Biotechnology

Mitochondrial genome editing gets precise

Magomet Aushev & Mary Herbert

A bacterial toxin has been found that allows DNA in a cellular organelle called the mitochondrion to be precisely altered. This development could help to combat diseases caused by mutations in mitochondrial DNA. **See p.631**

The DNA in a cellular organelle called the mitochondrion encodes just 13 proteins, all of which are involved in generating the cell's energy supply. Mutations in mitochondrial DNA (mtDNA) can cause a range of incurable, life-limiting metabolic diseases in humans[1]. The development of tools for editing mtDNA has therefore been a long-sought goal in mitochondrial genetics. On page 631, Mok *et al.*[2] report a molecular tool that for the first time enables precise editing of mtDNA. Key to this achievement was the discovery of a toxin secreted by bacteria to kill neighbouring bacteria.

The bacterial toxin discovered by Mok *et al.* is a cytidine deaminase enzyme called DddA, which catalyses the conversion of the nucleotide base cytosine (C) to another base, uracil (U). A remarkable feature of DddA is that it targets double-stranded DNA, whereas all previously identified[3] cytidine deaminases target single-stranded DNA. Crucially, although conventional genome-editing approaches involve nuclease enzymes that act as molecular scissors to cut DNA on both strands, DddA converts C to U without inducing double-strand DNA breaks. This makes it particularly well suited to editing the mitochondrial genome, which lacks efficient mechanisms for repairing double-strand DNA breaks[4].

The researchers had to overcome several challenges to repurpose DddA for mitochondrial genome editing. Chief among these is the fact that cytidine deaminase is toxic to mammalian cells. Mok *et al.* split the toxin domain of DddA into two inactive parts called split-$DddA_{tox}$ halves. They fused these halves to TALE proteins, which can be engineered to bind to specified DNA sequences. Binding of the two TALEs to mtDNA brings together, and so activates, the split-$DddA_{tox}$ halves.

To reach mtDNA in the mitochondrial matrix, TALE–split-$DddA_{tox}$ must cross two mitochondrial membranes. Mok and colleagues therefore tagged the construct with an amino-acid sequence that acts as a mitochondrial-targeting signal. The ability to exploit existing protein-import machinery[5] gives this approach a major advantage over RNA-guided systems for genome editing such as CRISPR–Cas9. CRISPR methods do not work efficiently on mtDNA, possibly because the cell has no mechanisms for importing RNA into mitochondria[6].

Another challenge arises from the fact that cytidine deaminase converts C to U, rather than to the DNA-specific base thymine (T). Although U has the same base-pairing properties as does T, it belongs in RNA. The base is normally cut from DNA with the help of an enzyme called uracil-DNA glycosylase and replaced with C (ref. 7).

Mok *et al.* therefore fused the TALE–split-$DddA_{tox}$ halves with a uracil glycosylase inhibitor (UGI). This protects U from the glycosylase until the next round of DNA replication or repair occurs, at which point the guanine (G) base from the complementary strand – which was paired with C before editing – is replaced by adenine (A), the base that pairs with T. Incorporation of the UGI increased the efficiency of cytosine base editing about eightfold.

The final construct, dubbed a DddA-derived cytosine base editor (DdCBE), therefore consists of a mitochondrial-targeting signal, a TALE protein, a split-$DddA_{tox}$ half and a UGI (Fig. 1). Mok *et al.* demonstrated that the construct is efficiently imported into mitochondria in human cells and can modify a selection of mitochondrial genes. The edit,

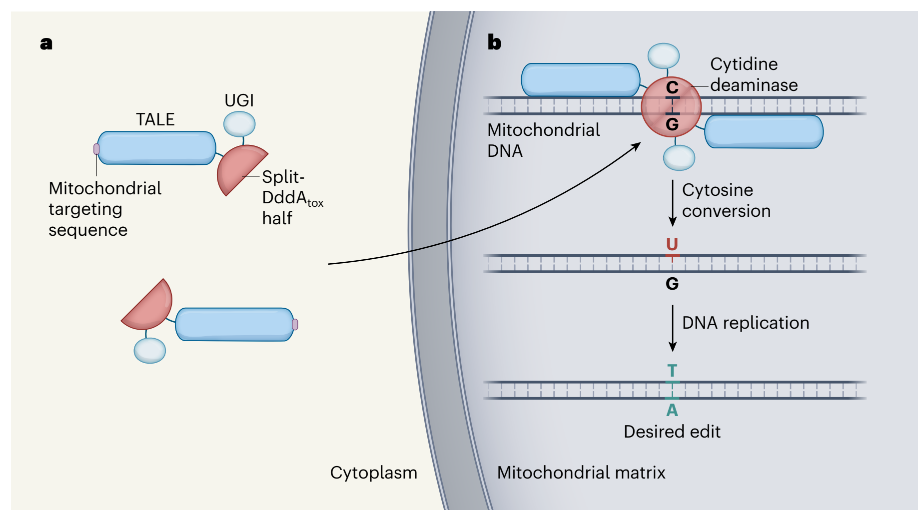

Figure 1 | A tool for correcting the mitochondrial genome. Mok *et al.*[2] have developed a molecular tool, DdCBE, that changes base pairs of cytosine and guanine (C–G) to pairs of thymine and adenine (T–A) in the DNA of cellular organelles called mitochondria. **a**, In the authors' approach, DdCBE is initially split into two constructs, each of which contains: an inactive portion of a bacterial toxin, called split $DddA_{tox}$; a TALE protein that binds to a specific mitochondrial DNA sequence; an amino-acid sequence that targets the construct to the mitochondrial matrix; and a uracil glycosylase inhibitor (UGI) protein. **b**, The mitochondrial-targeting sequence is lost when the construct is imported into the mitochondrial matrix. The TALE proteins bind to adjacent mitochondrial DNA sequences, bringing together and so activating the two split-$DddA_{tox}$ halves. The toxin acts as an enzyme that converts the targeted C base to another base, uracil (U). Normally, U is removed from DNA, but the UGI protein protects the edited base until DNA replication occurs (not shown). At this point, it is replaced with T, which forms a pair with A.

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from a C–G base pair to T–A, occurred about 5–50% of the time. The efficiency of editing was influenced by various factors: the spacing between the two DdCBE subunits; TALE design; orientation of the split-$DddA_{tox}$ halves; and the position of the target cytosine relative to the TALE bindings sites.

A major consideration for all genome-editing tools is whether they modify DNA at unintended sites. Mok and colleagues compared treated and untreated cells, and found no off-target effects in the nuclear genome. Off-target activity in mtDNA was low, except in the case of one gene, in which off-target edits were linked to the TALE design.

Next, Mok *et al.* examined the therapeutic potential of DdCBE. The authors reported that cytosine base editing has the potential to correct 49% of known harmful mtDNA mutations. However, in its current form, DdCBE can efficiently edit only C bases that are preceded in the genome by a T, narrowing its range.

The reliance of DdCBE on DNA replication to implement the C–G to T–A conversion implies a theoretical maximum editing efficiency of 50%. To explain, the two newly replicated mtDNAs each receive a parental DNA strand, one of which will be unedited, containing G, which becomes paired with a C. However, Mok *et al.* find that the activity of DdCBE persists over several days, potentially offering the opportunity for further editing during subsequent replication events. Whether off-target effects increase during prolonged exposure to DdCBE will be a key consideration for the future.

These caveats mean that DdCBE might cause a reduction in — rather than complete elimination of — mtDNA mutations. But given that the severity of the symptoms of mtDNA diseases increases with mutation load[8], the ability to reduce the mutation level in itself holds therapeutic promise.

Mitochondrion-targeted nucleases have previously been used to eliminate specific mtDNA mutations in mice[9,10]. This is possible because the double-strand breaks they create lead to mtDNA degradation. Cells contain many copies of their mtDNA, and only the copies that carry the harmful mutation are degraded. But there is a risk that, in cases of high mutation load, elimination of mutated mtDNA could reduce the mtDNA copy number to harmfully low levels. And the nuclease approach could not be used if all copies of mtDNA carry the same mutation. By contrast, base editing could reduce the fraction of mtDNA that carries a mutation without reducing the copy number. It might therefore be the preferred (or the only) option when the mutation load is high.

Does DdCBE have the potential to prevent the transmission of mtDNA disease? MtDNA is typically inherited only from mothers, and current mitochondrial-replacement procedures reduce the transmission of mtDNA mutations by transplanting the nuclear genome from the egg of a woman who carries the mutated mtDNA into an unaffected donor egg[11]. Base editing to reduce the mutation load in eggs or early embryos could theoretically be an alternative approach. However, mtDNA replication is thought not to occur during the first five to six days of human development[12], and so success might hinge on prolonged protection of U.

Mok and colleagues' work is a key advance towards the development of gene therapies for mtDNA diseases. In addition, by using the tool to experimentally alter the mitochondrial genome, we could gain a better understanding of the relevance of mtDNA mutations in complex diseases, cancer and age-related cellular dysfunction. The study is also likely to inspire further developments in protein engineering and evolution that increase the range and efficiency of DdCBE, and to intensify the search for other promising candidate base editors.

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Historical climatology

A flood history of Europe

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Europe's rich heritage of historical documents has been used to reconstruct the flooding history of the continent for the past five centuries. This could help policymakers to develop flood-management strategies for the future. **See p.560**

On page 560, Blöschl *et al.*[1] capitalize on a vast assembly of written historical observations to provide a history of flooding for 103 major European river reaches between AD 1500 and 2016. In doing so, they reveal nine flood-rich periods that affected extensive regions in distinct areas of Europe — and find that the most recent of these periods, which might not yet be over, differs in key respects from the others.

Some 0.03% of the European population, on average, are thought to have been affected by flooding annually between 1870 and 2016, at a yearly average cost of 0.8–0.9% of gross domestic product[2]. Increased flood hazards are widely expected in the future for a substantial area of Europe as a result of climate change[2], and so, without effective management and adaptation, these losses will potentially be even greater.

Such measures must be based on the best available knowledge, and require an understanding of long-term flooding patterns. Decision makers must know whether they are living in a flood-rich period (more-frequent flooding, of higher magnitudes or greater extent than usual) or a flood-poor one (fewer floods, with lower magnitudes or less-than-usual extent). Extreme floods in any one river basin in a given year are inherently rare, but the risk is cumulatively higher across large regions such as Europe — so the longer and more spatially extensive these flood histories are, the better.

Fortunately, Europe hosts some of the most abundant and diverse historical documentary sources for any world region, ranging from annals and chronicles to administrative and legal records, correspondence and newspapers (Fig. 1). These sources are replete with observations of extreme weather and hazards such as flooding, given their often severe human impacts, spectacular effects and religious significance as portents or vehicles of divine retribution[3]. For example, the Gaelic Irish *Annals of Connacht* for AD 1471 reports[4]: "Showers of hail fell [on] each side of Beltaine [1st May], with lightning and thunder, destroying much blossom and beans and fruits in all parts of Ireland where they fell. One of these showers, in the east, had stones two or three inches long, which made large

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