Methods 69 (2014) 22–31

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

TALEN-mediated *Drosophila* genome editing: Protocols and applications

Jiyong Liu [a,b], Yixu Chen [a], Renjie Jiao [a,b,*]

[a] State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, China
[b] Sino-French Hoffmann Institute, Guangzhou Medical University, Dongfengxi Road 195, Guangzhou 510182, China

ARTICLE INFO

Article history:
Received 31 October 2013
Revised 8 April 2014
Accepted 10 April 2014
Available online 19 April 2014

Keywords:
Drosophila
TALEN
TALE
Genome editing

ABSTRACT

TALEs (transcription activator-like effectors) are a family of natural transcriptional activators originally isolated from the plant pathogen of *Xanthomonas* spp. The DNA binding motif of TALEs can be re-designed in such way that they bind specific DNA sequences other than their original targets. Fusion of customized TALEs with an endonuclease, *Fok* I, generates artificial enzymes that are targeted to specific DNA sites for cutting, allowing gene specific modification of both animal and plant genomes. Previously, we reported the use of TALEN (transcription activator-like effector nuclease) for the highly specific and efficient modification of the two *Drosophila* loci *yellow* and *CG9797*. Here, we describe a detailed protocol for TALEN-mediated genomic modification in *Drosophila*, with the aim of providing a practical bench guide for the *Drosophila* research community.

1. Introduction

The functional dissection of a genome greatly depends on the precision of tools available for modification of DNA at specific sites of interest, at the required size and the type of genomic modification, including nucleotide replacement, deletions, insertions, inversions or even translocations. Ever since the establishment of modern genetics over 100 years ago, finding such precision tools that allow for targeted genome modification has been a long-standing goal for generations of scientists. Over the course of the last century, a wide range of genome-modifying methods has been developed for *Drosophila*, including forward and reverse genetic techniques. However, one major obstacle for advancing novel insights into gene function has been the fact that EMS (ethylmethane sulphonate)- and transposon-mediated methods are not ideal methods for sequence specific modifications [1]. And whilst HR (homologous recombination)-based gene targeting (ends-out or ends-in) methods can be employed for gene specific mutations [2,3], their low efficiency of successful targeting of only about 1/500 to 1/800 gametes [4], together with the fact that they are time-consuming procedures, limits their prevalence in the fly community, especially for large-scale and high-throughput studies. Lastly, even though a customizable method based on ZFN (zinc-finger nuclease) has been developed specifically for *Drosophila* mutagenesis, an approach distinguished by high sequence specificity and considerable efficiency [5–7], the complexity of constructing DNA-binding zinc-finger arrays, as well as toxicity-related issues present a major drawback for wider application [8,9].

TALEs (transcription activator-like effectors) are a group of transcriptional activators specifically found in *Xanthomonas* spp. [10]. The plant pathogen translocates TALEs into host plant cells through a type III secretion system, resulting in the activation of target gene expression. Characteristically, a naturally occurring TALE is composed of three parts: a type III translocation signal-containing N-terminal, a C-terminal containing both a nuclear localization signal (NLS) and a transcriptional activation domain (AD), and a central DNA-binding domain [10] (Fig. 1A). The DNA binding domain consists of a variable number of highly repeated units (between 1.5 and 33.5), with each unit specifically recognizing one nucleotide. Each unit is composed of 33–35, but typically 34 amino acids, except for the last unit, which is composed of 20 amino acids. The amino acid sequences of all repeats are nearly identical, except for the hyper-variable residues in position 12 and 13, which are therefore called repeat-variable di-residues (RVDs) [11]. The DNA-binding specificity of a TALE is determined by its RVDs, with a simple cipher of HD to C, NG to T, NI to A, NN to A or G, and NS to A, C, G or T [12,13] (Fig. 1A and B). By fusing an artificially designed TALE, containing specific RVDs, with the endonuclease *Fok* I, it is possible for scientists to generate a customized fusion protein that can target this enzyme activity to any site within the genome.

The resulting fusion protein, composed of a TALE and the C-terminal *Fok* I cleavage domain, is termed TALEN (transcription activator-like effector nuclease), which combines the features of both specific DNA binding and DNA cleavage activity. TALENs are

* Corresponding author at: Institute of Biophysics, CAS, Datun Road 15, Beijing 100101, China.
E-mail address: rjiao@sun5.ibp.ac.cn (R. Jiao).

http://dx.doi.org/10.1016/j.ymeth.2014.04.010
1046-2023/

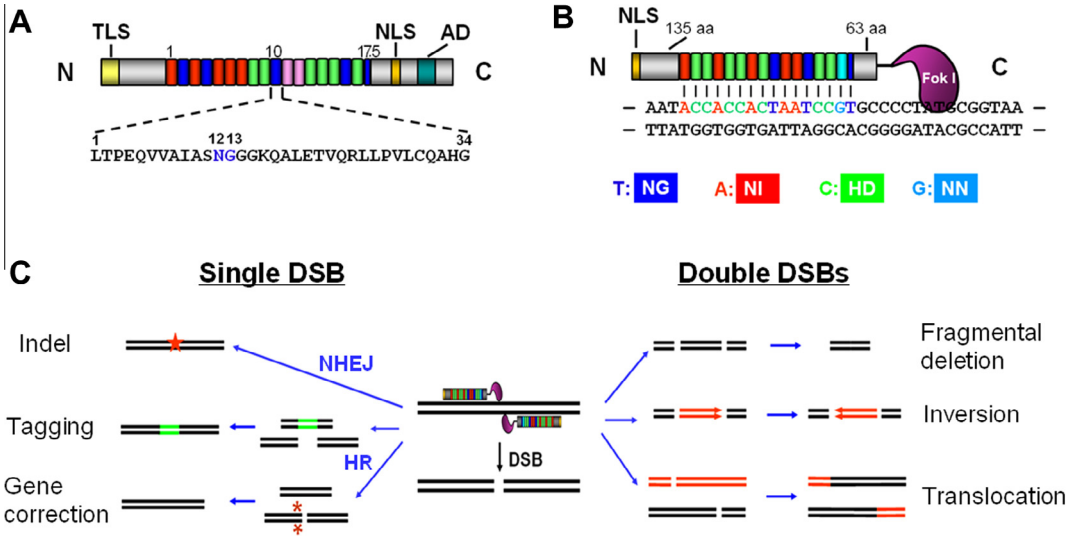

Fig. 1. Schematic view and principle of TALEN-mediated genome editing. (A) The structure of *Xanthomonas* TALE protein, with the amino acids sequence of a typical repeat depicted underneath. (B) Structure of a TALEN, with its recognized DNA sequence presented underneath. (C) TALEN-mediated genome editing. Left and right TALENs bind to the target sequence before the *Fok* I dimer induces a DSB in the spacer region. NHEJ repair produces small insertions and/or deletions (indels), and HR-mediated repair can be employed for gene tagging and gene correction. Double DSBs induced in the same chromosome can promote the generation of a large fragment deletion or an inversion. Double DSBs induced in different chromosomes may induce a chromosomal translocation. NLS, nuclear localization signal. AD, activation domain. TLS, translocation signal. Modified from Liu and colleagues [16].

designed in such way that they work in pairs: a left and a right TALEN bind to their respective target sequences, which are separated by a short DNA spacer region. In turn, the two *Fok* I form a dimer, which is required to cleave efficiently the spacer DNA, generating a DSB (double-strand break) as a result. The DSB is then repaired by one of two pathways: non-homologous end joining (NHEJ) or homologous recombination (HR). NHEJ is an error prone pathway that results in small insertions and/or deletions (indels) around the DSB. HR uses a homologous template to repair the broken DNA with high precision. When an exogenous homologous template containing modifications such as GFP tag is available, the desired sequence can then be incorporated into the genome, achieving a precise genome edition [14,15] (Fig. 1C). Recently, we have used this novel TALEN technique to target successfully two *Drosophila* genes, *yellow* and *CG9797*, with efficiencies ranging from 17.2% to 66.7% [16]. Thus far, TALEN has been employed in various species, including human cells [17], mouse [18], zebrafish [19], *Xenopus* [20], *Caenorhabditis elegans* [21], plants [22], bovine [23], silkworm [24], cricket [25] and mosquito [26].

The option of customizing this method for unique target regions makes TALEN an excellent tool to manipulate almost any genomic sequence. However, the high degree of similarity of the TALE repeats challenges the construction of customized TALENs. To overcome this constraint, several methods of constructing TALE repeats have been developed, which can be classified into three main types [14]: (1) The traditional, enzymatic digestion–ligation-based methods, including Unit Assembly [19] and REAL (restriction enzyme and ligation) [27]. Unit Assembly makes use of a collection of RVD mono-/di-/tri- and tetra-units. Taking advantage of the isocaudameric nature of enzymes such as *Nhe* I and *Spe* I, this method adds, step by step, *Spe* I/*Hind* III-digested fragments to the *Nhe* I/*Hind* III linearized vector, resulting in a complete set of TALE repeats. The construction of a TALE of no more than 17 RVD repeats can be achieved through 2 rounds of digestion–ligations (for experimental details, see Section 3.3). This method is suitable for small-scale construction of TALEs in most labs [16,28,29]. (2) Golden Gate-based methods employ an overhang generated by a type IIS endonuclease, from which the orderly assembly of up to 10 DNA fragments is directed in a single step [30]. Usually, a full-length TALEN construct can be generated within a timeframe of approximately 1 week [22,31,32]. (3) Other methods include Solid-phase-supported methods such as FLASH (Fast Ligation-based Automatable Solid-phase High-throughput) system [33], ICA (Iterative Capped Assembly) [34], etc. [35]. Here, ligation is carried out on solid-phase magnet beads, avoiding manual PCRs and the requirement for cumbersome gel isolation steps. FLASH is a high-throughput method, which allows the automatic production of 96 different TALE arrays in less than 1 day [33]. Another high-throughput strategy is called Ligation-Independent Cloning (LIC). This method can handle samples in a high-throughput manner, which can process more than 600 different TALE arrays in 1 day [36]. Customized TALEs can now also be obtained commercially, greatly reducing time and man power at the bench.

In addition to genomic modifications, TALE/TALEN has also been used in other research areas, including studies of gene expression regulation in *Drosophila* [37], DNA recombination in bacterial and human cells [38], as well as live visualization of chromatin dynamics in mouse cells [39] and others. In this article, we describe in detail a step-by-step protocol for gene mutagenesis in *Drosophila* using TALEN, from selection of target sites to molecular identification of designed mutations. In addition, we will discuss its use for other applications, including HR-based genomic modification.

2. Materials

2.1. Husbandry and handle of Drosophila

The flies are fed with common corn yeast food as described in Stocker and Gallant [40], raised at 25 °C in an incubator or a room with the humidity of 60% and a 12 h each of light and dark cycle. Other materials for handling flies include:

(1) Stereomicroscopes (Leica SE4);
(2) Forceps (Dumont, #55);
(3) Incubators (Percival, CAT# I-36VL);
(4) Fly-sleeping facility equipped with CO_2.