TRANSCRIPTION ACTIVATOR-LIKE EFFECTOR NUCLEASES-AMAZING TOOL FOR GENOME EDITING

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ABSTRACT

Transcription Activator-Like Effector Nucleases (TALENs) are used to cut specific target DNA sequence in order to knock out a gene or to change its sequence. They are formed by the fusion of TALE protein serving as DNA binding domain along with non-specific DNA cleaving nucleases. Its specificity towards DNA bases in the target sequence is derived from the structure of the DNA binding domain, consisting of variable pair of amino acids in middle of the tandem array of repeated segment. Earlier targeted genome editing was performed using Zinc Finger Nucleases (ZFN). Recently TALENS have rapidly emerged as alternative to Zinc Finger Nucleases for genome editing and introducing targeted Double Stranded Break. The TALEN approach improves on tools currently available for genome modification. This breakthrough could eventually make it possible to efficiently modify plant, animal and even human genomes. Targeted nucleases offer the potential to correct or disrupt the gene product or sequences that causes the disorder and thus motivates the strategies for treatment of wide range of genetic and other diseases. The road to practical use of TALENs could still contain potholes. But particularly to academics, the potential of TALENs seems limitless.

Keywords: TALENs, TALE Proteins, Zinc Finger Nucleases, Genome Modification

1. INTRODUCTION

1.1. TALENS

Newly developed engineered nuclease Transcription Activator-Like Effector Nucleases (TALENs) can efficiently modify any sequence of interest in living cells or organisms. It can make replacement and gene-editing therapies, Double-strand DNA breaks at specific sites in living cells, possibly leading to better gene. TALENs are hybrid protein containing a DNA binding domain and nonspecific fokI nuclease domain. DNA binding domain is derived from TALE protein secreted by a plant pathogen- Xanthomonasspp that attacks more than 350 plant species and causes diseases such as citrus canker and black rot (Kay and Bonas, 2009). TALE protein from bacteria alters the gene transcription in host plant (Ky a et al., 2007). The DNA binding domain binds specifically to the target sequence to be cleaved and the nuclease introduce double-stand breaks, thus providing an efficient tool for targeted genome engineering (Christian et al., 2010; Boch et al., 2009).

1.2. Structure of TALENs

The fundamental building block of DNA binding region of TALENs i.e., TALE protein consists of multiple sets of highly conserved repeat domain of same or almost same 34 amino acids occurring in tandem (Schornack et al., 2006; Ackerveken et al., 1996; Zhu et al., 1998). As shown in the Fig. 1, each repeat targets a specific base of DNA, identity of which is determined by a hyper variable pair of amino acid typically found at position 13 and 14 of the repeat. Each of these pair of amino acids binds to specific nucleotide in the target DNA (Boch et al., 2009; Moscou and Bogdanove, 2009; Herbers et al., 1992; Yang et al., 2005). Rest of the 34 amino acids in the repeats is nearly always the same. Thus the overall number of repeats matches an equal number of bases. In 2010, first nucleases were attached to TALE proteins for the purpose of genome engineering.
1.3. Similarity with Zinc Finger Nucleases

Zinc fingers are finger like sections of certain DNA binding proteins found in mammals (Elrod-Erickson et al., 1996; Isalan et al., 1997). They home in on specific target sequences of DNA. A different Zinc finger recognizes different sets of three bases (Bibikova et al., 2003). When combined with nucleases, they can knock out genes and also introduce new versions of genes at specific location in genome (Urnov et al., 2005; Wood et al., 2011). Several types of genomic alterations can be introduced with ZFNs, including point mutations, deletions, insertions, inversions, duplications and translocations, thus providing researchers with unprecedented tools to perform genetic manipulations (Wood et al., 2011; Townsend et al., 2009).

1.4. Problems with ZFN

ZFN are difficult to design and are expensive. TALENs could provide a cheaper alternative. As TALENs have high rate of cleavage activity and limitless targeting range, genome engineering using TALENs was named the 2011 method of the year by nature publishing group. Almost everybody who has once worked on ZFN is now shifting to TALENs, not only because of ease of making TALENs (Boch et al., 2009; Moscou and Bogdanove, 2009) but unlike ZFN, Sangamo Gregory doesn’t have a lock on TALENs. Sangamo controls almost all of the IPR to ZF technology, which is the main reason for the high cost of ZFN. But no patents have been issued yet in the field of TALENs.

1.5. Applications

Researchers using TALENs have engineered genomes in yeast, nematodes, tobacco, Arabidopsis, fruitfly, roundworm, cricket, zebrafish, frog, rat, pig, cow, thale cress, rice and silkworm. TALENs have also been used to introduce specific insertions in human somatic and pluripotent stem cells using double-stranded donor templates.

1.6. Gene Therapy

In contrast to therapies that treat symptoms of genetic diseases, the ability to target essentially any DNA sequence with TALENs will undoubtedly motivate the exploration of both gene-correction and gene-disruption strategies for the treatment of a wide range of genetic and other diseases (Miller et al., 2010). TALENs can correct the genetic defect underlying sickle cell disease, α1-antitrypsin disease and Parkinson's disease. TALENs can also go into a cell and turn on a specific gene, giving finer control over gene expression (Joung and Sander, 2013).

1.7. Livestock

TALENs have also been a welcome addition to the toolbox of those seeking to genetically modified pigs, cows and livestock to make versions that are more useful for biomedical research or food production. Precision crossbreeding can be done using TALENs that can greatly speed up the introduction of new traits to livestock. TALENs have been used to inactivate the gene encoding Low-Density Lipoprotein (LDL) receptor in pigs, thereby generating a model for familial hypercholesterolemia (Carlson et al., 2012).

1.8. Agriculture

Nuclease-mediated editing of agricultural plants having long reproductive cycles may greatly decrease the time required to generate new agriculturally-relevant varieties compared with traditional breeding strategies. To date, TALENs have been used to introduce knockout mutations in Arabidopsis thaliana (Morbitz et al., 2010) and to confer resistance to infection by Xanthomonas bacteria in rice by disrupting the target sites of naturally occurring TALEs that contribute to pathogenicity.

1.9. Miscellaneous

Targeted insertions could be used to fuse endogenous genes to genes encoding fluorescent proteins or epitope
tags to visualize protein expression, distribution and interactions. In addition to the generation of such fusions, HDR-based approaches might be used to create isogenic human or other mammalian cell lines bearing specific Single Nucleotide Polymorphisms (SNPs), thereby potentially enabling studies to determine the functional significance of these sequence variants (Geißler et al., 2011).

1.10. Limitations

TALENs are bigger and bulkier than zinc finger nucleases, so getting them to their target DNA may sometimes be tricky. Also the repetitive nature of TALEN means that the gene encoding it, once delivered into the cell, may be more susceptible to DNA rearrangements where the nuclease cut the unintended DNA sequence.

2. CONCLUSION

Formation of effective genome editing tools is requisite for fundamental research, genetic engineering and gene therapy. Efficient construction and application of Transcription Activator-Like Effector Nucleases (TALENs) in several organisms precede an exciting new era for genome editing. TALENs have already begun to revolutionize research and medicine and their potential over the coming years seems enormous. Basic biologists can study molecular pathway gene-by-gene, protein by protein. Plant biologists will be able to introduce multiple traits at once. It will just become a tool that every molecular biologist has in the lab. Another potential area for future exploration will be the creation of fusion proteins that harbor domains others than nucleases.

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