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REVIEW

Tell Me a Tale of TALEs

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Abstract Pathogenic bacteria of the *Xanthomonas* and *Ralstonia* genus have developed resourceful strategies creating a favorable environment to multiply and colonize their host plants. One of these strategies involves the secretion and translocation of several families of effector proteins into the host cell. The transcription activator-like effector (TALE) family forms a subset of proteins involved in the direct modulation of host gene expression. TALEs include a number of tandem 34-amino acid repeats in their central part, where specific residues variable in two adjacent positions determine DNA-binding in the host genome. The specificity of this binding and its predictable nature make TALEs a revolutionary tool for gene editing, functional analysis, modification of target gene expression, and directed mutagenesis. Several examples have been reported in higher organisms as diverse as plants, *Drosophila*, zebrafish, mouse, and even human cells. Here, we summarize the functions of TALEs in their natural context and the biotechnological perspectives of their use.

Keywords TAL effector · *Xanthomonas* · Pathogenicity target · Genome editing

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Introduction

In order to gain access to the nutrients present in plant cells, plant pathogenic bacteria need to overcome preformed structural barriers such as the cell wall [1]. After defeating this first obstacle, bacteria have to suppress induced defense responses triggered upon the recognition of the so-called conserved microbial associated molecular patterns (MAMPs) that pathogens produce. MAMPs are recognized as non-self by many multicellular organisms, including humans. In plants, this recognition event depends on membrane-anchored receptors named pattern recognition receptors (PRRs). The suppression of this first branch of plant immunity referred to as MTI (MAMP-triggered immunity) is achieved upon the injection of pathogen effector proteins. In bacteria most of these proteins are Type 3 Effectors (T3Es) which are secreted and translocated into the host cell cytoplasm through the type three-secretion system (T3SS) [1, 2]. Plants employ a second strategy based on the recognition of specific effectors to activate an efficient defense response. This second branch of plant immunity is referred to as effector-triggered immunity (ETI) and depends on the dual presence of a single resistance (R) protein and its matching recognized effector, classically defined as avirulence (Avr) determinant [3]. This recognition is direct or mediated by a third part defined as pathogenicity target, which is often a protein guarded by the R protein (Guard model) [4].

Plant pathogenic bacteria contain approximately 20–40 T3Es which are grouped in about 30 different families (http://www.xanthomonas.org/t3e.html). These T3Es may interact different plant targets with the purpose of interfering with host physiology, metabolism, and plant defense. In general, T3Es display specific activities but some of them show overlapping functions [5]. Relatively little is known about the mechanisms upon which bacterial effectors contribute to disease, although

important progress has been achieved in the last 5 years. Some T3Es target plant PRRs, R or guarded proteins, lead to their degradation or prevent the interaction with MAMPs or other effector proteins, thereby interfering with plant immune responses [6, 7]. Several *Pseudomonas* spp. T3Es interfere with proteins regulating signal transduction pathways [8–11] or locate to the chloroplast and remodel its structure [12]. Similarly, some *Xanthomonas* outer proteins (Xop) also act as suppressors of plant defense. For example, XopN targets a receptor-like kinase implicated in the response of tomato to *Xanthomonas axonopodis* pv. vesicatoria [13]. Another example is XopD which enhances bacterial growth and delays symptoms development at late infection stages, acting thereby as a positive regulator of defense responses [13, 14]. A particular class of T3E proteins predominantly found in *Xanthomonas* localize to the host cell nucleus where they modulate gene expression. These effectors originally named AvrBs3/PthA-like proteins are also referred to as transcription activator-like effectors (TALEs) [15].

Transcription Activator-Like Effectors

TALEs constitute a group of effector proteins with particular features. The *C*-terminus carries nuclear localization signals (NLSs), allowing import of the protein to the nucleus. Downstream of the NLSs an acidic activation domain (AD) is also present, which is probably involved in the recruitment of the host transcriptional machinery [16]. The central region harbors the most fascinating feature of TALEs and the most versatile. It is made of a series of nearly identical 34/35 amino acids modules repeated in tandem. Residues in positions 12 and 13 are highly variable and are, therefore, referred to as repeat-variable di-residues (RVDs) [17]. Most of the variation between TALEs relies on the number (ranging from 5.5 to 33.5) and/or the order of the quasi-identical repeats, which also specify TALE virulence and/or avirulence activities. The number of TALEs present in a particular *Xanthomonas* species or strain varies from none (i.e., *X. campestris* pv. campestris strain 8004 or *X. axonopodis* pv. vesicatoria strain 85–10) to several (7–28 in *X. axonopodis* pv. malvacearum, *X. oryzae* pv. oryzae, *X. oryzae* pv. oryzicola) [15]. Why do *X. oryzae* pathovars or *X. axonopodis* pv. *malvacearum* carry a high number of TALEs, and what is the function of each of them are questions that still remain unsolved. A running hypothesis is that most of the copies act as reservoir genes facilitating the generation of new virulence alleles to escape *R* genes and/or unresponsive susceptibility alleles [18–21].

The *Boch–Bogdanove* Code

AvrBs3 from *X. axonopodis* pv. vesicatoria which is one of the best-studied TALE, induces the expression of several host genes once injected into pepper mesophyll cells [18, 22, 23]. A particular promoter element is required for AvrBs3 binding and is conserved in most *UPA* (upregulated by AvrBs3) genes. Upon infection of susceptible plants, AvrBs3 binds to the promoter and activates the expression of *UPA20*, which encodes a basic helix-loop-helix (bHLH) transcription factor. Single transient expression of *UPA20* in *N. benthamiana* phenocopies the cell hypertrophy phenotype naturally provoked by avrBs3-expressing *Xanthomonas*. In turn, UPA20 activates the transcription of *UPA7,* a gene coding for a putative α-expansin, which is potentially involved in plant cell expansion [18, 22]. In *Bs*3 resistant pepper lines, AvrBs3 induces the expression of *Bs*3, which encodes a flavone monooxygenase and triggers a hypersensitive response [24]. The analysis of *UPA* promoters allowed the detection of an element similar in length to the number of repeats present in the AvrBs3 protein [22]. This, together with additional pairs of TALEs and cognate DNA target sequences supported studies conducted by two teams which resulted in the revolutionary discovery that each RVD in a repeat of a particular TALE determines the interaction with a single nucleotide, thereby cracking the mysterious recognition code [25, 26]. Careful analysis of the nucleotide binding specificity of each RVD demonstrated that some are highly specific while others may recognize several nucleotides with varying interaction levels. Given that TALEs may induce or repress the expression of target genes, it has been suggested to rename the *UPA* box as TAL box, replacing the word TAL by the name of the referred protein [15]. Resolving the 3D structure of the TALE repeats is a challenging issue but could contribute to the complete elucidation of binding specificities. Although limited, data from NMR analysis on a 1.5 repeat polypeptide provided initial insights into this protein–DNA interaction [27]. Recently, two studies provided a very detailed model from a natural and an artificially engineered TALE, both in a complex with their target DNA box [28, 29].

TALEs Break the Old Paradigm in Plant–Pathogen Interactions

Classical models on the recognition of pathogens by plant resistance proteins involve a direct or indirect interaction between the Avr and the matching R proteins. However, the discovery of TALE function as virulence factors and the nature of their recognition in resistant plants led to some adjustments of the classical models. Indeed and with the exception of the AvrBs4/Bs4 interaction, TAL effector-triggered immunity occurs via binding to DNA [30] (Fig. 1). It is also noteworthy that "*R genes*" in the case of TALEs-mediated resistance do not match classical NBS-LRR proteins [24, 31], but rather correspond to proteins with no apparent function in resistance [32].

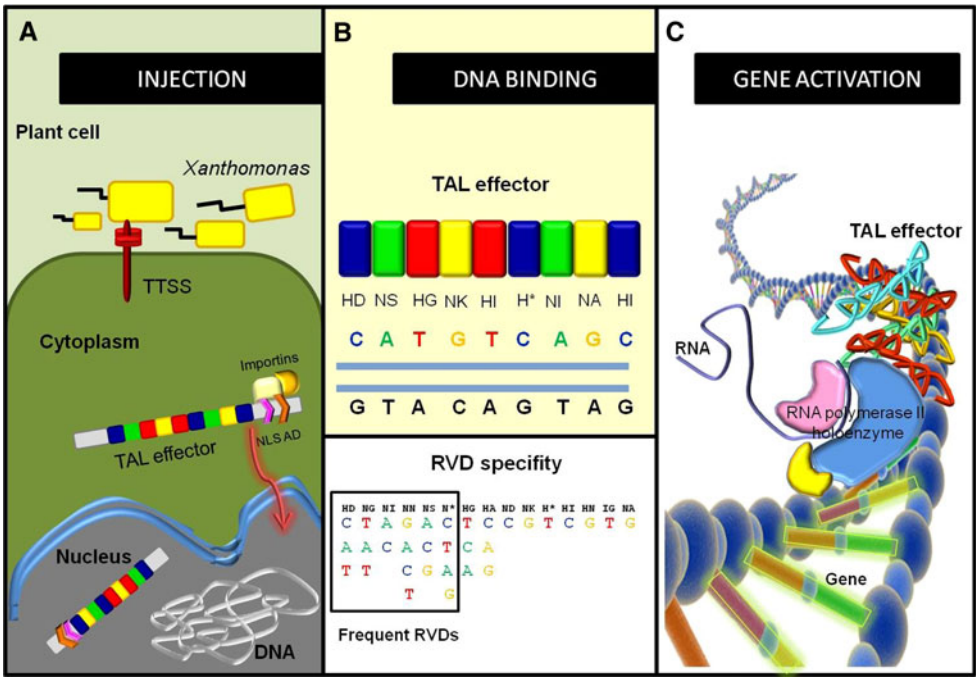

Fig. 1 Schematic representation of TAL effector activity. **a** Once in contact with the cell, bacteria deliver a multitude of effectors into the host cytoplasm using a TTSS. The TAL effector is then transported to the nucleus via α or β importins. **b** Once in contact with the DNA each RVD (positions 12 and 13 in each repeat) matches with a specific nucleotide. Only 15 RVD couples are found in nature and six of them are more frequent than the others. **c** RVDs bind to the DNA, other aminoacids repeats provide stability to this binding. Together with the activation domain, the transcription machinery is recruited to activate (or repress) gene expression

TALEs and More TALEs

Back in the 90s, TALEs provided the first insights in our understanding of the true function of the so-called avirulence genes during pathogenesis [33]. It was early hypothesized that these genes must fulfill some important functions in the fitness of the pathogen in the absence of the corresponding *R* gene. Indeed *pthA* from *Xanthomonas axonopodis* pv. citri [21, 34, 35] and *avrB6* from *Xanthomonas axonopodis* pv. malvacearum were the first TALEs reported to be required for full virulence in their respective hosts. Introduction of *pthA* in non aggressive citrus strains of *X. axonopodis* pv. citri confers the ability to cause canker on citrus plants [36], while *avrb6* enhances water soaking symptoms in susceptible cotton [37]. *hax2*, *hax3*, and *hax4* from *Xanthomonas campestris* pathovars infecting *Brassicaceae* were later demonstrated to have an additive effect in symptom development on radish, with *hax2* showing the strongest effect [38].

Together with the seminal work performed on AvrBs3, the elucidation of TALE molecular function and targets *in planta* mainly derives from studies on *Xoo* which is the causal agent of Bacterial leaf Blight of rice. Upon translocation into the host, PthXo1 induces the expression of *Os8N3,* a susceptibility gene in rice [20]. Interestingly, rice lines carrying recessive alleles of this gene previously referred to as *xa13*, are "resistant" to *Xoo* strains relying on PthXo1 for virulence. This is due to nucleotides changes in the promoter at positions −86 to −69 leading to a non-induction of *Os8N3* [39]. In consequence, *xa13* accessions of rice are resistant (or not susceptible) to *pthXo1*-expressing *Xoo* strains, while *Xa13* accessions are susceptible. In addition, naturally occurring rice accessions with an *xa13* genotype show reduced fertility. High levels of *Xa13* (*Os8N3*) expression have been detected in pollen, which may be related to the fertility problem observed in plants defective for *Xa13* expression (*xa13* lines). Recently, it was shown that Xa13 interacts with COPT1 and COPT5, two copper transporter proteins [40]. Copper is a toxic element for bacteria and the strain of *Xoo* PXO99 is particularly sensitive to it. In this manner, bacteria would induce the accumulation of Xa13, which upon interaction with COPT1 and COPT5 promotes the reduction in the concentration of copper in xylem vessels [40]. This results in an increase in the ability of the PXO99 strain to multiply in xylem vessels, leading to susceptibility.

Resistance mediated by *xa13* can be overcome by *Xoo* strains containing the major virulence TALEs AvrXa7 or PthXo3 [41]. The defeat of *xa13*-mediated resistance is due to the activation of the alternative susceptibility gene *Os11N3* gene by AvrXa7 or PthXo3, which are binding to a specific target box in the *Os11N3* promoter. Interestingly, an African strain of *Xoo* was found to contain a major virulence TALE (denominated TalC) also inducing the expression of *Os11N3*. A *talC* mutant can multiply at the inoculation point but is unable to successfully colonize the vascular tissues, a novel phenotype that is suggestive of a new function for TALEs in vascular colonization [42]. Recently, *Os8N3* and *Os11N3* encoding proteins (renamed OsSWEET11 and OsWEET14) were demonstrated to act

as passive sugar transporters [43] and hypothesized to redirect the accumulation of nutrients in the vascular tissues at the site of *Xoo* multiplication.

Similar to AvrBs3, other TALEs from *X. oryzae* also activate the expression of transcription factors, which in turn activate a set of genes in the host. For example, *Xoo* TALEs PthXo6 and PthXo7 induce the expression of *OsTFX1* and *OsTFIIAγ1*, respectively [44]. In particular, *TFIIAγ1* encodes a general transcription factor of the RNA polymerase II and a paralog of *TFIIAγ5* present on the chromosome 5 of rice. Bacteria containing *avrXa5* are avirulent on rice plants containing *xa5*, an allele of *TFIIAγ5* containing a substitution of a single residue. However, when *pthXo7* is expressed in *avrXa5*-containing bacteria and inoculated on *xa5* rice plants, the virulence is restored as a consequence of the induction of *TFIIAγ1* [44].

Another *Xoo* TALE of interest is AvrXa27 from strain PXO99, due to its ability to elicit resistance in IRBB27 rice lines as a consequence of the induction of the *Xa27* resistance gene [31]. The coding sequence for *Xa27* is 100 % identical in susceptible and resistant plants, but differences lie in the promoter region. In the case of an incompatible interaction, AvrXa27 binds to the promoter of *Xa27,* activating its expression [31]. In susceptible plants, mutations at the promoter do not permit binding of the AvrXa27 protein. As for *Bs3*, the coding sequence of *Xa27* does not provide clues about the function of this protein in plant resistance.

From Basic Knowledge to Biotechnological Engineering

As stated before, TALEs contain a new and unique kind of DNA-binding motif with high sequence specificity. Based on the TALE code, this remarkable feature can be exploited to artificially design TALE proteins interacting specifically with DNA sequences of interest to modify them by insertion, deletion, or other targeted rearrangements [26, 45]. Collectively, these approaches can be referred to as genome editing. Genome editing is a promising therapeutic avenue to replace spiteful or aberrant DNA sequences and/or introduce genes coding for desired traits in a specific region of the genome assuring their expression [46]. The basis of genome editing rests on the DNA repair systems present in eukaryotic cells when breaks occur in the DNA molecule. The two most important DNA breaks repair mechanisms are the non-homologous end-joining (NHEJ) and homologous recombination (HR) [47]. NHEJ produces DNA sequence changes such as deletion/insertion and/or nucleotide substitutions in the target sequence, which could correspond to a particular gene. On the other hand, HR occurs when an exogenous DNA sequence is added, whereby recombination will take place between this and the target region allowing the incorporation of the exogenous sequence in the genome. Technologically, the activation of the NHEJ and HR and targeting of particular sequences depend on the possibility to create, in a particular genome region and in a controlled manner, double stranded DNA breaks (DSB) which are the substrate for the aforementioned reparation systems [47] (Fig. 2).

This has already been achieved through the use of chimeric endonucleases such as zinc-finger nucleases (ZFNs) [46]. ZFNs are hybrid proteins containing the zinc-finger DNA-binding domain present in transcription factors and the non-specific cleavage domain of the endonuclease Fok1. In order to be active, Fok1 has to bind DNA and dimerize. The most critical aspect for the use of ZFNs as a tool for genome editing is their specificity and affinity to particular target DNA regions. The ability to assemble ZFNs in a modular manner increases their ability to bind specific DNA sequences. The use of ZFNs has not been as widespread as anticipated, mainly due to the difficulty of producing proteins with high selectivity for a target DNA sequence and low failure rates, which is a laborious and time-consuming process [46]. Considering the mode of action of TALEs, one can anticipate that their use will overcome some of these difficulties and become a true alternative. Once a gene sequence is defined, a TALE can be designed to target this particular gene. This highly specific TALE can be coupled with an endonuclease that will produce DSB and in consequence the gene will be edited (Fig. 2). Alternatively, a TAL can be constructed to be directed to a particular promoter region of an specific gene which allow the induction or repression of the gene. This can be considered as a new option for gene therapy. As a matter of fact, the potential of using TALE Nucleases (TALENs) for genome targeting was reported shortly after the elucidation of the TAL code [48]. This first report on TALENs is based on the use of a yeast assay and the TALEN-dependent reconstruction of a functional *lacZ* reporter system. Two plasmids were introduced into yeasts, the first one containing the *lacZ* reporter gene with a duplication of a 125-bp sequence flanked by the target sequence of a particular TALE, and the other plasmid carrying the sequence coding for this TALE fused to the Fok1 endonuclease. Once in yeast, TALENs produce a DSB within *lacZ* leading to its repair and the generation of a functional *lacZ* gene [48]. Recently, new design and methods were developed to construct specific and desired TALENs employing repeat arrays in a faster and easier way [49]. Artificial TALENs have been reported to also function in plants [50] and other eukaryotes like yeast [51], zebrafish [52], rat embryos [53], and human cells [54, 55]. For example, it was possible to disrupt two

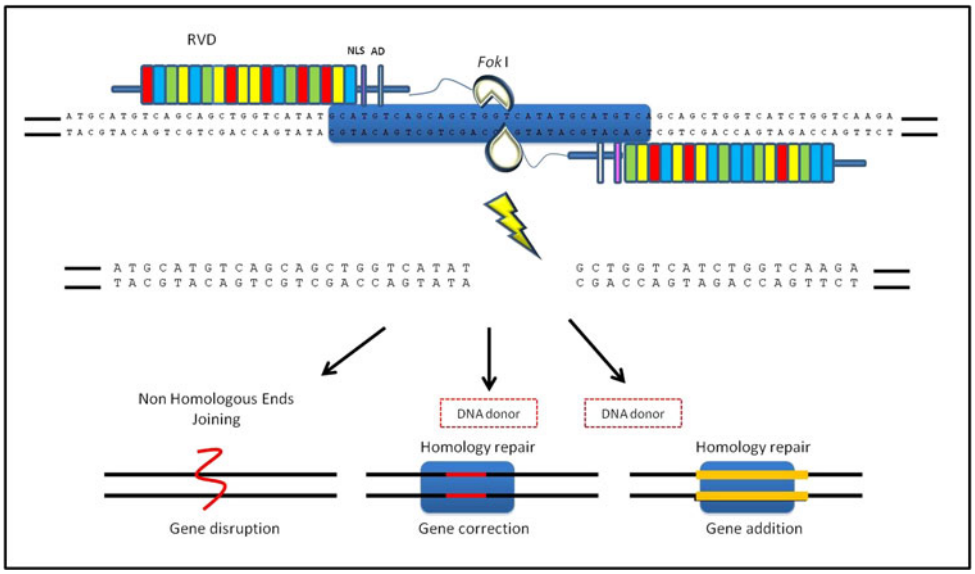

Fig. 2 Genome editing. A pair of forward and reverse artificial TALs are designed to target specifically the genome region of interest. As a result of the fusion with the FokI domain, the TALEN will produce DSB. In order to overcome these breaks, two possible reparations can be done: NHEJ or HR. By HR via a DNA donor it leads to a DNA correction or to a gene addition

zebrafish genes by means of artificial TALENs that caused directed mutations in a very specific manner. The mRNAs coding for the TALs were injected into zebrafish causing mutations on the target genes and interestingly, these mutations were transmitted through the germ lines. Efficiencies of up to 25 % were reported in human cells [54] and mutation frequencies of 11–33 % in somatic zebrafish cells [52] (Table 1). In most of the cases, mutation are due to small indels but deletions as large as 3,003 bp have also been reported [55]. The success of the TALEN technology is such that it is now possible to create and order specific TALEN (http://www.cellectis-bioresearch.com/talen). Another application provided by TALEs is the control of the expression of genes of interest [48, 55]. For example, fusion of a specific TALE to the strong VP16 trans-activation domain enabled a 70-fold induction of the target gene [55]. The fact that TALEs can induce or repress host genes opens the possibility to use them for controlled expression of target genes involved in disease or other traits.

Table 1 TAL-mediated genome editing advances

Organism	Main results
Zebrafish	Heterodimeric TALENs had better toxicity profiles and better transmission rates than homodimeric TALENs. Demonstrated the feasibility of this technology for the in vivo generation of knockout zebrafish efficiently [58]. TALEN had a high efficiency in inducing locus-specific DNA breaks in somatic tissue in vivo and germline tissues [52, 59]. Successful germline transmission [59]. TALENs showed a higher success rate, when compared to ZFNs, for obtaining active nucleases capable of inducing mutations [60]. First demonstration of heritable gene-targeting in zebrafish using customized TALENs [61]
Rats	Efficient gene-targeting in embryonic stem cells using TALEN [62]. Generation of knockout rats by microinjection of TALEN in embryos [53]
Drosophila	Efficient gene-targeting using TALEN mRNA injected into *Drosophila* embryos. 31.2 % of the injected embryos had inheritable modifications of the target gene [63]
Yeast	Custom-designed TALEN used for effective gene repair at the beta-globin locus in yeast [64]. Generation of designer TALENs (dTALENs) with the potential of specific recognition of unique DNA sequence in any yeast gene. Low levels of cytotoxicity [65]
Human	TALENs designed for gene modification in human cells. TALENs were as efficient and precise as do ZFNs [54]. Gene modification in genes involved in cancer and/or epigenetic regulation [66]
Arabidopsis	TALEN directed to ADH1 in Arabidopsis thaliana protoplasts [49]
Rice	Rice lines with resistance to Xoo by directed mutagenesis of susceptibility genes employing TALEN-based gene editing [67]
Hemimetabolous insect	A TALEN and non-transgenic approaches to control insect pests [68]
HIV-derived DNA–RNA hybrid	Potential control of DNA replication and retroviral infections by a specific DNA–RNA hybrid recognition employing TALENs [69]

Since the discovery of the TAL code, several approaches have been taken to improve the use of TALEs for genome editing in several organisms. This table collects the latest studies where TALE-based engineering has been applied

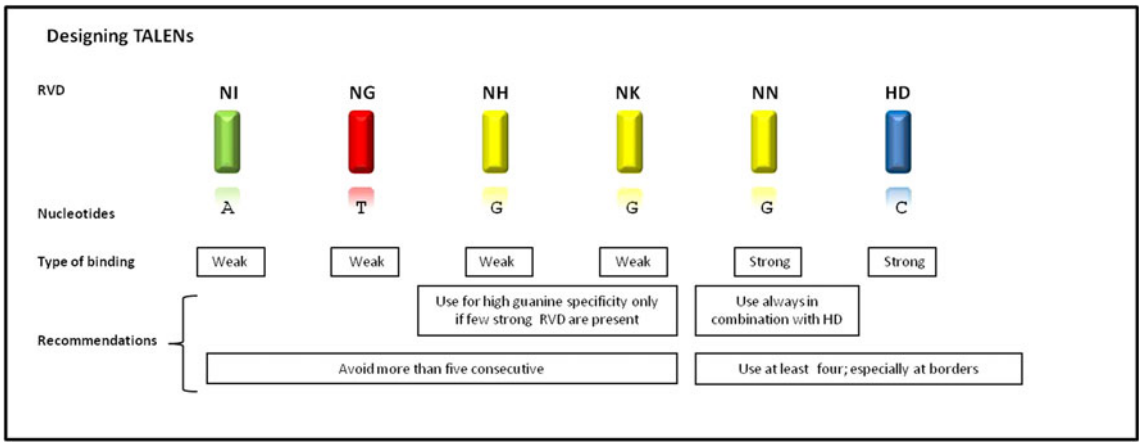

Fig. 3 Designing artificial TALs. In order to improve the use of engineered TALs for genome editing, the study about each RVD specificity and their optimal position into the construct have been recently described [56]. This figure collects the major advices to accomplish successfully an artificial TAL design

TALEs play often but not always a crucial role during plant–bacteria interactions. Their fascinating ability to interact with the eukaryotic transcription machinery is suggestive of an interesting co-evolution phenomenon between plants and bacteria. Direct target gene expression reprogramming by TALEs reveals a new and original strategy for bacteria to conquer their host, it also unveiled more adaptive mechanisms deployed by plants to avoid infection.

The predictable and modular nature of TALE repeats for DNA-binding makes them a useful tool for diverse biotechnology applications. The possibility to create artificial TALEs is a tempting solution for this and other aspects in biotechnology. A recent study on the specific contribution of RVDs to DNA-binding [56] provide a few recommendations to optimally design artificial TALs (Fig. 3). Particularly interesting, some approaches have been taken to couple artificial TALs with other protein domains such as activators, repressors, methylases or nucleases to edit the genome specifically with the most appropriate activity [57]. However, the widespread use of TALEs will imply to clarify some unanswered questions: how many TALENs is it possible to use in parallel to improve gene regulation? Are TALEs able to work in a chromosomal context in mammalian cells? What would be the consequences of the eventual non-specific binding of TALEs on organisms? What is the effect of DNA methylation on TALEs activity? All these questions, together with ethical and sociological discussions concerning the application of TALEs should be considered globally to improve their use as a tool for biological engineering.

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