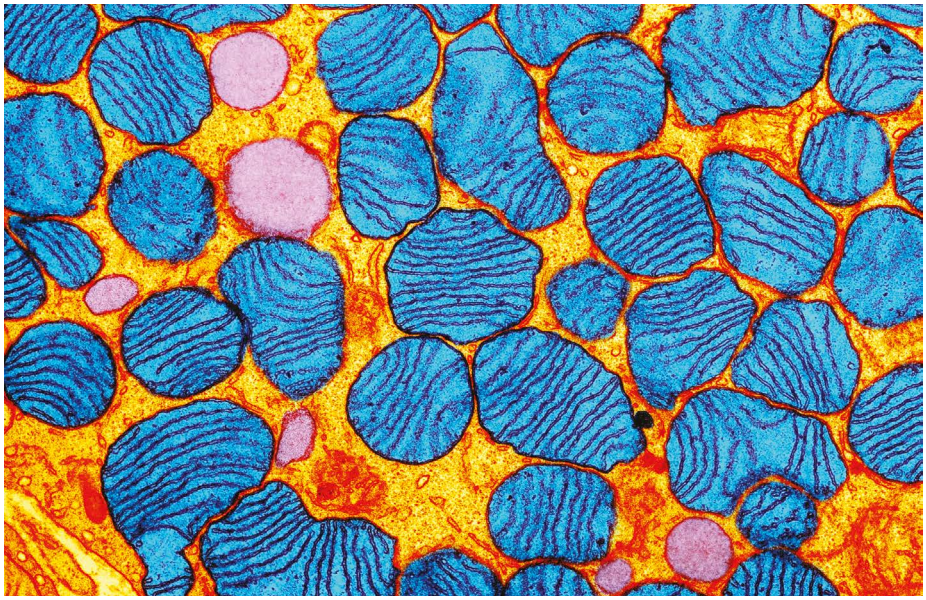

CNRI/SPL

Mitochondria (blue) are the cell's energy-producing organelles.

GENE EDITORS MAKE FIRST PRECISE CHANGES TO MITOCHONDRIAL DNA

Weird enzyme enables researchers to study — and potentially treat — deadly diseases.

By Heidi Ledford

A peculiar bacterial enzyme has allowed researchers to achieve what even the popular CRISPR–Cas9 genome-editing system couldn't manage: targeted changes to the genomes of mitochondria, cells' crucial energy-producing structures.

The technique — which builds on a super-precise version of gene editing called base editing — could allow researchers to develop ways to study, and perhaps even treat, diseases caused by mutations in the mitochondrial genome. Such disorders are most often passed down maternally, and impair the cell's ability to generate energy. Although there are only a small number of genes in the mitochondrial genome compared with the nuclear genome, these mutations can particularly harm the nervous system and muscles, including the heart, and can be fatal to people who inherit them.

But it has been difficult to study such disorders, because scientists lacked a way to make animal models with the same changes to the mitochondrial genome. The latest technique marks the first time that researchers have made such targeted changes. "It's a very exciting development," says Carlos Moraes, a mitochondrial geneticist at the University of Miami in Florida. "The ability to modify mitochondrial DNA would allow us to ask questions that, before, we could not." The work was published on 8 July (B. Y. Mok *et al. Nature* http://doi.org/d3gd; 2020).

Expanded toolbox

CRISPR–Cas9 has allowed researchers to tweak genomes to their liking in many organisms. But the tool uses a strand of RNA to guide the Cas9 enzyme to the region of DNA that scientists wish to edit. This works well for DNA in the cell's nucleus, but researchers have no way of shuttling that RNA into mitochondria, which are surrounded by membranes.

In late 2018, chemical biologist David Liu at the Broad Institute of MIT and Harvard in Cambridge, Massachusetts, received an e-mail from across the country: in Seattle, a team led by microbiologist Joseph Mougous at the University of Washington had discovered a strange enzyme. It was a toxin made by the bacterium *Burkholderia cenocepacia* — and when it encountered the DNA base C, it converted it to a U. Because U, which is not commonly found in DNA, behaves like a T, the enzymes that replicate the cell's DNA copy it as a T. This effectively converts a C in the genome sequence to a T.

Liu had harnessed similar enzymes in base editing, which allows researchers to use components of CRISPR–Cas9 to change one DNA base to another. But those enzymes, called cytidine deaminases, normally act only on single-stranded DNA. DNA in human cells consists of two strands wound together and, in the past, Liu had to rely on the Cas9 enzyme to break the DNA and create a region of unwound, single-stranded DNA for his enzymes to act on. Because of its reliance on the strand of RNA that guides Cas9, this technique wouldn't be able to reach the mitochondrial genome.

The enzyme that Mougous's team had found, called DddA, could act directly on double-stranded DNA without relying on the Cas9 enzyme to break it. This, Liu and Mougous reasoned, could make DddA suitable for reaching the mitochondrial genome.

But to turn DddA into a genome-editing tool, Liu first needed to "tame the beast" — the ability to modify double-stranded DNA also makes the enzyme deadly because, if set loose, it would change every C it came across. To prevent this, the team split the enzyme into two pieces that would change DNA only when brought together in the right orientation. And to control which DNA sequence the enzyme modified, the team then linked each half of DddA to proteins that were engineered to bind to specific sites in the genome.

Exploring diseases

The work is a long way from being used in the clinic, Liu cautions. Although his team's initial studies found few off-target DNA changes — a common problem in CRISPR–Cas9 gene-editing — more studies in different cell types are needed, he says (see page 332).

The technique could ultimately complement existing methods used to prevent or treat mitochondrial disorders. Some countries already allow a procedure called mitochondrial replacement, in which the nucleus of an egg or embryo is transplanted into a donor egg or embryo that contains healthy mitochondria.

Researchers are also trying to correct mitochondrial mutations by taking advantage of the fact that cells can contain thousands of copies of the mitochondrial genome, and that, often, a fraction of these do not contain the mutation linked to disease. Moraes and others have been developing enzymes that will enter mitochondria and cut the DNA at the site of the harmful mutation. Rather than repairing the cut, mitochondria often simply degrade DNA that has been damaged. The result would be mitochondria that have been depleted of the mutated copy of the genome, eventually allowing the normal copy to repopulate the structure.

The latest approach could allow researchers to correct such mutations even when the mitochondria lack sufficient normal copies of the gene, says Michal Minczuk, a mitochondrial geneticist at the University of Cambridge, UK. "It's an amazing step forward."