Application and Challenge of CRISPR System to Mitochondrial Genetic Disorders

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Abstract. A distinct class of genetic illnesses known as mitochondrial genetic disorders is brought on by genetic mutations in the mitochondrial DNA. The lowest prevalence of mtDNA mutations is 1 in 5,000, leading to mitochondrial genetic disorders for which there are yet no economical diagnostic methods or therapeutic interventions. The CRISPR system is an immune defense system of prokaryotes that can recognize and cut foreign DNA, inhibit the expression of foreign genes, and fend off viral interference. It is because of its precise targeting ability that the CRISPR/Cas system has been transformed into a highly effective gene editing technique. Future treatments for mitochondrial illnesses may benefit from the CRISPR system, as well as the successful development of mitochondrial gene editing instruments. By referring to both domestic and international literature, this paper introduces Crispr/Cas technology and mitochondrial genes, summarizes specific cases of CRISPR system applied to mitochondrial gene, and focuses on the technical limitations of Crispr system for mtDNA modification. There is also discussion of the application prospect of CRISPR system in mtDNA modification. At present, it is found that the main challenge impeding the advancement of mtDNA editing technology within the CRISPR system is the technology for gRNA to gain entry into the mitochondria.

Keywords: Mitochondrial gene, CRISPR/Cas9, Cas12, Cas13, gRNA.

1. Introduction

Mitochondrial genetic disorders are a class of diseases resulting from gene mutations in the mitochondrial DNA, whose transmission and expression are completely different from those caused by nuclear gene mutations. Mutations in mtDNA are often inherited maternally in humans, affecting tissues that require a lot of energy, such as the brain and muscles. Trichloroglycerol etheria (MELAS), Kern-Gayle syndrome, Leighs syndrome, Parkinson's disease, and others are common mitochondrial gene diseases. mtDNA has been reported to have nearly 90 disease-causing point mutations, with the lowest prevalence of mtDNA mutations being 1 in 5,000 [1].

Existing gene sequencing methods for diagnosing mtDNA diseases laborious, costly, and inappropriate for clinical testing and mass genetic screening. Additionally, because there is currently no effective cure or therapy for mitochondrial genetic illnesses, clinicians can only provide symptom management to patients. While vitamins and supplements are routinely used to treat, they are rarely beneficial to patients. And a large number of patients exhibit drug insensitivity or resistance, just as palliative care. Therefore, mtDNA detection and editing is a promising research field that could theoretically diagnose, treat or even cure mitochondrial genetic disorders [2].

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and nearby CRISPR-associated (Cas) genes make up the CRISPR system. The system can precisely identify and sever alien nucleic acids, silence the expression of foreign genes, and maintain the stability of its own genetic system. CRISPR technology is advantageous in the realm of genetic modification due to its simple structure, ease of design, and accurate and efficient modification of the DNA sequence. The utilization of the CRISPR system contributes to the development of efficient methods for mitochondrial gene modification and treatments for mitochondrial diseases. This review summarizes the principle and specific cases of CRISPR system applied to mitochondrial genetic diseases, and discusses the application prospects and technical limitations, so as to contribute to the innovation of diagnosis and treatment of mitochondrial genetic diseases.

2. CRISPR System

CRISPR is a short palindromic repeat sequence of prokaryotic cells, and the genes associated with it are called CRISPR-Assoassociated genes (cas genes).

The following is the main process of CRISPR/Cas9 gene editing technology. First, the sgRNA is designed in accordance with an appropriate target sequence that is located next to the PAM site in the genome. This target sequence is roughly 18–20 nucleotides in length. Second, vectors are used to transport sgRNA and Cas9 genes. Then, sgRNA is integrated into Cas9 protein in vivo. After the Cas9-guide RNA ribonucleoprotein complex forms a R ring, the Cas9 protein uses sgRNA guidance to locate the target DNA sequence. The target DNA strand that is bound by the guide RNA is then cut by the HNH nuclease domain. And the non-targeted DNA strand containing PAM is cut by the RUVC-like nuclease domain. The blunt end cutting results in DNA double-strand breaks [3]. Cas9 can also produce interleaved cuts, resulting in predictable 1-bp insertions; Finally, the DNA repair system within the cell repairs double-strand breaks, using different mechanisms depending on the desired change, with the results classified as gene disruption, gene correction, gene insertion, and large gene deletion (Figure 1) [4].

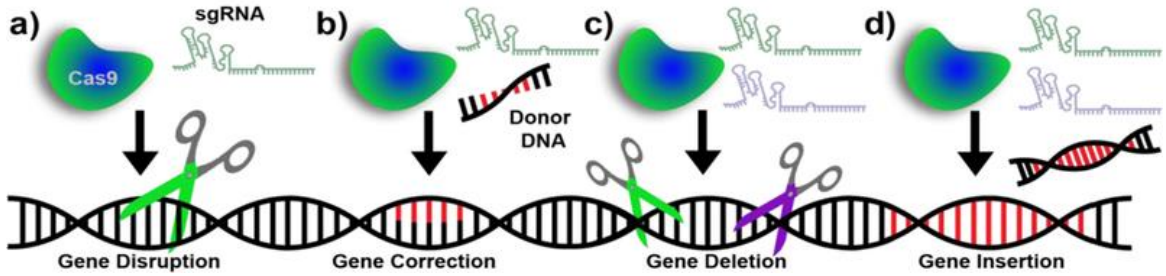

Fig. 1 Multiple components of CRISPR/Cas9 system are delivered into cells to achieve a specific function [4]

There are two kinds of CRISPR/Cas systems: type 1, including types I, III, and IV,uses multiple Cas proteins to function, while type 2, which includes types II (Cas9), V (Cas12), and VI (Cas13), uses just one Cas protein. Cas9 and Cas12 are RNA-directed DNA remodeling instruments, while Cas13 has RNA-directed RNA enzyme activity.

Cas9 recognizes downstream 5 '-NGG-3' PAM sequences, and Cas12 detects PAM sequences upstream of 5 '-TTTN-3' commonly. Genome editing can be done in AT-rich regions using the T-rich PAM of Cas12, broadening the target selection of RNA-guided nucleases. In contrast to Cas9, many Cas12s are capable of generating single or multiple CRISPR Rnas (CrRnas) due to their nuclear endogenous RNase activity [5]. By using a single, unprocessed crRNA array, Cas12 can associate with multiple CrRnas, which makes it possible to target multiple different genomic sites. This is known as multiplexing. Another important difference is in the division pattern. Cas12 nucleases have only one RUVC-like nuclease domain that facilitates the rupture of aimed ssDNA, resulting in an interlaced DSB cleavage in the region distal to the PAM sequence of the original spacer [3], producing sticky ends, while Cas9 causes blunt-ended cuts. In the appropriate position, the sticky ends produced by Cas12 can improve the accuracy of gene insertion or replacement. A characteristic of the V-type Cas12 family is the ability to cleave RNA and ssDNA non-specifically. This property is known as trans-fission that is activated only in the presence of a stimulator that contains complementary base pairs with the guide crRNA. Furthermore, CRISPR/Cas12 is also extensively used in genetic testing. The existence of the target stimulator may be verified when CRISPR/Cas12 unites with a FRET-based reporter protein and fluorescence linked to the quencher by a short oligonucleotide sequence. SHERLOCK (a particular very sensitive enzyme Reporter unlocked) and DETECTER (a DNA-targeted CRISPR Trans Reporter) effectively leverage this mechanism to dependably identify nucleic acids [6].

Cas13, unlike Cas9 and Cas12 targeting DNA, has RNA-directed intracrnas nuclease activity that particularly targets ssRNA. Cas13 can also be multiplexed. Once its linked crRNA and target mRNA

are supplemented, the two nucleotide-binding RNase domains (HEPN-1, HEPN-2) found in Cas13 proteins normally form an enzymatic site that cuts RNA when activated. Interestingly, several Cas13 orthogonals like LwaCas 13a and RfxCas13d, still function in the absence of PFS sequence limitations. These Cas13 variations greatly broaden the spectrum of targeted sequences by increasing the selectivity of sgRNA [7]. Using the Cas13-sgRNA complex, complementary mRNAs could be targeted and destroyed, providing a method to decreasing or suppressing gene translation. Cutting mRNA can be a more effective way to directly reduce protein expression than gene-editing techniques since changes arising via NHEJ mediated by Cas9/12 do not always result in gene collapse. Moreover, targeting genes at the level of transcription can avoid irreversible DNA alterations, which might be helpful in preventing untargeted mutations in the genome [8]. The programmability of Cas13 makes them an appealing original point for developing technique for RNA binding and perturbation applications. An overview of CRISPR-Cas9/cas12/cas13 is shown in Figure 2 [3].

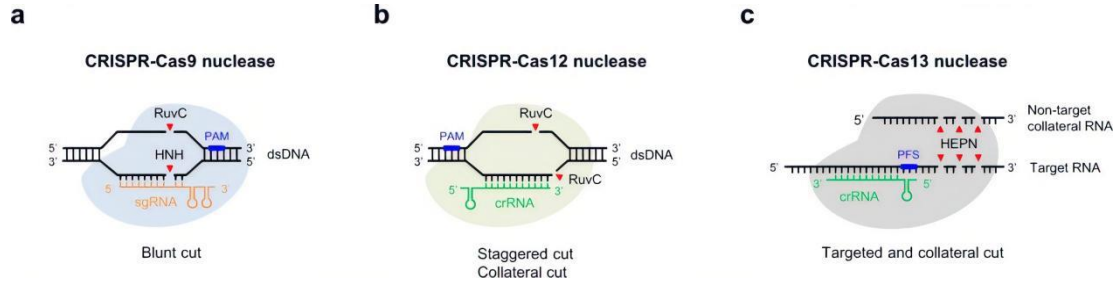

Fig. 2 CRISPR -Cas9/cas12/cas13 [3]

Although this new technique imposes modest limits on the number of genomic sites suitable for genome editing due to PAM requirements, it used simple Watson-Crick base pairing to design new guide Rnas for each genomic site of interest, replacing the complex protein design and engineering [9].

3. Mitochondrial gene

Mitochondria are the energy and metabolic centers of cells and play a key role in regulating cell signaling pathways, respiration, catabolism, and apoptosis. Mitochondria are bilayer semi-autonomous organelles with mtDNA independent of nDNA. Instead of being arranged into chromosomal pairs, mtDNA exists as compacted nuclides that proliferate apart from nuclear DNA (nDNA) [10].

Human mtDNA is a 16,569-bp looped dsDNA, 93% of which is the coding region, encoding a total of 37 genes, including 22 trnas, 13 proteins, 1 12S rRNA and 1 16S rRNA [11]. The 13 encoded proteins are seven subunits of complex I (ND1, ND2, ND3, ND4, ND4L, ND5, ND6) and one subunit of complex III (CYB) on the mitochondrial transport chain, three subunits of complex IV (COX1, COX2, COX3) and two subunits of complex V (ATP6, ATP8).

The copy number of mtDNA is widely known to be rigorously controlled. Each cell has 100-1000 mitochondria, each of which contains 2-10 mtDNA molecules. Owing to the presence of mutations, different copies of mtDNA molecules differ, resulting in heterogeneity. Heterozygous is a term used to describe mitochondria that contain mutated mtDNA and wild type (WT) mtDNA in individual cells [12]. Unlike nDNA variation, which is only heterozygous (50%) and homozygous (100%), mitochondrial variation can range from 0 to 100%. Since symptoms of mitochondrial genetic diseases depend on the degree of heterogeneity in the affected tissue and each tissue has a different biochemical threshold for the proportion of mutated genomes and WT mtDNA molecules. So, there are individuals who have low amounts of mtDNA mutations without symptoms [13].

Account of the long-term oxidative internal environment of mitochondria, the mutation rate of mtDNA is 100 times higher than that of nDNA [14]. The rise of mutated mtDNA happens through various ways, involving replicative segregation, relaxed replication or replicative dominance [15]. Replicative segregation applies to both oocyte development and the division of somatic cells in the

body. During cell division, mitochondria are separated at random. If there is heterogeneous variation, every daughter cell may get a variable ratio of WTmtDNA and mutated mtDNA. If cells with greater levels of modified mtDNA proliferate faster, the "founder effect" occurs, leading in the production of cell clones with higher amounts of mutated mtDNA. However, the average proportion of altered mtDNA in a cell population may remain constant [15]. Relaxed replication means that mtDNA continues to replicate during the cell cycle, even in post-division period, compared to nDNA which replicates only once throughout the cell cycle. mtDNA is constantly turned over throughout the cell cycle, which may bring about heterometastasis [15].

Replicative dominance is the last strategy. mtDNA molecules with a certain replication advantage are replicated more often than WT mtDNA. This has been utilized for mtDNA molecules with a number of disease-causing deletions that have thousands of bases fewer than WT mtDNA. Theoretically, this permits smaller circles to replicate faster and more frequently. This theory is also a possible interpretation for the accumulation in mtDNA mutation of patients [15]. The types of mtDNA variations include point mutation, deletion, duplication and loss. So far, about 200 pathological mtDNA point mutations have been discovered. The most vulnerable genes were tRNA genes, with A3243G causing MELAS, A8344G producing MERRF, and T8993G generating Leigh syndrome. Additionally, the amount of fragment deletion can be thousands of nucleotides, shortening the genome. Hundreds of deletions, including single and multiple deletions, have been found. Most single deletion illnesses, such as CPEO and KSS syndrome, are sporadic. Multiple deletions are mtDNA secondary alterations caused by nuclear gene mutations, such as familial PEO. Fragment repetition is the insertion of thousands of additional mtDNA nucleotides into the genome. Fragment loss means a decrease in the quantity of mtDNA copies.

In terms of heredity, unlike nDNA which has a parental inheritance, mtDNA follows a strict maternal inheritance. Due to the mtDNA bottleneck, which exhibits a more random propagation pattern than Mendelian genetics. mtDNA from sperm is eliminated by ubiquitination during zygotic formation [16]. People with elevated mutated mtDNA levels are likely to descend from families without a history of the illness and healthy mothers. Often, the mother has a portion of the defective mtDNA molecule, but her heterogenous proportions do not meet the biochemical threshold for disease expression. Conversely, infants born with subthreshold proportions may suffer from heterogenic mutations that increase in level throughout their lives, eventually developing the disease in adulthood [17].

4. Application of CRISPR/Cas system in mitochondrial genetic disorders

At least 1 in 5,000 people are affected by mitochondrial genetic disorders [1]. The pathogenesis of mtDNA disorders is multiple single nucleotide polymorphisms (SNPS). Because of the heterogeneity of these mutations, wild and mutant types coexist and may change over time [18]. The severity of mitochondrial disease is strongly correlated with the degree of heterogenicity because the percentage of mutant genomes and WT mtDNA molecules has different biochemical thresholds for each tissue [13]. Through the detection and editing of mitochondrial genes, CRISPR system has great promise in the mechanism of mitochondrial genes, diagnosis and cure of mitochondrial diseases.

4.1. Diagnosis of mitochondrial genetic disorders

The current molecular diagnostic method for mitochondrial diseases, whole exome and whole genome sequencing, is time-consuming and expensive, and is unfit for clinical testing and large-scale genetic screening [18]. The PCR-based assay methods for clinical diagnosis, such as Taqman-MGB probes (PCR-RFLP) and ARMS-PCR, can quantify the heterogeneic level of SNPS in mtDNA to less than 1%, but the clinical sample is requiring, the operation is complex, and the equipment is demanding. However, CRISPR technique for detecting disease has the benefits of being fast, accurate, sensitive, highly specific and easy to operate. Leber inherited optic neuropathy (LHON), for example, is the most prevalent maternally inherited mitochondrial disease. More than 90% of LHON cases are

caused by one of three primary mitochondrial DNA point mutations. Wan et al show how to discover LHON variations using CRISPR/Cas12a. There were five steps in total. Briefly, a finger drop of blood was obtained, and 100μL of nucleic acid releaser was used to liberate the gDNA. The target gDNA was then magnified and crRNA induced Cas12a reaction was carried out. Lastly, a blue light was used for judging the outcome. One of three typical variations was present in the patient if there was a green fluorescence signal at 485 nm. The absence of green fluorescence signal indicated an adverse outcome. Without the use of specialized instruments or costly reagents, the period from sampling to outcome was less than 30 minutes.The method is convenient, accurate, highly specific and sensitive, needing only one drop of blood to complete within 30 minutes, and can reach a heterosensitivity of 1%[19].

However, diagnosis of mitochondrial genetic disorders concentrates on accurately measuring the abundance of SNPS. Recently, Wu et al. demonstrated Ratio CRISPR, a CRISPR/Cas12a-based automatic ratio metric biochip sensor that was used to identify multiple heterogeneous SNPS in mtDNA to accurately quantify the level of heterogeneity in SNPS [20].

4.2. Understand the basic mitochondrial molecular mechanism

Mitochondria are bilayer membrane semi-autonomous organelles with many molecular mechanisms still unknown. The Crispr system can be used to investigate the molecular mechanisms of mitochondria. As an illustration of the specific repair mechanism of mitochondrial DNA and its regulation, Jo et al. found that using the CRISPR/Cas9 system to split and truncate specific loci of mtDNA may be possible to determine compensatory amplification of intact circular mtDNA by increasing the number of copies of uncut loci [21].Recently, Sack et al. used mtDNA damage agents and CRISPR/Cas9 screening to reveal nuclear genes critical to mtDNA damage response, including *WRNIP1*. *MCM8/9, C17orf53*(*HROB*)complex [22].

4.3. Generation of specific cell lines to investigate examine mitochondrial dysfunction and pathogenic mutations

CRISPR technology enables the production of viable cell lines and animal models for investigating the roles of various gene products in mitochondrial biology and illness. Hussain et al. created a hybrid sgRNA that joined the RP Loop, encoding the 11205G region of the mouse mtND4 gene. Then they transfected this structure into cells together with the MLS-Cas9. Based on the results, there was a significant drop in both the expression of ND4 in the cells and the number of mtDNA copies carrying 11205G in the mtND4 sequence [23]. Intragenic reversal mutation in MT-ND1 gene m.3902_3908inv (m.3902_3908ACCTTGC>GCAAGGT) can cause fatal lactic acid poisoning and mitochondrial myopathy in infants. BiR et al. developed a mito-Cas9 system and introduced m.3902_3908inv mutation into HEK293T cells and co-transfected with ssODN1ND4 and sODN3902. The m.3902_3908inv mutation had a knocking efficiency of roughly 0.05% [24].

These findings suggest that the Crispr system is employed as a feasible instrument for precisely editing mtDNA and can be refined as a feasible method for building cell lines to study mitochondrial dysfunction and disease-causing mutations.

4.4. Provide new directions to cure mitochondrial genetic disorders

There is presently no effective therapy for mitochondrial genetic illnesses, and doctors can only aid patients with symptom management and palliative care with vitamins and supplements.

Mitochondrial gene editing using the CRISPR/Cas system is a promising therapeutic strategy with three methods. The first idea is to reduce mtDNA heterogeneity levels by triggering aimed rupture of mutated mtDNA. Due to the absence of the restoration mechanism for DSB in human mitochondria, linear mtDNA will degrade uickly, allowing WT mtDNA to replicate to reconstruct the mtDNA pool [25]. For example, Bian and colleagues tested the mito-CRISPR/Cas9 system and ssDNA template for knockin in HEK293T cells and zebrafish embryos by microinjection, reducing mtDNA copy number in human cells and zebrafish [26]. The second concept is to employ base editors to reverse

mutations without disrupting mtDNA. A third approach is that the CRISPR/Cas13 system aims at single-stranded RNA, targeting genes at the transcriptional level. Furthermore, after employing the CRISPR/Cas system to understand and identify potential therapeutic targets, targeted medications to treat the disease can be produced. The CRISPR system could theoretically treat disorders resulting from mtDNA mutations.

5. Challenges in applying CRISPR system to mitochondrial genetic disorders

Compared with nDNA editing, the application of CRISPR technology in mtDNA editing is not smooth. Because mtDNA differs from nDNA, there are two challenges to operating mtDNA with the CRISPR system. The first is to assemble the CRISPR-Cas complex inside the mitochondria that needs the transportation of protein and RNA directly into the mitochondrial matrix [27]. Second, CRISPR/Cas9 itself only mediates DNA double-strand breaks, completing gene editing through NHEJ and HDR, while mitochondria lack HDR and NHEJ [28].

5.1. Localization of Cas protein to mitochondria

Proteins can be effectively introduced into mitochondria by joining the N-terminal of the relevant protein to mitochondrial targeting sequences (MTS) [29]. Then two mitochondrial localization signals (MLS) are installed on either side of the Cas9 sequence. MTS are identified by the outer membrane transphyllase receptor, which directs proteins to the mitochondrial matrix. A 3'-UTR can also be attached to promote this procedure by facilitating mRNA migration to the mitochondria-bound ribosome [30]. It should be noted that the function of the Cas9 protein can be impacted by codon bias. When editing mouse mt DNA, Hussain et al. selected the Cas9 coding sequence optimal for mouse expression [23]. Numerous studies have demonstrated that this method is particularly effective for Cas protein targeting mitochondria, as opposed to Cas9, which is scattered throughout the cytoplasm and nucleus with no localization sequence [31].

5.2. RNA delivery to mitochondria

Introducing gRNA components into mitochondria is a significant obstacle to use CRISPR systems. The traditional RNA introduction method, which is inefficient and difficult to duplicate, can only fix abnormalities caused by mitochondrial tRNA mutations. First, the use of plasmids that express gRNA in the nucleus is well established for nuclear DNA applications. Due to the need for efficient gRNA export from the nucleus, it is inconvenient for gRNA mitochondrial targeting in the absence of RNA export signals. Second, the sgRNA of the Cas9 system is quite long (about 100 bp) and strongly reduces its mitochondrial targeting [32].

The first idea to solve this problem inspired by the mechanism of transferring Cas9 protein to mitochondria is to add MLS to sgRNA. Four rings and three stem rings make up the T-shaped structure of the sgRNA. Stem ring 1 is indispensable in forming functional Cas9/sgRNA complexes. Stem rings 2 and 3 promote steady complex formation and improve the stability of sgRNA [33]. The inserted MLS should not significantly disrupt the function of the sgRNA. As some researchers reported, expression entirely failed when MLS is inserted into the interior of the sgRNA. So, MLS can only be inserted into the 5'or 3' ends of the RNA molecule. The structure of sgRNA is shown in Figure 3 [34].

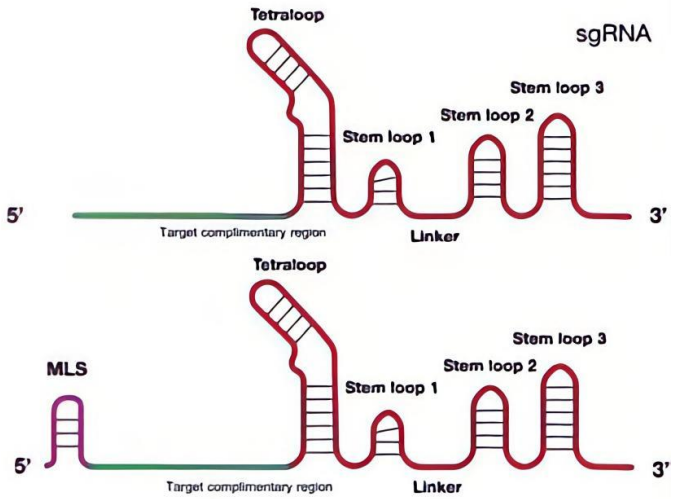

Fig. 3 The original sgRNA and sgRNA with MLS secondary structures [34]

The 20-nucleotide RNA dry ring structure of PNPase, F-arm or D-hairpin motifs from the yeast cytosolic tRNALys (CUU) have been suggested as possible MLS. PNPase is a mitochondrial intermembranous RNA transport enzyme that is involved in the entry of tiny Rnas into the mitochondria [35]. Hussain et al. attached the 20-nucleotide RNA dry ring structure of PNPase to the 5' end of the sgRNA sequence successfully delivered this sgRNA into the mitochondria [23]. Ludwig Schmiderer, on the other hand, added RP ring sequence to gRNA and tried to transport sgRNA to mitochondria too, but failed [31]. The effectiveness of RP rings in delivering RNA to mitochondria has yet to be further demonstrated.

Loutre et al. examined the ability of F-arm modified sgRNA to target mitochondria and found that F-arm motif did not improve mitochondrial input of sgRNA, which also requires further validation. Nikitchina et al. demonstrated the ability of D-hairpin motif to transport RNA molecules to mitochondria [36]. It should be noted that the F-arm and D-hairpin motif may affect how well sgRNA binds to Cas9 nucleases and how well sgRNA recognizes targeted DNA. When examining the capacity of sgRNA to target mitochondria in the experiment, it is necessary to eliminate the mitochondrial outer membrane to exclude the outer membrane pollutants. The mitochondria used to check the ability of sgRNA to target mitochondria did not eliminate the outer membrane to exclude outer membrane contaminants. A schematic of CRISPR/Cas9 delivery to mitochondria is shown in Figure 4 [23].

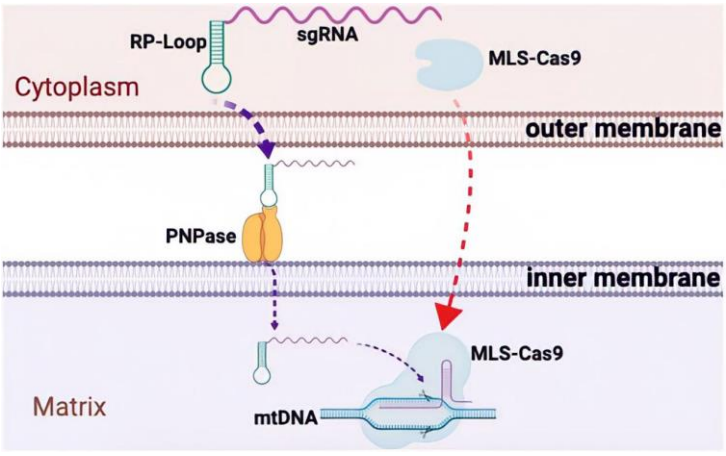

Fig. 4 Graphical summary of CRISPR/Cas9 transportation into the mitochondria [23]

5.3. Others

Cas9 cuts nucleic acid using two protein domains: HNH and RuvC. However, Cas12a has only one RUVC-like domain. Thus, the size of Cas12a is much smaller than Cas9. Cas12a may be a better substrate, which needs a significantly shorter crRNA (~41 nucleotides) than fusion crRNA-tracrRNA (~100 nucleotides) of Cas9, According to Antón et al., also proved that, compared with other Cas nucleases such as SpCas9, Cas12a efficiently localized to mitochondria and caused less mitochondrial damage [37]. Therefore, CRISPR/Cas12a has a much higher propensity to be introduced into mitochondria than CRISPR/Cas9.

The mtDNA repair mechanism is exceptional, and the mechanism is not clear yet. Existing research has found that mtDNA rarely or never occurs HDR and NHEJ [27]. Therefore, gene editing techniques based on DSB appear to be weak, and cutting mRNA might be more effective in straightly reducing protein expression. Unlike Cas9 and Cas12 which target DNA, Cas13 possesses RNA-directed RNA enzyme activity that specifically targets ssRNA. Targeting genes at the transcriptional level with CRISPR/Cas13 can avoid permanent DNA alterations, allowing precise gene expression control, but CRISPR/Cas13 needs to reduce its non-targeted and incidental effects.

The straightforward, efficient and precise single-base editing technique can also bypass the limitations of the double-strand break repair mechanism. Cas9 mutations form dCas9 or nCas9 proteins that lose nuclease activity but retain the ability to bind gRNA. Cytosine base editor (CBE), adenine base editor (ABE), and guanine base editor (GBE) are fusion proteins composed of dCas9 or nCas9 and deaminase with the ability to change a single base, directly acting on the base to achieve point mutation, and finally through base excision repair to achieve gene editing. In practice, however, attention should be paid to random base insertion caused by base excision repair; Prime Editor is a fusion protein that combines dCas9 incision enzyme domain and engineering reverse transcriptase domain, which carries template chain to initiate reverse transcription-induced point mutation [38]. It is strongly related to mismatch repair, and its repair mechanism is still unclear.

6. Conclusion

Mitochondrial genetic disorders are a distinct class of genetic illnesses resulting from mtDNA mutations, and diagnostic methods suited for clinical testing and large-scale genetic screening, and effective treatment strategies are lacking. The CRISPR system is an accurate and efficient gene editing technique with simple structure and easy design. Its use will aid in the future research of effective strategies for mitochondrial gene modification and feasible treatments for mitochondrial disorders. However, there are many limitations in the widespread practical application of this technology, such as the technology of gRNA delivery into mitochondria, the unclear repair mechanism of mtDNA, and the pseudogene influence of genomic mtDNA. Now utilizing CRISPR technology, researchers have developed an automated ratio biochip sensor that accurately quantifies SNP abundance and a LHON mutation diagnostic system. Researchers have also generated specific cell lines to examine mitochondrial dysfunction and disease-causing mutations. In this paper, the author proposes three potential future therapeutic approaches for mitochondrial gene diseases: reducing mtDNA heterogeneity by inducing targeted cleavage of mutated mtDNA, using base editors to edit genes, and targeting transcriptionally specific genes with the CRISPR/Cas13 system.

In conclusion, CRISPR system, as a potent genome editing technique, offers new methods for the study and therapy of mitochondrial gene diseases, but there are also some challenges. The CRISPR system needs to be further refined and optimized in the future to enhance its application in mitochondrial genetic disorders.

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