nature reviews bioengineering

https://doi.org/10.1038/s44222-026-00488-6

Review article

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Mitochondrial genome editing

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Abstract

Mitochondria possess small, maternally inherited genomes that encode indispensable components of the oxidative phosphorylation machinery. Mitochondrial DNA (mtDNA) mutations underlie a broad spectrum of metabolic and degenerative disorders, yet therapeutic options remain limited. Advances in mitochondrial genome engineering, including programmable nucleases and mitochondria-compatible base editors, enable the selective depletion of mutant genomes and the installation of defined C-to-T and A-to-G substitutions. These technologies have enabled the development of cellular and animal models for mechanistic studies of mitochondrial biology and opened up avenues for potential therapeutic intervention. In this Review, we discuss the development and application of current mtDNA-editing platforms and examine the key technical barriers and opportunities for their clinical translation.

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Key points

- Mitochondrial DNA (mtDNA) mutations disrupt oxidative phosphorylation and perturb cellular homeostasis. Disease phenotypes arise when mutant loads surpass critical heteroplasmic thresholds.
- Approaches such as mitochondrial replacement therapy and allotopic expression provide preventive or partial corrective benefits but are limited by residual mutant carryover and inefficient mitochondrial protein import.
- Programmable nucleases for heteroplasmy shifting — including mitoREs, mtZFNs, mitoTALENs and mitoARCUS — selectively introduce double-strand breaks in mutant mtDNA, triggering its degradation to promote wild-type dominance, but these nucleases do not enable precise base correction.
- Mitochondrial base editors — such as DdCBEs, TALEDs, mitoBEs and mtCyDENT — enable precise C-to-T and A-to-G conversion within the mitochondrial genome, although concerns persist regarding off-target editing, editing window constraints and delivery challenges.
- Mitochondrial base editing has enabled accurate modelling of pathogenic variants, mechanistic dissection of mitochondrial gene function and therapeutic rescue in cellular, organoid and animal models.
- Key priorities for future research include expanding the chemical scope of mitochondrial editing, improving efficiency and fidelity, and developing safe, tissue-targeted delivery platforms to enable clinical applications.

(DdCBEs, double-stranded DNA deaminase A (DddA)-derived cytosine base editors; mitoARCUS, I-CreI homing endonuclease-derived mitochondrial targeted nucleases; mitoBEs, mitochondrial base editors; mitoREs, mitochondrially targeted restriction endonucleases; mitoTALENs, mitochondrial transcription activator-like effector nucleases; mtCyDENT, mitochondrial cytidine deaminase–exonuclease–nickase–TALE; mtZFNs, mitochondrial zinc-finger nucleases; TALEDs, transcription activator-like effector (TALE)-linked deaminases).

Introduction

Mitochondria are essential cytoplasmic organelles responsible for adenosine triphosphate (ATP) production through oxidative phosphorylation and regulate processes such as apoptosis and cellular metabolism[1]. Their abundance correlates with cellular energy demands; neurons, cardiomyocytes and skeletal muscle cells have high numbers of mitochondria, but they are absent in mature erythrocytes[2]. Although mitochondria contain their own circular genome (16,569 bp in humans and 16,299 bp in mice), encoding 13 respiratory chain subunits, 22 transfer RNAs (tRNAs) and two ribosomal RNAs (rRNAs), they rely on the import of over a thousand nuclear-encoded proteins[3,4] (Fig. 1a). Unlike nuclear DNA, mitochondrial DNA (mtDNA) exists in hundreds to thousands of copies per cell and is maternally inherited[5]. Additionally, mtDNA undergoes a genetic bottleneck during maternal transmission, meaning that only a small and stochastic subset of maternal mtDNA molecules is passed to the offspring. Owing to limited repair capacity, an absence of histone protection and the proximity to reactive oxygen species, mtDNA accumulates mutations at a rate approximately 10–20 times higher than that of nuclear DNA[6–11], and the mtDNA genetic bottleneck can result in substantial variation in the proportion of inherited mutant mtDNA among siblings. The coexistence of wild-type and mutant mtDNA molecules (heteroplasmy), or the exclusive presence of a single variant (homoplasmy), is a key determinant of the variable penetrance of mitochondrial disorders[12], although additional genetic and environmental factors can also contribute. For example, in Leber's hereditary optic neuropathy (LHON), which is primarily caused by mutations in the mitochondrial complex I genes, disease penetrance is incomplete despite homoplasmic mutations, and is modulated by the nuclear genetic background, mitochondrial haplogroup differences and environmental factors such as smoking and alcohol intake[13,14].

Mitochondrial diseases collectively affect or place at risk approximately 1 in 5,000 individuals and can be caused by pathogenic mutations in mtDNA or in nuclear genes encoding mitochondrial proteins, with mtDNA mutations accounting for more than 75% of adult-onset cases[15,16]. In addition to classical mitochondrial disorders[5] such as Leigh syndrome, LHON, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), mtDNA mutations can contribute to neurodegenerative diseases[17,18], ageing[19] and cancer[20–22]. Mitochondrial diseases often involve multiple organ systems, such as the nervous system, digestive tract, eyes and ears (Fig. 1b), and clinical symptoms manifest only when mutation load exceeds a specific threshold, which varies for different mutations[23]. MITOMAP lists 130 confirmed pathogenic mtDNA variants, of which 116 are point mutations, 12 are insertions or deletions (indels) and two are inversions[24] (Fig. 1c). Large-scale mtDNA deletions (1.8–8 kb) also represent a common cause of mitochondrial disease.

Mitochondrial replacement therapyinvolves transferring the nuclear genetic material from an affected mother's oocyte or zygote into a donor oocyte or zygote that contains healthy mitochondria, has been approved for clinical use in the UK and offers an effective means of preventing the maternal transmission of pathogenic mtDNA mutations[25–30]. Mitochondrial replacement therapy is primarily recommended for women carrying high levels of pathogenic mtDNA heteroplasmy, who have a high risk of transmitting severe mitochondrial disease to their offspring. However, many women carrying pathogenic mtDNA mutations remain undiagnosed or asymptomatic, limiting the broader preventive application of mitochondrial replacement therapy. Additionally, residual carryover of mutant mtDNA during mitochondrial replacement therapy can reach up to 16%, and the long-term clinical consequences of such carryover remain unclear[29,31]. Furthermore, there are at present no effective ways to reverse or prevent disease progression in individuals carrying pathogenic mtDNA mutations. Allotopic expression (nuclear delivery of recoded mitochondrial genes followed by protein import) offers a potential therapeutic route, but it faces major hurdles, including inefficient protein import and incomplete functional integration[32]. Gene therapies based on this strategy have been developed for the treatment of LHON, including GenSight Biologics' GS010/LUMEVOQ and Neurophth's NFS-01, both of which are currently undergoing clinical evaluation and use adeno-associated virus (AAV) vectors to deliver a wild-type *MT-ND4* gene into retinal ganglion cells via intravitreal injection to restore respiratory chain function and improve visual outcomes[33–35]. However, allotopic expression is not suitable

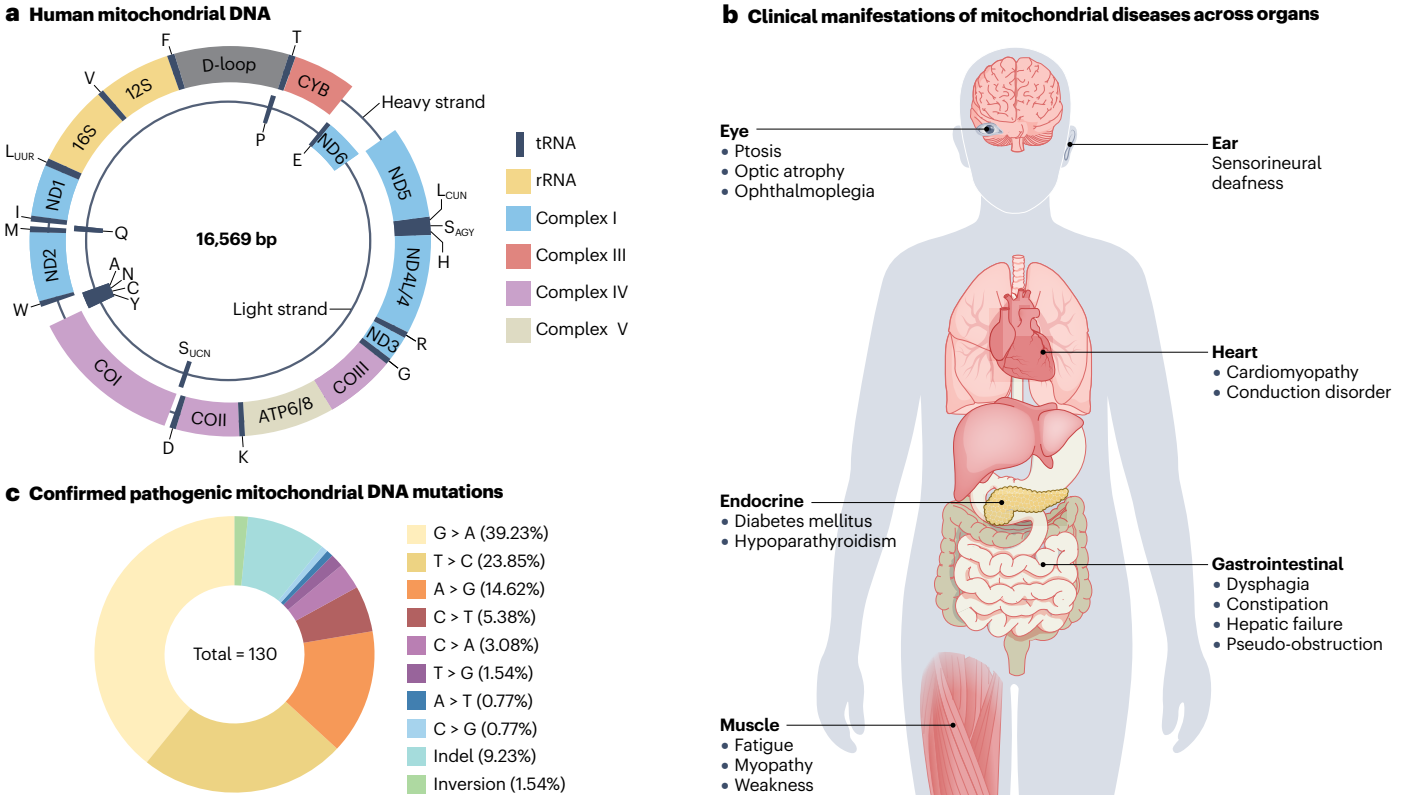

Fig. 1 | Mitochondrial genome mutations and human disease. a, Mitochondrial genes are encoded by the heavy and light strands of the human mitochondrial genome. Single letters around the circles denote the transfer RNA (tRNA) genes for the corresponding amino acids. 12S, 12S ribosomal RNA (rRNA); 16S, 16S rRNA; ATP6/8, ATP synthase membrane subunits 6/8; COI–III, cytochrome *c* oxidases I to III; CYB, cytochrome *b*; L_{UUR}, tRNA leucine 1 (UUR); L_{CUN}, tRNA leucine 2 (CUN); ND1–ND6, NADH dehydrogenases 1 to 6; ND4L, NADH dehydrogenase 4L; S_{AGY}, tRNA serine 1 (AGY); S_{UCN}, tRNA serine 2 (UCN). **b**, Mitochondrial mutation-associated diseases affect diverse tissues and organs. **c**, Confirmed pathogenic mitochondrial DNA (mtDNA) mutations have varied distribution, as curated in the MITOMAP database.

for pathogenic variants located in non-protein-coding regions of the mitochondrial genome.

The emergence of mtDNA-editing tools offers opportunities for both mechanistic studies and therapeutic development. mtDNA-editing technologies can be divided into two categories: mitochondria-targeted sequence-specific nucleases that induce double-strand breaks (DSBs) to selectively eliminate mutant mtDNA, and DSB-independent base editors that directly mediate precise nucleotide conversions. Unlike the nuclear genome, mitochondria lack efficient DSB-repair pathways such as non-homologous end joining (NHEJ) or homologous recombination, meaning that DSBs are largely irreparable and often result in degradation of damaged mtDNA rather than precise sequence correction. Consequently, nuclease-based approaches, such as mitochondrial transcription activator-like effector nucleases (mitoTALENs), primarily act by shifting heteroplasmy through selective depletion of mutant genomes, and base editors enable direct installation or correction of pathogenic variants without disrupting mtDNA integrity. In this Review, we summarize the development of these tools, their applications in human disease modelling and potential therapeutic strategies. We also outline the key technical challenges and future directions that can guide the optimization and clinical translation of mitochondrial genome-editing platforms.

Targeted degradation of mutant mitochondrial DNA

Pathogenic mtDNA mutations are typically heteroplasmic and manifest clinically only when their proportion exceeds a mutation-specific heteroplasmy threshold. Manipulating heteroplasmy is therefore an attractive therapeutic strategy for a broad spectrum of mitochondrial disorders. Owing to the lack of efficient DSB-repair mechanisms in mitochondria, nuclease-induced breaks yield linear mtDNA that is rapidly degraded[36–38]. Following this depletion, mtDNA copy number is restored through replication of the remaining genomes, although the mechanisms governing depletion sensing and replication control remain unclear. Thus, selective cleavage of mutant mtDNA depletes the pathogenic species and enables repopulation by the residual, predominantly wild-type genomes[39–41].

Early efforts employed mitochondrially targeted restriction endonucleases (mitoREs) (Fig. 2a), such as PstI, ApaLI, ScaI, XmaI and SmaI, to selectively deplete specific mtDNA haplotypes in cell lines and animal models[42–49]. However, these enzymes require the presence of highly specific and often palindromic recognition sequences of fixed length and composition, which are rarely present at or near diverse pathogenic mtDNA variants, thus limiting their broader applicability.

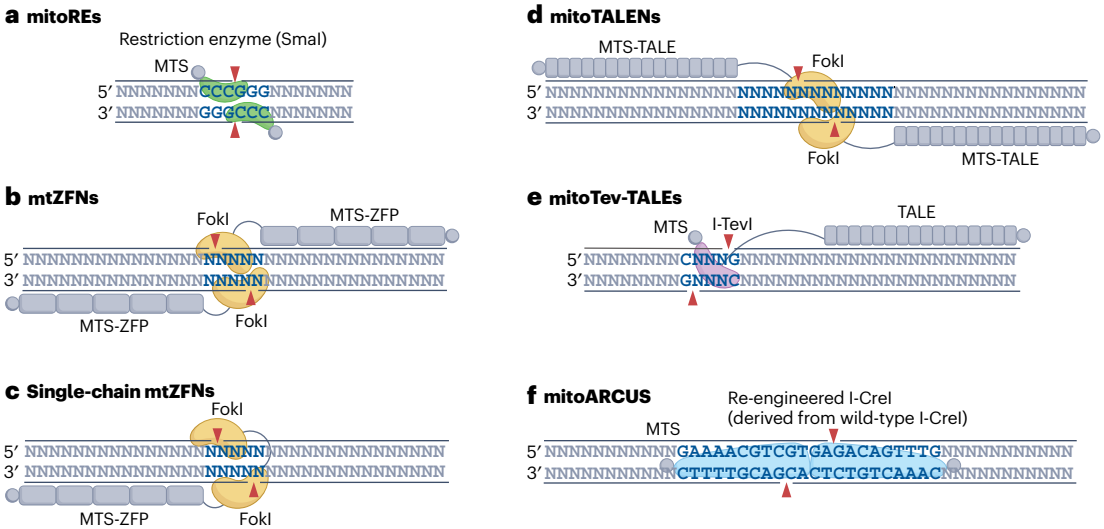

Fig. 2 | Mitochondrial genome-editing tools that induce double-strand breaks. a, Mitochondrially targeted restriction endonucleases (mitoREs), such as SmaI, cleave mitochondrial DNA (mtDNA). **b**, In mitochondrial zinc-finger nucleases (mtZFNs), each monomer comprises a zinc-finger protein (ZFP) DNA-binding domain fused to a FokI nuclease. **c**, Single-chain mtZFNs consist of a zinc-finger array linked to two FokI endonuclease domains. **d**, Each mitochondrial transcription activator-like effector nuclease (mitoTALEN) has two monomers, both of which contain a transcription activator-like effector (TALE) DNA-binding domain fused to the FokI endonuclease domain. **e**, MitoTev-TALEs comprise a TALE DNA-binding domain fused to the homing endonuclease I-TevI. **f**, Mitochondrially targeted engineered I-CreI (mitoARCUS) and mitochondrially targeted wild-type I-CreI induce double-strand breaks in mtDNA. In **a**–**f**, all proteins include a mitochondrial targeting signal (MTS). Red triangles mark the positions at which DNA nicks are introduced.

To expand the targeting scope, programmable DNA-binding proteins that can be rationally designed to recognize user-defined DNA sequences, such as zinc-finger proteins and transcription activator-like effectors (TALEs), were adapted for mitochondrial genome targeting[50–52]. Zinc-finger proteins contain tandem Cys_2His_2 motifs, each comprising approximately 30 amino acids and recognize specific three-base sequences via a conserved ββα fold[53]. TALEs comprise modular repeats of 33–35 amino acids, in which each repeat module recognizes a single nucleotide, with base specificity determined by the repeat-variable di-residues at positions 12 and 13 (refs. 54,55). TALEs generally provide comparatively superior modularity and binding specificity; by contrast, zinc-fingers offer compactness[56].

Fusion of these DNA-binding modules to a mitochondrial targeting signal (MTS) and a nuclease domain — either the FokI nuclease domain or the homing endonuclease I-TevI (5′-CNNN↓G-3′, where C represents cytosine, G represents guanine, N represents any nucleotide, and the arrow represents the cleavage point) — can yield mitochondria-targeted zinc-finger nucleases (mtZFNs) (Fig. 2b), single-chain mtZFNs (Fig. 2c), mitoTALENs (Fig. 2d), mitochondria-targeted I-TevI–transcription activator-like effector nucleases (mitoTev-TALEs) (Fig. 2e) and compact TALENs[49,57–62]. Subsequent engineering of an obligate heterodimeric FokI variant (ELD−/KKR+) reduced homodimerization and improved cleavage specificity compared with wild-type FokI[63,64]. Despite their versatility, mtZFNs and mitoTALENs are constrained by their limited single-nucleotide discrimination and their large sizes, which hinders efficient in vivo delivery. For example, specific recognition of a 15-bp DNA sequence typically requires approximately five zinc-finger repeats or fifteen TALE repeats, resulting in a single DNA-binding domain of roughly 150 or 500 amino acids, respectively, before inclusion of mitochondrial targeting sequences, linkers and nuclease domains.

Meganuclease-based approaches offer an alternative. Mitochondria-targeted mitoARCUS is derived from the compact I-CreI homing endonuclease, originally discovered in *Chlamydomonas reinhardtii*, which recognizes long and specific DNA sequences and can be reprogrammed to induce DSBs at a wide range of mtDNA sites (Fig. 2f). Its compact size (about 40 kDa), which facilitates delivery, together with its high specificity at single-nucleotide resolution[65–67], make mitoARCUS an attractive platform for heteroplasmy shifting.

Nevertheless, degradation-based strategies are ineffective for treating diseases caused by homoplasmic mtDNA mutations, because indiscriminate cleavage of all copies of the mitochondrial genome would be detrimental to cellular viability. Furthermore, these nucleases do not enable precise sequence correction, limiting their broader applicability for mitochondrial genome engineering.

Mitochondrial DNA base editing

Approximately 90% of pathogenic variants associated with mitochondrial diseases are single-nucleotide substitutions and mitochondrial base-editing technologies could offer precise correction of these mutations (this estimate does not include large-scale deletions). Cytosine base editors (CBEs) and adenine base editors (ABEs) were originally developed for nuclear genome editing and are derived from the CRISPR–Cas system[68,69]. Guided by single-guide RNAs (sgRNAs), Cas nucleases generate R-loops at target loci, exposing the non-target DNA strand for deamination. Single-stranded DNA (ssDNA)-specific deaminases convert cytosine (C) to uracil (U) or adenine (A) to inosine (I), which are interpreted as thymine (T) and guanine (G), respectively, during replication or repair, ultimately producing C•G-to-T•A or A•T-to-G•C conversions. In this nomenclature, C•G-to-T•A indicates a cytosine–guanine base pair converted to a thymine–adenine base pair or vice versa.

Although attempts have been made to use CRISPR-based systems to edit mtDNA, reported editing efficiencies typically remain below 1%, primarily owing to poor sgRNA import into mitochondria[70–76], a consequence of the hydrophobic mitochondrial inner membrane having a strong membrane potential. However, attempts to engineer sgRNAs to facilitate mitochondrial import, such as by appending RNA motifs including RNA transport-derived stem–loop structures to enhance mitochondrial localization, have shown only modest improvements in mtDNA-editing efficiency[72,74,77].

CRISPR systems can unwind and generate locally exposed ssDNA through R-loop formation, enabling coupling with ssDNA deaminases to achieve base editing in the nuclear genome. By contrast, TALEs and zinc-finger proteins bind to double-stranded DNA (dsDNA) without actively unwinding it, and therefore cannot generate ssDNA substrates on their own. Because previously characterized DNA deaminases primarily act on ssDNA, this limitation precluded straightforward fusion of TALE- or zinc-finger-based platforms with ssDNA deaminases for mtDNA base editing. A major breakthrough came with the identification of double-stranded DNA deaminase A (DddA), a bacterial toxin and double-stranded DNA cytidine deaminase that catalyses the conversion of cytosine to uracil in dsDNA[78,79]. By fusing DddA with a TALE protein and a uracil glycosylase inhibitor (UGI), researchers developed DddA-derived cytosine base editors (DdCBEs), enabling efficient C-to-T editing in mtDNA[80] (Fig. 3a). To reduce the inherent cytotoxicity of DddA, the enzyme was split into N- and C-terminal halves in the DdCBE system, each fused to a TALE protein containing an MTS and a UGI. When both halves bind adjacent sites on the mtDNA, DddA reassembles into an active enzyme capable of catalysing cytosine deamination. Building on this principle, FusXTBE was created using the FusX TALE assembly system to enable mitochondrial cytosine editing[81,82].

DdCBE-editing outcomes are strongly sequence-dependent[83]: the original editor preferentially targeted cytosines in 5′-TC contexts. Through phage-assisted evolution, DddA6 and DddA11 variants of the toxin were generated, with DddA6 improving editing efficiency by approximately 3.3-fold at canonical 5′-TC targets and DddA11 expanding compatibility to 5′-HC motifs (H denotes A, C or T)[84]. Incorporation of activation-induced cytidine deaminase from *Xenopus laevis* further broadened editing to previously inaccessible 5′-GC sites and enhanced activity at 5′-TC and 5′-CC contexts compared to DddA6- and DddA11-based DdCBEs[85]. Additional homologue screening and mutagenesis-based engineering yielded multiple DdCBE variants, including Ddd_Ss (a DddA homologue from *Simiaoa sunii*)-derived cytosine base editors (DdCBE_Ss), Ddd_Bc (a DddA homologue from *Burkholderia cenocepacia*)-derived cytosine base editors (DdCBE_Bc), riDddAtox (a dsDNA deaminase originated from a *Roseburia intestinalis* interbacterial toxin)-mediated mitochondrial cytosine base editor (mitoCBE), FZY2 (a DddA homologue from a *Lachnospiraceae bacterium sunii NSJ-8*)-derived cytosine base editor (FZY2-DdCBE) and Q2L7 (a DddA homologue from a *Streptomyces* sp. *BK438*)-derived cytosine base editor (Q2L7-DdCBE), expanding the editable sequence space to 5′-NC motifs (N denotes A, C, G or T)[86–88].

However, DdCBEs can induce the unintended integration of mtDNA fragments into the nuclear genome[89] and exhibit off-target activity[90,91], largely caused by spontaneous reassembly of split DddA halves or unintended interactions between DddA and the chromatin insulator CCCTC-binding factor. Strategies to mitigate off-target effects include lowering the affinity between DddA fragments (for example, by using high-fidelity DddA-derived cytosine base editors, HiFi-DdCBEs), introducing charge-reversal mutations near the DNA-binding interface, incorporation of nuclear export signals and nuclear expression of the DddA inhibitor $DddI_A$. These improvements have increased editing specificity[88,91–94]. The combination of structure-guided DddA engineering and WinPred (a model that predicts and guides high-precision DdCBE editing outcomes)-guided TALE design produced more accurate DdCBEs with a narrowed editing window and enhanced precision compared to sDdCBE11 (G1397-split-DddA11-containing DdCBEs, in which G1397 denotes a split site at Gly1397 of DddA)[95]. Furthermore, a de novo protein-design strategy has yielded a rigidly integrated TALE-oriented deaminase (TOD) that tightly constrains the editing window by limiting deaminase flexibility. In both full-length and split DdCBE–TOD configurations, this design enhances the specificity of DdCBE_Ss by reducing bystander off-target editing[96].

The large size and dimeric nature of DdCBEs complicate delivery via size-constrained vectors, with the two editor halves together encoding proteins of approximately 200 kDa. To overcome this limitation, multiple mutations (S1326G, G1348S, A1398V and S1418G) were introduced into full-length DddA to attenuate toxicity, enabling monomeric DdCBEs[97]. Replacing bulky TALEs with compact zinc-finger proteins yielded mitochondria-targeted zinc-finger deaminases (mitoZFDs), a highly compact editing platform in which the DNA-binding domain is approximately one-third the size of that used in conventional DdCBEs[98].

Furthermore, a mutant of the catalytic domain of single-stranded DNA deaminase toxin A ($SsdA_{tox}$), a DYW-like deaminase derived from *Pseudomonas syringae*, has been reported to enable mitochondrial base editing when fused to a TALE protein, a UGI and an MTS, although its editing efficiency remains relatively low, typically not exceeding 5%[99]. Structure-based protein clustering, a technique involving the prediction of protein structures followed by grouping proteins into families based on structural similarity, has further identified several new dsDNA cytosine deaminase variants[100], and subsequent engineering of one such enzyme, Ddd1, enabled efficient mitochondrial base editing, with an editing efficiency of approximately 10–20%[101]. Because conventional TALEs preferentially bind target sites with an upstream thymine, optimization of the TALE N-terminal domain led to the development of αDdCBEs, which exhibit broadened target compatibility and enhanced editing efficiency compared to DdCBEs. Additionally, repositioning TALE binding sites can modulate editing outcomes by adjusting the relative positions of the two TALEs on the target DNA, thereby narrowing or expanding the editing window and controlling which cytosine residues are editable[102,103].

TALE-linked deaminases (TALEDs) were developed to enable A-to-G base editing in mitochondrial DNA by combining TALE DNA-binding domains with the cytosine deaminase DddA and the engineered adenine deaminase TadA8e (an evolved variant of the *Escherichia coli* tRNA adenosine deaminase, TadA)[104] (Fig. 3b). TALEDs have been implemented in three main architectures: split, momomeric and dimeric. In split TALED (sTALED), DddA is divided into two inactive halves that reconstitute activity upon target binding, analogous to DdCBEs, with one DddA half-fused with the adenine deaminase on one TALE and the other DddA half-fused to a second TALE. In monomeric TALED, a full-length DddA carrying an E1347A mutation (a Glu-to-Ala substitution at residue 1347 that attenuates DddA toxicity) and the adenine deaminase are fused to a single TALE. Finally, in dimeric TALED, one TALE is fused to full-length DddA(E1347A) and the other to the adenine deaminase. In this system, DddA induces C-to-U deamination in double-stranded DNA, which subsequently activates endogenous base excision repair and generates transient single-stranded DNA intermediates that serve as substrates for TadA8e-mediated adenine

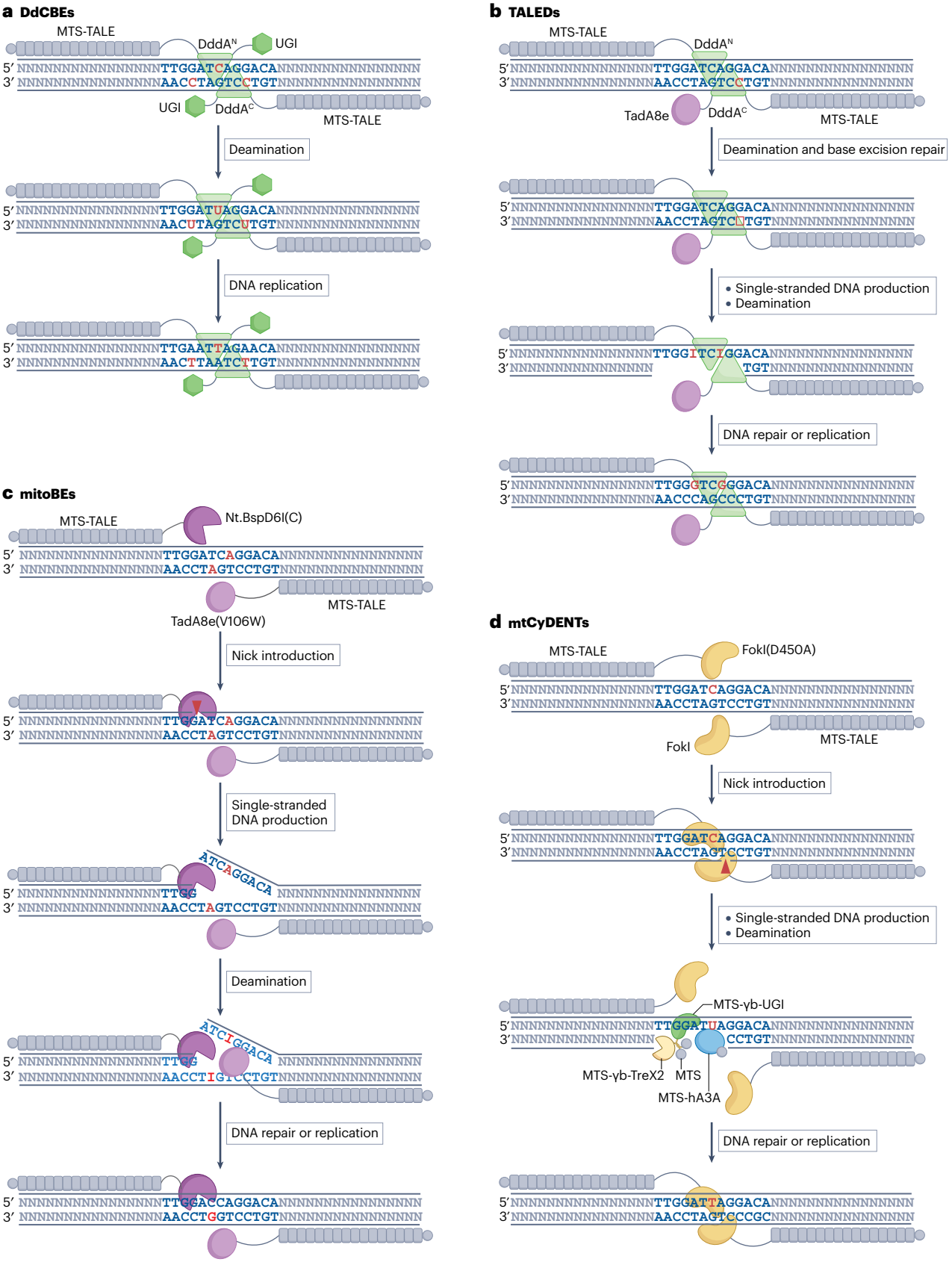
a DdCBEs
MTS-TALE
DddA^N
UGI
UGI
DddA^C
MTS-TALE
Deamination
DNA replication
b TALEDs
MTS-TALE
DddA^N
TadA8e
DddA^C
MTS-TALE
Deamination and base excision repair
• Single-stranded DNA production
• Deamination
DNA repair or replication
c mitoBEs
MTS-TALE
Nt.BspD6I(C)
MTS-TALE
TadA8e(V106W)
Nick introduction
Single-stranded DNA production
Deamination
DNA repair or replication
d mtCyDENTs
MTS-TALE
FokI(D450A)
FokI
MTS-TALE
Nick introduction
• Single-stranded DNA production
• Deamination
MTS-γb-UGI
MTS-γb-TreX2
MTS
MTS-hA3A
DNA repair or replication

Fig. 3 | Mitochondrial DNA (mtDNA) base editors for C-to-T and A-to-G conversions. a, In double-stranded DNA deaminase A (DddA)-derived cytosine base editors (DdCBEs), the DddA is split into two inactive halves, each fused to a transcription activator-like effector (TALE) bearing an mitochondrial targeting signal (MTS) and a uracil glycosylase inhibitor (UGI). These two TALEs bind adjacent sites, and the DddA halves reconstitute to catalyse C-to-U deamination, which is resolved as a C-to-T transition upon mtDNA repair or replication. $DddA^{C}$, the C-terminal half of DddA; $DddA^{N}$, the N-terminal half of DddA. **b**, In TALE-linked deaminase (TALED) systems, DddA-mediated deamination generates uracil, which is excised through an endogenous base excision repair pathway, producing an abasic site (red box). Subsequent processing exposes single-stranded DNA, enabling TadA8e-mediated A-to-I deamination and resulting in A-to-G editing after repair or replication. TadA8e is an evolved variant of the *Escherichia coli* transfer RNA adenosine deaminase (TadA). **c**, A proposed working model for mitochondrial base editors (mitoBEs) involves a nickase that introduces a single-strand break, exposing single-stranded DNA accessible to deaminases such as TadA8e(V106W) for A-to-G editing; the same principle applies to C-to-T editing. Nt.BspD6I(C), the C-terminal domain of Nt.BspD6I; TadA8e(V106W), a variant of the TadA8e protein containing a mutation at position 106 from Val to Trp. **d**, Mitochondrial cytidine deaminase–exonuclease–nickase–TALE (mtCyDENT)-based mtDNA editing involves a FokI heterodimer that generates a nick that is further processed by the exonuclease TreX2 (3′ repair exonuclease 2) to expose single-stranded DNA. In the presence of hA3A (human apolipoprotein B mRNA editing catalytic polypeptide-like 3A) and UGI, C-to-T editing is achieved. FokI(D450A), a variant of the FokI protein containing a mutation at position 450 from Asp to Ala. In **a**–**d**, all proteins include an MTS; red triangles indicate nicking positions.

deamination and conversion to ionosine[105]. This mechanism contrasts with DdCBE-mediated C-to-T editing, where UGI suppresses base excision repair to preserve the C-to-U intermediate. TALEDs use the endogenous base excision repair pathway by leveraging cellular uracil DNA glycosylase to process uracil-containing DNA intermediates, therefore facilitating formation of the required single-stranded DNA substrates.

TALEDs exhibit editing efficiencies below 10% at some target sites and can induce widespread transcriptome off-target effects as well as bystander editing within the editing window. Subsequent engineering efforts further improved the efficiency and precision of TALED systems. For example, introduction of the V28R (Val at position 28 replaced by Arg) or R111S (Arg at position 111 replaced by Ser) mutation into the substrate-binding pocket of TadA8e yielded sTALED variants (sTALED-V28R and sTALED-R111S) with over 99% reduction in RNA off-target activity and a narrower editing window compared to sTALED[106]. Replacing DddA with the hyperactive DddA6 variant also enhanced TALED editing efficiency[85]. Building on this improvement, the incorporation of uracil DNA glycosylase and engineered TadA variants (TadA8e or TadA8e(V28R)) further produced improved mitochondrial A-to-G editors, eTALED6s and eTALED6Rs, with higher efficiency and precision[105]. Introducing the previously characterized T1391A mutation (Thr at position 1391 replaced by Ala; known to reduce off-target DNA editing)[93] into DddA6, together with TadA8e(V28R), yielded high-fidelity split TALED (HiFi-sTALED), which enabled highly efficient and more precise adenine base editing in mouse embryos, with nearly a twofold increase in efficiency compared to sTALED-V28R. Incorporation of the αTALE N-terminal domain further produced an αTALE N-terminal domain-containing high-fidelity split TALED (αnHiFi-sTALED), with an additional ~1-fold increase in editing efficiency[107].

In parallel, DddA-independent mitochondrial editors have also been developed. Fusing TALE proteins to DNA nickases and single-stranded DNA deaminases, either TadA8e(V106W) (a variant of the TadA8e protein containing a mutation at position 106 from Val to Trp) or APOBEC1 (apolipoprotein B mRNA editing catalytic polypeptide 1)-UGI, enabled mitochondrial A-to-G and C-to-T editing, respectively. These systems, termed mitochondrial adenine base editors (mitoABEs) and mitochondrial cytosine base editors (mitoCBEs), are collectively referred to as mitochondrial base editors (mitoBEs)[108–110]. Their proposed mechanism involves single-stranded DNA generation, with editing occurring preferentially on the non-nicked DNA strand (Fig. 3c). Further engineering of the deaminases, such as introducing the V28F (Val at position 28 replaced by Phe) mutation into TadA8e(V106W) and replacing APOBEC1 with the TadA-derived cytosine deaminase CBE6d, yielded a more precise platform known as mitoBEs v2 (ref. 111). The strong strand selectivity of mitoBEs, coupled with the absence of detectable nuclear off-target effects, makes them an attractive alternative to DddA-based systems[108].

Strand-selective C-to-T mitochondrial editing has also been achieved using mtCyDENT (mitochondrial cytidine deaminase–exonuclease–nickase–TALE), which co-expresses TALE-FokI and catalytically inactive TALE-FokI(D450A) (a variant of the FokI protein containing a mutation at position 450 from Asp to Ala) dimers along with an exonuclease, a single-stranded DNA-specific cytosine deaminase, and UGI, all of which are targeted to mitochondria[112] (Fig. 3d). Mechanistically, mtCyDENT introduces a nick in double-stranded mtDNA via the nickase module, after which the exonuclease degrades one DNA strand from the nick site, thereby exposing single-stranded DNA, which serves as a substrate for the ssDNA-specific cytidine deaminase to catalyse C-to-T base conversion. Collectively, these tools provide a diverse and expanding mitochondrial genome engineering toolkit, enabling researchers to select the most appropriate system based on specific experimental or therapeutic requirements (Table 1).

Mitochondrial DNA base editors for disease modelling

Accurate animal models carrying defined mtDNA mutations are essential for understanding pathogenic mechanisms, phenotypic heterogeneity and maternal inheritance in mitochondrial disorders. These models enable investigation of mitochondrial dysfunction in multisystem diseases and offer platforms to advance targeted therapies and inform genetic counselling. Prior to the advent of programmable mtDNA base editing, mouse models were generated using non-targeted strategies, including in vitro mutagenesis combined with cell fusion. In this approach, enucleated cells carrying naturally occurring or induced mtDNA mutations are fused with mtDNA-depleted embryonic stem cells (pretreated with rhodamine 6G) to generate cytoplasmic hybrid cells, which are then injected into blastocysts to generate transmitochondrial mice (commonly referred to as 'mito-mice') (Fig. 4a). This method has produced models carrying chloramphenicol resistance (CAP^{R}) mutations[113,114], a 4,696-bp deletion (nucleotides (nt) 7,759–12,454)[115], and point mutations including m.6589T>C[116], m.13997G>A (for LHON)[117], and m.7731G>A[118], where the nomenclature indicates a nucleotide substitution at the specified mtDNA position. The generation of these models enabled early studies of mtDNA mutation-associated diseases in animal models and provided valuable platforms for investigating mitochondrial disease pathogenesis.

Table 1 | Tools for mitochondrial genome editing

Tool	Editing	Treatable target	Advantages	Limitations	Refs.
Targeted mitochondrial DNA degradation tools					
MitoREs	DSB	Contains specific restriction sites; heteroplasmic mutation	High specificity; small size	Limited target diversity	42–49
MtZFNs	DSB	Any targeting sequence; heteroplasmic mutation	Programmable; relatively small size	Limited specificity for SNVs	58,61
Single-chain mtZFNs	DSB	Any targeting sequence; heteroplasmic mutation	Programmable; smaller than mtZFNs	Limited specificity for SNVs	57
MitoTALENs	DSB	Any targeting sequence; heteroplasmic mutation	Programmable; higher specificity and easier assembly than zinc-fingers	Limited specificity for SNVs; large size limits delivery	59
MitoTev-TALEs	DSB	Contains 5′-CNNNG-3′; heteroplasmic mutation	High specificity	Sequence restrictions	62
Compact TALENs	DSB	Any targeting sequence; heteroplasmic mutation	Programmable; smaller than mitoTALENs	Limited specificity for SNVs	60
MitoARCUS	DSB	Any targeting sequence; heteroplasmic mutation	Small size; high specificity for SNVs	Different enzymes need to be designed for specific sites	65–67
Mitochondrial DNA base-editing tools					
DdCBEs	C-to-T editing	A-to-G or T-to-C mutations	Programmable	Strong sequence preference (5′-TC); nuclear off-target effects; large size limits delivery; susceptible to bystander editing	80
DdCBE-DddA6; DdCBE-DddA11	C-to-T editing	A-to-G or T-to-C mutations	Programmable; improved editing efficiency and extended compatibility to 5′-HC contexts	Potential nuclear off-target effects; large size limits delivery; susceptible to bystander editing	84
DdCBE_Ss; DdCBE_Bc; riDddAtox-mediated mitoCBEs; FZY2-DdCBEs; Q2L7-DdCBEs	C-to-T editing	A-to-G or T-to-C mutations	Programmable; improved editing efficiency and extended compatibility to 5′-NC contexts	Potential nuclear off-target effects; large size limits delivery; susceptible to bystander editing	86–88
HiFi-DdCBEs	C-to-T editing	A-to-G or T-to-C mutations	Programmable; improved editing specificity	Retain sequence preference (5′-TC); large size limits delivery; susceptible to bystander editing	93
Accurate DdCBEs	C-to-T editing	A-to-G or T-to-C mutations	Programmable; narrowed editing window	Large size limits delivery; susceptible to bystander editing	95
Monomeric DdCBEs	C-to-T editing	A-to-G or T-to-C mutations	Programmable; smaller than DdCBEs	Retain sequence preference (5′-TC); susceptible to bystander editing	97
MitoZFDs	C-to-T editing	A-to-G or T-to-C mutations	Programmable; relatively small size	Lower editing specificity than TALE-based editors; susceptible to bystander editing	98
TALEDs	A-to-G editing	C-to-T or G-to-A mutations	Programmable	Large size limits delivery; RNA off-target effects; susceptible to bystander editing	104
sTALED-V28R or R111S	A-to-G editing	C-to-T or G-to-A mutations	Programmable; reduced RNA off-target effects; narrowed editing window	Large size limits delivery; susceptible to bystander editing	106
Engineered TALED6s and TALED6Rs	A-to-G editing	C-to-T or G-to-A mutations	Programmable; enhanced editing efficiency and precision	Large size limits delivery; susceptible to bystander editing	105
HiFi-sTALED; αnHiFi-sTALED	A-to-G editing	C-to-T or G-to-A mutations	Programmable; further improved editing efficiency; reduced RNA off-target effects	Large size limits delivery; susceptible to bystander editing	107
MitoBEs	C-to-T; A-to-G editing	A-to-G, T-to-C, C-to-T or G-to-A mutations	Programmable; strand-selective editing; minimal nuclear off-target effects	Large size limits delivery; susceptible to bystander editing	108–110
MitoBEs v2	C-to-T; A-to-G editing	A-to-G, T-to-C, C-to-T or G-to-A mutations	Programmable; strand-selective editing; minimal nuclear off-target effects; reduced RNA off-target effects for mitoABE and reduced mtDNA off-target effects for mitoCBE	Large size limits delivery; susceptible to bystander editing	111
MtCyDENT	C-to-T editing	A-to-G or T-to-C mutations	Programmable; strand-selective editing	Moderate editing efficiency; large size limits delivery; susceptible to bystander editing	112

DdCBEs, DddA-derived cytosine base editors; DdCBE-DddA6, DddA6-derived cytosine base editors; DdCBE-DddA11, DddA11-derived cytosine base editors; DdCBE_Ss, Ddd_Ss (a DddA homologue from *Simiaoa sunii*)-derived cytosine base editors; DdCBE_Bc, Ddd_Bc (a DddA homologue from *Burkholderia cenocepacia*)-derived cytosine base editors; DSB, double-strand break; FZY2-DdCBEs, FZY2 (a DddA homologue from *Lachnospiraceae bacterium sunii NSJ-8*)-derived cytosine base editors; HiFi-DdCBEs, high-fidelity DddA-derived cytosine base editors; HiFi-sTALED, high-fidelity split TALEDs; mitoARCUS, I-CreI homing endonuclease-derived mitochondrial targeted nucleases; mitoBEs, mitochondrial DNA base editors; mitoREs, mitochondrially targeted restriction endonucleases; mitoTALENs, mitochondrial transcription activator-like effector (TALE) nucleases; mitoTev-TALEs, mitochondria-targeted I-TevI–transcription activator-like effector nucleases; mtCyDENT, mitochondrial cytidine deaminase–exonuclease–nickase–TALE; mitoZFDs, mitochondria-targeted zinc-finger deaminases; mtZFNs, mitochondria-targeted zinc-finger nucleases; Q2L7-DdCBEs, Q2L7 (a DddA homologue from *Streptomyces* sp. *BK438*)-derived cytosine base editors; riDddAtox-mediated mitoCBE, riDddAtox (a dsDNA deaminase originated from a *Roseburia intestinalis* interbacterial toxin)-mediated mitochondrial cytosine base editors; SNVs, single-nucleotide variants; sTALED-V28R, TadA(V28R)-derived split TALEDs; sTALED-R111S, TadA(R111S)-derived split TALEDs; TALEDs, TALE-linked deaminases; αnHiFi-sTALED, an αTALE N-terminal domain-containing high-fidelity split TALEDs.

A second non-targeted strategy uses *Polg*$^{D257A/WT}$ female mice, in which *Polg*, the nuclear gene encoding mtDNA polymerase γ, carries a proofreading-deficient D257A (Asp at position 257 replaced by Ala) mutation that increases mtDNA mutagenesis during oogenesis and early embryonic development. These mice are subsequently bred and screened over generations to isolate offspring carrying desired mtDNA mutations. Once a desired mtDNA mutation is identified, animals harbouring a wild-type *Polg* nuclear genotype are selected to establish the mutant line, preventing the further accumulation of mtDNA mutations (Fig. 4b). This approach has yielded a mouse line carrying both the m.5024C>T and m.13715C>T mutations[119]. These mutations do not correspond to specific human pathogenic variants but serve as experimental tools with which to model mtDNA dysfunction in vivo and enable the study of mitochondrial genetic variation. However, offspring of *Polg*$^{D257A/WT}$ mice often harbour multiple linked mutations within the same mtDNA molecule, complicating genotype–phenotype analysis and limiting the ability to assess the pathogenicity of individual mutations. Additionally, these traditional approaches are constrained by technical complexity, low efficiency, random mutational spectra, high inter-individual variability and purifying selection during maternal transmission.

The development of mitochondrial base editors along with delivery technologies has enabled the construction of precise and stable disease models. Microinjection of mRNAs encoding paired DdCBE into

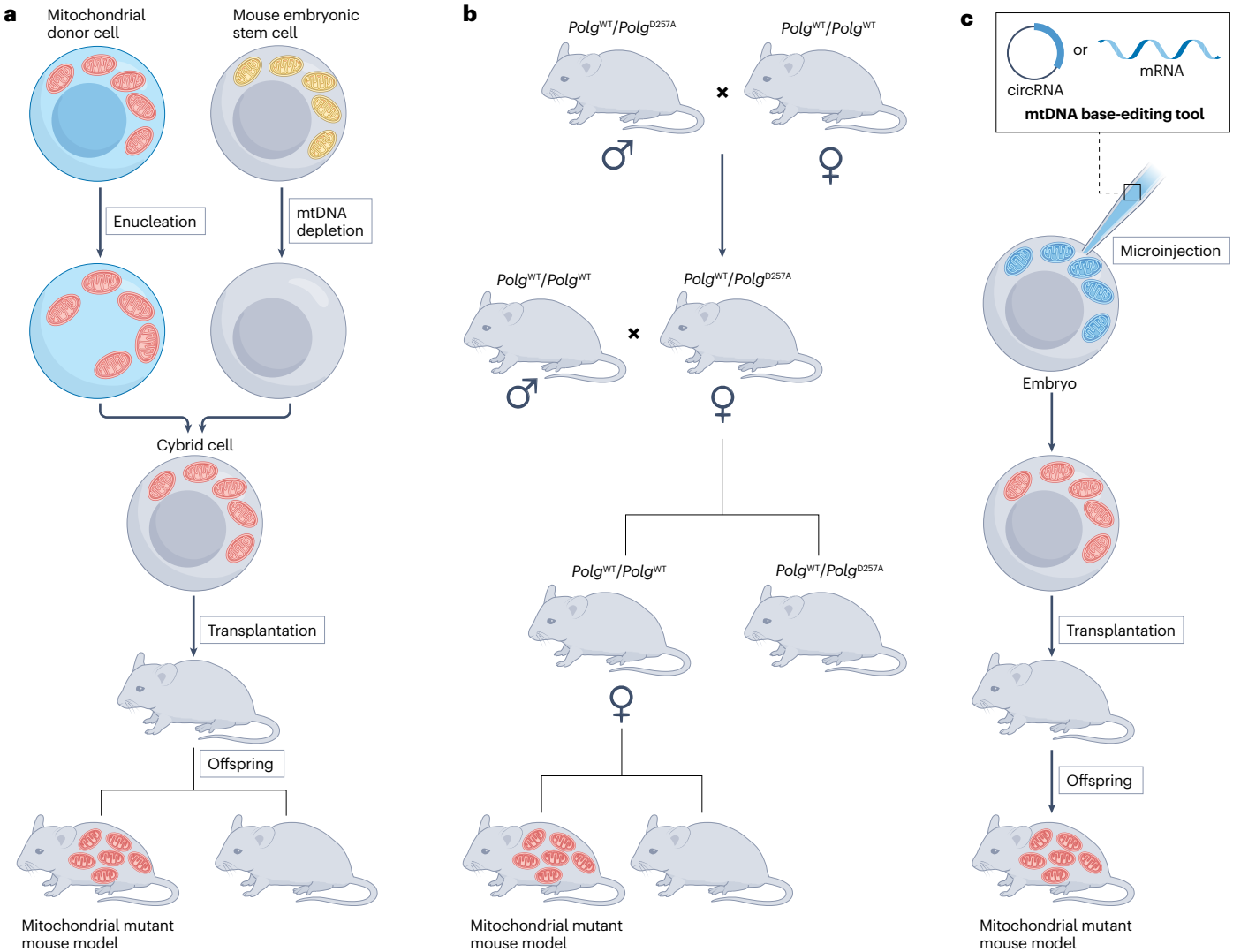

Fig. 4 | Approaches for generating mitochondrial mutant mouse models. **a**, Generation of a mitochondrial mutant mouse via mitochondrial replacement involves enucleation of donor cells carrying induced or naturally occurring mitochondrial DNA (mtDNA) mutations, which are then fused with rhodamine 6G-treated embryonic stem cells to produce cytoplasmic hybrids. These cybrid cells are injected into blastocysts to generate founder (F_0) chimeras. Mutant lines can be derived from F_0 animals or subsequent progeny. **b**, Generation of mitochondrial mutant lines by breeding *Polg* mutant mice, with desired mtDNA mutations identified and enriched across different generations. *Polg* encodes DNA polymerase γ, the enzyme responsible for replicating and repairing mtDNA. **c**, Generation of mitochondrial mutant mice by microinjecting mRNA or circular RNA (circRNA) encoding mitochondrial base editors into early embryos. Following translation of the editor, targeted mtDNA mutations are introduced during embryonic development. Edited embryos are transferred to surrogate females to generate founder mice, which are screened for the desired mtDNA mutations.

secondary follicle oocytes or early embryos has been used to generate multiple founder mouse lines, in which the transiently expressed base editor introduces targeted mtDNA mutations during early development that can be transmitted to the offspring, such as a mouse model carrying concurrent m.12539C>T and m.12542G>A mutations in the mitochondrial *mt-Nd5* gene[96,120–125] (Fig. 4c), and similar approaches have been applied to rats and zebrafish[126,127]. Furthermore, electroporation of DdCBEs has been used to introduce the m.15150G>A mutation into human liver organoids, generating organoid models with heterogeneous mutation loads and reduced ATP production compared to wild-type organoids[128]. Combining DdCBEs fused with nuclear export signals (which promote cytoplasmic retention of the base editor to enhance mitochondrial localization and editing efficiency) with mitoTALENs enables the generation of a m.12918G>A mouse model. In this dual-system approach, mRNAs encoding both DdCBEs and mitoTALENs are co-injected into mouse embryos, where DdCBE mediates the targeted mtDNA base substitution, while mitoTALENs selectively cleave and eliminate non-edited mitochondrial genomes, thus enriching for edited mtDNA. This strategy achieved editing efficiencies of up to 39.6%, accompanied by mitochondrial damage resulting in aberrantly enlarged brain ventricles[92]. In vivo delivery of DdCBEs via adeno-associated viruses also enables targeted mtDNA editing in both adult and neonatal mice[129], and a miniaturized zinc-finger DdCBE achieved up to 83% editing efficiency across multiple tissues following intravenous administration in newborn mice[130]. DdCBEs have also been used to generate knockout-type mouse models, including *mt-Nd5* and *mt-Atp6* knockouts (via m.12336C>T or m.8069G>A)[120,131], as well as conditional knockout rat models targeting *mt-Atp8* or *mt-Nd1*, using Cre/loxP-mediated activation of m.7783G>A or m.2996G>A[132].

According to MITOMAP, approximately 45% of confirmed pathogenic mtDNA mutations are amenable to modelling via C-to-T editing, and around 38% via A-to-G editing. Optimized TALEDs enabled the generation of a Leigh-syndrome-related *mt-Atp6* mouse model with around 20% editing efficiency, as well as sensorineural hearing loss and Leigh syndrome rat models targeting *mt-Ts1* and *mt-Nd6*, with editing efficiencies up to 44% and 74%, respectively. These models exhibited relevant phenotypes, including hearing loss and bradycardia[106,133,134]. The use of αnHiFi-sTALED enabled the generation of *mt-Rnr1* mutant mice carrying the m.978A>G mutation, which corresponds to the human pathogenic m.1555A>G variant associated with aminoglycoside-induced hearing loss, displaying impaired mitochondrial function[107]. Other engineered sTALEDs enabled the generation of *MT-ATP6* mutant rabbits carrying the m.8465T>C mutation, which corresponds to the human m.T9035C variant in *MT-ATP6*, which has been associated with ataxia syndromes[135].

Further improvements in accuracy enabled by mitoBEs v2, engineered from the original mitoBE system[108] and encoded using circular RNA (circRNA), facilitated the generation of multiple A-to-G or T-to-C and C-to-T or G-to-A disease models. These include m.8591T>C, m.12784A>G, m.978A>G, m.1029G>A, m.7741G>A, m.8251T>C, m.8576T>C and m.12499T>C, all of which correspond to pathogenic human mtDNA variants associated with distinct mitochondrial disorders and serve as disease models, with the m.12784A>G model achieving up to 82% editing in F_0 founder mice[111]. Phenotypic assessments revealed decreased heart rate in m.8591T>C mice, resembling Leigh syndrome, and reduced photoreceptor function in m.12784A>G mice, resembling LHON[111]. Owing to the mitochondrial DNA genetic bottleneck, offspring derived from the same maternal mouse can inherit markedly different levels of heteroplasmy. For example, progeny carrying the m.12784A>G mutation can exhibit a wide range of mutation loads, including homoplasmic states, enabling researchers to investigate how heteroplasmy influences disease onset and severity. Furthermore, animals carrying this mutation in the absence of additional mtDNA variants provide a valuable system for establishing direct genotype–phenotype relationships between individual mtDNA point mutations and mitochondrial disease[111].

Collectively, mitochondria-targeted base editors have improved the efficiency, precision and versatility of mtDNA disease-model generation, providing platforms for mechanistic studies and the development of targeted therapeutics. However, identical mtDNA mutations may produce divergent phenotypic outcomes in humans and animal models, reflecting species-specific differences in mitochondrial biology. Additionally, the efficiency and tissue specificity of mtDNA editing in animal models are strongly influenced by the choice of delivery strategy, including mRNA (or circRNA) microinjection, electroporation, viral vectors such as AAV and emerging nanoparticle-based systems. Each approach presents distinct advantages and limitations: for example, mRNA (or circRNA)-based delivery enables transient and precise embryonic editing but is restricted to early developmental stages. By contrast, viral delivery can achieve broader in vivo distribution but may suffer from packaging constraints and variable tissue tropism. Electroporation provides a relatively simple ex vivo or organoid-compatible method but is less suitable for systemic in vivo applications. These delivery-related challenges, together with issues such as mosaicism, off-target effects and variability in heteroplasmy levels, remain key factors that must be further optimized for the generation of robust and clinically relevant mtDNA disease models.

Mitochondrial DNA editing for disease treatment

Advances in mitochondrial genome editing and the development of disease animal models have accelerated efforts towards therapeutic interventions for mitochondrial disorders. One major approach involves the selective elimination of mutant mtDNA, enabling residual wild-type genomes to repopulate the mitochondrial network and restore oxidative phosphorylation (Fig. 5a). Early studies showed that mitochondria-targeted restriction endonucleases, such as SmaI or XmaI, could selectively deplete mutant mtDNA in human cells harbouring the heteroplasmic m.8993T>G mutation associated with neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP) and with maternally inherited Leigh syndrome (MILS), by increasing steady-state ATP production and oxygen consumption in cybrid cells, which are hybrid cells generated by fusion of mtDNA-depleted cells with patient-derived cytoplasts containing mutant mitochondria[43,46]. Similar heteroplasmy shifts have also been achieved in cell-based models and heteroplasmic mouse lines using other mitochondria-targeted restriction enzymes[42,44,45,47–49]. However, because suitable restriction sites are absent near most pathogenic mutations, the broader therapeutic use of mitoREs is limited.

Programmable nucleases have expanded the spectrum of targetable variants by enabling sequence-specific cleavage independent of restriction sites. mtZFNs and mitoTALENs can selectively eliminate mutant mtDNA variants, including ‘the common deletion’ (a frequent 4,977-bp mtDNA deletion associated with mitochondrial syndromes and accumulated in ageing tissues), m.8993T>G, m.14459G>A, m.8344A>G and m.13513G>A, in cytoplasmic hybrid cells, restoring mitochondrial function[57,59,61,136,137]. In vivo studies further demonstrate the therapeutic potential of mtZFNs and mitoTALENs: AAV-mediated delivery of either editor can shift heteroplasmy in the cardiac and

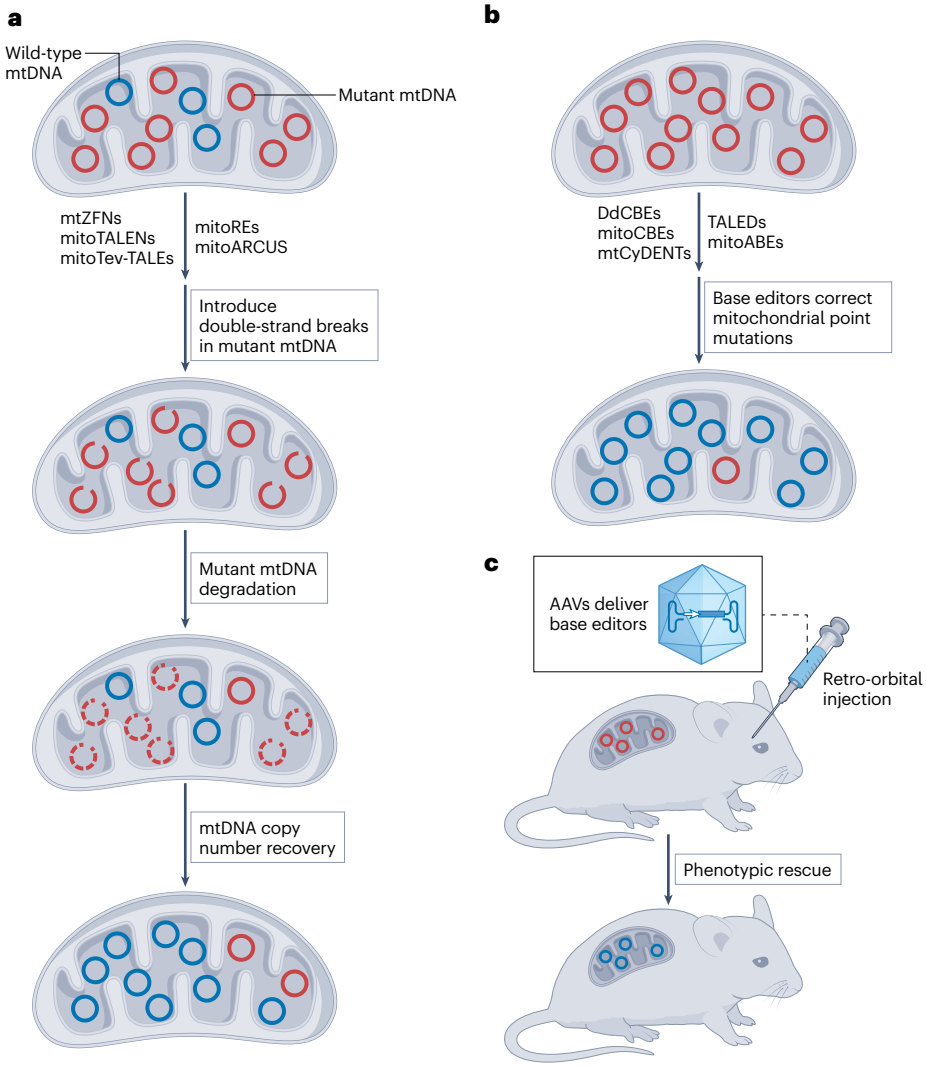

Fig. 5 | Therapeutic applications of mitochondrial genome-editing tools. a, Mitochondrial genome-targeted nucleases selectively degrade pathogenic mitochondrial DNA (mtDNA). mitoARCUS, I-CreI homing endonuclease-derived mitochondrial targeted nucleases; mitoTALENs, mitochondrial transcription activator-like effector (TALE) nucleases; mitoTev-TALEs, mitochondria-targeted I-TevI–TALE nucleases; mitoREs, mitochondrially targeted restriction endonucleases; mtZFNs, mitochondria-targeted zinc-finger nucleases. **b**, Mitochondrial base editors correct pathogenic mtDNA mutations. DdCBE, DddA-derived cytosine base editor; mitoABE, mtDNA adenine base editor; mitoCBE, mtDNA cytosine base editor; mtCyDENT, mitochondrial cytidine deaminase–exonuclease–nickase–TALE; TALEDs, TALE-linked deaminases. **c**, m.C5024T mutant mice are treated with an AAV9-delivered DdCBE pair. AAVs, adeno-associated viruses.

skeletal muscles of m.5024C>T mice and reverse associated biochemical defects[138–140], such as the decrease in $tRNA^{Ala}$ levels. Furthermore, an AAV-delivered mitoARCUS has also been used to selectively eliminate mutant mtDNA in m.5024C>T mice[141,142].

However, several challenges remain for clinical translation, including the risk of excessive mtDNA depletion when mutant genomes approach homoplasmy, and the difficulty of achieving efficient and tissue-specific delivery of editing systems across affected organs.

Current mitochondrial base editors can target a broad range of homoplasmic and heteroplasmic C-to-T (G-to-A) and A-to-G (T-to-C) mutations associated with human disease (Fig. 5b). DdCBEs have been used to correct the homoplasmic m.4300A>G mutation in induced pluripotent stem cells derived from a person with hypertrophic cardiomyopathy, restoring both $tRNA^{Ile}$ levels and mitochondrial function, and the m.4291T>C mutation in fibroblasts derived from a patient with Gitelman-like syndrome, rescuing mitochondrial membrane potential[128,143]. Similarly, circRNA-encoded mitoABE was used to correct the m.11778G>A mutation in cells from a patient with LHON, restoring mitochondrial function[108]. Furthermore, microinjection of precision-optimized DdCBEs into zygotes of rats carrying the m.13875T>C mutation restored an average of 53% wild-type mtDNA, ameliorating Leigh syndrome-related phenotypes[134].

For in vivo applications, delivery dosage, tissue distribution and the potential immunogenicity of viral vectors or editor components can influence editing efficiency, safety and the stability of edited mtDNA populations. For example, a DdCBE pair designed to install a compensatory m.5081G>A mutation (a C-to-T change in *mt-Ta* that restores the aminoacyl stem of $tRNA^{Ala}$) was packaged into AAV9 vectors and administered by retro-orbital injection into m.5024C>T mice (Fig. 5c). Although this treatment restored total $tRNA^{Ala}$ levels in the heart and skeletal muscle, it also caused severe in vivo toxicity, probably due to high systemic exposure and broad tissue distribution following AAV9-mediated delivery, which may have increased cumulative off-target editing events across multiple organs[144]. These findings highlight both the therapeutic potential of DdCBEs and the need for tight control of dosage, delivery and off-target risks.

TALEDs and mitoABEs can also be delivered using AAV9 to enable mitochondrial adenine base editing in vivo[145]. For example, sTALED-V28R restored both genotype and visual function in a LHON mouse model[125]. More accurate mitoBEs with minimal or undetectable off-target activity may therefore provide safer and more precise options for future mitochondrial gene therapies.

Ensuring the safety and reliability of mitochondrial genome editing for therapeutic applications requires rigorous quality control of off-target editing. Genomic off-target mutations can be assessed using high-depth genomic DNA sequencing with sensitive off-target prediction algorithms, such as TALENoffer, and bystander edits within the target window are detectable using targeted amplicon sequencing. Off-target editing in the nuclear genome can be caused by mitochondrial editing tools or integration of mtDNA fragments into the nuclear genome; both of which can be detected using whole-genome sequencing and digital polymerase chain reaction. Unintended RNA modifications are quantified by transcriptome-wide RNA sequencing. Although several platforms, including optimized mitoBEs and TALEDs, have been extensively characterized in cellular and animal models, systematic comparisons across different mtDNA-editing technologies remain limited. Comprehensive assessment of these risks is essential to guide the development of safer editors, to inform dosage and delivery strategies and ultimately to facilitate clinical translation.

Outlook

Selective elimination of pathogenic mitochondrial DNA initially appeared as a promising therapeutic strategy for mitochondrial diseases and continues to be crucial for addressing mutations such as large-scale deletions. However, this method does not directly repair the underlying mutations. Therefore complementary strategies, such as mitochondrial base editing, have gained interest in the field. Optimization of mitochondrially targeted C-to-T and A-to-G base-editing tools has enabled detailed analysis of mtDNA mutation dynamics during cell division[146,147] and facilitated the generation of diverse mitochondrial disease models, such as m.8591T>C mouse models, m.12784A>G mouse models, and m.13875T>C rat models[92,106,111,121,122,124,126,127,131–134]. These models have provided valuable insights into tissue-specific mutation distribution and intergenerational transmission, including the impact of heteroplasmy dynamics across tissues and the maternal inheritance patterns of mtDNA mutations[111,121,122,131]. Furthermore, selective elimination of deleterious mutations during germline transmission has been observed in some cases, such as m.8591T>C and m.2820G>A mutations, suggesting that purifying selection operates in a mutation-specific manner during oogenesis, thereby limiting the transmission of highly deleterious mtDNA variants[111,122]. Collectively, these advances provide a strong foundation for future studies of disease pathogenesis and the development of genetic interventions.

Among the known pathogenic mtDNA variants, the m.3243A>G mutation is one of the most prevalent, accounting for about 15–20% of all mitochondrial disease cases. Its carrier frequency is estimated to be 1 in 400–500 individuals, and around 30–40% of carriers develop clinical symptoms during their lifetime[148–152], including a broad spectrum of disorders such as MELAS, Leigh syndrome, diabetes mellitus and deafness, maternally inherited diabetes and deafness, sensorineural hearing loss, chronic progressive external ophthalmoplegia, myopathy and myoclonus, focal segmental glomerulosclerosis, autism spectrum disorder as well as cardiac and multi-organ dysfunction. This mutation causes severe multi-organ dysfunction, yet no successful animal model has been generated, largely because current mitochondrial base-editing tools have very low editing efficiency at this locus. Establishing a mouse model carrying this mutation would be extremely valuable for identifying pathogenic mechanisms and developing targeted therapeutic strategies.

At present, mitochondrial base editing is limited to C-to-T (G-to-A) and A-to-G (T-to-C) transitions, leaving many pathogenic mtDNA variants — including C-to-A, T-to-G, A-to-T and C-to-G transversions, as well as indels — beyond the reach of current methods. Although large-scale mtDNA fragment deletions can be induced by co-expressing mtDNA-targeted endonucleases and end-joining factors[153], more versatile mtDNA-editing systems capable of mediating base transversions and small insertions and/or deletions are urgently needed. In the nuclear genome, diverse CRISPR-based tools have already been developed for base transversions, including deaminase-dependent base editors such as CGBEs[154,155], GBEs[156], AYBEs[157] and ACBEs[158], and deaminase-independent base editors such as gGBEs[159], DAF-BEs[160], TSBEs[161] and TBEs[162]. However, adapting these strategies to mitochondrial DNA remains challenging owing to limited delivery efficiency and the absence of some canonical DNA repair pathways in mitochondria. Although similar approaches could, in principle, enable mtDNA base transversions, expanding the toolkit to include more versatile manipulation of mtDNA to treat a broader spectrum of mitochondrial diseases, the editing outcomes will ultimately depend on the distinct DNA repair environment within mitochondria. This environment is characterized by limited DNA repair pathway diversity, the absence of robust DSB repair mechanisms and a reliance on replication-driven resolution of DNA lesions, thus posing unique challenges for precise and efficient editing.

Mitochondrial base editors also frequently generate bystander edits within the editing window and often exhibit suboptimal efficiency. Although high-efficiency, single-site-edited mouse models can sometimes be isolated by narrowing the editing window or leveraging the mtDNA genetic bottleneck[111], precision remains a major limitation, particularly for therapeutic applications. Because many mitochondrial disorders arise from high heteroplasmy of a single-nucleotide variant, unintended edits could disrupt essential genomic elements or introduce novel pathogenic mutations, whereas insufficient editing efficiency could fail to restore normal mitochondrial function. As such, the development of next-generation mitochondrial base editors with single-nucleotide resolution, high specificity and high editing efficiency is a key priority for both mechanistic studies and clinical translation. In parallel, the development of mitochondria-compatible prime editing systems[163] offers a strategy to overcome limitations in edit types and bystander activity, potentially expanding the editing repertoire beyond transition mutations.

Mitochondrial diseases typically involve multiple tissues and organs, manifesting systemic and highly heterogeneous clinical phenotypes. Consequently, the success of mitochondrial genome-editing therapies will depend heavily on the development of effective and precise delivery systems. Although current mitochondrial base editors demonstrate strong activity in cultured cells, achieving efficient in vivo delivery of editors to mitochondria across diverse tissues remains a major obstacle. Tissue-specific differences in mitochondrial abundance, metabolic requirements and physiological barriers pose additional challenges to delivery. Therefore, designing next-generation delivery platforms that integrate cargo size, tissue tropism, long-term safety and low immunogenicity represents a central challenge and a key research direction for advancing the clinical application of mitochondrial genome-editing technologies. In this context, emerging

approaches such as engineered AAV capsids, lipid-nanoparticle-mediated mRNA delivery, and non-viral nanoparticle systems may offer improved tissue specificity and payload flexibility, while lessons from nuclear gene therapy, particularly in optimizing immune evasion and repeat dosing strategies, could provide additional guidance for overcoming current delivery limitations.

Published online: 26 August 2026

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Acknowledgements
This project was funded by various sources, including Changping Laboratory, the National Science Foundation of China (NSFC82341207 and NSFC32530017), and the Peking-Tsinghua Center for Life Sciences (to W.W.). We acknowledge that papers authored by scholars from historically excluded groups are systematically under-cited. Here, we have made every attempt to reference relevant papers in a manner that is equitable in terms of racial, ethnic, gender and geographical representation.

Author contributions
All authors contributed to writing and revising the paper.

Competing interests
The authors declare no competing interests.

Additional information
Peer review information *Nature Reviews Bioengineering* thanks Carlos Moraes and the other, anonymous reviewer(s) for their contribution to the peer review to this work.

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