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

James G. Tidball[1,2,3,*] and Carmen Bertoni[4]
[1]Molecular, Cellular and Integrative Physiology Program, University of California, Los Angeles, Los Angeles, CA 90095-7239, USA
[2]Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, University of California, Los Angeles, Los Angeles, CA 90095-1732, USA
[3]Department of Integrative Biology and Physiology, University of California, Los Angeles, Los Angeles, CA 90095-7239, USA
[4]Department of Neurology, David Geffen School of Medicine at UCLA, University of California, Los Angeles, Los Angeles, CA 90095-1769, USA
*Correspondence: jtidball@lifesci.ucla.edu
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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

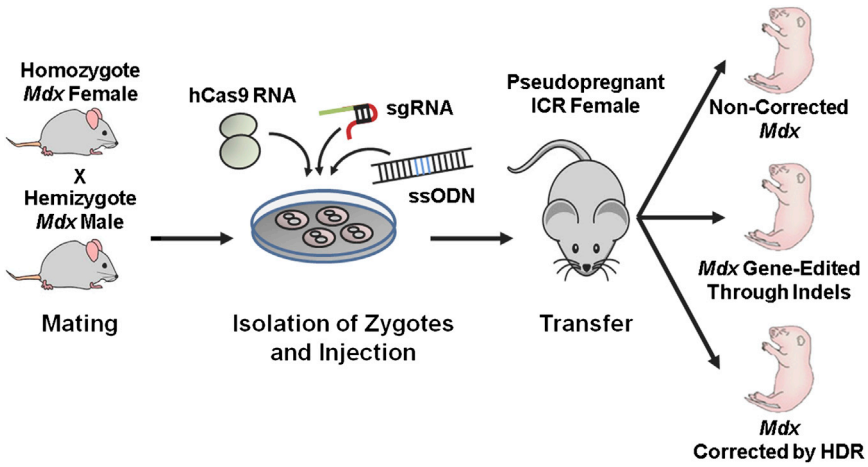

Figure 1. Gene Editing Mediated by CRISPR/Cas9 in *mdx* Zygotes

Schematic representation of the strategy used to induce correction of the dystrophin gene defect in the germline of dystrophin-deficient mice. hCas9 RNA and single guide RNA (sgRNA) were coinjected with an ssODN template designed to target and correct the genetic defect in mouse zygotes. Injected zygotes were then transferred into the oviducts of pseudopregnant ICR female mice. The majority of the littermates that experienced editing of the dystrophin gene contained indels generated by NHEJ. Correction mediated by HDR occurred in approximately 5% of all newborn *mdx* mice.

strategies has been explored for the treatment of the disease. Among those, techniques for editing of the dystrophin gene in vivo are most appealing because they may permanently correct the defect. However, only limited, local gene correction has been achieved by intramuscular injection of RNA/DNA oligonucleotides or single-stranded oligodeoxynucleotides (ssODNs) in animal models of DMD (Rando et al., 2000; Kayali et al., 2010), leaving the field of experimental therapeutics in search of mechanisms for systemic and persistent correction of endogenous mutations that cause DMD. Now, a team of investigators has ridden the crest of the wave of recent discoveries that demonstrate that bacterial gene editing mechanisms using Cas9 endonuclease can be used to modify the structure of vertebrate genes, this time to cure muscular dystrophy (Long et al., 2014) (Figure 1).

Many bacteria excise viral DNA from invasive viruses, which is then interspersed within the bacterial DNA at a clustered, regularly interspaced, short palindromic repeats (CRISPR) locus, from which RNAs can be later transcribed to guide an endonuclease to viral DNA during subsequent infections. Cas9 then cleaves double-stranded DNAs if directed to the sequence by a guide RNA containing the sequence, providing the bacterium with a form of innate immunity. Double-strand breaks can then be repaired by two mechanisms. In one, the break is repaired by nonhomologous end joining (NHEJ), which can lead to insertion/deletion mutations (indel). Alternatively, homology directed repair (HDR) occurs if an exogenous template is provided so that engineered sequences can be inserted at the targeted site. By injecting Cas9 with the appropriate guide RNA and HDR template into *mdx* zygotes at the one-cell stage (Figure 1), Long et al. (2014) were able to correct the *mdx* point mutation in some zygotes, producing mice that were free of pathology. In particular, *mdx* mice that experienced more than 40% gene correction at the target site by NHEJ or HDR displayed normal dystrophin expression at the cell membrane. Mice with those relatively high levels of gene repair also showed normalization of muscle histology, recovery of muscle strength, and an absence of pathological leakiness of the muscle cell membrane, which is a characteristic of dystrophin deficiency. Because the guide RNA may cause undesirable, unintended mutations, the investigators also tested for off-target mutations in the treated *mdx* mice. However, none of the 32 most likely off-target sites showed an increase in indel mutations (Long et al., 2014). The findings show unequivocally that the CRISPR/Cas9 system can be exploited to permanently repair the genetic defect that causes *mdx* dystrophy.

Two therapeutic strategies are available to attempt to use CRISPR/Cas9 technology to treat DMD. The first would apply the tools to human, one-cell zygotes, as used in *mdx* mice. However, the specific dystrophin mutation of the maternal carriers of the disease would have to be known so that guide RNA could be designed to target the nuclease to the mutation site. Unfortunately, about one-third of dystrophin mutations are spontaneous mutations that cannot be addressed by this strategy (Davie and Emery, 1978). In addition, large deletion mutations could exceed the size of functional templates; the *mdx* mutation that was repaired by the CRISPR system is a point mutation that could be corrected by HDR or NHEJ, but point mutations comprise only about 15% of DMD mutations. Mosaicism also presents a challenge. Individual pups generated from treated *mdx* zygotes varied from 2% to 100% in the proportion of dystrophin genes that were repaired by treatment. Low levels of mosaicism are attributable to insufficient time between RNA injection into the zygote and the first cell division to permit translation of enough Cas9 to mediate biallelic mutagenesis (Yen et al., 2014). In mice, the first division occurs in about 24 hr. In human zygotes in vitro, only about 18% of the zygotes reach the first division in 24 hr (Shoukir et al., 1997), so mosaicism may be a larger problem with DMD treatments.

Despite the challenges for developing the CRISPR/Cas9 strategies for correcting DMD mutations in zygotes, the approach offers unique advantages. First, by correcting the mutation in the zygote, the embryo will develop tolerance for dystrophin during normal development of the immune system, thereby avoiding the possibility that the newly restored dystrophin protein would be seen as foreign and initiate a cellular immune response. More importantly, the treatments at the one-cell zygote stage can lead to a correction of the defect in the germline. That correction can cure the disease not only in the males who would otherwise be born with DMD, but also correct the mutation in females who would otherwise be carriers transmitting the mutation to their future progeny. In fact, recent findings show that gene correction using CRISPR technology will persist in the progeny of treated animals. Gene correction of the

mutation in the *Crygc* gene responsible for causing cataracts was achieved by the CRISPR/Cas9 system in mouse zygotes and persisted in the progeny of treated mice (Wu et al., 2013).

The second strategy would be to use CRISPR/Cas9 technology to treat DMD postnatally. This approach has the advantage of applicability to all DMD patients, regardless of whether the mutation was inherited or spontaneous. However, the challenges of mosaicism, off-target effects, and immunogenicity would remain. In addition, an efficient mechanism to deliver the Cas9/guide RNA/template to patients requires development, and this is an almost completely unexplored issue in adult mammals. Recent findings showed that successful gene editing with Cas9 could be achieved in hepatocytes after intravascular injections of Cas9/guide RNA/template into mice carrying a mutation for fumarylacetoacetate hydrolase, which causes a fatal liver disease (Yin et al., 2014). That finding suggests that postnatal genome editing for DMD may also hold promise for functional recovery. Although muscle fibers are postmitotic and lack proteins required for homologous recombination and are therefore refractory to HDR-mediated correction (Hsu et al., 2014), a population of muscle stem cells (satellite cells) that reside in mature skeletal muscle are viable targets for Cas9-mediated gene editing.

The exciting findings and clever experimental design of Dr. Olson and his colleagues have illuminated a path for correction of dystrophin mutations not only in somatic cells to alleviate the disease in individuals, but also for correction in the germline, which could feasibly eliminate the disease in the progeny, though the road to clinical application may be long.

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Enteroendocrine MC4R and Energy Balance: Linking the Long and the Short of It

Sean Manning[1,2] and Rachel L. Batterham[1,3,4,*]
[1]Centre for Obesity Research, Rayne Institute, Department of Medicine, University College London, London WC1E 6JJ, UK
[2]School of Medicine, University College Cork, Cork, Ireland
[3]UCLH Centre for Weight Loss, Metabolic and Endocrine Surgery, University College London Hospitals, Ground Floor West Wing, 250 Euston Road, London NW1 2PG, UK
[4]National Institute of Health Research University College London Hospitals Biomedical Research Centre, London W1T 7DN, UK
*Correspondence: r.batterham@ucl.ac.uk
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Central MC4R pathways are well established as integrators of peripheral signals in the control of energy balance. In this issue, Panaro et al. (2014) identify that MC4Rs expressed on intestinal enteroendocrine L cells regulate PYY and GLP-1 secretion, two gut hormones implicated in the regulation of energy and glucose homeostasis.

Feeding bouts are regulated by complex, integrated neural and endocrine networks in a process that attempts to optimize use of nutrient availability in the context of changing energy requirements. Hormonal signals of "long-term" energy storage, such as the adipokine leptin, act centrally to modulate food intake (Morton et al., 2014). Feeding behavior is also modulated centrally by an array of gut-derived hormones that either promote initiation (orexigenic) or termination (anorexigenic) of feeding, thus serving as "short-term" signals of energy availability and regulators of meal size (Morton et al., 2014). For example, secretion of the orexigenic hormone ghrelin from gastric mucosal endocrine cells progressively increases in the absence of nutrients. In contrast, enteroendocrine L cells release the anorexigenic hormones peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) in response to nutrient stimulation. These "short- and long-term" energy signals