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Minireview

A TALE of Two Nucleases: Gene Targeting for the Masses?

Karl J. Clark,[1] Daniel F. Voytas,[2] and Stephen C. Ekker[1]

Abstract

Genome editing appears poised to enter an exciting new era. Targeted double-stranded breaks due to custom restriction enzymes are powerful nucleating events for the induction of local changes in the genome. The zinc finger nuclease (ZFN) platform established the potential of this approach for the zebrafish, but access to high quality reagents has been a major bottleneck for the field. However, two groups recently report successful somatic and germline gene modification using a new nuclease architecture, transcription activator-like effector nucleases (TALENs). TALEN construction is simpler, potentially more reliable, and in the few cases examined, shows fewer off-target effects than corresponding ZFNs. TALENs promise to bring gene targeting to the majority of zebrafish laboratories.

Introduction

The zebrafish (*Danio rerio*) has a completed genome (www.sanger.ac.uk/Projects/D_rerio/Zv9_assembly_information.shtml), but mutations in only about 10% of the genome are currently available to the zebrafish researcher. Custom zinc finger nuclease (ZFN) technology has proved to be a viable approach for the generation of targeted mutations in this animal model,[1–3] with dozens of mutant alleles published in the past 2 years.[4] The main challenge to large-scale use of ZFNs has been the difficulty in manufacture and the diverse range of targeting efficacy of these reagents.

Transcription Activator-Like Effector Nucleases

Recent advances in DNA targeting have focused on transcription activator-like effector (TALE) sequence-specific DNA binding domain proteins from plant pathogenic bacteria.[5,6] When fused to the FokI nuclease domain (the same nuclease used in ZFNs), TALE nucleases (TALENs) recognize specific DNA sequences using a straightforward DNA base recognition cipher (Fig. 1). Binding of two TALENs to DNA allows FokI to dimerize and create a targeted chromosome break.

Zebrafish Gene Targeting Using TALENs

Two recent reports show that TALENs can effectively recognize targeted loci in both somatic[7] and germline[8] cells in the zebrafish. Germline frequencies from this initial report appear comparable to that seen by ZFNs.[8] Importantly, for those sites successfully targeted by ZFNs, TALENs appear to be readily able to induce cleavage and subsequent mutations.[7] Thus, switching a sequence-specific DNA binding platform from zinc fingers to TALEs appears to be a straightforward technology transition.

Gene Targeting for the Masses?

In addition to this very promising functional utility for constructed TALENs, four groups have reported molecular toolboxes for the ready construction of TALENs.[9–12] The DNA base recognition cipher for TALE proteins is much simpler than zinc fingers (Fig. 1A), with an individual base recognized by two amino acids in an individual TALE repeat unit. Rapid assembly of custom TALENs is very accessible to even small laboratories, as the entire platform can be initiated with clones from a single 96-well plate, and construction takes less than 1 week once the system is operational.

Pioneering Data from ZFNs—A Cautionary TALE?

The future looks bright for TALENs and their use in zebrafish and other model organisms, and the current trajectory is heavily dependent on the rich history of ZFN science. ZFNs have been the leading technology for custom nuclease-catalyzed genome editing applications. However, ZFNs are not without limitations. Several papers also report that ZFNs can induce unintended changes within the host genomes in addition to the target location.[13–15] Notably, with unbiased discovery approaches, some of the double-stranded breaks

[1]Department of Biochemistry and Molecular Biology, Mayo Addiction Research Center, Mayo Clinic, Rochester, Minnesota.
[2]Department of Genetics, Cell Biology, and Development and Center for Genomic Engineering, University of Minnesota, Minneapolis, Minnesota.

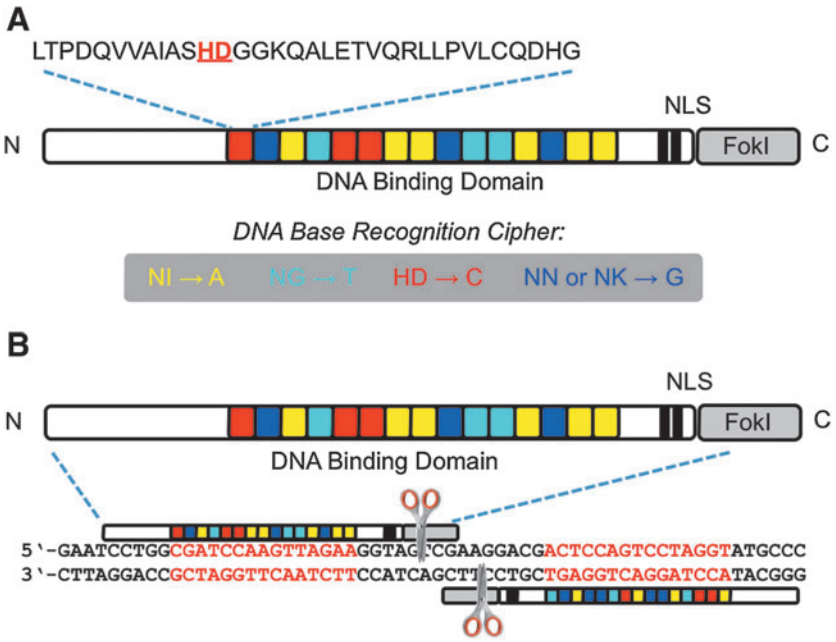

FIG. 1. Engineered transcription activator-like effector Nuclease (TALEN) cartoons. **(A)** Engineered TALE DNA binding domain recognizes specific bases by using a known cipher involving two key amino acid residues. These amino acids are embedded in a 33–35 amino acid repeat. Nuclear access is provided by the native nuclear localization signal (NLS). **(B)** Total sequence specificity and formation of a fully functional nuclease is achieved from each of the two TALEN monomers after dimerization on the target sequence in the genome.

were not predicted based on *in silico* predictions.[15] Whether such issues will be encountered with TALENs is only beginning to be investigated, but it does appear that they, too, will create off-target mutations at some frequency.[16] However, one recent TALEN paper reports reduced off-target effects for a TALEN compared with ZFNs that recognize the same chromosome site.[17] For genetics research, the take-home message is that having multiple alleles at the same locus is a worthy goal even with the potential for complications due to background off-target effects.

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Disclosure Statement

D.F. Voytas consults for Cellectis, a company that markets TALENS, and he is a named inventor on a TALEN patent.

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Address correspondence to:
Stephen C. Ekker, Ph.D.
Department of Biochemistry and Molecular Biology
Mayo Addiction Research Center
Mayo Clinic
Rochester, MN 55905

E-mail: ekker.stephen@mayo.edu