CRISPR-free, strand-selective mitochondrial DNA base editing using a nickase

The fusion of a programmable transcription-activator-like effector (TALE) protein with a nickase, in conjunction with a deaminase, enables efficient and strand-selective DNA base editing. This approach has the potential to advance our understanding and treatment of diseases associated with mutations in the mitochondrial or nuclear genome.

This is a summary of:
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The problem

The physical and chemical characteristics of the mitochondrial inner membrane present a challenge for the CRISPR system, which is commonly used for base editing the nuclear genome, as its guide RNA cannot easily enter mitochondria to facilitate the editing of mitochondrial DNA (mtDNA)[1]. Furthermore, previously identified DNA deaminases mainly target single-stranded DNA (ssDNA), which limits their use in the development of mitochondrial DNA base editors. However, the discovery of the DddA deaminase, which can modify cytosine in double-stranded DNA (dsDNA), enabled the development of mitochondrial DNA base editors such as DddA-derived cytosine base editors (DdCBEs) and transcription-activator-like effector (TALE)-linked deaminases (TALEDs)[2,3]. These tools rely on DddA and are limited by its sequence preference and the risk of it having off-target effects on the nuclear genome through its interaction with the transcriptional repressor CTCF[4]. Moreover, DdCBEs and TALEDs edit both strands of the target sequence[2,3], leading to inaccuracies. These limitations hinder the application of these tools to the study and treatment of diseases caused by mutations in mtDNA.

The solution

With the exception of DddA deaminase, previously discovered DNA deaminases have been limited to ssDNA substrates. We hypothesized that inducing a nick in mtDNA could generate ssDNA, overcoming this limitation to enable deaminase-mediated base editing. Our experiments confirmed this hypothesis, as we achieved up to 77% efficiency in A-to-G editing by co-transfecting the nickase MutH (which nicks 5′ of the G in 5′-GATC-3′) and the adenine deaminase TadA8e-V106W, both of which were fused to a mitochondrial targeting sequence and a TALE (Fig. 1a,b). We introduced mutations in MutH to expand its scope for nicking ssDNA. We also identified other nickases, including the C-terminal domain of Nt.BspD6I and FokI-FokI-D450A, that can effectively edit mtDNA when combined with TadA8e-V106W. Additionally, combining a nickase with a cytosine deaminase enabled C-to-T editing of mtDNA, and fusing the nickase and deaminase with the same TALE also worked efficiently. Notably, the G11778A mutation in *MT-ND4* (the gene encoding mitochondrially encoded NADH dehydrogenase 4) in cells derived from patients with Leber hereditary optic neuropathy was corrected using mitochondrial adenine base editors (mitoABEs), significantly restoring mitochondrial function.

Our study demonstrates that combining a nickase with a deaminase enables strand-selective base editing in mtDNA. By studying the rules underlying MutH-mediated cleavage, we also discovered that editing tends to occur on the non-nicked strand. The speculative working principle of this strategy is as follows: the nickase induces ssDNA at the target site, which is then deaminated by a deaminase (namely, TadA8e-V106W in this model) that targets the same site. After mtDNA repair and replication, the deaminated adenine remains on the non-nicked strand, leading to strand-selective A-to-G conversion (Fig. 1c). This model also applies to mitochondrial cytosine base editors (mitoCBEs). Furthermore, whole-genome high-throughput sequencing data showed that mitoBEs (mitochondrial DNA base editors, our collective term for mitoABEs and mitoCBEs) had no detectable off-target effects on either the mitochondrial or nuclear genome, and no large insertions or deletions of mtDNA were observed. Thus, mitoBEs represent precise and efficient tools for editing mtDNA, with broad potential application in treating mitochondrial genetic diseases.

Future directions

We have achieved strand-preferential A-to-G and C-to-T base editing in mtDNA using mitoBEs, with minimal off-target effects. We expect that this technique will have broad applications in the study and treatment of mitochondrial diseases caused by mutations in mtDNA. In addition, we have applied this approach (Fig. 1c) to nuclear genome base editing. These findings demonstrate the potential of CRISPR-free nickases for DNA base editing.

The working model of mitoBEs requires further validation. We have hypothesized that nicked mtDNA preferentially forms ssDNA, facilitating efficient deamination. However, this hypothesis still requires experimental confirmation. Furthermore, despite the improved precision of mtDNA base editing using mitoBEs, multiple bases are often edited within the editing window on a single strand of DNA, making precise editing of the target site challenging. Additionally, the average editing efficiency achieved by mitoBEs is lower than that of current nuclear genome base editing tools. These areas all require optimization in future research.

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EXPERT OPINION

"The authors have successfully created a new class of all-protein base editors able to efficiently introduce targeted sequence changes in human mitochondrial DNA with low off-target editing, by combining nickases with ssDNA deaminases. The inclusion of nickase functionality in mitoBEs leads to a level of strand preference that favors the editing of non-nicked strands. This feature is an important development that is not present in existing technologies." **An anonymous reviewer.**

FIGURE

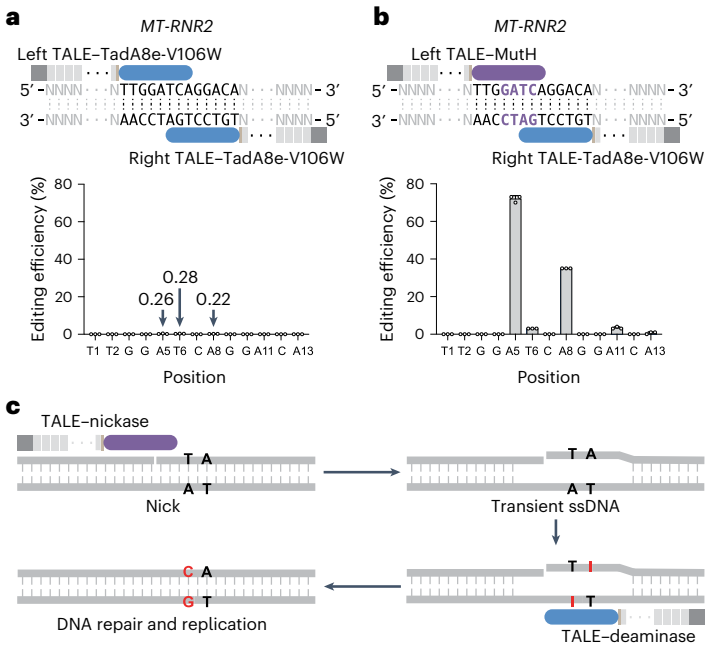

Fig. 1 | CRISPR-free, strand-selective DNA base editing with the introduction of a nickase. **a**,**b**, Mitochondrial A-to-G editing efficiency, after treatment with paired TALE-TadA8e-V106W (**a**) and Left-TALE-MutH and Right-TALE-TadA8e-V106W (**b**), at the target site mitochondrially encoded 16S rRNA *(MT-RNR2)*. Blue and purple rounded rectangles represent TadA8e-V106W and the nickase, respectively. **c**, A speculative working model for mitoBEs. TALE-nickase binds and nicks the dsDNA. The nicked dsDNA is prone to form ssDNA, which allows the TALE-deaminase to deaminate. Following DNA repair and replication, editing results on the non-nicked strand are preserved. TadA8e-V106W was used as part of a mitoABE in this model; this model also applies to mitoCBEs.

BEHIND THE PAPER

Mitochondria are at the center of regulating cellular energy homeostasis, and mutations in mtDNA (including C-to-T and A-to-G mutations) can lead to serious genetic diseases[5]. Our research started in 2021, when DdCBEs had been developed to achieve C-to-T base editing of mtDNA; filling the gap in mitochondrial adenine base editing in the field was our starting point. However, no deaminase that could perform adenine deamination on dsDNA had been reported. Therefore, if we wanted to use the existing ssDNA adenine deaminase to achieve mitochondrial adenine base editing, we reasoned that we needed to first generate a ssDNA substrate. We first tried to use the nickase MutH combined with TadA8e-V106W to see if we could achieve efficient editing, and it is surprising that we got very good results (Fig. 1a,b). Our subsequent results are relatively logical. We would like to thank all of the researchers involved in this study for their hard work and collaboration. **X.X.Z.**

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FROM THE EDITOR

"The key advance here is demonstration of strand-specific editing in mitochondria, which the authors accomplish by pairing TALE-fused deaminases with TALE-fused nickases." **Editorial Team, *Nature Biotechnology*.**