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Unraveling the Power of Transcription Activator-Like Effector Proteins

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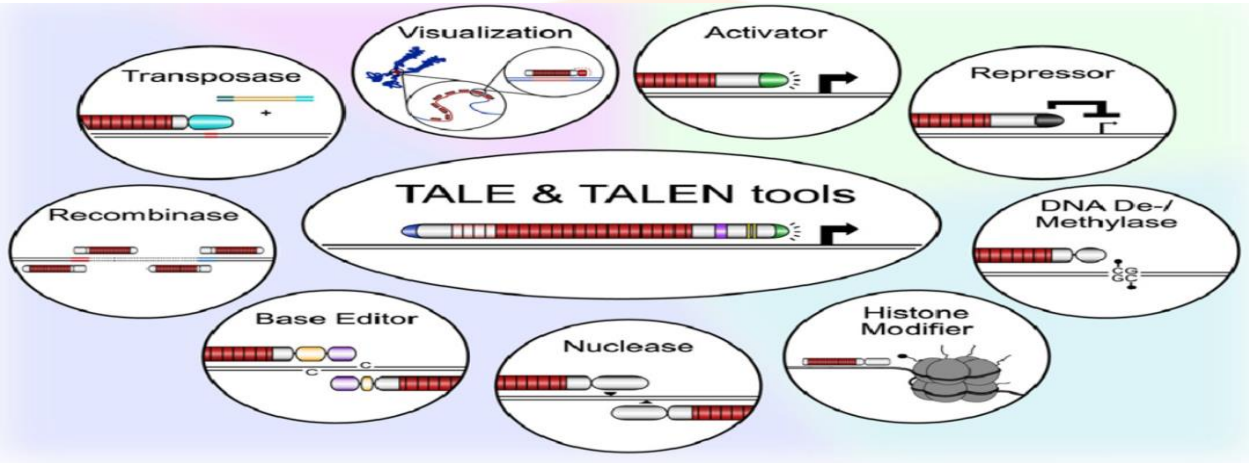
Abstract:

Precise study of genomes of Plant and Animal have provide us evidences for collecting data for biotechnology and medicine, however nucleotide sequence of genomes is not enough to perceive the function and relationship in phenotype formation and disease etiology. In post-genomic era methods allowing genomic DNA sequences manipulation, visualization and regulation of gene expression are rapidly evolving. Their application in multiple species brought targeted mutagenesis to the attention of scientists worldwide. Key breakthrough successes of genome editing have since been achieved using TALEN, among these, the first commercialization of an edited crop and the first human cured from cancer. TALEN have since been largely replaced by the CRISPR technologies which are somewhat easier to build, much easier to multiplex, and have spawned multiple derived techniques. Nevertheless, the flexible and precise positioning of TALEN is unmatched, and thus they have continued to evolve to new functionalities. Here, we assemble essential facts, design guidelines as well as important past and exciting novel developments..



Introduction:

Genetic engineering emerged in the laboratory of Paul Berg in 1972 in the form of a recombinant DNA technology, when scientists combined the *E. coli* genome with the genes of a bacteriophage and the SV40 virus. Since then, this science has achieved tremendous success; the molecular genetic mechanisms and phenomena that can now be reproduced *in vitro* have been discovered and studied in detail. Studies in the field of molecular genetics and biochemistry of bacteria and viruses have allowed the development of methods to manipulate DNA, generate various vector systems and methods for their delivery to the cell. All of this has enabled not only transgenic microorganisms production, but also genetically modified plants and animals. The application area of genetic engineering has experienced rapid development, which provided the impetus for

progress in selection and biotechnology. However, the conventional genetic engineering strategy has several drawbacks and limitations, one of which is the complexity of manipulations with large animal and human genomes.

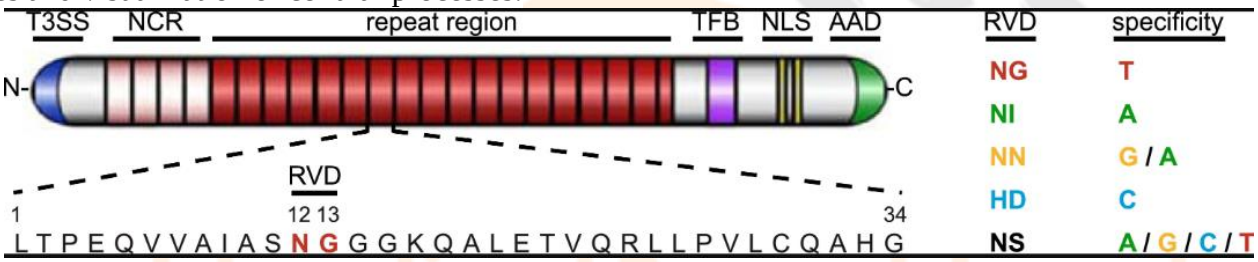
THERE ARE SOME GENETIC TOOL APPEARED IN EARLY 2011 AND 2013

1. CRISPR-Cas9: This system is the most well-known and widely used genetic editing tool. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and Cas9 (CRISPR-associated protein 9) allow scientists to target specific DNA sequences and make precise edits, such as insertions, deletions, or replacements.
2. TALENs (Transcription Activator-Like Effector Nucleases): TALENs are customizable enzymes that can also be used for targeted gene editing. They work by binding to specific DNA sequences and inducing double-strand breaks, which can then be repaired by the cell's own DNA repair machinery.
3. ZFNs (Zinc Finger Nucleases): Like TALENs, ZFNs are customizable enzymes that can be engineered to target specific DNA sequences. They consist of a zinc finger DNA-binding domain fused to a nuclease domain, which induces double-strand breaks in the DNA.

Base Editors: Base editors are a newer class of genetic editing tools that allow for more precise changes to individual DNA bases without inducing double-strand breaks. They typically consist of a catalytically inactive Cas protein fused to a deaminase enzyme, which can convert one DNA base to another without cutting the DNA strand.

4. Prime Editing: Prime editing is a recent advancement in genetic editing that enables precise DNA sequence modifications without the need for double-strand breaks. It uses a modified Cas9 protein fused to a reverse transcriptase enzyme, allowing for targeted insertions, deletions, and substitutions of DNA bases

Here we generally review application of these systems for genome editing in conventional model objects of current biology, functional genome screening, cell-based human hereditary disease modeling, epigenome studies and visualization of cellular processes.



HISTORY AND DEVELOPMENT OF TALE EDITING TOOL

TALEN were the first easy-to-use genome editing technology and sparked the genome editing revolution. It have since been largely replaced by the CRISPR technologies which are somewhat easier to build, much easier to multiplex, and have spawned multiple derived techniques.

TALE proteins were initially discovered in plant pathogenic bacteria of the genus Xanthomonas, which cause diseases in various crops such as rice, citrus, and peppers. Researchers observed that these bacteria secrete proteins called transcription activator-like effectors (TALEs) into plant cells to manipulate gene expression and promote infection.

Strucuture and Function of TALE

TALE is a special class of proteins that can bind DNA. TALEs offers flexible applications in genetic engineering due to its compatibility with many functional domains. Different associations of TALE proteins with transcriptional activators, repressors, or endonucleases give them potential transformation from transcriptional modulators to genome editing tools. A typical TALEN unit comprises a central DNA-binding domain of 12-28 repeats, a nuclear localization signal (NLS), an acidic domain for target gene transcription activation, and Fok1 nuclease. The DNA-interacting region is a preserved sequential arrangement of significantly constant 33-35 amino acids with polymorphic 12 and 13 repeat variable di-residues (RVDs). Each repeat uniquely binds to a single nucleotide in the 5′ to 3′ orientation on the target. The biochemical

structure-function studies suggest that the amino acid present at the place 13 uniquely identifies a nucleotide on the DNA target major groove. This DNA-protein interaction unit is stabilized by the amino acid at place 12. At the 3′-end of target locus, a half repeat of only 20 amino acids exists to bind the DNA sequence.
The four most common RVDs identified by various experimental validations are NN, NG, HD, and NI with unique preferential binding affinity toward G/A, T, C, and A respectively bestowing target specificity. Remarkably screening of all 400 possible combinations of RVDs is also reported, which are considered as non-conventional RVDs because of their rare existence in nature.
The popularly used TALEN system comprises 2 units of DNA binding domain (DBDs) from TALE proteins. Each unit is attached with a catalytic domain from Folk1 restriction enzyme. Fok1 nuclease of the TALENs dimerizes which generates a cleavage on both the strands of DNA-double helix, activating the DNA repair machinery to fix disruption.
As we align repeat modules (RVDs) in a particular structure, it is possible to create TALENS with the required sequence precision. There is, however, a limit to the option of target sites for TALEN. A thymine at position 0, i.e., immediately precursory to the TALE-repeat bound sequences is invariably required. The complete gene activation is ensured by the weak van der Waal forces acting between the C5 methyl group of thymine and the extremely conserved tryptophan in the N-terminal. Newer versions of TALEs are also reported in nature that replaces thymine with cytosine at position 0 with no effect on their activity. These scaffolds are independent of the prerequisite 5′T Nonetheless, customizing TALE-based tools to alter any genome is quite versatile but simple. The crystal structure of TALE proteins bound to target DNA reveals that each repeating unit forms a v-shaped structure consisting of two alpha helix assembled to form a solenoid-like structure wrapped around the major groove of DNA via the hypervariable 12 and 13 amino acids

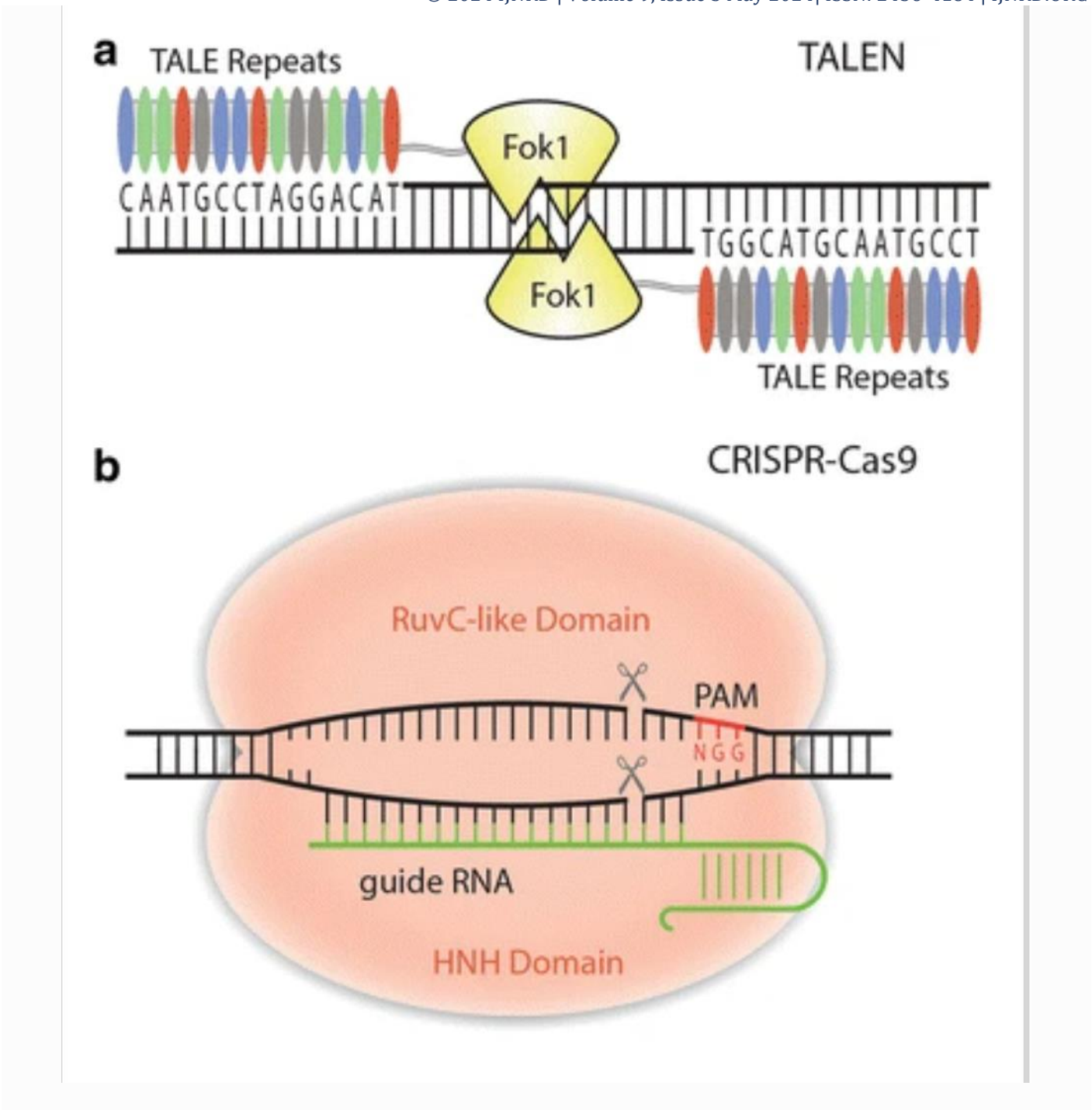
TALE as a precise and versatile editing tool compare to other gene editing tools

OFF TARGET EFFECTS

A CRISPR gRNA targets ~20 bp sequence, whereas a TALEN pair binds to a total of ~36 bp target sequence. In addition, Cas9/gRNA complex has higher tolerance for sequence mismatches (up to 5 bp mismatches) than TALEN does. Therefore, TALEN-mediated cleavage has better specificity than CRISPR, and off-target cleavage in the genome by TALEN is unlikely. In contrast, off-target effects have been reported for CRISPR in cell lines, though analyses of CRISPR knockout mice suggest lower off-target frequency in vivo

TARGET SITE

TALEN can be generated at a specific position of the gene. To precisely edit a specific genomic site using CRISPR, a homologous recombination donor vector or long oligo containing the desired edit sequence flanking by the immediate upstream and downstream TALEN can be generated to specifically target nearly any sequence in the genome. In contrast, target site selection for CRISPR is limited by the requirement for a PAM sequence (typically NGG) sequence located on the immediate 3’ end of the gRNA target sequence. This is no barrier to knocking out genes because cleavage anywhere in the gene is potentially effective, but may present difficulties in generating site-specific mutations or insertions that require cleavage homology arms of the target site can be delivered to the cells together with gRNA(s) and Cas9, in order to guide HDR (homology directed repair)-mediated DNA repair at the target site.



Application

1. Genome Editing with TALE Nucleases (TALENs):
   - TALENs are engineered nucleases composed of a TALE DNA-binding domain fused to a nuclease domain (e.g., FokI).
   - TALENs can be designed to target specific genomic loci, inducing double-strand breaks (DSBs) at desired sites.
   - These breaks stimulate cellular repair mechanisms, such as non-homologous end joining (NHEJ) or homology-directed repair (HDR), enabling precise genome editing.
   - TALENs have been utilized in various organisms, including plants, animals, and microorganisms, for applications such as gene knockout, gene correction, and gene insertion.
2. Gene Regulation and Transcriptional Control:
   - TALE-based transcription factors (TALE-TFs) can be designed to modulate gene expression by targeting specific promoter or enhancer regions.
   - By fusing transcriptional activation or repression domains to TALE DNA-binding domains, researchers can control the transcriptional activity of targeted genes.
   - TALE-TFs have been employed to study gene function, manipulate cellular pathways, and engineer synthetic gene circuits for biotechnological applications.

3. Epigenome Engineering:
   - TALE proteins can be customized to target specific epigenetic marks, such as DNA methylation or histone modifications.
   - By fusing effector domains with epigenetic-modifying activities to TALE DNA-binding domains, researchers can precisely modify the epigenetic landscape of targeted genomic regions.
   - Epigenome engineering using TALE proteins enables the study of epigenetic regulation and potential therapeutic interventions for epigenetic disorders.
4. Disease Modeling and Drug Discovery:
   - TALE-based genome editing tools have been utilized to generate cellular and animal models of human diseases.
   - These models facilitate the study of disease mechanisms, screening of potential therapeutic compounds, and development of personalized medicine approaches.
   - TALENs and TALE-TFs have been employed in drug discovery pipelines for target validation, compound screening, and mechanism elucidation.
5. Agricultural Biotechnology:
   - TALENs have been used for targeted genome modifications in crop plants to confer desirable traits, such as disease resistance, improved yield, and enhanced nutritional content.
   - TALE-based transcriptional regulators have been applied to modulate the expression of endogenous genes involved in plant development, stress response, and metabolic pathways.
6. Therapeutic Applications:
   - TALENs hold potential for therapeutic genome editing in the treatment of genetic disorders, viral infections, and cancer.
   - TALE-based gene regulation strategies are being explored for the modulation of disease-related pathways and the development of gene therapy approaches.

Conclusion

In conclusion, the extensive research conducted on Transcription Activator-Like Effector (TALE) proteins underscores their profound impact and versatility in genetic engineering, molecular biology, and biotechnology. Through their remarkable ability to precisely bind to specific DNA sequences, TALE proteins have revolutionized the field of genome editing, transcriptional regulation, and epigenome engineering.

The development of TALE nucleases (TALENs) has enabled precise genome modifications with wide-ranging applications, from generating disease models to engineering crop plants with improved traits. Similarly, TALE-based transcriptional regulators have provided powerful tools for studying gene function, manipulating cellular pathways, and developing novel therapeutic strategies.

Despite the remarkable progress achieved, challenges such as off-target effects and optimization of TALE-based tools persist, underscoring the need for ongoing research and innovation in this field. Additionally, the integration of TALE technology with other genome editing platforms, such as CRISPR-Cas systems, holds promise for further enhancing precision and efficiency in genetic manipulation.

Looking ahead, the future of TALE research is promising, with potential applications extending to areas such as synthetic biology, personalized medicine, and environmental biotechnology. Continued advancements in TALE protein engineering, delivery methods, and bioinformatics tools will undoubtedly unlock new possibilities and broaden the scope of their applications.

In essence, the journey of exploring TALE proteins has not only deepened our understanding of molecular biology but also paved the way for groundbreaking discoveries and transformative technologies with profound implications for human health, agriculture, and beyond. As we embark on the next chapter of TALE research, it is imperative to remain vigilant, collaborative, and innovative, leveraging the power of these remarkable proteins to address pressing challenges and realize the full potential of genetic engineering and biotechnology.

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