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TAL effector-mediated genome visualization (TGV)

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ABSTRACT

The three-dimensional remodeling of chromatin within nucleus is being recognized as determinant for genome regulation. Recent technological advances in live imaging of chromosome loci begun to explore the biological roles of the movement of the chromatin within the nucleus. To facilitate better understanding of the functional relevance and mechanisms regulating genome architecture, we applied transcription activator-like effector (TALE) technology to visualize endogenous repetitive genomic sequences in mouse cells. The application, called TAL effector-mediated genome visualization (TGV), allows us to label specific repetitive sequences and trace nuclear remodeling in living cells. Using this system, parental origin of chromosomes was specifically traced by distinction of single-nucleotide polymorphisms (SNPs). This review will present our approaches to monitor nuclear dynamics of target sequences and highlights key properties and potential uses of TGV.

1. Introduction

1.1. Nuclear architecture

DNA is packaged by forming nucleosomes, called chromatin. Chromatin fibers are organized non-randomly within the nuclear space and move continually even in interphase, not only due to temperature-dependent Brownian motion [1,2] but also involving active movement [3–5]. Spatiotemporal organization of chromatin within nucleus is suggested as an emerging key player to regulate genome functions including gene expression [6]. The developmental program which requires a precise control of gene expression accompanies nuclear remodeling, resulting in the generation of cell-type specific nuclear architecture. Moreover, defects in nuclear reorganization lead to developmental aberrations and several human diseases [7]. Despite the drastic change of nuclear structure during cell differentiation, its functional role in cell-fate decision remains largely unexplored.

1.2. Applications to study nuclear organization

DNA fluorescent *in situ* hybridization (DNA-FISH) is currently the method of choice to study subnuclear positioning of target sequences with microscopy. Since it is performed with fixed cells, live imaging of nuclear remodeling cannot be addressed by DNA-FISH. Fluorescent Lac repressor (LacI) system allows imaging of target genomic regions in living cells, where integration of exogenous array of LacO DNA binding sequences into the genome is required [8]. In this system, LacI fused with monomeric fluorescent protein specifically binds to LacO array, resulting in detection of subnuclear positioning of the target region as a visible fluorescent spot. However, since introduction of the large exogenous LacO array (~10 kb) into the genome usually occurs at random positions, the technique is in fact inapplicable for *in vivo* imaging of specific endogenous genomic loci. In 2007, Lindhout et al. reported a new approach for live visualization of endogenous genomic sequences using zinc finger-DNA recognition codes [9]. Expression of zinc finger proteins fused with GFP, which are designed to bind specific repetitive DNA sequences, allows imaging of subnuclear localization of the target sequences in living cells. Based on this concept, we developed TAL effector-mediated genome visualization (TGV), which offers several advantages including ease of design, its potential ability to be applied to any sequences and simpler optimization [10].

1.3. TAL effector

TALEs, proteins discovered in the plant pathogenic bacteria Xanthomonas, have emerged as powerful scaffolds for engineering DNA binding proteins [11,12]. TALE proteins are composed of tandem repeat of ~34 amino acids (TALE repeats) (Fig. 1A). Their sequences are nearly identical between these repeats except for two variable amino acids, referred as repeat-variable diresidue (RVDs), that define the base-recognition specificity of each unit [11,12]. Therefore arrays of four different repeat units allow us to

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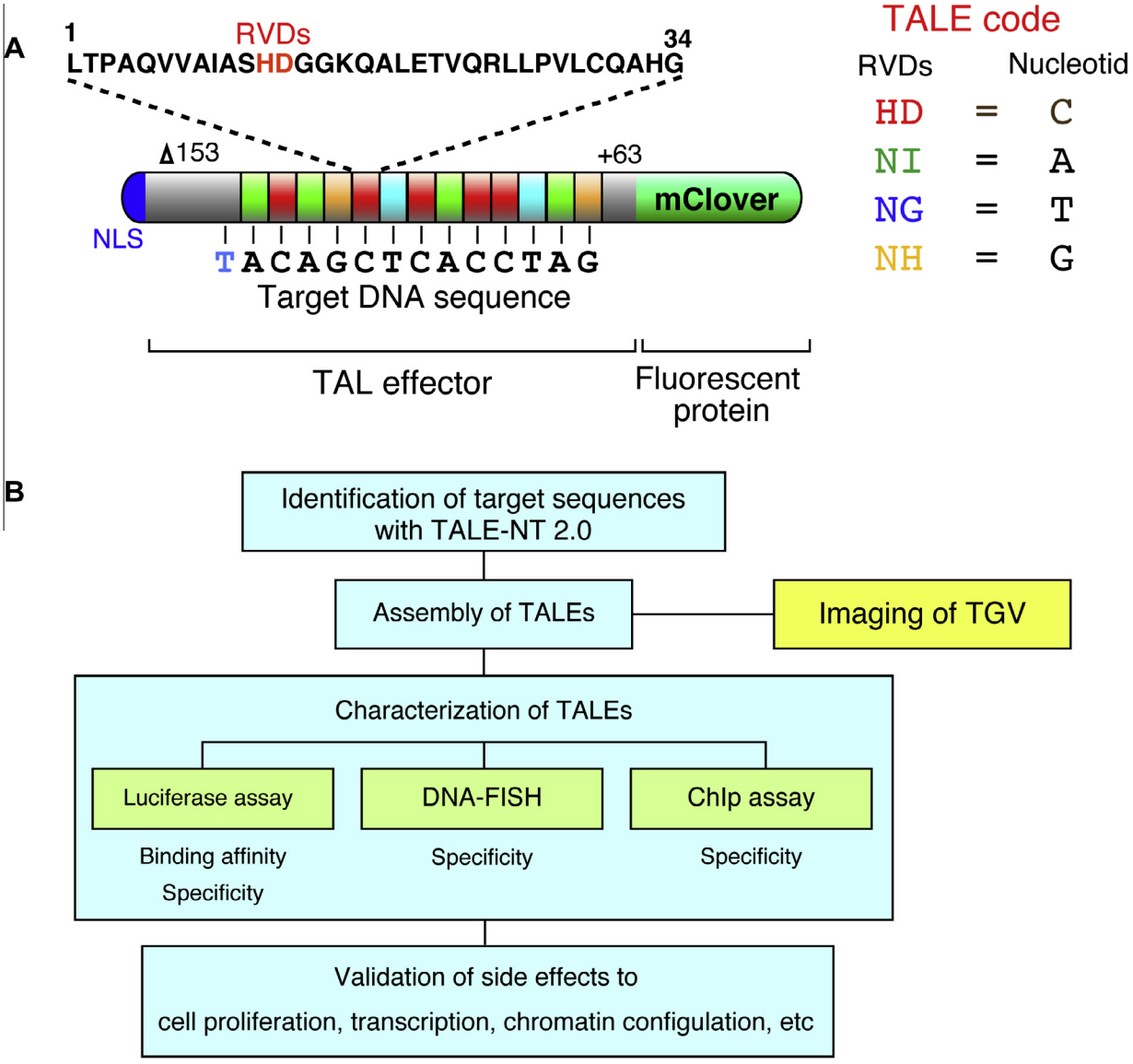

Fig. 1. (A) Schematic diagram of a fluorescent TALE and target sequence. Truncated TALE (N153AA and C63AA) was fused with nuclear localization signal (NLS) at N-terminus and monomeric fluorescent protein such as mClover at C-terminus. Central domain of TALE is composed of tandem array of 34 aa TALE repeat harboring repeat-variable diresidue (RVDs). TALE code representing specificity of RVDs to corresponding nucleotide is shown on the right side. (B) Work flow for selection of fluorescent TALEs for TGV.

generate TALEs with user-defined specificity. Using this simple code, TALE is a versatile platform for engineering DNA-binding proteins with specific functionality upon fusion with operative proteins such as nucleases [13], transcriptional modulators [14–16], recombinases [17], and epigenetic modifiers [18,19]. Here I will highlight the application that we developed, and that we refer to as TGV, using TALEs fused with fluorescent proteins to visualize the subnuclear positioning of repetitive sequences in living cells [10]. I also discuss the potential target sequences of TGV and alternative approaches to visualize repetitive sequences and unique loci.

2. Materials and methods

2.1. Design of TALEs for TGV

2.1.1. Identification of target sequences

Target DNA sequences of TALEs were identified from sequences of interest using the TAL effector Nucleotide Targeter (TALE-NT) 2.0 website (https://tale-nt.cac.cornell.edu/about) (Fig. 1B) [20,21]. Sequences of 15 nt or longer are appropriate for specific labeling of target sequences, since TALE against shorter sequence displayed higher background signal in the nucleoplasm as compared with TALE recognizing 15 nt or longer, possibly due to lower binding affinity or lower specificity to the target sequences [10]. It should be noted that DNA methylation on target sequences could affect binding affinity of TALEs, since TALE repeat with the HD RVD is sensitive to methylation at cytosine [22]. Hence, it is safer to select DNA sequences without CpG sites which are potentially methylated. Alternatively, N* RVD repeats could be used for recognizing methylated cytosine [22]. NH RVD module was used for recognizing guanine, since it displays higher specificity than substituting NN RVD module, which has affinity for both guanine and adenine [23,24].

2.1.2. Structure of fluorescent TALEs

TALEs for TGV were fused with nuclear localization signal (NLS) at N-terminus and fluorescent protein at C-terminus [10] (Fig. 1A).