INNOVATION

TALENs: a widely applicable technology for targeted genome editing

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Abstract | Engineered nucleases enable the targeted alteration of nearly any gene in a wide range of cell types and organisms. The newly-developed transcription activator-like effector nucleases (TALENs) comprise a nonspecific DNA-cleaving nuclease fused to a DNA-binding domain that can be easily engineered so that TALENs can target essentially any sequence. The capability to quickly and efficiently alter genes using TALENs promises to have profound impacts on biological research and to yield potential therapeutic strategies for genetic diseases.

Targeted genome editing is a broadly applicable approach for efficiently modifying essentially any sequence of interest in living cells or organisms. This technology relies on the use of engineered nucleases; artificial proteins composed of a customizable sequence-specific DNA-binding domain fused to a nuclease that cleaves DNA in a non-sequence-specific manner. These targetable nucleases are used to induce double-strand breaks (DSBs) into specific DNA sites, which are then repaired by mechanisms that can be exploited to create sequence alterations at the cleavage site. Nuclease-mediated genome editing enables genetic studies that were previously difficult or impossible to perform. This technology also has potential as a therapeutic approach for genetic disorders, including monogenic diseases such as sickle cell anaemia or cystic fibrosis. Reflecting its broad importance, genome editing with engineered nucleases was named the 2011 'Method of the Year' (REF. 1).

The vast majority of targeted genome editing described in the literature (including initial foundational studies) has been performed using zinc-finger nucleases (ZFNs) (BOX 1). ZFNs have been used to modify endogenous genes in a wide range of organisms and cell types[2]. 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. Furthermore, ZFNs can potentially be used for therapeutic purposes; for example, ZFNs designed to disrupt the expression of the HIV host co-receptor *CCR5* (chemokine receptor 5) gene[3] have entered Phase 2 clinical trials for the treatment of HIV/AIDS.

Recently, transcription activator-like effector nucleases (TALENs) have rapidly emerged as an alternative to ZFNs for genome editing and introducing targeted DSBs. TALENs are similar to ZFNs and comprise a nonspecific *Fok*I nuclease domain fused to a customizable DNA-binding domain. This DNA-binding domain is composed of highly conserved repeats derived from transcription activator-like effectors (TALEs), which are proteins that are secreted by *Xanthomonas* spp. bacteria to alter gene transcription in host plant cells[4] (FIG. 1a,b).

TALENs have generated much interest and excitement because they can be very easily and rapidly designed using a simple 'protein–DNA code' that relates modular DNA-binding TALE repeat domains to individual bases in a target-binding site. Over the last two years, leveraging technologies and methodologies previously developed for the use of ZFNs, several groups have used TALENs to modify endogenous genes in yeast[5], fruitfly[6], roundworm[7], cricket[8], zebrafish[9–11], frog[12], rat[13], pig[14], cow[14], thale cress[15], rice[16], silkworm[17] and human somatic[15,18,19] and pluripotent stem cells[20] (see Supplementary information S1 (table)), and presumably the technique will continue to extend to additional organisms. Furthermore, a recent large-scale test demonstrated that TALENs have a very high success rate and can be used to target essentially any DNA sequence of interest in human cells[18] (see Supplementary information S2 (box)). Although ZFNs and TALENs have not been directly compared, studies have shown that they can cleave DNA with similar efficiency when targeted to the same gene[9,13,18,20]. Thus, the ease of design, high rates of cleavage activity and the essentially limitless targeting range of TALENs make them suitable for use by non-specialist researchers.

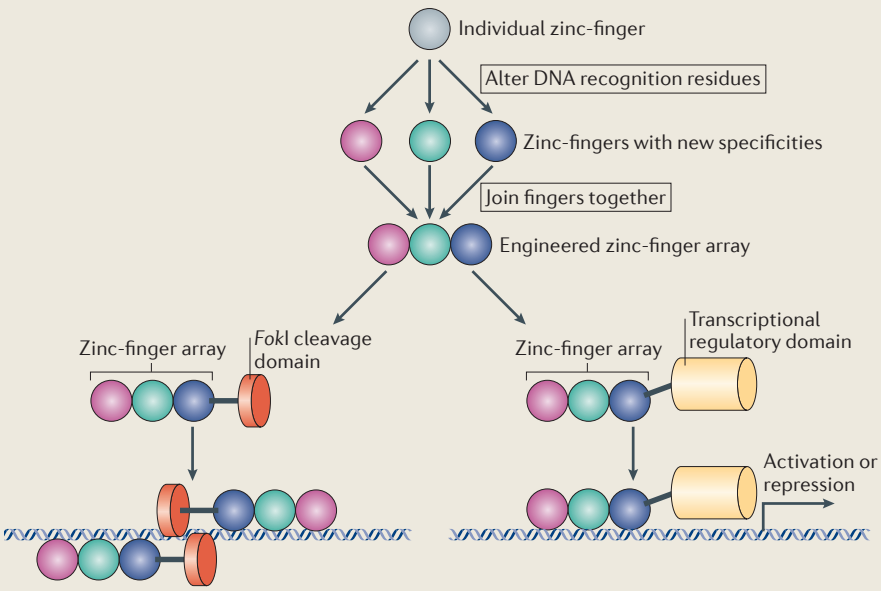
In this Innovation article, we briefly describe the simple modular strategy that is used to design customized TALE repeat DNA-binding domains and review recent progress on the use of TALENs to introduce different types of genome alterations in a wide range of organisms and cell types. In addition, we provide a comparison of the various publicly available methods for constructing TALENs. Finally, we outline important goals for future research to further enhance the utility of these tools for research and therapeutic applications.

Custom TALE DNA-binding domains

The fundamental building block that is used to engineer the DNA-binding region of TALENs is a highly conserved repeat domain derived from naturally occurring TALEs encoded by *Xanthomonas* spp. proteobacteria. These TALEs are injected into host plant cells via a type III secretion system and bind to genomic DNA to alter transcription in host cells, thereby facilitating pathogenic bacterial colonization[4]. DNA binding is mediated by arrays of highly conserved 33–35 amino acid repeats that are flanked by additional TALE-derived domains at the amino-terminal and carboxy-terminal ends of the array (FIG. 1c).

Box 1 | **Engineered zinc-finger proteins**

Cys2His2 zinc-fingers are DNA-binding domains that recognize approximately three base pairs of DNA. Alteration of a small number of residues in or near an α-helix within this domain can lead to changes in its DNA-binding specificity[83,84]. Engineered zinc-fingers can be joined together into more extended arrays that are capable of recognizing longer DNA sequences (see the figure). However, to obtain proteins with high affinities and specificities, context-dependent effects among fingers in an array should be considered[84–87]. Sangamo BioSciences has developed a proprietary and highly effective platform for engineering zinc-finger arrays[36], and customized proteins that are generated by this method can be purchased from Sigma-Aldrich. In addition, various publicly available methods for engineering zinc-finger proteins have been described. Modular assembly of individual pre-selected zinc-finger domains provides a simple and rapid method for creating customized arrays[88], but the success rate of this strategy has been reported to be low[89]. Additional methods that explicitly account for context-dependent effects among zinc-fingers in an array include: oligomerized pool engineering (OPEN)[90], context-dependent assembly (CoDA)[52], and a bacterial one-hybrid (B1H) selection-based system[37]. However, OPEN and B1H selections can be challenging for some non-specialist laboratories, and OPEN, B1H and CoDA have limitations in the range of sequences that they can target. A large number of zinc-finger arrays that have been engineered using these various methods have been fused to a nonspecific nuclease domain from the *Fok*I restriction enzyme to create zinc-finger nucleases (ZFNs)[91]. The *Fok*I nuclease functions as a dimer, and therefore two zinc-finger arrays must be designed for each target site (see the figure; bottom left). Early ZFNs used wild-type homodimeric *Fok*I nuclease domains, which can form unwanted dimers of the same monomeric ZFN. More recent studies have described obligate heterodimeric *Fok*I nuclease domains that reduce the formation of unwanted homodimeric species and therefore have improved specificities[76]. Engineered zinc-finger arrays have also been fused to transcriptional regulatory domains to create artificial transcription factors that activate or repress the expression of endogenous genes[92] (see the figure; bottom right).



Individual TALE repeats in an array specifically bind to a single base of DNA, the identity of which is determined by two hypervariable residues typically found at positions 12 and 13 of the repeat (FIG. 1c,d). Experimental evidence for this simple recognition code was first provided by Bonas and colleagues in 2009 (REF. 21). The researchers observed that the number of repeats in an array corresponded to the length of its target site, and this insight enabled them to deduce a simple correlation between the hypervariable residues and the base bound by each repeat. Moreover, they found that a thymine is conserved at the position just 5′ to the base bound by the first repeat in the array (FIG. 1d). This group provided experimental evidence for the TALE repeat code by constructing the first examples of engineered TALE repeat arrays with novel specificities[21]. The TALE repeat code was also confirmed by another group by performing a computational analysis of the binding specificities of naturally occurring TALEs[22]. Subsequent reports provided additional evidence that engineered TALE repeats with desired specificities can be created using the code[19,23–25].

More recently, co-crystal structures of TALE DNA-binding domains bound to their cognate sites have shown that individual repeats comprise two-helix v-shaped bundles that stack to form a superhelix around the DNA, and the hypervariable residues at positions 12 and 13 are positioned in the DNA major groove. The residues at position eight and position 12 within the same repeat interact with each other, thereby possibly stabilizing the structure of the domain whereas the residue at position 13 can make base-specific contacts with the DNA[26,27].

Nearly all engineered TALE repeat arrays published to date use four domains that contain the hypervariable residues NN, NI, HD and NG for the recognition of guanine, adenine, cytosine and thymine, respectively. Another repeat with the hypervariable residues NK has been reported to be more specific for guanine than the NN containing repeat (which can also recognize adenine)[19,22], but TALE repeat arrays using the NK repeats show less activity than those using NN containing repeats[10,28]. More recently, a repeat with the hypervariable residues NH has been suggested to be more specific than the NN repeat but with slightly lower activity[28,29]. Additional studies with a greater number of repeat arrays are needed to determine the optimal repeat domain for the recognition of guanine. It will also be interesting to explore whether repeats bearing other hypervariable residue combinations will have higher or different specificities for one or more DNA nucleotides.

Nuclease-mediated alterations

A substantial body of literature demonstrates that normal cellular repair of ZFN-induced DSBs by non-homologous end-joining (NHEJ) or homology-directed repair (HDR) can be exploited to introduce targeted genome alterations in a wide range of organisms and cell types[2,30]. NHEJ-mediated repair of a nuclease-induced DSB leads to the efficient introduction of an insertion or deletion (indel) mutation of variable length that originates at the site of the break (FIG. 2a). Thus, NHEJ-mediated repair of DSBs introduced into gene coding sequences will often yield frameshift mutations that can lead to the knockout of gene function.

Alternatively, if a double-stranded DNA 'donor template' is supplied, HDR of a nuclease-induced DSB can be used to introduce precise nucleotide substitutions or insertions of up to 7.6 kb at or near the site of the break[31] (FIG. 2a). Recent work has also shown that oligonucleotides can be used

together with ZFNs to introduce precise alterations, small insertions and large deletions[32]. ZFNs have been used to introduce NHEJ- or HDR-mediated gene alterations in fruitfly[33,34], roundworm[7,35], zebrafish[36,37], rainbow trout[38], catfish[39], sea urchin[40], frog[41], pig[42], cattle[43], cricket[8], rabbit[44], silkworm[45], butterfly[46], mouse[47,48], rat[49,50], soybean[51,52], thale cress[53,54], corn[55], tobacco[56], petunia[57], hamster cells[58] and human somatic[59,60] and pluripotent stem cells[61–63]. In most of these organisms and cell types, the high absolute rates of mutagenesis that can be achieved with ZFNs have enabled researchers to screen for mutations without the need for selective markers.

ZFNs and the I-SceI homing endonuclease have also been used to induce other more complex types of genome alterations in mammalian cells (FIG. 2b). These include large deletions induced by the introduction of two DSBs with subsequent deletion of an intervening sequence of up to 15 Mb in length[64], translocations induced by two DSBs on different chromosomes[65,66] and inversions of a chromosomal sequence between two DSBs on the same chromosome[67]. Given the requirement to introduce two DSBs, it is not surprising that these more complex alterations are obtained with lower efficiencies compared to alterations that are dependent on a single DSB.

Although TALENs were first described only two years ago, these nucleases have already been utilized in a large number of applications. TALENs have been used to generate NHEJ-mediated mutations in various organisms with generally high efficiencies (see Supplementary information S1 (table)). TALENs have also been used to introduce specific insertions in human somatic and pluripotent stem cells using double-stranded donor templates[19,20].

Applications of genome editing

As noted above, the rapid development of customized ZFNs has substantially expanded the scope of genetic research that can be performed in a broad range of organisms and cell types. The high efficiencies of alterations observed have already inspired efforts to use ZFNs as a potential therapeutic approach for genetic-based diseases. The relative simplicity with which TALENs can be engineered will further spur efforts to explore the research and therapeutic applications of customized nuclease technology. In this section, we review recent progress on the use of TALENs in various organismic and cellular contexts and briefly discuss prospects for their future applications.

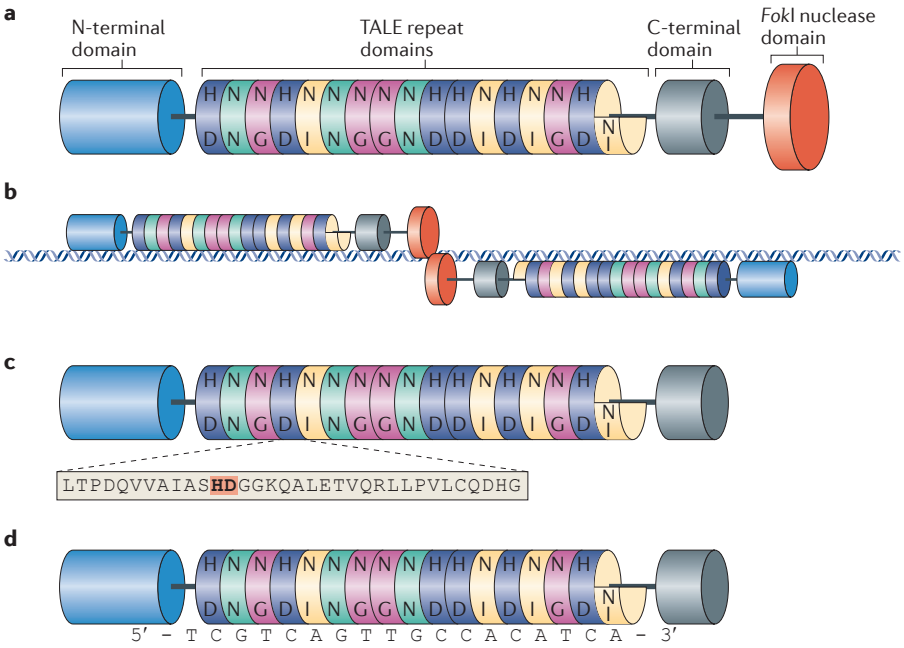


Figure 1 | **Overview of TALENs and TALE repeat arrays.** **a** | Schematic diagram of a transcription activator-like effector nuclease (TALEN). TALE repeats are shown as coloured discs with a final carboxy-terminal truncated half repeat. Letters inside each repeat represent the two hypervariable residues. Transcription activator-like effector (TALE)-derived amino-terminal and C-terminal domains that are required for DNA-binding are indicated. The nonspecific nuclease domain from the *FokI* endonuclease is shown in red. **b** | TALENs bind and cleave as dimers on a target DNA site. Note that the TALE-derived N-terminal and C-terminal domains flanking the repeats may also contact the DNA. Cleavage by the *FokI* nuclease domains occurs in the 'spacer' sequence that lies between the two regions of the DNA bound by the two TALEN monomers. **c** | Schematic diagram of a TALE-derived DNA-binding domain. The amino acid sequence of a single TALE repeat is expanded with the two hypervariable residues highlighted in orange and bold text. **d** | TALE-derived DNA-binding domain aligned with its target DNA sequence. Note that the repeat domains bind to single bases in the target sequence according to the TALE code. Also note the presence of a 5′ thymine preceding the first base bound by a TALE repeat.

Model organisms. As with ZFNs, TALENs have enabled the efficient introduction of targeted alterations in a number of model organisms that were previously difficult or impossible to genetically manipulate such as fruitfly[6], roundworm[7], zebrafish[9–11], frog[12], rat[13] and pig[14]. In addition, TALENs have also been used to modify endogenous genes in cow[14], cricket[8] and silkworm[17]. Most of these studies used a single TALEN pair to generate NHEJ-induced knockout mutations, but two of these reports also described the use of two TALEN pairs that are targeted to the same chromosome to generate deletions and/or inversions of large chromosomal segments[14,17]. In addition, another recent study also used TALENs together with short single-stranded DNA oligodeoxynucleotide donors to make precise insertions into the zebrafish genome[11]. The ability to efficiently induce mutations in various organisms should lead to the development of new animal models of human diseases. For example, TALENs have been used to inactivate the gene encoding low-density lipoprotein (LDL) receptor in pigs, thereby generating a model for familial hypercholesterolemia[14].

Plants and livestock. Unlike model organisms, which are typically selected for their short life cycles, many agricultural plants and animal species have long reproductive cycles and therefore can take many years to breed successfully. Nuclease-mediated editing of these organisms may greatly decrease the time required to generate new agriculturally-relevant varieties compared with traditional breeding strategies. For example, using micropropagation strategies, some plant species can be modified at the single cell level and then grown into mature plants. ZFNs have been used to make targeted indels in soybean[51,52] and to introduce specific mutations and transgene insertions that confer herbicide resistance in tobacco[56] and corn[55], respectively. To date, TALENs have been used to introduce knockout mutations in *Arabidopsis thaliana*[15] and to confer resistance to infection by

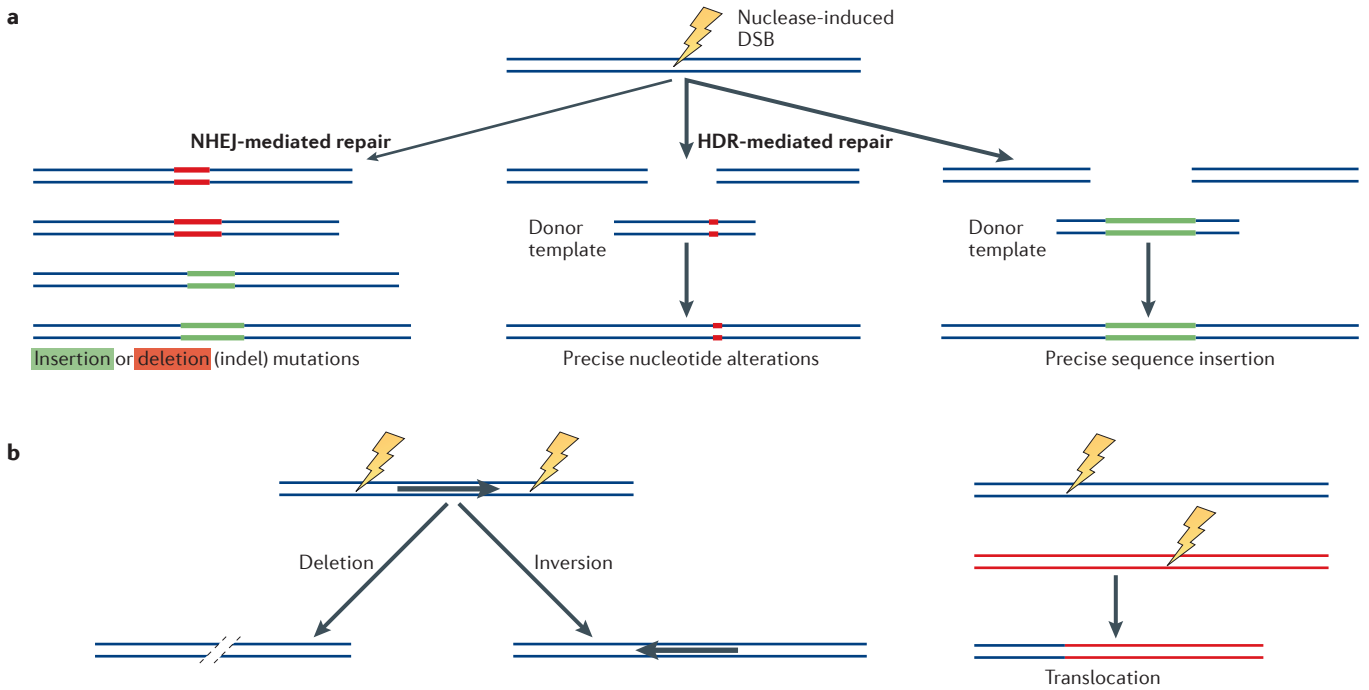


Figure 2 | **Nuclease-induced genome editing.** **a** | Single nuclease-induced double-strand breaks (DSBs) in a gene locus can be repaired by either non-homologous end-joining (NHEJ; thin black arrow) or homology-directed repair (HDR; thick black arrows). NHEJ-mediated repair leads to the introduction of variable length insertion or deletion (indel) mutations. HDR with double-stranded DNA 'donor templates' can lead to the introduction of precise nucleotide substitutions or insertions. **b** | Introduction of two nuclease-induced DSBs in cis on the same chromosome can lead to the deletion or inversion of the intervening sequence (left panel). The introduction of two nuclease-induced DSBs on two different chromosomes can lead to the creation of a translocation (right panel).

Xanthomonas bacteria in rice by disrupting the target sites of naturally occurring TALEs that contribute to pathogenicity[16]. Gene-editing nucleases have also been used to introduce targeted NHEJ-induced indel mutations in pigs and cows[14]. In addition, large deletions and inversions of sequences that are longer than 6 kb have been obtained in pigs by targeting two TALEN pairs to the same chromosome[14].

Cell-based disease modelling. Gene-editing nucleases offer the potential to directly assess the impact of gene disruption and of specific sequence variants on gene function in somatic cell-based models of disease. To date, TALENs have primarily been used to disrupt human genes by introducing NHEJ-induced indels into the coding sequence[15,18,19,68–71]. In principle, such loss-of-function mutations could be used to create somatic cell-based models of disease. Moreover, precise insertions have also been introduced into endogenous human genes using TALEN-induced HDR and a double-stranded homologous donor template plasmid[19,20]. 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) that have been identified by large-scale genome-wide association study (GWAS), encyclopedia of DNA elements (ENCODE) or other sequencing projects, thereby potentially enabling studies to determine the functional significance of these sequence variants.

Therapeutics. In contrast to therapies that treat symptoms of genetic diseases, targeted nucleases offer the potential to correct or disrupt gene products or sequences that cause the disorder. For example, recent studies have shown that ZFN-induced HDR can be used to correct genetic mutations that are responsible for sickle cell anemia[72] or α1-antitrypsin disease[73] or mutation in the Parkinson's disease-associated alpha-synuclein (*SNCA*) gene[74] in patient-specific induced pluripotent stem (iPS) cells that have been reprogrammed from fibroblasts. These reports provide important proof-of-principle for autologous transplant strategies in which patient-derived cells might be corrected *ex vivo* and then be reintroduced into patients with presumably reduced probability of immune complications. Although these studies were performed with ZFNs, TALEN-induced HDR in human pluripotent stem cells and somatic cells has also been achieved[19,20]. Another potential therapeutic strategy is to use nuclease-induced disruption via NHEJ-mediated repair to abolish the activity of a gene. This approach is currently being tested as a therapy for AIDS in which ZFNs are used to disrupt the expression of the *CCR5* gene product, which is required by certain HIV strains as a co-receptor to infect cells[3]. 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.

Platforms for engineering TALENs

The construction of DNA encoding engineered TALE repeat arrays can be challenging due to the requirement to assemble multiple, nearly identical repeat sequences. Different platforms have been designed to facilitate the assembly of plasmids that encode TALE repeat arrays. These methods (see Supplementary information S3 (figure)) can be grouped into three broad categories:

standard restriction enzyme and ligation-based cloning; 'Golden Gate' cloning; and solid-phase assembly (for a detailed descriptions of these methods, see Supplementary information S4 (box)).

These platforms vary in their throughput, the molecular cloning technique used, numbers of plasmids required (and time required to prepare these DNAs), use of potentially mutagenic PCR, flexibility in the length of arrays that can be constructed, ease with which the required reagents and detailed protocols can be acquired and the availability of author-supported web-based software (for a summary, see Supplementary information S5 (table)). Reagent kits for three of these platforms are available to academics by the non-profit plasmid distribution service Addgene. We have established and maintain an active and open newsgroup (currently with nearly 700 members) for discussion of TALE-related projects and a 'one-stop' comprehensive website with links to protocols, reagents, software and other information about engineered TALE technology (TALengineering.org).

The specific architecture of a TALEN is an important factor for users to consider when choosing an assembly method. Various TALEN architectures have been used to date, and one difference among these is the length and sequence composition of the N-terminal and C-terminal TALE-derived sequences that flank the TALE repeat array. In the earliest TALENs described in the literature, large segments of naturally occurring TALE sequences were used to join the *Fok*I nuclease domain to the C-terminal end of engineered TALE repeat arrays[24]. The TALEN framework was then refined by showing that nuclease activities could be greatly enhanced by truncating the length of this C-terminal TALE-derived sequence[11,19,68]. In addition, although early studies used wild-type homodimeric *Fok*I nuclease domains, more recent reports[10,20,75] have used various obligate heterodimeric domains originally developed and used with ZFNs[76] (BOX 1).

Therefore, because not all architectures are the same, we suggest that users should carefully consider the reported activity levels and potential specificities of TALENs that were generated on the basis of these different frameworks when choosing a method of assembly. We note that as of the writing of this review, the most extensively tested and validated TALEN framework remains that described by Rebar and colleagues[7,9,13,18–20,75,77] (see Supplementary information S1 (table)).

Box 2 | TALE-based transcription factors

Similarly to zinc-fingers (BOX 1), transcription activator-like effector (TALE) repeat arrays have been fused to transcriptional regulatory domains to create artificial transcription factors that can activate or repress gene expression. To date, various studies have shown that TALE-based activators and repressors can be used to modulate expression of endogenous genes in plants and in human cells, with changes in gene or protein expression in the range of twofold to 30-fold[15,19,29,93–98]. However, the vast majority of these TALE-based transcriptional regulators show relatively modest levels of activity. Although the high success rate and robust targeting range of dimeric TALE nucleases (TALENs) have been recently established[18], less is known about these parameters for monomeric engineered TALE-based transcriptional activators or repressors. The epigenetic status (for example, chromatin and DNA methylation) of the target sites may influence the activities of TALE-based transcription factors, as has been noted previously for zinc-finger-based activators[99]. In addition, it has been suggested that users should apply computationally derived design guidelines (originally proposed for dimeric TALENs[15]) to generate monomeric TALE-based transcription factors[100], but no published experimental support yet exists for this recommendation. Guidelines both for the selection of potential target sites and for the use of particular TALE repeat domains (harbouring NH residues at the hypervariable positions) for the recognition of guanine bases have been proposed[28]. Large-scale, systematic studies should prospectively determine whether the activities and specificities of engineered TALE-based transcription factors are influenced by adherence to these recommendations (or by the effects of varying other parameters such as the number of repeats, the length and composition of TALE-derived protein sequences that flank the TALE repeat array or the nature of the transcriptional regulatory domain used).

Future directions

Although the development of engineered TALE technology has proceeded at an extremely rapid pace over the past three years, many important questions remain to be addressed if these proteins are to be used routinely for research and therapeutic applications. First, although TALENs and ZFNs can induce specific HDR events, competing mutagenesis by NHEJ can still lead to unwanted mutation of the original and, in some cases, the HDR-altered allele. It will therefore be important to develop generalizable methods that tip the balance away from NHEJ- and towards HDR-mediated repair. For example, recent work[78,79,80] has demonstrated that ZFN-derived nickases that cleave only one DNA strand, instead of both strands, can shift this balance, although the absolute frequencies of HDR-mediated repair can be lower than those induced by the ZFNs from which such nickases are derived. Second, developing methods that enable definition of the genome-wide specificities of TALENs will be crucial to minimize off-target NHEJ-mediated mutagenesis. Third, the optimization of methods for efficiently delivering TALENs or nucleic acids encoding them into cells will also be an important area for future research. It will be interesting to investigate whether purified native or modified TALENs, like ZFNs[81], might be efficiently taken up directly by cells.

Another potential area for future exploration will be the creation of fusion proteins that harbour domains others than nucleases. TALE-based activators and repressors have already been described (BOX 2). However, one can also envision that engineered TALE repeat arrays might be used to direct functional domains that induce epigenetic alterations (such as covalent histone or DNA modifications) to specific genomic loci to induce stable, heritable alterations in gene expression. TALE repeat arrays fused to a recombinase domain have recently been described[82], raising the exciting possibility of enabling targetable site-specific recombination events.

Engineered TALE technology promises to facilitate and enhance genetic manipulations in different organisms and cell types. The simplicity with which TALENs can be designed together with their robust success rates has already spurred much broader adoption of genome-editing technology. Although many interesting and challenging questions remain, the accessibility and power of TALENs make this technology an exciting and important subject for future research and development.

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OPINION

Protein homeostasis: live long, won't prosper

Brandon H. Toyama and Martin W. Hetzer

Abstract | Protein turnover is an effective way of maintaining a functional proteome, as old and potentially damaged polypeptides are destroyed and replaced by newly synthesized copies. An increasing number of intracellular proteins, however, have been identified that evade this turnover process and instead are maintained over a cell's lifetime. This diverse group of long-lived proteins might be particularly prone to accumulation of damage and thus have a crucial role in the functional deterioration of key regulatory processes during ageing.

Ageing is a universal phenomenon that challenges all biological systems at multiple levels and ultimately results in their functional decline. Age-dependent changes can be seen in a wide range of organisms, from the decrease in replicative potential observed in unicellular yeast[1] to the reduced performance of vital organs in more complex organisms such as humans. The rate by which cells and organisms age varies widely, and genetic and environmental factors have been shown to be involved in the age-dependent decline of cell and tissue function. Although ageing is a complex phenomenon, it is becoming clear that a cell's failure to maintain proper protein homeostasis has a major role in ageing and age-related disease[2].

Constant protein turnover is one of the key strategies used to maintain this homeostasis and has been the focus of much work. Recent studies on ageing have now placed a new emphasis on literally old culprits: long-lived proteins which evade turnover[3,4]. In this Opinion article, we discuss the different contexts in which long-lived proteins have been characterized and the possible functional consequences of their persistence. We argue that these long-lived proteins have a larger role in organismal ageing than previously appreciated.

Exceptions to the rule

Proteins are constantly being degraded and subsequently replaced with newly synthesized copies. This turnover process ensures a constant supply of new and functional proteins, allowing non-functional, damaged or even toxic species to be destroyed. The rate of turnover, however, can vary widely from protein to protein, with half-lives spanning orders of magnitude within the same cell. Studies in budding yeast (which have a cell cycle of ~1.5 hours) have found the median and mean protein half-life under normal growth conditions to be ~43 minutes[5]. This figure increases to 0.5–35 hours in dividing mammalian cells (which have a cell cycle of ~24 hours) and ~43 hours in non-dividing cells[6,7]. Turnover studies in mice found the average half-lives of proteins in the brain, liver and blood to be 3–9 days[8]. Although half-lives for different proteins in the cell may range from minutes to days, protein turnover rates often correlate with their function or subcellular localization. For example, proteins within the mitochondria and endoplasmic reticulum (ER) on average have longer half-lives than other proteins[8]. Large complexes, such as ribosomes and proteasomes, also have highly similar rates of turnover for each of their components[6,8].

Of all the studies on protein turnover to date, most have concentrated on turnover during relatively short timescales (that is, timescales that are significantly shorter than cellular and organismal lifespan). However, the existence of long-lived proteins has been a well-established fact for several decades. As early as 1966, using radioisotope pulse-labelling (BOX 1), histones were found to have long half-lives[9]. Later studies from the 1970s also used radioisotopes to identify myelin and myelin proteolipid protein as long-lived[10,11]. An alternative technique, L-/D-Asp racemization (BOX 1), was also used to monitor protein turnover, and collagens, elastins, eye lens crystallins, tooth enamel and tooth dentine were identified as proteins that have half-lives on the order of years[12–17] (TABLE 1).

Recent advancements in pulse-chase labelling strategies provide strong evidence that more long-lived proteins remain to be discovered. In two studies, it was found that nuclear pore complex (NPC) proteins and a