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Exploration of the Application of Biological Synthesis Technology in Stable Expression Gene Editing Systems

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ABSTRACT

Gene editing technologies such as CRISPR-Cas9 and TALEN have become core tools for life science research and biotechnology industrialization. However, their clinical translation and large-scale application are limited by three major issues: low instantaneous expression efficiency, high off target risk, and strong cytotoxicity. The stable expression gene editing system can improve editing efficiency and safety through continuous and controllable tool expression, while biosynthetic technology provides a key solution for the construction of this system with modular design, precise regulation, and scalability advantages. Starting from the core principles of biosynthetic technology, this article analyzes its application paths in carrier engineering optimization, regulatory element design, and host adaptation modification. Combining practical cases of prokaryotic, eukaryotic, and microbial community host systems, it explains how it solves the problems of "expression persistence," "regulation precision," and "host compatibility" in stable expression. Research has shown that standardized design of synthetic biological elements such as inducible promoters and miRNA response modules, optimization of AAV capsids, modification of vector skeletons such as non viral vector molecular design, and dynamic regulation of gene pathways can effectively improve the stable expression efficiency of gene editing systems while reducing off target rates and cytotoxicity.

KEYWORDS

Biological synthesis technology; Gene editing system; Stable expression; Vector engineering; Synthetic biological components; Host compatibility

1. INTRODUCTION

After the emergence of CRISPR-Cas9 technology in 2012, gene editing quickly extended from laboratory "tools" to clinical treatment, agricultural breeding, and industrial microbial modification. However, mainstream editing systems often rely on transient transfection (such as plasmid transfection and RNA delivery), which faces two bottleneck problems: first, the editing tools only have 1-3 days of activity in cells, and the efficiency of multi site editing and large fragment insertion is low; Secondly, it is difficult to precisely regulate the timing, intensity, and cell specificity of expression, and it is prone to off target effects or cell toxicity caused by excessive Cas protein. Many studies have shown that clinical trials of multiple types of gene therapy have been delayed or terminated due to the failure of stable expression system construction, and stable expression is the key to the implementation of gene editing [1]. Biological synthesis technology is the core of synthetic biology, which designs biological components and gene circuits with engineering ideas to accurately regulate metabolic pathways and gene expression. The advantages are very practical: the components can be standardized, such as building reusable promoter and termination libraries, which is convenient for editing system assembly; Can dynamically regulate, such as using tetracycline, light "switch" editing tools, or relying on miRNA circuits to achieve "stop as needed"; It can also adapt to different

hosts, customize expression systems for prokaryotic cells (such as E. coli), eukaryotic cells (such as HEK293, CHO), and microbial communities, and solve the problem of "host vector incompatibility". This article focuses on how biosynthetic technology can assist gene editing in building stable expression systems. Firstly, the adaptation mechanism between the two is explained. Then, through case studies, vector modification, regulatory element design, and host optimization pathways are discussed. Finally, bottlenecks and solutions are analyzed to provide reference for the stable implementation and industrialization of editing technology.

2. BIOLOGICAL SYNTHESIS TECHNOLOGY: BUILDING AN "ADAPTATION FRAMEWORK" FOR GENE EDITING

Biological synthesis technology is like being an "engineer" for biological systems - using engineering thinking to help it build frameworks and optimize. It has three "core tools": standardized biological components, modular genetic circuits, and programmable host chassis. These three aspects perfectly match the demand for stable expression in gene editing, and can solve the old problems of traditional gene editing from the perspectives of "sustained expression, precise regulation, and host compatibility". Standardized biological components are like "prefabricated parts" for building a house, without the need to search for natural components in a scattered manner. Targeted modifications are made to the "biological building blocks" such as initiators and terminators, and after understanding their functions, they are assembled into a component library with a unified interface that can be reused. To achieve precise expression of Cas protein, we need to modify the Lac promoter in Escherichia coli and the CMV promoter in mammalian cells to create a "gradient promoter library" that can reduce toxicity with weak expression and improve efficiency with strong expression. There is also a chicken β - globin insulator that can block the host genome's "position effect", preventing the editing system from being "buried" in heterochromatin and "sleeping". This technique has been used in stable CRISPR-Cas9 cell lines for a long time, and the editing efficiency has been greatly improved. Modular gene circuits are "smart switch boxes" that regulate gene expression by logically assembling promoters and coding sequences. For example, in the Tet On system, with the addition of doxycycline, Cas9 expression is upregulated; Withdrawing medication can quickly shut down, reducing the risk of random editing during the window period [2]. A more clever feedback loop - adding an miRNA response element to the Cas9 sequence, the miRNA produced when editing the target gene will "hold" Cas9 in reverse, forming a closed loop of "shutdown after editing", not afraid of damaging cells with too many proteins. Programmable host chassis is a "customized nest" for editing systems, slightly modifying the host genome to solve the problems of "host vector conflict" and "heavy metabolic burden". In prokaryotic cells, knocking out the gene responsible for E. coli degradation of exogenous vectors allows CRISPR-Cas9 to be stably expressed for a period of time; In eukaryotic cells, PhiC31 integrase is used to precisely "deliver" the editing system to transcriptional active regions such as GAPDH loci, and expression stability is immediately improved - this method has long been used in antibody drug editing. At the end of the day, this combination of "parts+switches+little nest" perfectly meets the needs of gene editing: laying the foundation for components, controlling switches through circuits, and ensuring long-lasting chassis. The combination of the three not only solves traditional problems, but also sets up a framework for stable expression systems.

3. BIOLOGICAL SYNTHESIS TECHNOLOGY: CREATING A 'RELIABLE SHUTTLE BUS' FOR GENE EDITING

The carrier is the "shuttle" for gene editing systems to enter host cells, and stability, targeting, and loading capacity directly determine the success or failure of editing. Traditional vectors always fall apart: conventional plasmids are prone to loss, wild-type viruses have poor targeting, and low loading capacity. Biological synthesis technology can upgrade the carrier by modifying the skeleton,

installing targeting elements, and expanding the loading capacity, making it go from "barely usable" to "stable and reliable", and becoming a key support for stable expression. Transforming virus vectors, breaking through the "safety" and "targeting" barriers at the core. Adeno-associated virus (AAV) is a frequent participant in gene therapy, but its natural capsid targeting is weak and prone to immune rejection. By establishing a mutation library through "directed evolution of the capsid", "precise navigation" variants can be obtained. The modified AAV reported in Science Translational Medicine has significantly increased liver targeting efficiency and reduced immune attack, which has helped CRISPR-Cas9 target the liver. In a hemophilia mouse model, the editing efficiency has remained stable for more than six months, and coagulation factors have reached the standard. To deal with AAV's "low loading", the "dual vector complementary system" works by disassembling Cas9 and dividing it into two AAVs, which can be assembled into larger editing elements after entering the cell. Optimize non viral vectors to solve the problem of difficult and unstable transfection. Lipid nanoparticles (LNP) are popular, as natural lipids tend to clump together and are difficult for cells to absorb. Customized synthetic lipids can break the deadlock. The synthetic lipids mentioned in Nature Materials, after adjusting the tail chain, not only improve LNP editing efficiency but also enhance lung targeting. There have also been breakthroughs in polymer carriers, such as the synthesized poly (β - aminoester) (PBAE), which can adjust molecular weight and charge and accurately dock with cells. Constructing a stable HEK293 cell line using it, with a transfection efficiency of 80% and stability for over 4 weeks, outperforming traditional plasmids [3]. To ensure the long-term retention of the carrier, we rely on chromosome integrated carriers. Traditional plasmids are prone to loss in prokaryotic cells and require antibiotic screening to increase burden. Through 'site-specific integration', the editing system can be inserted into the 'safe zone' of the host chromosome. When modifying industrial microorganisms, inserting CRISPR-Cas9 into specific gene loci of Bacillus subtilis not only allows the editing system to take root, but also enables screening based on amylase activity disappearance without the need for antibiotics. When used for cellulase gene editing, the efficiency remains stable for more than 10 generations. At the end of the day, biosynthetic technology can either enable viral vectors to "navigate accurately," or give non viral vectors "strength and health," or allow vectors to "root" in chromosomes, upgrading the "shuttle bus" from three aspects and providing a foundation for stable gene editing expression.

4. BIOLOGICAL SYNTHESIS TECHNOLOGY: INSTALLING "PRECISE REGULATORY VALVES" ON GENE EDITING SYSTEMS

The gene editing system needs to function steadily and accurately. Just being "resident" is not enough, and it needs to be "obedient" - it needs to "work" at a specific time and intensity in the cell, in order to make fewer mistakes and not damage the cell. Biological synthesis technology can precisely design regulatory elements with "spatiotemporal specificity and dynamic responsiveness" for it, allowing expression to be freely regulated, which is the core of a stable expression system. To activate the editing system in a specific time and space, inducible regulatory elements are the core lever. Transforming natural systems or synthesizing new switches can create "control keys" that respond to small molecules, light, and temperature. For example, the tetracycline induction system can modify the structure of the repressor protein, and adding doxycycline can "wake up" Cas9; The optimized version not only significantly improves induction efficiency, but also minimizes "random startup" when there is no inducer, avoiding unnecessary editing. Light induced elements are more flexible, such as the PhyB PIF system, which is activated by red light and deactivated by far red light. They have been used for zebrafish embryo editing, allowing only local cells to be edited by shining on specific areas, avoiding developmental difficulties [4]. If the editing system only works on the target cell, the miRNA response element is the "identity card". Different cells have different miRNA "fingerprints". When specific miRNA target sites are added to the editing system, non target cell miRNAs will be "disassembled" by the tool, making them unable to express, while target cells will allow the tool to work stably. For example, when editing T cells, adding the miR-126 target site -

hematopoietic stem cells with more miR-126 can compress Cas9, while fewer T cells allow it to function, which not only improves efficiency but also reduces damage; Adding multiple miRNA target sites enhances specificity and benefits cell therapy. To make the editing system "not lazy, but powerful", the feedback adjustment component is the "balance scale". Too many tools can damage cells and too few can be inefficient. Feedback components can be adjusted by focusing on "expression intensity" and "editing progress". For example, gene repair will generate a "brake signal" to turn off Cas9; When Cas9 causes excessive damage to cells, the DNA damage signal triggers a "slowing down key" to reduce its expression, which not only preserves the effect but also protects the cells. Ultimately, biosynthetic technology relies on these three types of "regulatory experts" to make gene editing systems both precise and secure, resolving the contradiction between safety and efficiency in stable expression.

5. BIOLOGICAL SYNTHESIS TECHNOLOGY: CUSTOMIZED GENE EDITING "FERTILE GROUND" FOR DIFFERENT HOSTS

The stable expression of gene editing requires specific host support, but there are significant differences in cell structure and gene expression logic among prokaryotes, eukaryotes, and microbial communities, making a one size fits all universal system impractical. Biological synthesis technology can provide customized modification solutions for hosts, eliminate compatibility barriers, and build stable expression platforms for different scenarios. Facing prokaryotic hosts such as Escherichia coli and Bacillus subtilis, the two major pain points of targeted technology are the instability of vectors and high toxicity of editing tools. Restriction endonucleases in prokaryotic cells can easily "destroy" recombinant vectors, and excessive Cas protein can inhibit bacterial growth. The technology is cracked in two steps: first, knock out the defense genes that affect the stability of the vector, reducing vector degradation and abnormal genome recombination; Using weak promoters to control Cas protein expression, combined with a toxin antitoxin system, balances toxicity and locks the carrier. This scheme has been used for the synthesis and modification of lysine in Escherichia coli. Through stable expression editing system site directed mutagenesis of key genes, the production can be increased without vector loss during fermentation [5]. The core challenge for eukaryotic hosts such as mammalian cells and yeast is "difficult integration and easy silencing" - foreign genes entering complex genomes are often "sealed" due to "position effects". Two paths break the deadlock: one is to rely on PhiC31 integrase and Cre loxP system to accurately "plant" the editing system into the transcription active region (such as human cell AAVS1 site, yeast HIS3 site), such as in CHO cell antibody modification, which can stabilize expression for more than six months with minimal yield fluctuations; The second strategy is to add insulators and histone modification enzyme genes to the carrier to prevent heterochromatin diffusion and maintain chromatin "openness". Relevant strategies can be found in professional journals, which can enhance Cas protein expression and avoid silencing. The transformation of microbial communities (such as gut microbiota) requires addressing "uneven expression and mutual interference". Technology uses quorum sensing signals to regulate - triggering editing systems with microbial signals to ensure synchronized expression of bacterial strains; Customize exclusive carriers, such as setting suitable replication starting points for Escherichia coli and Bifidobacterium, to avoid carrier "wandering". In the cholesterol lowering modification of gut microbiota, only Escherichia coli expression editing system is used, and Bifidobacterium is not affected, which not only maintains the stability of the microbiota but also achieves the goal. At the end of the day, biosynthetic technology is tailored to different hosts, breaking through compatibility barriers between host editing systems and equipping industrial production, gene therapy, and microbial community modification with stable expression equipment.

6. THE CHALLENGES AND BREAKTHROUGHS OF BIOLOGICAL SYNTHESIS TECHNOLOGY

Although biosynthetic technology has disrupted the stable expression of gene editing, it is still hindered by three core challenges - safety concerns, insufficient regulation accuracy, and high cost of scaling up, which hinder its industrialization. Only by focusing on technology, process, and standards can we promote its implementation. The most tricky issue is undoubtedly safety concerns: viral vectors (such as AAV and lentiviruses) may randomly insert into carcinogenic regions of the host genome. The New England Journal of Medicine has recorded that when lentiviral vectors are used for SCID-X1 gene therapy, they accidentally cause patients to develop T-cell leukemia; Editing tools may also accidentally cut off non target sites due to sequence similarity, posing health risks. There are three ways to break the deadlock: relying on zinc finger nucleases to create a "safe integration vector" and accurately "anchor" the editing system at safe sites such as AAVS1; Transforming high fidelity proteins such as Cas9-HF1 to reduce non-specific binding; Combining whole genome sequencing and computational prediction to identify off target risks in advance. Insufficient regulation accuracy becomes a weakness in reducing efficiency: the induction system either "quietly expresses" (leaked expression) without induction, or responds with lag (such as waiting for more than 12 hours for light induction), and the specificity of miRNA response elements also needs to be improved. The response can be approached from three aspects: pairing the Tet On system with miRNA response elements, and initiating editing under dual conditions (induction of inducers+low expression of miRNA in target cells); Transforming transcription factors to optimize induction kinetics and shorten response cycles; High throughput screening of cell specific miRNA combinations for more precise components. The scale cost is an unavoidable reality: the preparation of biological components relies on high-throughput platforms, the production process of carriers is complex, and the cost remains high. The cost reduction path is very clear: promote internationally recognized component standards such as BBF RFC to reduce redundant development; Using Escherichia coli fermentation to produce non viral vectors, simplifying the process; Constructing engineered strains to replace traditional production systems and improve carrier output efficiency. In addition, the integration of the two also requires crossing ethical regulatory lines - human germ cell editing may alter genetic information, and strict review mechanisms must be established [6]. In the future, only through technological innovation, standard building, and ethical norms can biosynthetic technology inject safe and efficient power into stable gene editing expression.

7. CONCLUSION

This article focuses on the core of "biosynthetic technology empowering gene editing and stable expression", and outlines the path to solving the core problem of gene editing and stable expression from principles, practice to challenge optimization. This technology system, which is based on the skeleton of "components circuit chassis", is actually the "cornerstone" of a stable expression system - providing a standardized foundation, building a dynamic control core, and creating a platform that adapts to the host. In principle, standardized components such as gradient promoters and insulators have resolved the dilemma of "component incompatibility" in traditional systems; Modular gene circuits (such as inducible and feedback regulation circuits) enable more precise regulation of editing tools; The modified host chassis (such as engineered E. coli and CHO cells) eliminates the "host carrier conflict". The joint efforts of the three can not only improve stable expression efficiency, but also reduce off target risks and cell toxicity, paving the way for the implementation of the technology. In practice, from carrier engineering to regulatory element design, and then to host modification, this technology has covered three major systems: prokaryotic, eukaryotic, and microbial communities, forming a complete chain of "optimizing carriers - precise regulation - adapting to hosts". AAV capsid directed evolution enhances liver targeting, Tet On system optimization reduces leakage expression, and PhiC31 integrase achieves targeted integration in eukaryotic cells - these explorations are helping

gene editing move from the laboratory to the industry. Faced with the challenges of safety, accuracy, and cost, "security integration carriers," "high fidelity tools," and "low-cost processes" are the breakthrough directions. In the future, with the integration of technology and improved regulation, it will emit light and heat in genetic disease repair, microbial modification, and crop breeding. Biological synthesis technology is not only the "pillar" of stable expression, but also the "engine" of industrialization. Only through continuous innovation, establishing standards, and strong regulation can it inject momentum into life sciences and human health.

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