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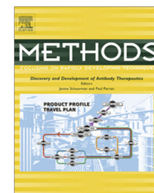

The application of transcription activator-like effector nucleases for genome editing in *C. elegans*

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A B S T R A C T

The nematode *Caenorhabditis elegans* has been a powerful model system for biomedical research in the past decades, however, the efficient genetic tools are still demanding for gene knockout, knock-in or conditional gene mutations. Transcription activator-like effector nucleases (TALENs) that comprise a sequence-specific DNA-binding domain fused to a FokI nuclease domain facilitate the targeted genome editing in various cell types or organisms. Here we summarize the recent progresses and protocols using TALENs in *C. elegans* that generate gene mutations and knock-ins in the germ line and the conditional gene knockout in somatic tissues.

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1. Introduction

The newly-developed transcription activator-like effector (TALE) nucleases (TALENs) enables the targeted genome editing in a variety of living cells or organisms (reviewed in [1]). TALENs comprise a customizable sequence-specific DNA-binding domain fused to a nonspecific DNA cleaving FokI nuclease domain (Fig. 1). The DNA binding domain of TALE typically contains tandem repeats of highly conserved 34 amino acids: LTPEQVVAIASNIGGKQALETVQALLPVLCQAHG [2]. Each repeat differs only at the 12th and 13th amino acids (underlined) which are termed as repeat variable diresidues (RVDs) that dictate the nucleotide-binding specificity [3]. Hypervariable residues NN, NI, HD and NG are widely used for the recognition of guanine, adenine, cytosine and thymine, respectively. The specific DNA recognition domain directs the FokI nuclease to induce DNA double-strand breaks (DSBs) at desired genomic locations. DSBs are primarily repaired by erroneous nonhomologous end joining (NHEJ) [4] or precise homology-directed repair (HDR) [5]. The NHEJ-mediated DNA repair often introduces an insertion or deletion (indel) mutation of variable length at the site of the break, whereas HDR can generate precisely specified changes at the target site when a homologous DNA template is provided. TALE has been proved versatile in genomic editing across species, including gene knockout [6–11], precise knock-in [12,13], gene activation [14,15], gene repression [16], DNA and histone modification [17,18].

The nematode *Caenorhabditis elegans* is an excellent metazoan model for the study of various biologic processes, owing to its small size, fast development, optical transparency and easy transgenesis. Although the entire genome sequences and a large collection of mutant strains are available for *C. elegans* [19,20], its potential has been limited by a lack of conditional genetic tools to modify the wild-type genome. This problem can be overcome by making conditional knockout animals, which allows a gene to be inactivated in a spatial- or temporal-specific fashion. In *C. elegans*, the frequency of spontaneous homologous recombination is low, and this technique is rarely used to generate targeted conditional deletions. The Cre/LoxP system has been reported to produce conditional knockout nematodes, however, it requires a null mutant which is not necessarily available for every single gene [21,22].

TALEN has been successfully applied to edit genomes in *C. elegans* and other nematode species [6]. It enables researchers to create viable and targeted mutants (*ben-1* [6], *rex-1* [13]) via germ line delivered mRNAs, or conditionally knock out genes (*dpy-5*,

Abbreviations: TALENs, transcription activator-like effector nucleases; GFP, green fluorescent protein.

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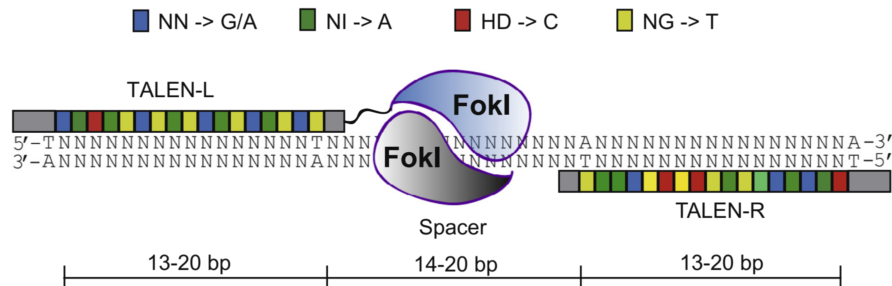

Fig. 1. Design of TALEN target sites. A typical TALEN target contains two binding sites on the left and right flanking a spacer. The binding site is 13–20 base pair (bp) in length, specifically following a T at the 5′ end. The repeat at the 3′ end is manually fixed to target T. The spacer is 14–20 bp in length. Four repeat variable diresidues (RVDs) recognizing G (NN), A (NI), C (HD), T (NG) are labeled as blue, green, red and yellow boxes. Since NN repeats also recognizes A, low G content in the target is recommended. Each subunit of heterodimer FokI is fused to the C-terminal of a pair of TALEs and named as TALEN-L and TALEN-R.

lon-2, *mab-5*, *cor-1*, *gfp*) in a subset of somatic cells using the heat shock or tissue specific promoters [23]. TALEN also has been proved efficient in targeted genomic insertion of short templates through HDR in *C. elegans* and other nematode species [13]. Nematode genes edited by TALENs are summarized in Table 1.

Mutagenesis in the germ line or somatic cell lineages by TALENs facilitates *C. elegans* genetics. The gene knockout in the germ line enables the disruption of the gene at the desired domain [6,13]. The somatic TALEN strategy that expresses TALEN in somatic cell lineages induces mutations efficiently at expected developmental stages and enables the dissection of the function of embryonic essential genes at the post-embryonic development [23]. For example, the conserved actin-binding protein Coronin has been implicated in cell migration and cell viability in mammalian cells, while its homolog *cor-1* in *C. elegans* was not explored due to the embryonic lethal phenotype [23]. Cheng et al. knocked out *cor-1* specifically in Q cell lineage, and revealed its essential role in regulating Q cell migration competence other than cell viability. Different from the TALEN mutagenesis in the germ line, mutations in somatic cells generated by somatic TALEN technique may not be heritable. However, due to the indel mutation caused by TALENs, null mutations are very likely to be produced in different animals, resulting in the consistent phenotype observed across generations [23].

This method provides an overview of the TALEN application in *C. elegans*. The details of generating mutations and knock-ins in *C. elegans* germ line have been recently reported [13], and this method describes the protocol that conditionally edits *C. elegans* genome by somatically expressed TALENs.

2. Principles for TALE target selection

To disrupt the function of the target gene, the target sites should be chosen in the exon that is shared by all the splice variants, coding for critical function domains and close to N terminal of the protein. Once the exon of the target gene is selected, online tools for the selection of TALEN target can be used to identify potential TALEN sites. Several online tools are available: https://tale-nt.cac.cornell.edu/ [24], http://www.e-talen.org/E-TALEN/ [25], http://www.talendesign.org/ [12] and http://eendb.zfgenetics.org/ [26]. The selected target sites should be checked following the below guidelines, which can be also used for manual selection of the target site.

2.1. The 5′ and 3′ ends of the target sequence have to be thymines. The DNA binding site of the natural TALE always begins with a thymine which is required for the recognition of the target site but not part of the TALE binding site [27]. To simplify the construction of TALE repeats, the last half-length repeat at the 3′ end of each TALE binding site is fixed to recognize thymine [7].

2.2. The target site is 13–20 nt in length to ensure the specificity. The increase of the length of the target site can increase its specificity, but more time and efforts are required. Therefore, the search pattern for a TALEN target site is 5′-T$(N)_{12-19}$T-3′ (N = A, C, G, or T).

2.3. Target sites with the low guanine content are preferred because the NN RVD that recognizes guanine also binds to adenine [3].

2.4. A pair of TALE sites on the opposite strands of target dsDNA is required. To avoid the potential off-targets, TALEN is engineered as a heterodimer, the subunits of which are referred as TALEN-L (Left) and TALEN-R (Right).

2.5. Two TALE binding sites are separated by a 14–20 bp spacer (Fig. 1) to ensure a proper space for FokI nuclease dimerization [28].

2.6. A unique restriction site is recommended within the spacer to facilitate the further molecular detection of the mutation.

Table 1
Summary of TALEN targeted genes in nematode species.

Target genes	Species	Injection compounds	Expressing tissue	Type of editing	References
ben-1	*C. elegans*	mRNAs	Germ line	Knockout	[6]
ben-1	*C. elegans*	mRNAs + ssOligo	Germ line	Knock-in	[13]
rex-1	*C. elegans*	mRNAs/mRNAs + ssOligo	Germ line	Knockout/knock-in	[13]
smo-1	*C. elegans*	mRNAs + ssOligo	Germ line	Knock-in	[13]
rex-32	*C. elegans*	mRNAs + ssOligo	Germ line	Knock-in	[13]
sdc-2	*C. sp. 9*	mRNAs	Germ line	Knockout	[13]
unc-119	*P. pacificus*	mRNAs/mRNAs + ssOligo	Germ line	Knockout/knock-in	[13]
dpy-5	*C. elegans*	Plasmids	All somatic cells/epithelia/neurons	Knockout	[23]
lon-2	*C. elegans*	Plasmids	All somatic cells	Knockout	[23]
gfp	*C. elegans*	Plasmids	Q cell lineage derived neurons	Knockout	[23]
mab-5	*C. elegans*	Plasmids	Q cell lineage derived neurons	Knockout	[23]
cor-1	*C. elegans*	Plasmids	Q cell lineage derived neurons	Knockout	[23]

2.7. The specificity of the TALEN target should be assessed by BLASTing the TALE target sequence against the genome.

3. Construction of customized TALENs

3.1. Assembly of TALE repeats

Several techniques have been described to construct TALE repeats, including the Golden Gate, the Unit Assembly, fast ligation-based automatable solid-phase high-throughput (FLASH) and ligation-independent cloning (LIC) [7,28–34]. The Golden Gate method relies on type IIS endonucleases whose recognition sites are directional and distal from their cut sites. The ligation products do not contain the recognition sequence and cannot be recut. Therefore, the Golden Gate reaction allows digestion and ligation in a single reaction and enables the rapid assembly of multiple TALE repeats with the unique ligation adaptors in a directional manner. In the Unit Assembly method, TALE repeat arrays are assembled through a series of double-digestion and ligation cycles using the (NheI + HindIII) and (SpeI + HindIII) pairs of restriction enzymes. FLASH system is designed for the high-throughput production of TALENs [33]. TALE repeat units are iteratively ligated on solid-phase magnetic beads until a repeat array of desired length is assembled. In contrast to traditional digestion-ligation cloning method, LIC technique relies on TALE repeat units containing long, unique single-stranded DNA overhangs [34]. Complementary overhangs of two fragments are annealed and then transformed to bacteria where two TALE repeat fragments are ligated by bacterial ligases. Both the Golden Gate and Unit Assembly were used to construct TALEN plasmids in *C. elegans* [13,23], and the details of Gold Gate protocol have been described [13,28]. This method focuses on the Unit Assembly strategy, which is extensively used in our laboratory.

A library of the single-unit, double-units and 4-unit TALE repeat vectors encoding all the possible combinations of NN, NI, HD and NG was built by Dr. Bo Zhang Laboratory [7], and the library is assessable to researchers for free. Each TALE repeat unit is flanked by a SpeI site (A^CTAGT) at the 5′ end and a NheI (G^CTAGC) site tightly close to a HindIII (A^AGCTT) site at the 3′ end (Fig. 2A) [7]. The desired number of ordered TALE repeats can be constructed through several rounds of digestion-ligation cycles using (NheI + HindIII) and (SpeI + HindIII) pairs of restriction enzymes. For example, a TALE targeting 5′-TGTACTACACAGTATACT-3′ can be divided into tetramers as GTAC, TACA, CAGT and ATAC. The first T is not included in the binding domain and the last T is pre-assembled into the empty TALEN expression vector backbone. The starting vectors for constructing this TALE target sequence are four 4-unit vectors referred as pGTAC, pTACA, pCAGT and pATAC. Here is a stepwise protocol.

3.1.1. Double digest vector pGTAC and pCAGT with NheI + HindIII at 37 °C for 4 h. Recover the DNA fragment containing TALE repeats and the backbone (~3 kb in this case) from the agarose gel.

3.1.2. Double digest vector pTACA and pATAC with SpeI + HindIII at 37 °C for 4 h. Recover the DNA fragment containing TALE repeats insert (408 bp in this case) from the agarose gel.

3.1.3. Ligate the two DNA fragments of pGTAC and pTACA recovered from Steps 3.1.1 and 3.1.2 at 16 °C for 2 h by Solution I (Takara). The same ligation is performed for two DNA fragments of pCAGT and pATAC.

3.1.4. Transform the ligation products into TOP10 competent cells and incubate the LB/Amp plate at 37 °C overnight to get clones containing vector pGTACTACA and pCAGTATAC. Positive clones are identified by colony PCR using M13-47 (5′-CGCCAGGGTTTTCCCAGTCACGAC-3′) and RV-M (5′-AGCGGATAACAATTTCACACAGGA-3′) primers and a ~800 bp PCR product is expected. The positive clones are confirmed by DNA sequencing.

3.1.5. Double digest vector pGTACTACA with NheI + HindIII and pCAGTATAC with SpeI + HindIII at 37 °C for 4 h. Recover ~4 kb pGTACTACA DNA fragment and 816 bp pCAGTATAC DNA fragment from the agarose gel.

3.1.6. Ligate the two DNA fragments of pGTACTACA and pCAGTATAC recovered from Steps 3.1.5 at 16 °C for 2 h by Solution I (Takara).

3.1.7. Transform the ligation products into TOP10 competent cells and incubate the LB/Amp plate at 37 °C overnight to get clones containing vector pGTACTACACAGTATAC. Positive clones are identified by colony PCR using M13-47 and RV-M primers and a ~1.6 kb PCR product is expected. The identified clones are confirmed by DNA sequencing.

3.2. Construction of TALEN expression vectors

3.2.1. The two empty TALEN expression vectors driven by the heat shock promoter are available on Addgene (pOG2002 and pOG2003, http://www.addgene.org/49514/ http://www.addgene.org/49516/ Fig. 2B). To replace the heat shock promoter (P*hsp*) with the promoter of interest, pOG2002 or pOG2003 is PCR amplified using primers 5′-TTTTTCTACCGGTACCCTCAAGGG-3′ and 5′-ATGGCTCCAAAGAAGAAGCGTAAGG-3′. pOG2002 and pOG2003 template is removed by DpnI digestion. PCR product of 7 kb is gel purified. The promoter of interest can be PCR amplified from genomic DNA, fosmids or cosmids containing the promoter sequence, using the promoter-specific primers with 15 bp extensions homologous to pOG2002 and pOG2003 ends. Gel-purify the PCR product of the promoter. Set up two In-fusion cloning reactions (Clontech):

Reaction 1: 1 μl 5× In-fusion premix (Clontech), 1 μl pOG2002 PCR product and 3 μl promoter PCR product.

Reaction 2: 1 μl 5× In-fusion premix (Clontech), 1 μl pOG2003 PCR product and 3 μl promoter PCR product.

Incubate two reactions at 50 °C for 15 min and transform the two In-fusion reactions into TOP10 competent cells. Screen for clones containing the promoter sequence by PCR and positive clones are confirmed by sequencing.

3.2.2. Once the empty TALEN expression vectors with the desired promoter are obtained, digest/linearize the empty TALEN expression vector with NheI at 37 °C for 4 h and dephosphorylate to prevent self-ligation.

3.2.3. Digest plasmids with the full length TALE repeats with SpeI + NheI at 37 °C for 4 h. Ligate the fragment containing TALE repeats with the NheI linearized empty TALEN expression vector (Fig. 2C).

3.2.4. Transform the ligation product into TOP10 competent cells. Screen for clones containing the full length TALE repeats by colony PCR using primers 5′-GAGGCGACACACGAAGCGATCGT-3′ and 5′-AAGGCGACGAGGTGGTCGTTGGT-3′ and positive clones are confirmed by DNA sequencing.

3.2.5. If the custom promoter contains a NheI recognition site, linearize the empty TALEN expression vectors by PCR amplification with a pair of primers containing 15 bp extensions homologous to the ends of the full length TALE repeats. The plasmid template for PCR needs to be removed by DpnI digestion. The SpeI + NheI digested TALE repeat fragment is cloned to one of the linear empty TALEN expression backbones by the In-fusion cloning reaction: 1 μl 5× In-fusion premix (Clontech), 1 μl linear empty TALEN expression backbone and 3 μl SpeI + NheI digested TALE repeat.

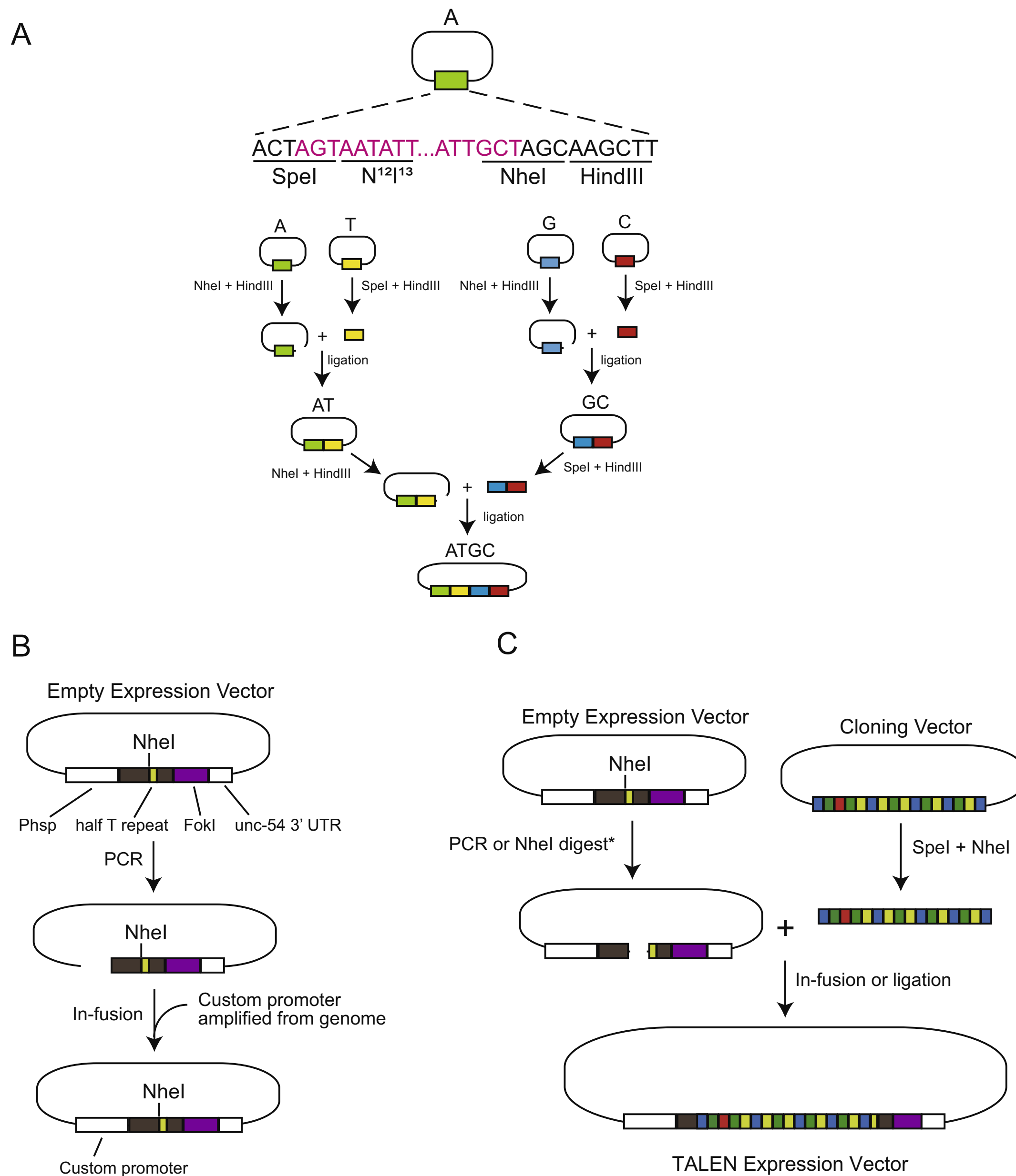

Fig. 2. Construction of TALEN expression vectors by the "Unit Assembly" strategy. (A) An example of digestion and ligation steps to construct a tetramer unit targeting ATCG. The DNA sequence of the single unit A is given. SpeI, NheI, HindIII recognition sites and the diresidues NI are underlined. The coding sequence of 34 amino acid (aa) unit is colored by pink. (B) Insertion of a custom promoter for TALEN expression using In-fusion cloning (Clonetech). The start plasmid contains a heat shock promoter and the *unc-54* 3′UTR. The 136 aa N-terminal and +63 aa C-terminal of *pthA* plus the last half-repeat are fused to the FokI endonuclease. A sole NheI recognition site is prior to the half-repeat. These plasmids to express each subunit of FokI are available from Addgene. (C) Full TALE DNA binding domains are inserted into linearized expression vectors via ligation or In-fusion. *NheI restriction enzyme recognition site should be mutated in the custom promoter.

3.2.6. Transform the In-fusion reactions into TOP10 competent cells. Screen for clones containing the full length TALE repeats by colony PCR using the same primers in Step 3.2.4 and positive clones are confirmed by sequencing.

4. TALEN expression and delivery

TALEN-mediated knockout in *C. elegans* was first achieved in Meyer lab by microinjecting gonads with TALEN-encoding mRNAs [6]. TALEN mRNA was synthesized *in vitro* and injected into the nematode germ line at the concentration of 1000–1500 ng/μl (Fig. 3A). Homology-directed insertion has also been accomplished by Meyer lab recently. In this assay, the 50 ng/μl single strand DNA template (ssOligo) with 20–50 nt of target homology was co-injected with TALEN mRNAs [13]. Germline delivered TALEN mRNA makes heritable mutations with targeted genomic indels [6,13].

To generate *C. elegans* conditional gene knockout by expression of TALENs in somatic cells, TALEN-encoding plasmid DNA can be used for microinjection [35]. TALEN-L and TALEN-R are co-injected with a selection marker to the gonads of wild-type worms using the standard methods [35]. The concentration of each transgene is 10–30 ng/μl. The selection markers can be pRF4(rol-6(su1006) (roller worms), P*odr-1::dsRed* (red fluorescence in the head AWC neurons), P*egl-17::mCherry::his-24* or P*egl-17::Myri-mCherry* (red fluorescence in *C. elegans* vulva). F1 progenies expressing the selection marker are singled as positive transformants and stable F2 transgenic lines with high transmission rates are maintained

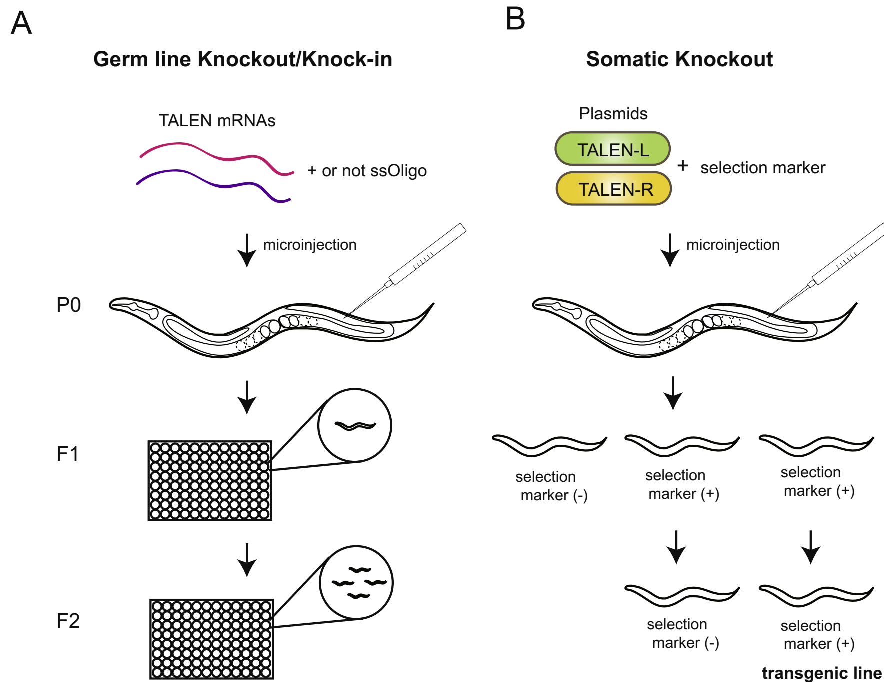

Fig. 3. Generation of TALEN-mediated knockout or knock-in worms. (A) TALEN mRNAs (1000–1500 ng/μl) with or without ssOligo (50 ng/μl) are injected into gonads of wild type young adult worms for the heritable knockout or knock-in. F1 progenies are singled into 96-well plates. Mutants from F2 progenies are screened by CEL-1 nuclease assay [6,13]. (B) Generation of transgenic lines for somatic expression of TALENs. TALEN-L and TALEN-R driven by customized promoters were injected into the gonad of young adult worms with co-injection markers. The concentration of each plasmid is 10–30 ng/μl. F1 progeny with selection markers are picked. Once F2 progeny inherit selection markers, a transgenic line expressing somatic TALENs is generated.

(Fig. 3B). TALENs in transgenic worms exist as extra-chromosomal assays which are usually silenced in the germ line but highly expressed in somatic cells. Tissue specific promoters and the heat shock promoter allow the edition of the somatic genome in a spatial or temporal fashion.

5. Phenotype analysis of conditional knockout mutants

5.1. To temporally knock out a target gene in somatic cells, TALENs are expressed by a heat shock inducible promoter [23].
The heat shock promoter turns on the gene expression in various tissues including neurons, muscles, epithelia but not the germ line. The efficiency of heat shock promoter to induce mutations in somatic cells depends on the time point of heat shock. Heat shock at the early developmental stage would induce expression of TALENs in more founder cells and generate the higher mutation frequency compared to heat shock at the late stage. However, the transgenes generated by microinjection are usually silenced in the germ line, causing the inefficiency of TALEN expression in the early embryos. Previous results showed the penetrance of mutant phenotypes was highest if the expression of TALENs was induced in eggs 12 h after laying or at the L1 larval stage. Detailed steps are below (Fig. 4).
1. Pick 100 transgenic adult worms to a seeded NGM plate to lay eggs for 2 h.
2. Transfer the adult worms out, and culture the eggs at 20 °C for 12 h.
3. Heat shock the eggs at 33 °C for 1 h.
4. Culture the eggs at 20 °C till the adult stage.
5. Examine the phenotypes. The mutant penetrance is determined by the percentage of mutants among all transgenic siblings [23].

5.2. To spatially knock out a target gene in somatic cells, TALENs are expressed by tissue specific promoters [23].
Tissue specific promoters restrict the expression of TALENs in a subset of somatic cells, which enables somatic knockout in any tissues of interest and avoids toxicity of continuously and extensively expressed nucleases. Phenotypes can be examined even in the F1 generation of transgenic worms. Once transgenic lines are obtained, mutants are available across generations and the penetrance of the mutant phenotype is consistent.

6. Molecular detection of mutations introduced by TALENs

To screen for mutants created by germ line knockout or knock-in, F2 progenies of injected worms in 96-well plates are pooled to amplify the TALEN target site. The products are melted and reannealed. If the mutation occurs, the products form mismatched double strand DNAs which can be specifically cut by CEL-1 nuclease, and the corresponding positive wells are selected. Singled F2 from positive wells are sequenced to determine mutations (Fig. 3A) [13].

To detect mutations generated by somatic TALENs, ten transgenic or heat-shocked transgenic worms are lysed for PCR amplification of a fragment containing the spacer between two TALEN binding sites (Fig. 5). Worms at the L3 or early L4 stage are preferred, and we do not use adult worms because a large number of nuclei containing the wild-type genome are produced in their germ lines. The fragment is digested by the restriction enzyme that recognizes a unique site within the spacer. PCR products normalized to 60 ng/μl are digested overnight to ensure a complete digestion. We expect that mutations generated by TALENs disrupt the restriction site and a portion of PCR products amplified from mutant cells remains intact after digestion. Gel electrophoresis of digested PCR products from conditional mutants should reveal a full length band. The undigested fragment is then purified and

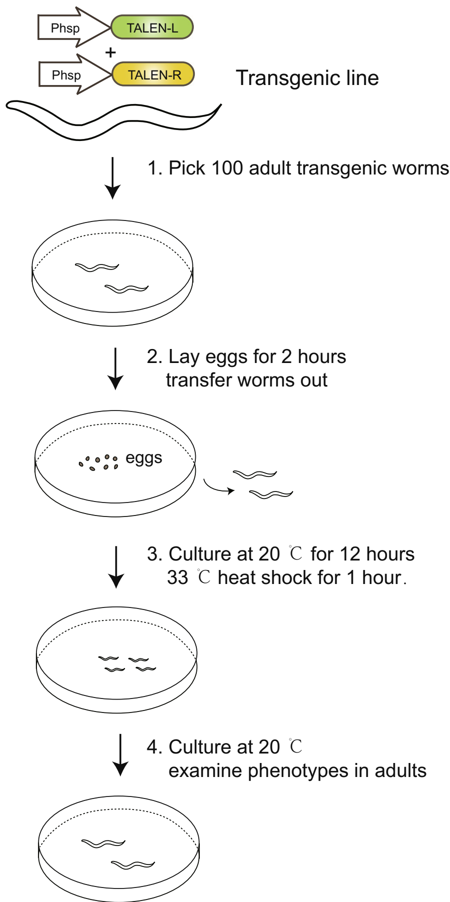

Fig. 4. Conditional gene knockout induced by somatic TALENs under the control of a heat shock promoter. Eggs 12 h after laying were heat shocked at 33 °C for 1 h and then cultured at 20 °C to the adult stage for phenotype analysis.

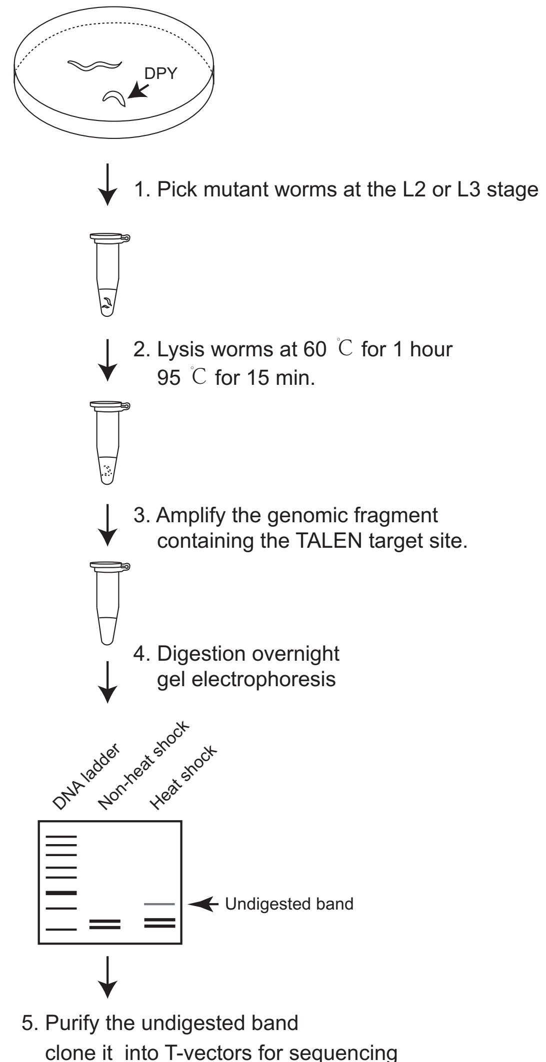

Fig. 5. Molecular detection of genomic mutations induced by somatic TALENs. Mutant worms (e.g. DPY) or randomly picked transgenic worms (after heat shock if P*hsp* used) are lysed to generate template DNA for amplification of a DNA fragment that contains the TALEN target site. Lysis buffer: 0.1 mg/ml proteinase K in 2× PCR buffer (Mg^{2+}plus). After digestion, the intact band is purified and cloned into T-vectors for sequencing. Worms older than L4 should not be used in the assay.

cloned to T-vectors. Single colonies are sequenced to detect molecular lesions. In contrast, PCR products from non-transgenic or non-heat-shocked animals are completely digested into two fragments (Fig. 5). Since only a small number of somatic cells may be edited by TALENs when TALENs are expressed by the tissue-specific promoters, an alternative way to examine whether the target site can be mutated is to express TALENs under the control of the heat shock promoter. Transgenic larvae 24 h after the heat shock can be used for the molecular assay.

To improve the sensitivity of molecular detection, T7 endonuclease I (T7E1) which cuts dsDNA at mismatched sites is recommended. T7E1 is commercially available from New England Biolabs and works reliably for this purpose. First, we amplify the DNA fragment containing the target site for each worm by PCR and use the PCR purification kit (Qiagen) to concentrate the PCR product to 200 ng/μl. Second, we set up a T7E1 reaction by mixing 1 μl 10× T7E1 buffer, 3 μl PCR products and 5.5 μl ddH_2O and denature the DNA at 95 °C for 5 min. Third, after reannealing the DNA at room temperature for 5 min, we add 0.5 μl T7E1 enzyme (10 Unit/μl) to digest the DNA at 37 °C for 30 min. Finally, we analyze the T7E1 digested PCR products by gel electrophoresis. When a mutation occurs, two smaller bands at expected sizes should be visible compared to a single intact band in the non-mutated animals.

Although it is not be applicable to determine the phenotype of each mutated worm generated by somatic TALENs if the molecular analysis is first performed, one can examine the phenotype of a transgenic worm at L3 or L4 stage and subsequently analyzes its molecular lesion by single worm PCR and T7E1 digestion. The somatic mutations generated by TALENs are not heritable, but null mutations are most likely produced by NHEJ. Consistently, all the five genes (*dpy-5*, *lon-2*, *gfp*, *mab-5*, *cor-1*) targeted by somatic TALENs, showed similar penetrance across generations [23]. This result indicates that somatic TALENs can induce mutations in a constant proportion of transgenic worms in each generation, allowing researchers to perform molecular detection and phenotypic analysis at different generations.

7. Conclusion and prospective

The methods presented here allow the genome editing by TALENs in *C. elegans* and other nematode species and enable the study of embryonic essential genes at the post-embryonic stages. Recent improvements in the engineering of TALENs continue to make genome editing more rapid and efficient, making TALENs potentially suitable for the large-scale functional genomic studies [33,34].

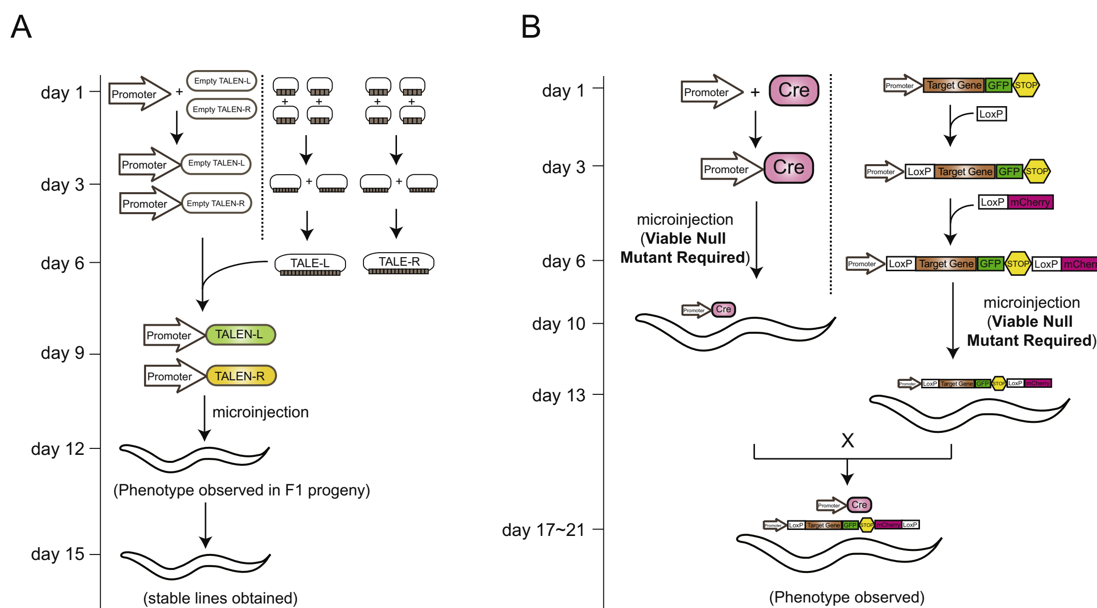

Fig. 6. Timeline Comparison between the Somatic TALENs (A) and the Cre/LoxP Induced (B) Conditional Gene Knock-out Strategies. Note that the Cre/LoxP system requires a null mutation of the target gene to start with.

In mammalian pluripotent cells [36] and zebrafish [7], deep sequencing results revealed no evident off-targets effects of TALENs. Although similar assays have not been performed in *C. elegans*, off-target phenotypes have not been reported using either the germ line delivered or somatically expressed TALENs [13,23]. *C. elegans* carrying somatic TALEN transgenes grow healthy if the TALEN expression is not induced, and the expression of TALNE-L or TALEN-R only did not generate any phenotypes [23], suggesting the specificity of TALENs on genome editing.

The Cre/LoxP system is another excellent tool that has been applied for *C. elegans* conditional gene knockout, but it requires a null mutation of the target gene to start with and is more time-consuming compared to somatic TALENs procedures (Fig. 6). Cell-specific RNA interference (RNAi) has been used to knock down target genes in *C. elegans* [37]. However, knockdown by RNAi is incomplete and refractory to worm neurons and has unpredictable off-target effects. Zinc finger nuclease (ZNF) is a useful tool to achieve targeted genome editing [6], but each module of ZNF recognizes three bases, restricting the flexibility in customizing the target site. Recently, the CRISPR/Cas9 system has been proved efficient in *C. elegans* genome editing [38–45] and its vector construction is much simpler than TALENs. However, the CRISPR/Cas9 system shows high off-target frequency [46]. Although the equivalent attention should be paid to TALENs, the confirmatory results obtained from CRISPR and TALEN should largely alleviate the concerns about off-target effects of both strategies. Moreover, TALENs tagged with a mitochondrial localization signal can be directed into mitochondria to edit mitochondrial genome [47], but it is unknown whether the single guide RNA (sgRNA) in the CRISPR/Cas9 system can be imported into mitochondria to guide the mutation of mitochondrial genome. As a summary, we expect the continued wide applications of TALEs to genomic/mito-genomic editing, chromatin modifications as well as gene expression regulations in *C. elegans* in the future.

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