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REVIEW

TALEN or Cas9 − Rapid, Efficient and Specific Choices for Genome Modifications

Chuanxian Wei [a,b], Jiyong Liu [a], Zhongsheng Yu [a,b], Bo Zhang [c,*], Guanjun Gao [d,*], Renjie Jiao [a,*]

[a] *State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Datun Road 15, Beijing 100101, China*
[b] *University of Chinese Academy of Sciences, Beijing 100080, China*
[c] *College of Life Sciences, Peking University, Beijing 100871, China*
[d] *College of Life Sciences, Tsinghua University, Beijing 100084, China*

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ABSTRACT

Precise modifications of complex genomes at the single nucleotide level have been one of the big goals for scientists working in basic and applied genetics, including biotechnology, drug development, gene therapy and synthetic biology. However, the relevant techniques for making these manipulations in model organisms and human cells have been lagging behind the rapid high throughput studies in the post-genomic era with a bottleneck of low efficiency, time consuming and laborious manipulation, and off-targeting problems. Recent discoveries of TALEs (transcription activator-like effectors) coding system and CRISPR (clusters of regularly interspaced short palindromic repeats) immune system in bacteria have enabled the development of customized TALENs (transcription activator-like effector nucleases) and CRISPR/Cas9 to rapidly edit genomic DNA in a variety of cell types, including human cells, and different model organisms at a very high efficiency and specificity. In this review, we first briefly summarize the development and applications of TALENs and CRISPR/Cas9-mediated genome editing technologies; compare the advantages and constraints of each method; particularly, discuss the expected applications of both techniques in the field of site-specific genome modification and stem cell based gene therapy; finally, propose the future directions and perspectives for readers to make the choices.

KEYWORDS: Genome editing; TALEN; CRISPR/Cas9; Gene therapy; Stem cells

INTRODUCTION

Modifications of genomes have laid the foundation of functional studies in modern biology and have led to significant discoveries (Esvelt and Wang, 2013). Since the time of Thomas Morgan, scientists, particularly geneticists, have been seeking methods to manipulate genetic materials in different organisms. For a long time, genome editing has largely relied on traditional forward genetic screens, such as chemical mutagenesis (Eeken and Sobels, 1983; Solnica-Krezel et al., 1994) and transposon-mediated mutagenesis (Marx, 1982; Rubin and Spradling, 1982). These screens are intrinsically limited, because (1) it is ineffective to map the mutations to a single gene due to the existence of functional redundancy of different genes; (2) not every mutation produces measurable phenotypes; (3) the biggest constraint is the inability to make specific targeted mutations. The completion of several model organisms' genome sequence has greatly facilitated functional studies of specific genes and opened the era of reverse genetics. Therefore, scientists developed in the past decades reverse genetic technologies that can be used to make precise genetic manipulations, including homologous recombination-based gene targeting (Thomas and Capecchi, 1987; Xu and Rubin, 1993; Melton, 1994; Golic and Golic, 1996; Xu

* Corresponding authors. Tel/fax: +86 10 6275 9072 (B. Zhang); Tel/fax: +86 10 6277 2955 (G. Gao); Tel: +86 10 6486 7568, fax: +86 10 6488 8474 (R. Jiao).
E-mail addresses: bzhang@pku.edu.cn (B. Zhang); gaogu@mail.tsinghua.edu.cn (G. Gao); rjiao@sun5.ibp.ac.cn (R. Jiao).

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et al., 2009; Chen et al., 2010; Du et al., 2010; Yu and Jiao, 2010; Huang et al., 2011a; Liu et al., 2011; Xie et al., 2012; Dui et al., 2012), ΦC31-mediated integration system (Groth et al., 2004) and zinc finger nucleases (ZFNs)-mediated genomic edition (Bibikova et al., 2002; Bibikova et al., 2003). However, these techniques are often inefficient, time consuming, laborious and expensive, which have been pushing the demand of developing new simpler, more rapid, more efficient and less expensive genome editing techniques to meet the new era of biomedical research.

The principle of genome editing relies on DNA repair system that works when DNA double strand breaks (DSBs) occur. In eukaryotic cells, there are two main types of DNA double strand breaks repair mechanisms, non-homologous end-joining (NHEJ) (Barnes, 2001; Lieber, 2010) and homologous recombinational (HR) repair (van den Bosch et al., 2002). NHEJ rejoins the broken ends and is often accompanied by loss/gain of some nucleotides, thus the outcome of NHEJ is variable: nucleotide insertions, deletions, or nucleotide substitutions in the broken region. HR uses homologous DNA as a template to restore the DSBs, and the outcome of this kind of repair is precise and controllable. For example, through HR repair an exogenous DNA sequence can be added at the break site in the genome. Scientists have been seeking to develop better genetic tools to manipulate the genome by creating a DNA binding domain that can recognize a specific DNA sequence and fusing it with a protein that can offer a nuclease activity. The discovery and application of zinc finger proteins made a revolutionary contribution to genomic editing toolbox. Based on the feature that different zinc fingers recognize different sets of nucleotide triplets, an hybrid protein containing specific zinc finger DNA binding domains and the endonuclease *Fok* I (ZFN) was generated to target specific DNA sequences (Kim et al., 1996; Urnov et al., 2010). Although considerable progress has been achieved, the use of ZFNs has not been picked up as widely as anticipated mainly due to: (1) there exist context effects on the specificities of individual finger in an array; (2) not all nucleotide triplets have got their corresponding zinc fingers discovered; (3) production of the ZFN proteins with high selectivity is costly, laborious, and time consuming (Bibikova et al., 2002; Bibikova et al., 2003; Urnov et al., 2010; Cradick et al., 2011).

In contrast, TALEN (transcription activator-like effector nuclease)-mediated specific genome editing has much more attractive advantages than ZFNs since its birth about 2−3 years ago (Morbitzer et al., 2010; Hockemeyer et al., 2011; Huang et al., 2011b; Tesson et al., 2011). It has been rapidly and widely used to perform precise genome editing in a wide range of organisms and cell types, including plants (Christian et al., 2010, Morbitzer et al., 2010, Li et al., 2012), frogs (Lei et al., 2012), fish (Huang et al., 2011b, 2012; Shen et al., 2013a; Zu et al., 2013), flies (Liu et al., 2012), worms (Wood et al., 2011), rats (Tesson et al., 2011; Tong et al., 2012), mice (Sung et al., 2013), livestock (Carlson et al., 2012), human somatic cells (Cermak et al., 2011) and human pluripotent stem cells (Hockemeyer et al., 2011). Interestingly, a very recent burst of publications (in the last 2−3 months) indicate that another new site-specific genomic editing tool is being developed, which borrows the CRISPR (clusters of regularly interspaced short palindromic repeats) system and the Cas9 endonuclease (Ishino et al., 1987; Hale et al., 2009; Jore et al., 2011; Carroll, 2012; Jinek et al., 2012). Unlike ZFN or TALEN, CRISPR/Cas9-mediated genome editing system adopts the Watson−Crick complementary rule to recognize and cleave target DNA sequence *via* a short RNA molecule and the endonuclease Cas9, respectively. It has appeared to be a very effective and promising genome editing tool in mammalian cells (Cho et al., 2013; Cong et al., 2013; Jiang et al., 2013; Jinek et al., 2013) and zebrafish somatic cells at the organismal level (Hwang et al., 2013). However, no success of inheritable Cas9-mediated genome modifications has been reported yet thus far although it is expected to come soon. Up to date, in the family of genomic editing toolbox, TALEN has shown to be an efficient, rapid, specific and economic method with a wide range of applications, and CRISPR/Cas9 system is emerging to be a new choice.

TALEN AND CAS9 OPEN THE NEW ERA OF GENOME EDITING

TALEN − an established genomic editing tool

TAL effectors (TALEs), originally discovered in the plant pathogen *Xanthomonas* sp., act as the bacteria invasion strategies to infect plant (Bonas et al., 1989). These effectors are injected into plant cells *via* the bacterial type III secretion system, imported into the plant cell nuclei, targeting effector-specific gene promoters to activate gene transcription (Kay et al., 2007; Romer et al., 2007), which may contribute to bacterial colonization. TALEs consist of a group of special effector proteins, which contain N- and C-termini for localization and activation and a central domain for specific DNA binding (Boch and Bonas, 2010; Miller et al., 2011). The central domain comprises of a variable number of tandem monomer repeats, varying from 5 to over 30 with an average of typically 17.5. Each repeat contains 34 amino acids that specifically recognize one target nucleotide (Boch and Bonas, 2010). The amino acid sequence of the repeats is highly conserved with its primary variation in the residues at position 12 and 13, a pair of residues termed as “repeat variable di-residues” (RVDs) that determines the nucleotide binding specificity of each TALE repeat (Boch and Bonas, 2010; Bogdanove and Voytas, 2011; Miller et al., 2011). Generally, the tandem repeat region ends with a truncated repeat, which is commonly referred to as a “half repeat”, containing the first 20 residues (including the RVDs) of a complete repeat (Boch and Bonas, 2010; Miller et al., 2011).

Naturally existing TALEs were first employed to manipulate genomic DNA in plants (Kay et al., 2007; Romer et al., 2007), which led to the initial decoding of the TALE DNA binding mechanism. The DNA binding specificity is determined by the RVDs with A, C, G, T recognized by NI (Asn Ile), HD (His Asp), NN (Asn Asn)/NK (Asn Lys) and NG (Asn

Gly), respectively (Boch et al., 2009; Moscou and Bogdanove, 2009). More recent studies have indicated that NH has a higher specificity than NN to target nucleotide G (Cong et al., 2012; Streubel et al., 2012). In addition, another common feature of the natural TALEs is that they always start with a repeat that recognize T in the 5′ end of their binding sequences (Boch et al., 2009; Moscou and Bogdanove, 2009; Boch and Bonas, 2010; Bogdanove and Voytas, 2011). The well-deciphered specificity of the RVDs for their target nucleotides makes it possible to develop TALE-based genome editing tools. By fusing the *Fok* I nuclease with an artificial TALE to create a specific TALEN, scientists have demonstrated that it is a powerful genome editing tool in a wide range of model systems and cultured human cells (Christian et al., 2010; Cermak et al., 2011; Hockemeyer et al., 2011; Huang et al., 2011b; Tesson et al., 2011; Wood et al., 2011; Lei et al., 2012; Liu et al., 2012; Tong et al., 2012; Sung et al., 2013; Zu et al., 2013). As shown in Fig. 1A, a single TALEN consists of an N-terminal domain including a nuclear localization signal, a central domain typically composed of tandem TALE repeats for the recognition of a specific DNA sequence (for natural TALENs, the length of the DNA sequence is usually 17−18 bp, and for artificial TALENs, it is usually 14−20 bp), and a C-terminal domain of the functional endonuclease *Fok* I. *Fok* I works in a dimeric fashion, and thus a pair of TALENs is needed to make a cut at a particular site of the genome. The pair of designed TALENs binds to their target DNA sequences flanking a spacer DNA (usually 14−18 bp), which facilitates the *Fok* I heterodimerization. The dimeric *Fok* I then cuts in the spacer DNA region, leading to a double strand DNA break (DSB). DSBs are generally repaired by NHEJ pathway, thus often generating small insertions and/or deletions (indels), which is essential for modifying the genome at a targeted site (Fig. 1B). The TALEN-induced indels are often variable in length, often leading to a frame-shift when it occurs in a coding region of the genome. It is worth noting that different pairs of TALENs yield indels with different efficiencies, ranging from 0% to about 65% in flies (unpublished data) and 0% to about 50% in zebrafish (unpublished data). The chance to get 0% indels for a given pair of TALENs in our hands thus far has been about 15% in zebrafish (unpublished data) and about 20% in flies (unpublished data). Alternatively, TALEN-mediated DSBs can also stimulate HR DNA pathway in the presence of homologous donor DNAs, enabling a site-specific insertion of an exogenous DNA sequence (Miller et al., 2011; Bedell et al., 2012; Zu et al., 2013), or a precise replacement of an "unwanted" endogenous nucleotide with a desired or corrected nucleotide in place (Li et al., 2011; Sun et al., 2012).

Artificial TALEN-mediated genome editing, with efforts in optimizing cloning strategies, has shown to be a rapid, efficient, specific and affordable method for the modification of most, if not all, of the genomes (Cermak et al., 2011; Hockemeyer et al., 2011; Huang et al., 2011b; Tesson et al., 2011; Bedell et al., 2012; Carlson et al., 2012; Garg et al., 2012; Lei et al., 2012; Li et al., 2012; Liu et al., 2012; Tong et al., 2012; Shen et al., 2013a, 2013b; Sung et al., 2013; Zhang et al., 2013; Zu et al., 2013). Applications derived from the TALEN-based genetic manipulation are being appreciated. For example, (1) it allows feasible introduction of exogenous sequences (for example, tagging FRT/loxP sequences) for any endogenous genes. *In vivo* tagging of genes of interest with fluorescent tags would enable scientists to trace any proteins easily in living organisms; (2) it will be easier to generate transgenic animals that have conditional expression of a given gene with TALENs and a specific promoter, and similarly to make cell or tissue specific knock-out of particular genes; (3) with TALEN-mediated genetic manipulations, large genomic rearrangements, such as deletions, inversions/reversions, can be generated in a controllable fashion. Large deletions are particularly important for studying the function of non-coding RNA genes, because frame-shift resulted from small indels may not be sufficient to inactivate the function of these genes; (4) it can also be used to build human disease models in human iPS (induced pluripotent stem) cells. Very recently, scientists have created mutations in 15 genes and performed detailed phenotypic analyses of four genes, *APOB*, *SORT1*, *AKT2*, and *PLIN1*, whose novel roles in disease biology are being elucidated (Ding et al., 2013). Inspired by the combination of TALEs with the nuclease *Fok* I, possibilities for TALEs to "guide" different enzymes/proteins to specific genomic sites for various purposes has also started to be explored. These proteins include transcriptional activators and chromatin remodeling factors. By the fusion of specific TALEs with epigenetic modulators, scientists will be able to manipulate local epigenetic states of the chromatin, aiming to control the expression of developmental pathways spatially and temporally during development (Song et al., 2007; Huang et al., 2010; Huang and Jiao, 2012). TALEs have been shown, in a synergistic manner with other proteins, to increase the expression of endogenous human genes over a wide range (Maeder et al., 2013). Two recent studies reported very detailed models for a natural and an artificially engineered TALE, respectively, both in complex with their DNA target sites (Deng et al., 2012; Mak et al., 2012), which will facilitate scientists to consider TALE in their experimental designs. These studies provided detailed mechanistic information of how RVDs make the contacts with their recognizing nucleotides, for example, they explained why the 13th amino acid has a more critical role in determining the specificity of their targets.

The full utilization of TALEs relies on the effective and rapid construction of customized TALE tandem repeats. However, due to the highly repetitive feature of these repeats, it was laborious and time consuming to construct long TALE repeats. Efforts have been made and several methods have been developed to solve this "problem", most of which are based on the Golden Gate cloning strategy (Weber et al., 2011, Reyon et al., 2012a; Sanjana, et al., 2012). A simple and rapid repeats-assembly method (referred as "Unit Assembly") which takes advantage of a pair of isocaudomers *Nhe* I and *Spe* I has been employed for constructions of TALE repeats by many researchers since it was reported (Huang et al., 2011b). Starting with four single-unit vectors that contain the coding sequence of unit-recognizing A, C, G or T, TALE repeats can then be easily assembled by standard molecular cloning to achieve "unlimited" number of repeats

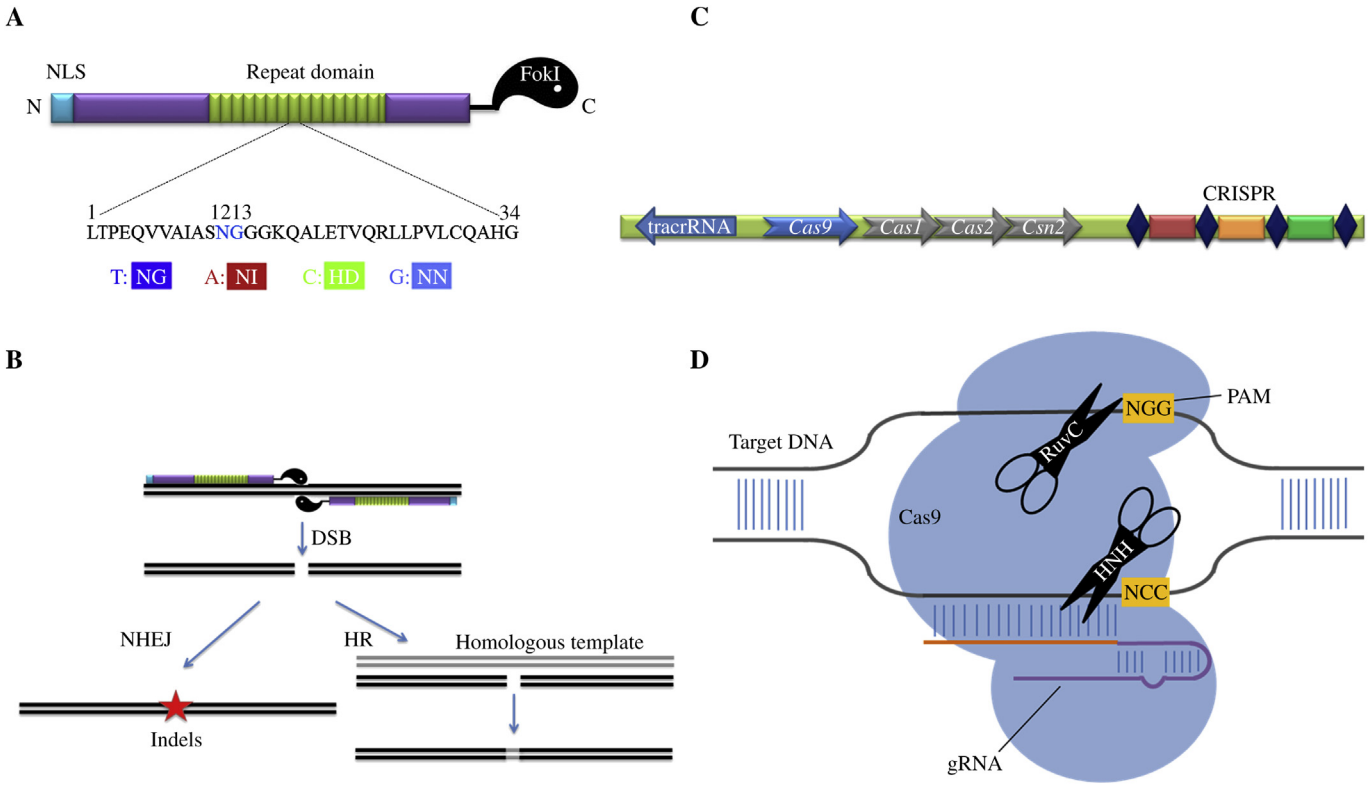

Fig. 1. Schematic principles of TALEN- and CRISPR/Cas9-mediated genomic modifications.
A: a single TALEN consists of an N-terminal domain including a nuclear localization signal (NLS, blue); a central domain typically composed of tandem TALE repeats (green) for the recognition of a specific DNA sequence; and a C-terminal domain of the functional endonuclease *Fok* I (black). Each TALE repeat comprises of a 34-amino-acid unit that differs at the position of 12th and 13th amino acids: NG (recognizing T), NI (recognizing A), HD (recognizing C), or NN (recognizing G) (color boxes). **B:** double-strand breaks (DSBs) that are resulted from the cut by dimeric *Fok* I can be repaired either by non-homologous end-joining (NHEJ) to yield indels or by homologous recombination (HR) with available homologous donor templates. The red star indicates where indels occur. **C:** the CRISPR/Cas9 system consists of a group of CRISPR-associated (*Cas*) genes (arrows with the direction to the right) and a CRISPR locus that contains an array of repeats (dark diamonds) − spacer (color boxes) sequences. All repeats are the same in sequence and all spacers are different and complementary to their target DNA sequences. The tracrRNA (trans-activating crRNA, arrow on the most left) can help to produce the crRNA (CRISPR RNA). **D:** the Cas9 protein (blue) binds to crRNA (orange) and tracrRNA (purple) to form a ribonucleoprotein complex. The crRNA sequence guides this complex to a complementary sequence in the target DNA (black). Then the HNH and RuvC domains of Cas9 nick the complementary and non-complementary strands, respectively, making a DSB. PAM: protospacer adjacent motif NGG (yellow box). gRNA: guiding RNA. NCC is a complementary motif of the PAM motif (NGG).

at a designed order (Huang et al., 2011b; Liu, et al., 2012). Meanwhile, to avoid standard cloning procedures, such as gel purification, selection and amplification of positive bacteria clones after each step of ligations, several solid-phase based TALE repeats assembly methods have been developed, including FLASH (fast ligation-based automatable solid-phase high-throughput) (Reyon et al., 2012b) and ICA (iterative capped assembly) (Briggs et al., 2012), which use solid-phase magnetic beads for enzyme digestions and ligations, thus allowing researchers to construct large numbers of TALENs effectively and rapidly.

Useful websites for TALEN designs and purchase of plasmids are listed here: (1) https://boglab.plp.iastate.edu/; (2) http://www.addgene.org/.

Cas9 − a rising star for genomic editing

CRISPRs were first found in the *Escherichia coli* genome more than 30 years ago (Ishino et al., 1987), and these sequences exist in about 40% of all sequenced bacterial genomes (Sorek et al., 2008). A CRISPR locus is defined as an array made up of a series of short direct repeats interspersed with short intervening regions (spacers). The number of CRISPR arrays varies among organisms, as does the number of repeats. The length of repeats varies from 21 to 47 bp, with an average of 32 bp. For a given CRISPR locus, the repeats are almost always identical in length and sequence. The spacers are also similar in length but have highly variable sequence (Karginov and Hannon, 2010). Computational analyses have indicated that the sequences of some CRISPR spacers match with sequences from existing phages or plasmids, raising the possibility that these spacers may enable the hosts to recognize invading genetic materials (Garneau et al., 2010; Horvath and Barrangou, 2010; Karginov and Hannon, 2010). Hosts that contain spacers with sequences homologous to foreign invaders are immune to the corresponding invaders, whereas closely related CRISPR-negative species are susceptible to these invaders (Bolotin et al., 2005). A major step forward in understanding the mechanism came with the discovery of the processed RNAs, the CRISPR RNAs (crRNAs). It is known

now that CRISPR loci are surrounded by a cohort of conserved CRISPR-associated genes (*Cas* genes) often adjacent to the cluster repeats (Fig. 1C). These genes form the elemental components of the CRISPR defense pathway. Accumulating evidence supports a model in which processed crRNAs serve as sequence-specific guides for the Cas proteins/nucleases in the resistance against invading elements (Garneau et al., 2010; Horvath and Barrangou, 2010; Karginov and Hannon, 2010). Very recent studies have discovered that the combination of CRISPR and Cas proteins is able to utilize the small crRNA molecules to target and destroy the DNAs of invading viruses and plasmids (Garneau et al., 2010; Jore et al., 2011; Gasiunas et al., 2012; Jinek et al., 2012).

There are three distinct types of CRISPR/Cas immune systems (Karginov and Hannon, 2010). Based on the principle of type II prokaryotic CRISPR/Cas9 adaptive immune system, molecular geneticists have smartly developed a state-of-the-art genome editing tool (Cong et al., 2013, Hwang et al., 2013, Jiang et al., 2013, Jinek et al., 2013, Mali et al., 2013). In this method, the crRNA and the trans-activating crRNA (tracrRNA) forms a double strand RNA structure that directs the Cas9 to generate DSBs in the target DNA. At the genomic site where is complementary to the crRNA, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the non-complementary strand (Fig. 1D). The RNA hybrid of tracrRNA:crRNA, when engineered as a single RNA chimera, named as guide RNA (gRNA), also directs sequence-specific double strand DNA cleavage by Cas9 (Jinek et al., 2012, Jiang et al., 2013). This principle lays the foundation for the Cas9-mediated genome editing tool (Karginov and Hannon, 2010; Carroll, 2012). In the past few months, four groups have successfully applied the CRISPR/Cas9 system to make genomic editing in cultured mammalian cells (Cho et al., 2013; Cong et al., 2013; Jinek et al., 2013; Mali et al., 2013). Cas9 nuclease assembles with engineered gRNA in human and mouse cells prior to being directed by gRNA to induce DSBs at a specific site complementary to the gRNA in genome. This cleavage activity requires both Cas9 and the complementary binding of the single-guide RNA (Cong et al., 2013; Jiang et al., 2013; Jinek et al., 2013; Mali et al., 2013). Thus, this engineered type II CRISPR/Cas9 system to induce precise cleavage with high efficiency for genome editing has been successful not only in mouse and human cell lines, including human pluripotent stem cells, but also in zebrafish embryos with efficiencies that are comparable to those obtained using ZFNs and TALENs (Hwang et al., 2013). Very recently, CRISPR interference (CRISPRi) platform was developed to control gene expression in bacteria and mammalian cells based on a modified Cas9 that is defective in its catalytic activity (Qi et al., 2013). The pre-requisite for being a gRNA complementary sequence in the genome is just that it needs to be preceded by a NGG (protospacer adjacent motifs, PAM), leading to that about 40% of the genome can be targets for Cas9 to cut. However, the success of this method to generate inheritable genomic modifications still waits to be reported although it would be surprising if this method applies only in somatic cells but not in germ cells. Scientists, including ourselves, are working in this direction.

Easy cloning strategy or no cloning makes Cas9-mediated genome editing tool more attractive. Cas9 protein can also be converted into a DNA nicking enzyme to facilitate HR-directed repair with minimal mutagenic activity (Cong et al., 2013). Multiple gRNAs can be manipulated to enable simultaneous editing of several sites within the mammalian genome (Cong et al., 2013). However, a handful of important questions remain to be answered. For example, what is the precise molecular mechanism for the gRNAs to bind to their target DNAs? Does CRISPR/Cas9 system depend on the transcription/DNA replication processes that unwind DNA to single strand DNA? How is the function of CRISPR/Cas9 dependent on the local chromatin context? What is the best length for the gRNA to function with the most efficiency? What is the off-target probability for a given gRNA? Once these issues are resolved, CRISPR/Cas9-mediated genome editing will be expected to contribute to the entire genome editing toolbox in a competitive and complementary manner with TALEN.

TALEN and Cas9 − two choices for one purpose in the genome editing toolbox

TALEN- and CRISPR/Cas9-mediated genomic editing techniques share some common features, but also have different aspects. A brief comparison between TALEN and CRISPR/Cas9 based genetic manipulations is shown in Table 1. (1) Both TALEN- and CRISPR/Cas9-mediated genomic modifications can either generate indels at a targeted locus in the genome *via* NHEJ, or replace a piece of endogenous DNA sequence with a homologous donor DNA *via* the HR-directed DNA repair system. This HR-based gene replacement has been well established in at least some of the model organisms with TALEN-based assay (Li et al., 2011; Miller et al., 2011; Bedell et al., 2012; Sun et al., 2012; Zu et al., 2013), but yet to be established in Cas9-mediated engineering assay. (2) TALEN-mediated genome editing requires engineering a pair of large repetitive sequences encoding domains for specific DNA recognition in the genome, whereas in the case of CRISPR/Cas9-mediated genome editing, it requires only a short RNA molecule, which is much easier to be manipulated than to assemble TALE repeats, to guide for site-specific DNA recognition and cleavage. (3) TALENs recognition sites always start with T (this rule has been proven to be not 100% strict); CRISPR/Cas9 recognizes genomic sequences that usually are preceded by the di-guanine residues NGG. These features make it more likely that the TALEN- and Cas9-mediated genome editing tools will be more complementary and alternative than competitive approaches in designing specific genomic modifications.

TALEN- and Cas9-based gene therapy

Undoubtedly, TALEN (likely Cas9 as well) will also bring a promising future for human gene therapy, particularly in combination with stem cell-based applications. Such ideas are

Table 1
Comparison of TALEN- and CRISPR/Cas9-mediated genomic modifications − principles and applications

	TALEN	CRISPR/Cas9
Target-binding principle	Protein-DNA specific recognition	Watson−Crick complementary rule
Working mode	TALE specifically recognizes the target DNA and dimeric *Fok* I makes the DSB, which is repaired by NHEJ or HR	Guide RNA specifically recognizes the target DNA and Cas9 makes the DSB, which is repaired by NHEJ or HR
Essential components	TALE-*Fok* I fusion protein	Guide RNA and Cas9
Efficiency	High but variable	High but variable
Off-target effects	Minor effects	Not determined
Target site availability	No restriction	PAM (NGG) motif restriction
Work in pair/dimmer	Yes	No
Inheritability in animals	Yes	Not determined
3D structure	Yes	Yes
Time consumption for construction	5−7 days	1−3 days
Multitask	Not determined	Yes
Target DNA length	~2 × 17 bp + spacer (14−18 bp)	~20 bp
Designed component	Protein	RNA
Origin of discovery	Plant pathogen	*E. coli*

based on a feasible and reliable (specific without any off-target) gene correction method for stem cells that are derived from the patients. Although some disease-specific iPS cells have been generated (Choi et al., 2013), there has been limited progress in iPS cells-based therapeutic strategies for human diseases, partially due to the low gene targeting efficiency in human iPS cells. TALEN and CRISPR/Cas9 systems represent a new level of technology that promises to be powerful tools for genome editing with potential therapeutic applications. TALEN-mediated genome correction has been successfully applied to correct several genes (*PPP1R12C* (*AAVS1*), *OCT4* (*POU5F1*) and *PITX3*) *via* the HR repair mechanism with high efficiency in human pluripotent cell lines including iPS cell lines (Hockemeyer et al., 2011; Miller et al., 2011; Choi et al., 2013). Similarly, CRISPR/Cas9 has also shown its applications in correcting gene mutations in human pluripotent cell lines (Mali et al., 2013). However, important questions remain to be addressed if TALEN- and/or Cas9-mediated gene corrections are to be used routinely for therapeutic applications. While TALENs and CRISPR/Cas9 can induce specific HR events, competing mutagenesis by NHEJ can still lead to unwanted mutation of the original and, in some cases, the HR-corrected allele. It will therefore be important to develop common methods that tilt the balance away from NHEJ, but toward HR-mediated repair. HR can be facilitated by blocking the activity of DNA ligase IV that is required for NHEJ (Ochiai et al., 2012), but additional concerns about the loss of ligase IV may arise in this case. Cas9 can be modified to become a nickase to facilitate HR-directed repair (Cong et al., 2013). In the TALEN-based HR strategy, it is more efficient to use single strand DNA template than to use double-stranded DNA as donors (Chen et al., 2011). In addition, the optimization of methods for efficiently delivering TALENs or CRISPR/Cas9 into cells will also be an important issue to improve the efficiency of gene correction on cells. The successful application of TALEN and Cas9 genome editing system in personalized iPS cells promises the hope of curing genetic diseases in the near future despite the fact that challenges still remain.

PERSPECTIVES

The availability of easily customizable DNA binding factors has offered scientists versatile tools to target specific genomic loci. The elegant TALE code for DNA recognition has been well exploited to artificially design TALEN proteins to make genome-wide targeted genetic modifications. The emerging CRISPR/Cas9 system may become (has the potential to be) more effective in artificial genomic editing than TALEN. Unlike the TALE code, the specificity of CRISPR/Cas9-mediated genome editing relies on RNA-based recognition. Nevertheless, both techniques have shown high success rate, high efficiency and specificity of genomic modifications without detectable off-targets or toxicity. TALEN (likely Cas9 as well) has opened an exciting way for scientists to, much more easily than ever, manipulate the genomes in almost all living organisms.

However, there is still room for improvements despite the big success of TALEN (and likely Cas9 as well) in editing the genomes. (1) The targeting efficiency varies for different TALEN pairs and in different loci. One cannot, at this moment, predict the efficiency of different TALENs. It is known that there are engineered TALENs which fail to mediate any genome modifications, indicating that possibly there is still sequence- and/or chromatin-dependent mechanisms that need to be discovered. In this regard, CRISPR/Cas9

could be an alternative choice for those genomic sites which are not modifiable with the TALEN-based strategy. However, as a newly developed genome editing tool, CRISPR/Cas9 system needs to be more intensively examined so that its full utilization may be achieved. (2) The more important thing for any site-specific genomic editing tools is their potential off-target effects. Developing methods and good off-target prediction software that enable to define the genome-wide specificities of TALENs and CRISPR/Cas9 will be helpful to minimize off-targeting problem and to choose the best strategy to make your desired gene modifications. Systematic analyses for the assessment of any off-target effects of TALENs and CRISPR/Cas9 remain to be investigated.

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