, \( P < 0.001 \). In (A), \( n = 6 \) mice per group; in (B) to (D) and (H) to (K), \( n = 3 \) to 6 mice per group; in (E) to (G), \( n = 10 \) mice fed the control diet and 7 NR-treated mice: in (L), \( n = 12 \) donor mice and 3 recipient mice for each treatment. For (E) to (J), corresponding young control data can be found in fig. S2, J to O, respectively.
isolated MuSCs from aged animals confirmed increased expression of proteins related to cell cycle and senescence that could not be detected by SWATH-MS (Fig. 3I).

Muscle SCs from aged mice treated with NR exhibited increases in oxidative respiration (Fig. 3J and K). NR-treated MuSCs from aged animals also showed increased mitochondrial membrane potential (Fig. 3L and fig. S3G) and increased abundance of ATP (Fig. 3M). To test whether this protective effect of NR on MuSC senescence relies on mitochondrial function, we created a tamoxifen-inducible sirtuin-1 (SIRT1) MuSC-specific knockout mouse (SIRT1\textsuperscript{MuSC/-/}) by crossing SIRT1\textsuperscript{lox/lox} mice with the Pax\textsuperscript{creER} strain. SIRT1 is a NAD+-dependent deacetylase that increases mitochondrial biogenesis (16). The beneficial effect of NR on muscle regeneration after CTX injection appeared to be attenuated in SIRT1\textsuperscript{MuSC/-/} mice (Fig. 3N). Supporting this qualitative observation, the act of knocking out SIRT1 in MuSCs blocked the beneficial effects of NR on MuSC activation (Fig. 3, O to Q) and senescence (Fig. 3R and fig. S3H) 7 days after regeneration. These data indicate that NR inhibits MuSC senescence by improving mitochondrial function in a SIRT1-dependent manner. This finding is consistent with a report linking activation of FOXO3, a SIRT1 target, to improved mitochondrial metabolism in hematopoietic SCs (34).

**Rejuvenating MuSCs by activating the UPR\textsuperscript{mt} and prohibitin pathways**

We further explored how UPR\textsuperscript{mt} might regulate senescence by examining the role of prohibitins, a family of stress-response proteins. Prohibitins sense mitochondrial stress and modulate senescence in fibroblasts in mammals (35), maintain replicative life span in yeast (36), and promote longevity in worms (37), animals that lack adult SCs. Expression of the prohibitins Phb and Phb2 was reduced in the bioinformatics analysis (fig. S1P), indicating that NR inhibited these proteins in aged MuSCs.

**Fig. 3.** NR treatment prevents MuSC senescence and improves mitochondrial function. (A and B) Immunostaining (A) and quantification (B) of γH2AX staining in freshly sorted MuSCs from aged mice. Insets (20 μm by 20 μm) show single MuSCs. (C) Single-cell gel electrophoresis (comet) assay of MuSCs from aged mouse. (D) Proteins levels in C2C12 myoblasts after NR treatment for 1, 3, or 6 hours. (E) Colony-formation ability assay in isolated MuSCs. (F and G) Quantification of transcript expression for cell cycle and inflammatory secretoome genes (F) or OXPHOS and TCA cycle genes (G) in MuSCs. (H) Abundance of proteins from MuSCs of young (Y) and aged (A) mice fed with a chow (C) or NR (N) diet. Protein abundances were calculated by dividing the intensities of peptides detected in the SWATH-MS maps. Roman numerals indicate corresponding OXPHOS complexes, T, TCA cycle. (I) Protein levels in MuSCs. (J and K) OCR (J) and extracellular acidification rate (ECAR) (K), in MuSCs after 16 hours of recovery at 37°C. (L) Mitochondrial membrane potential, as measured by a TMRM assay in MuSCs. (M) Cellular ATP concentration in MuSCs from wild-type (WT) or SIRT1\textsuperscript{MuSC/-/} mice 7 days after CTX-induced muscle damage. Scale bar, 100 μm. (O to Q) Representative images (O) and quantification of PAX7-positive MuSCs in random fields of view (160 μm by 160 μm) (P) and the percentage of SIRT1-positive MuSCs (Q) in immunostained TA 7 days after CTX-induced muscle damage. Arrows in (O) point to PAX7-positive MuSCs. Insets (20 μm by 20 μm) show single MuSC. Scale bar, 50 μm. (R) Quantification of γH2AX-positive MuSCs in immunostained TA 7 days after CTX-induced muscle damage. All statistical significance was calculated by Student’s t test or two-way ANOVA. All data are represented as mean ± SEM (error bars). *P < 0.05; **P < 0.01; ***P < 0.001. Main effects for treatment or age are denoted as † or ‡, respectively, with interactions denoted as ×, ††, or ‡‡. P < 0.05; ††× or ‡‡× or †‖ or ‡‖. P < 0.001. In (A) to (C) and (N) to (R), n = 3 to 6 mice per group; in (E), n = 24 repeats per group; in (F) and (G) and (J) to (M), n = 6 mice per group; in (H), protein was extracted and pooled from 6 mice in each group. For (A), (B), (E), (F), (G), and (L), corresponding young control data can be found in fig. S3. A, B, D, E, F, G, and R, respectively.
as well as in freshly isolated aged MuSCs (fig. S4A). The addition of NR increased the expression of prohibitin proteins in C2C12 myoblasts (fig. 4A) and transcripts in MuSCs of young and aged mice (fig. 4B and fig. S4B). NR treatment was also shown to increase the expression of prohibitins concurrent to markers of UPRmt and the cell cycle (fig. S4C). Moreover, the overexpression of prohibitins, in the absence of NR, likewise increased UPRmt and cell cycle protein expression (fig. 4C). Demonstrating the dependency of the NR effect on prohibitins, improvements in UPRmt and cell cycle protein expression were not observed with NR treatment after the knockdown of prohibitins (fig. 4D and fig. S4D). To confirm the regulation of prohibitins on cell cycle proteins and to explore the effect of prohibitins on MuSC function, Phb was depleted in vivo through an intramuscular injection of shPhb lentivirus (PHB and PHB2 are functional only as a heterozygous protein complex) (38). Impairment of muscle regeneration and a reduction in MuSC numbers were observed in shPhb lentivirus–injected mice 7 days after CTX-induced muscle regeneration (fig. 4, E and F). Quantifying these results, Phb knockdown is shown to block the NR-induced increase of MusCs upon regeneration (fig. 4, G and H). Phb knockdown does not induce more MuSC senescence in aged mice but does prevent the beneficial effect of NR on MuSC senescence (fig. 4I and fig. S4E). Initiation of the UPRmt by thiamphenicol also induced expression of prohibitins and cell cycle genes in C2C12 cells (fig. S4F). These results indicate that NR activates the UPRmt and the prohibitin signaling pathway as it inhibits MuSC senescence.

**NR reprograms senescence-prone MuSCs in mdx mice**

With continuous muscle regeneration, MuSCs in mdx mice are abnormally active at a young age, leading to MuSC depletion and dysfunction later in life. As a result, primary MuSCs isolated from 14-week-old mdx mice were more intensively and frequently stained with β-gal and had a larger cell size than those of control mice (fig. 5A and fig. S5, A and B). Similar to the effect in aged animals, NR treatment of mdx mice increased

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**Fig. 4. Effects of NR on prohibitins, UPRmt, and MuSC senescence.** (A) Expression of HSP60, CLPP, and prohibitins in C2C12 myoblasts upon NR treatment at the indicated time points. (B) Quantification of transcript expression for UPRmt and prohibitin genes in MuSCs from aged (22 to 24 months old) C57BL/6J mice after 6 weeks of chow or NR diets. (C) Expression of prohibitins and cell cycle proteins in C2C12 myoblasts with the combined overexpression of Phb and Phb2. (D) Expression of prohibitins and cell cycle genes with a 6-hour NR treatment in C2C12 myoblasts after a combined Phb and Phb2 short hairpin RNA knockdown. (E) H&E staining of TA muscle in NR-treated or intramuscular shScr lentivirus–injected C57BL/6J mice 7 days after CTX-induced muscle damage. Scale bar, 100 μm. (F to H) Representative images (F) and quantification of PAX7-positive MuSCs in randomly chosen fields of view (160 μm by 160 μm) (G) and the percentage of PHB-positive MuSCs (H) in immunostained TA muscle 7 days after CTX-induced muscle damage. Arrows in (F) point to PAX7-positive MusCs. Insets (20 μm by 20 μm) show a single MuSC. Scale bar, 50 μm. (I) Quantification of γH2AX-positive MuSCs in immunostained TA muscle cross sections taken from control and NR-treated mice 7 days after CTX-induced muscle damage. All statistical significance was calculated by Student’s t test or two-way ANOVA. All data are represented as mean ± SEM (error bars). *P < 0.05; **P < 0.01; ***P < 0.001. Main effects for treatment or Phb knockdown are denoted as † or ††, respectively. † or †, P < 0.05; †† or †††, P < 0.001. In (B), n = 6 mice per group; in (E) to (I), n = 3 mice per group. For (B), corresponding young control data can be found in fig. S4B.
MuSC numbers by a factor of ~1.8 in vivo (Fig. 5, B to D, and fig. S5, C and D), as also confirmed by Pax7 immunostaining (fig. S5E). Along with the increase in MuSCs, there was an increase in regenerated muscle fibers after NR treatment (Fig. 5E and fig. S5E). We extended this analysis by examining the self-renewal capacity of MuSCs in mdx mice. The cellular redox ratio decreases as MuSCs differentiate (39), which can be detected by an increase in 450-nm autofluorescence (40). In line with NR increasing the number of MuSCs in mdx mice, we found reduced autofluorescence from MuSCs isolated from these animals (Fig. 5, F to H). We performed β-Gal staining on primary MuSCs that had been isolated from mdx mice with or without NR treatment in vivo and then further cultured with or without NR in vitro. MuSCs isolated from NR-treated mice were less prone to senescence (Fig. 5I and fig. S5G). When MuSCs isolated from control mdx mice were treated with NR in vitro, there was also a reduction in senescence (Fig. 5I and fig. S5G). The inhibition of MuSC senescence in NR-treated mdx mice was confirmed by the attenuation of γH2AX and cleaved caspase-3 immunostaining (Fig. 5J). To evaluate MuSC function, CTX-induced muscle regeneration was examined in NR-treated mdx mice. Consistent with the prevention of MuSC senescence, muscle regeneration was improved with NR in both aged (Fig. 5K and fig. S5H) and young mdx mice (fig. S5I). We also examined the effect of NR on the FAP population and muscle regeneration in mdx mice. NR treatment increased MuSCs and reduced FAP numbers in basal conditions and 7 days after CTX-induced damage (fig. S5, J to L). Abnormal activation of FAPs in mdx mice contributes to fibrosis (31). mdx mice treated with CTX and then exposed to NR showed lower levels of macrophage infiltration 7 days after damage (fig. S5, M and N). Hence, our results indicate a beneficial effect of NR on MuSC function and regeneration in mdx mice.

NR attenuates senescence of neural and melanocyte SCs and increases mouse life span

Aging is accompanied by a decline in the number and function of neural SCs (NSCs) (23) and melanocyte SCs (MoSCs) (41). Therefore, to examine the generalized importance of NAD⁺ homeostasis in somatic SCs, we assessed the effect of NR in NSCs from aged mice. NR increased proliferation, as shown by 5-ethynyl-2-deoxyuridine (EdU) and

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**Fig. 5. Increased SC number and function in NR-treated mdx mice.** mdx mice received a dietary supplement with NR for 10 weeks. All results were compared with those of mdx mice given a control diet. (A) β-Gal staining of MuSCs isolated from C57BL/10SnJ or mdx mice and cultured in vitro for three generations. Scale bar, 10 μm. (B to D) FACS contour plots of Sca-1⁺/Lin⁻ cells isolated from muscle. Percentages of the CD34⁺/integrin α7⁺/Sca-1⁻ MuSC populations are noted in red in the contour plots (B) and are quantified relative to the total Lin⁻/Sca-1⁻ cell population (C) or muscle weight (D). (E) Immunostaining of eMyHC fibers in tissue sections of NR-treated mdx mice 7 days after CTX-induced muscle damage. Scale bar, 100 μm. (F to H) FACS contour plots (F), quantification (G), and distribution (H) of MuSC autofluorescence as a measure of the relative NAD(P)H concentration (where NADPH is the reduced form of nicotinamide adenine dinucleotide phosphate) upon ultraviolet light excitation. Autofluorescence emission was detected with a wavelength of 450 nm. The arrowhead in (H) points to the highly autofluorescent SC population. (I) Quantification of β-Gal staining of FACS-sorted MuSCs from C57BL/6J (B6 with CD), untreated (mdx with CD), or NR-treated mdx (mdx with NR) mice challenged with phosphate-buffered saline or NR for 6 hours in vitro. (J) Immunostaining showing γH2AX and cleaved caspase-3 in MuSCs cultured in vitro for three generations. The arrow points to a γH2AX-positive nucleus. Scale bar, 10 μm. (K) H&E staining of tissue sections from NR-treated aged mdx mice (16 months old) with 7 days of recovery after CTX-induced muscle damage. Scale bar, 100 μm. All statistical significance was calculated by Student’s t test or one-way ANOVA. All data are represented as mean ± SEM (error bars). *P < 0.05; **P < 0.01; ***P < 0.001. In (A) to (H), (J), and (K), n = 3 to 5 mice per treated group; in (I), n = 3 mice and n = 6 in vitro treatments. More than 500 cells were quantified for each condition.
conclusions}

Oxidative stress, potentially introduced by mitochondrial respiration, is thought to be circumvented in SCs by their reliance on glycolysis as a primary energy resource (42). However, our study demonstrates that mitochondrial oxidative respiration is important for the functional maintenance of multiple types of adult SCs during aging. In fact, the reduction in cellular NAD⁺ pools blunts the adaptive UPRmt pathway (18), ultimately leading to a loss of mitochondrial homeostasis with a concurrent reduction in the number and self-renewal capacity of MuSCs. Accordingly, by boosting the MuSC concentration of NAD⁺, proteotoxic stress resistance may be restored due to the activation of the UPRmt pathway, stimulating the prohibitin family of mitochondrial stress sensors and effectors. In turn, this effect will improve mitochondrial homeostasis, protecting MuSCs from senescence and safeguarding muscle function in aged mice (fig. S6E). With the use of a MuSC-specific loss-of-function model for Sirt1, an essential regulator governing mitochondrial homeostasis (43), the importance and vital nature of the relationship between the NAD⁺/SIRT1 pathway, mitochondrial activity, and MuSC function were unequivocally established in vivo. Furthermore, maintaining healthy mitochondria by replenishing NAD⁺ stores seems to have beneficial effects beyond MuSCs and also appears to protect NSC and McSC populations from aging.

Our results demonstrate that the depression of prohibitin signaling, leading to mitochondrial...
dysfunction, can be reversed in aging by using a nutritional intervention to boost NAD+ concentrations in SCs. Additionally, our findings suggest that NAD+ repletion may be revealed as an attractive strategy for lengthening mammalian life span.

REFERENCES AND NOTES


Covalently bonded single-molecule junctions with stable and reversible photoswitched conductivity

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Through molecular engineering, single diarylethenes were covalently sandwiched between graphene electrodes to form stable molecular conduction junctions. Our experimental and theoretical studies of these junctions consistently show and interpret reversible conductance photoswitching at room temperature and stochastic switching between different conductive states at low temperature under a single-molecule level. We demonstrate a fully reversible, two-mode, single-molecule electrical switch with unprecedented levels of accuracy (on/off ratio of ~100), stability (over a year), and reproducibility (46 devices with more than 100 cycles for photoswitching and ~105 to 106 cycles for stochastic switching).

REFERENCES AND NOTES

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A dietary supplement protects aging muscle

The oxidized form of cellular nicotinamide adenine dinucleotide (NAD\(^{+}\)) is critical for mitochondrial function, and its supplementation can lead to increased longevity. Zhang et al. found that feeding the NAD\(^{+}\) precursor nicotinamide riboside (NR) to aging mice protected them from muscle degeneration (see the Perspective by Guarente). NR treatment enhanced muscle function and also protected mice from the loss of muscle stem cells. The treatment was similarly protective of neural and melanocyte stem cells, which may have contributed to the extended life span of the NR-treated animals.

Science, this issue p. 1436; see also p. 1396