CRISPR/Cas9 Gene Therapy Delivery Strategies

The emerging CRISPR/Cas9 gene editing technology has the advantages of simple design, easy operation, good specificity and high efficiency, which greatly accelerates the R&D process in life sciences. Here, we briefly introduce CRISPR/Cas9 and its delivery strategies.

What is CRISPR/Cas9?

The **CRISPR/Cas9 system** consists of a single-stranded small guide RNA (sgRNA) and a Cas9 protein with nucleic acid endonuclease function, as shown in Figure 1. Under the specific recognition of sgRNA, Cas9 protein reaches genome-specific sites and cleaves double-stranded (dsDNA) DNA, generating dsDNA breaks (DSB). DSBs are then repaired by cell-autonomous non-homologous DNA end joining (NHEJ) or homology-directed repair (HDR), with the dominant NHEJ repair prone to mutagenesis.

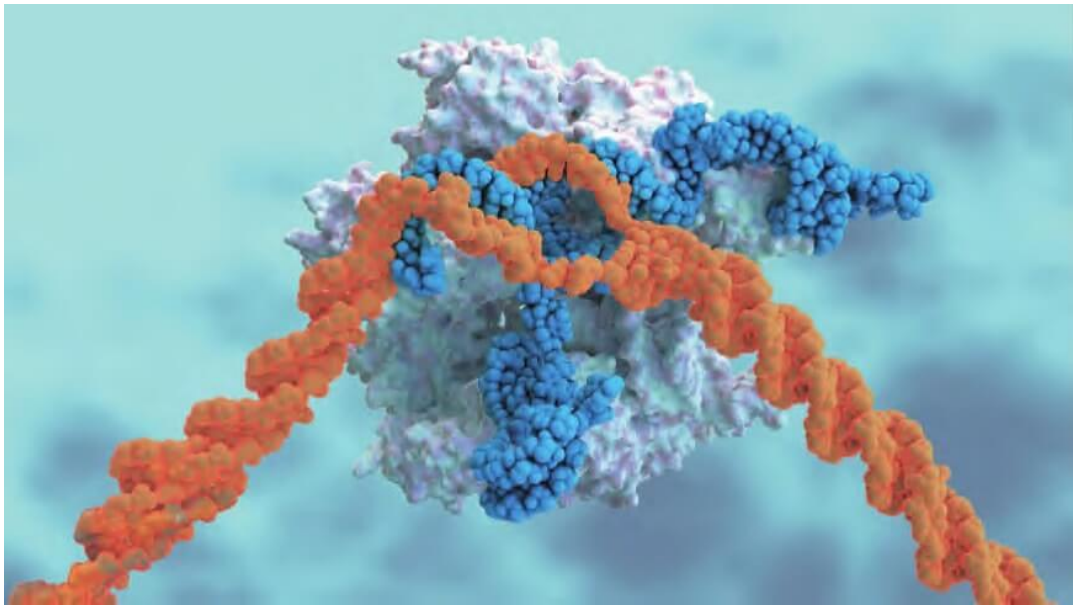

Figure 1. sgRNA (blue), CAS9 protein (white), target gene (orange) (Source: Science official website)

The innovative CRISPR technology generated widespread interest upon its introduction and was repeatedly named one of the **Top 10 Breakthroughs by Science** magazine in 2013, 2015, 2017, 2020 and 2021. In 2020, the Nobel Prize in Chemistry was awarded to CRISPR gene editing technology developer Jennifer Doudna, a professor at the University of California, Berkeley, and Emmanuelle Charpentier, a professor at the Max Planck Institute for Infection Biology in Germany.

Since its introduction, CRISPR has expanded its application areas as it continues to be optimized. CRISPR has shone in the fields of gene knockout & knock-in, repression & activation, multiplex editing, and functional genomic screening.

It has made remarkable contributions to the treatment of many genetic dysfunction-related diseases, such as **hereditary blood disorders (sickle cell anemia, β-thalassemia), hereditary neurological disorders (Huntington's disease), hereditary muscle disorders (Duchenne muscular dystrophy), AIDS, and eye diseases.** In addition, CRISPR/Cas tools delivered by lentivirus can also be used to modify patients' autologous T cells in tumor immune cell therapy to enhance T cell anti-tumor immune function, known as CAR-T therapy, which is a common application of CRISPR technology in in vitro gene therapy.

Delivery Strategies of CRISPR/Cas9 Gene Therapy

With the increasing clinical demand, the translation of CRISPR gene editing therapies for clinical application is also imminent. Therefore, the development of efficient and safe CRISPR delivery systems has become a major issue. There are three major challenges to the clinical translation of CRISPR: **editing efficiency, specificity, and delivery strategies.**

According to current research reports, there are three main **types of vector systems** that can be used for CRISPR tool delivery: **biological methods, chemical methods and physical methods**. Here is a brief inventory of several of these vector types to give you a quick overview of CRISPR tool delivery strategies commonly used in clinical research.

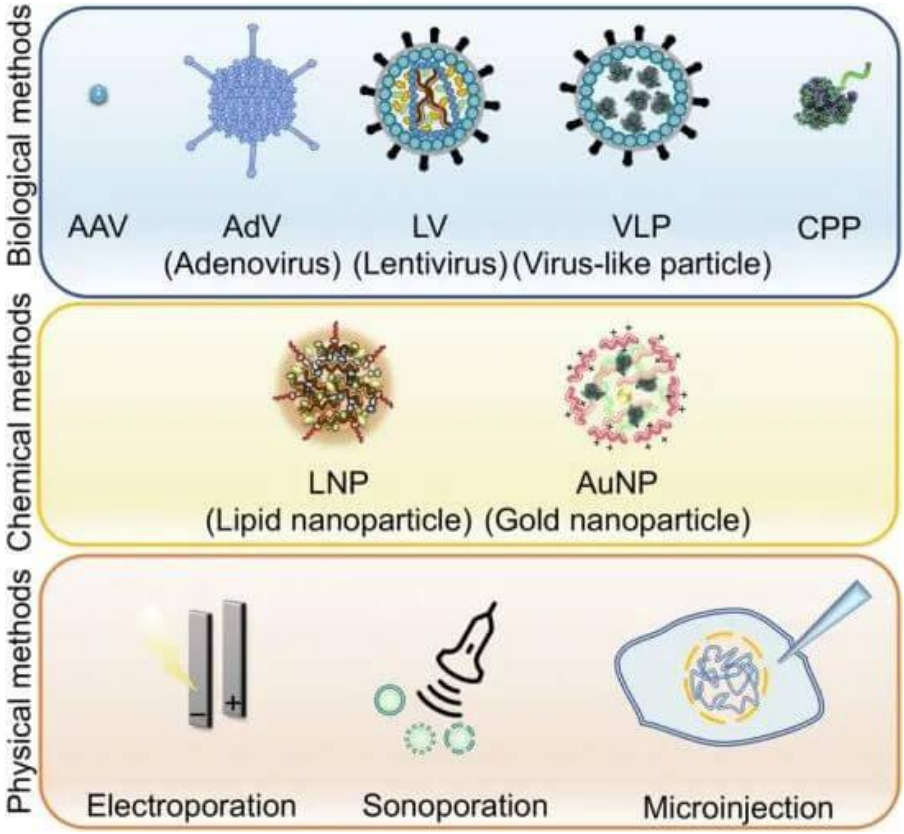

Figure 2. Various methods for delivering CRISPR-Cas9 components, source: reference [1]

Biological Methods - Viral Vectors For Gene Therapy

Viral vectors are still one of the more commonly used delivery systems, as reported in CRISPR-related clinical studies. Among them, **lentivirus (LV)** are a class of single-stranded RNA retroviral vectors modified from human immunodeficiency virus (HIV), whose virulence genes have been eliminated and replaced by exogenous target genes.

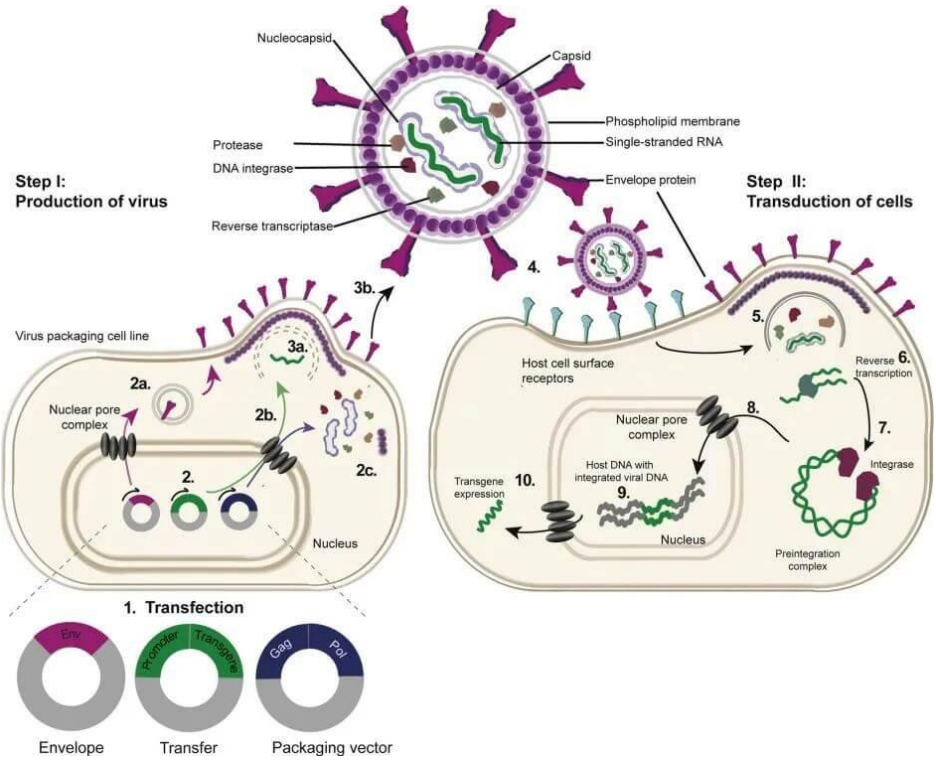

Figure 3. Lentiviral vector (LV) for transgene delivery, source: reference [2]

After entering the cell, the LV genome is reverse-transcribed into DNA in the cytoplasm, forming a preintegration complex (PIC). After the PIC enters the nucleus, the DNA is integrated into the cellular genome, and the integrated DNA is transcribed into mRNA, which returns to the cytoplasm to express the target protein or produce small RNA.

LV is able to efficiently integrate exogenous genes into the host chromosome, achieving persistent expression and dividing with the cellular genome. The integrative and persistent nature of its transgene expression makes LV unfavorable for in vivo delivery of CRISPR editing tools, as random integration of viral DNA may lead to activation of oncogenes or suppression of oncogenes, resulting in tumorigenesis. Also, the stable expression of Cas9 and gRNA may lead to a higher risk of off-target mutations, therefore LV is mainly used for in vitro delivery of CRISPR gene editing tools to edit and modify specific cell types, such as hematopoietic stem cells (HSC) and T-cell therapy.

Adeno-associated virus (AAV) vectors are also a common class of in vivo gene editing delivery systems used in clinical research and have been approved for delivery of CRISPR gene therapy to humans. The AAV viral vector consists of an icosahedral protein capsid of approximately 20-26 nm in diameter and a single-stranded DNA genome of approximately 4.7 kb, which cannot replicate autonomously and exists mainly in an additional form free of chromosomes. This is one of the major advantages of AAV vectors over LV vectors. AAV has been widely used for in vivo gene research and therapy due to its advantages of different tissue and cellular targeting, very low immunogenicity, high safety, long-time expression of genes in vivo, and the ability to infect both dividing and non-dividing cells.

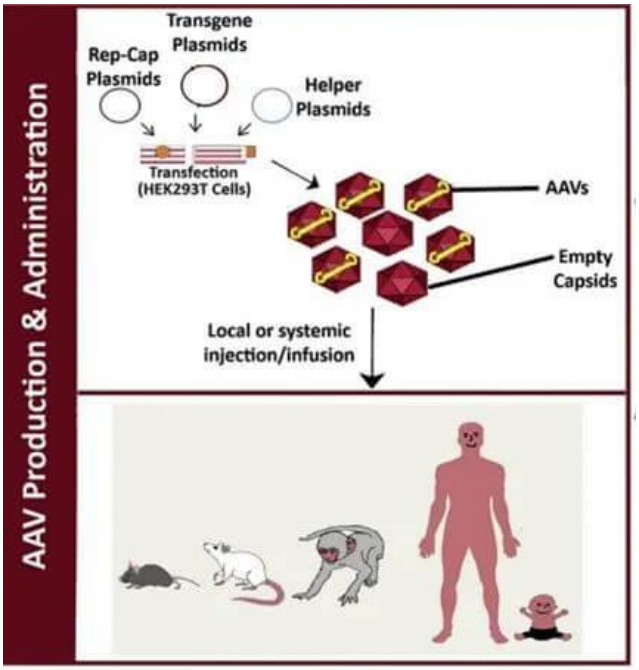

Figure 4. Adeno-associated virus (AAV) vectors for in vivo transgene delivery, source: reference [3]

AAV-mediated antibody gene transfer technique has been most studied for human immunodeficiency virus (HIV) treatments preclinically. Several successfully cured HIV patients have been treated with HIV-immune HSC or umbilical cord blood stem cell transplantation. However, in vitro CRISPR-edited HSC transplantation is usually

associated with the risk of immune rejection. In addition, the number of HIV patients successfully cured is only a minority, and most HIV patients still use antiretroviral drug therapy to alleviate HIV disease and cannot completely eradicate HIV from their bodies.

Can CRISPR gene editing be used to modify the immune cells in the patient's body so that they can continuously produce HIV antibodies on their own through a one-time treatment?

A recent study published in Nature Biotechnology used the CRISPR-Cas9 gene editing system to modify the genome of B cells by introducing a segment of the gene at the immunoglobulin locus, which encodes an antibody template against HIV. At the same time, they used two modified AAVs as vectors, carrying the gene encoding the antibody and encoding the CRISPR system, and delivered them to B cells in mice so that B cells could continuously secrete neutralizing antibodies against HIV in vivo.

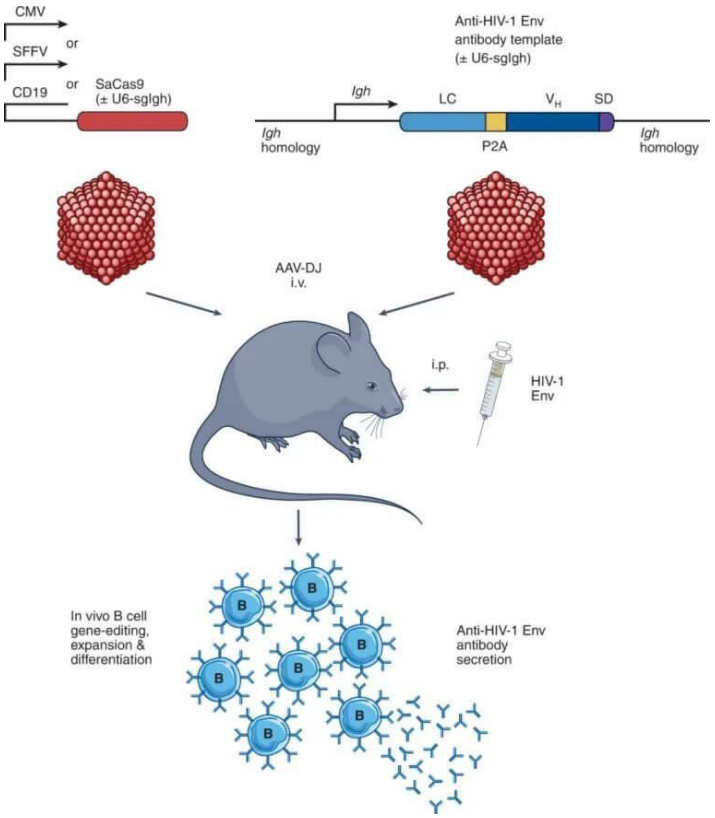

Figure 5. AAV in vivo delivery of CRISPR gene therapy modifies B cells to fight HIV, source: reference [4]

Chemical Methods - Lipid Nanoparticles (LNP) for Gene Therapy

In addition to viral vectors, chemical methods of delivering CRISPR tools, including **lipid nanoparticles (LNPs)**, a synthetic delivery technology that typically consists of four lipids: cationic or ionizable lipids, helper lipids, **polyethylene glycol (PEG) lipids,** and cholesterol, have been gaining attention in recent years. Compared with AAV and LV, synthetic LNPs are gradually showing some advantages for CRISPR gene therapy delivery, such as LNP delivery enables the transient expression of gene editing tools and is much less immunogenic than viral vectors, thus improving the safety of in vivo delivery; in addition, it can wrap large fragments of Cas proteins, etc.

PEG lipids have multiple effects on the properties of lipid nanoparticles, such as increased stability, prolonged blood circulation time, improved targeting ability, etc, **Biopharma PEG** is a dynamic science company dedicated to PEG derivatives. **We are capable of supplying small to large quantities of a rich selection of PEG derivatives with GMP & non-GMP standards for your mRNA vaccine R&D. We can produce and provide the following PEG products for your mRNA vaccines R&D.**

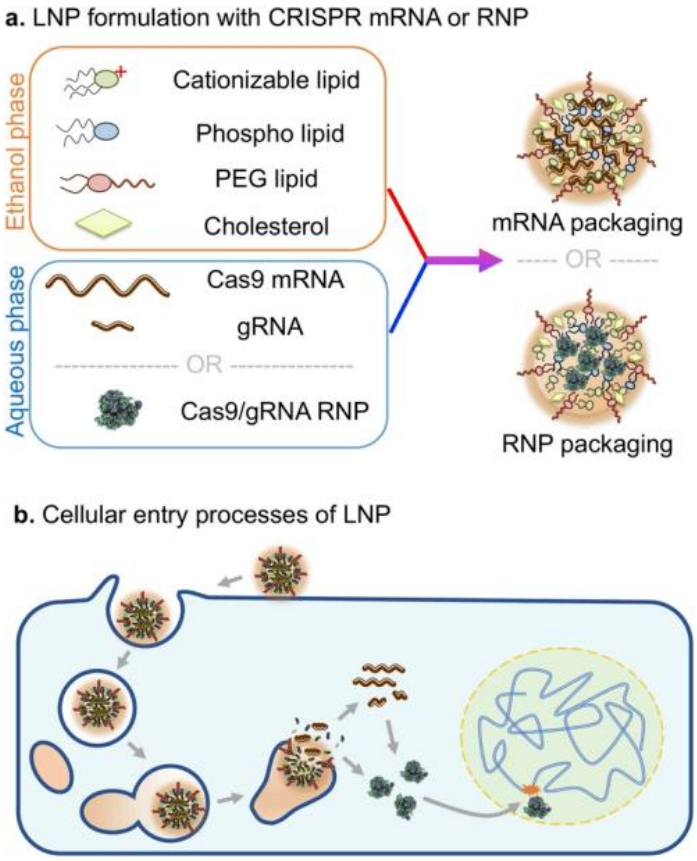

Figure 6. LNP delivery of CRISPR gene editing tools, source: reference [1]

In March 2022, Intellia Therapeutics, co-founded by Nobel Laureate Jennifer A. Doudna, announced again the latest clinical phase I trial data of its LNP in vivo delivery CRISPR gene therapy NTLA-2001 for the treatment of transthyretin amyloidosis (ATTR), developed in conjunction with Regeneron.

NTLA-2001 provides a rapid, profound and durable reduction in patient serum levels of the transthyretin (TTR). NTLA-2001 therapy is a novel CRISPR/Cas9-based in vivo gene editing therapy consisting of LNP and human codon-optimized Streptococcus pyogenes Cas9 protein mRNA sequences. The delivery system is hepatopathogenic and carries sgRNA targeting human TTR. The announcement of the first clinical results of NTLA-2001 last June attracted the attention of the global industry, and this update of NTLA-2001 data once again demonstrates the potential of CRISPR in vivo editing of liver delivery.

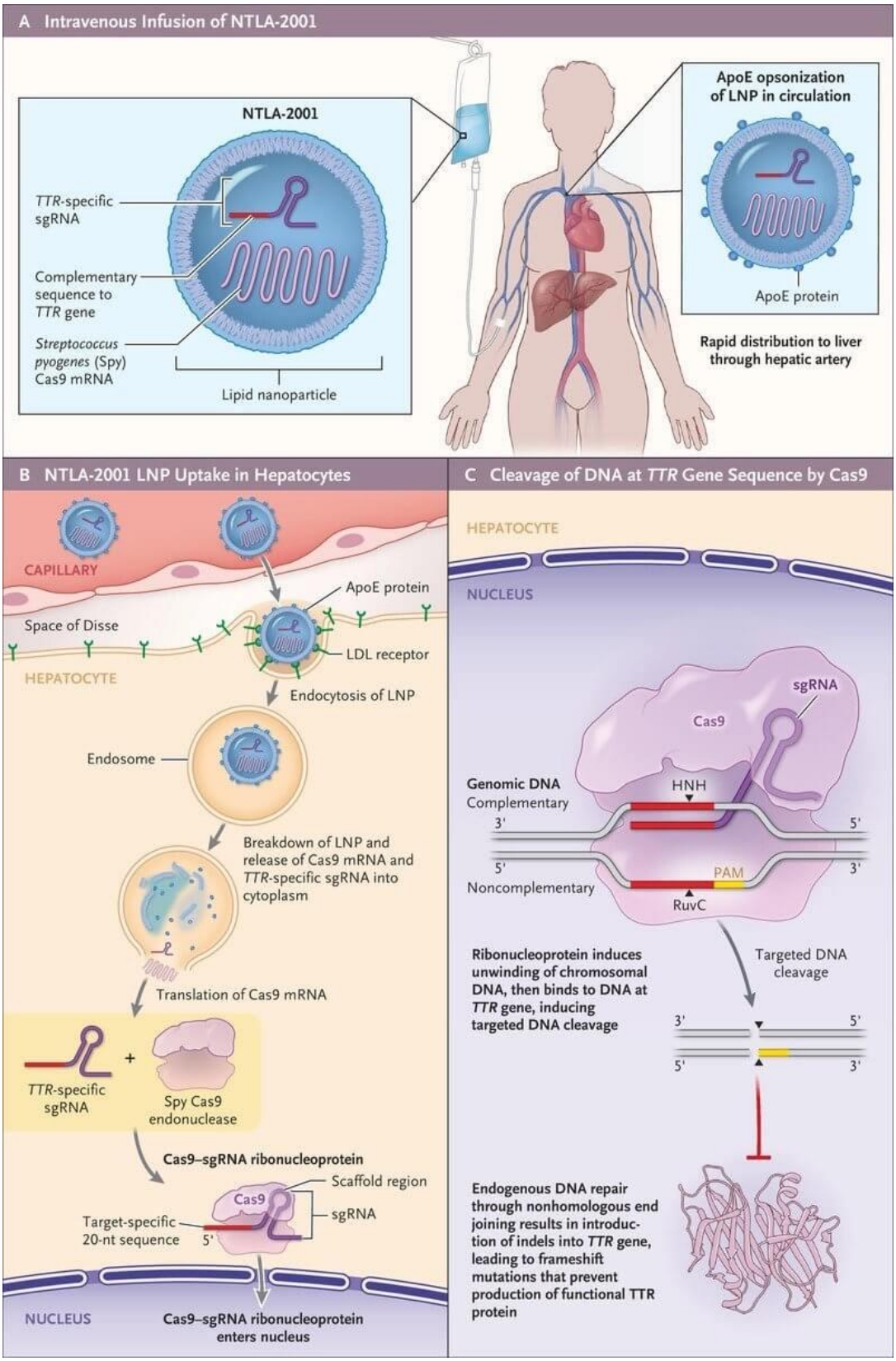

Figure 6. Mechanism of action of NTLA-2001 (LNP-delivered CRISPR gene therapy)

Of course, in addition to the commonly used biological and chemical delivery methods, scientists are gradually experimenting with some physical methods for the delivery of gene editing tools, and have made good research progress. Although the CRISPR/Cas system has shown excellent therapeutic potential in in vitro cells and in vivo animal models, the clinical translation application of CRISPR gene therapy is still difficult, so the continuous optimization and improvement of delivery strategies and vectors will open up unlimited possibilities for gene editing clinical therapies.

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