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TAL effectors: finding plant genes for disease and defense

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Transcription activator like effectors (TALEs) are injected via the type III secretion pathway of many plant pathogenic *Xanthomonas* spp. into plant cells where they contribute to disease or trigger resistance by binding to DNA and turning on TALE-specific host genes. Advances in our understanding of TALEs and their targets have yielded new models for pathogen recognition and defense. Similarly, we have gained insight into plant molecules and processes that can be co-opted to promote infection. Recent elucidation of the basis for specificity in DNA binding by TALEs expedites further discovery and opens the door to biotechnological applications. This article reviews the most significant findings in TALE research, with a focus on recent advances, and discusses future prospects including pressing questions yet to be answered.

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Introduction

Transcription activator like effectors (TALEs) are a structurally and functionally distinct class of proteins from plant pathogenic bacteria that are injected via the type III secretion pathway into plant cells. Once in the plant cell, they enter the nucleus, bind to TALE-specific DNA sequences, and turn on the downstream genes. Interaction with plants has selected TALEs that activate host genes that facilitate bacterial colonization and spread. Disease pressure has in turn selected plant adaptations that mount defense in response to TALEs. Thus, TALEs can be virulence factors, plant-recognized 'avirulence' factors, or both. Our understanding of TALEs has been shaped by 20 years of research, beginning with the discovery of the AvrBs3 protein of the pepper and tomato pathogen *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) [1]. Here, we review selected papers published during this period (for more thorough historical reviews, see [2–6]), with a focus on the most recent advances centering on the discovery of how TALEs find their DNA targets. These studies constitute a major leap forward in our understanding of plant–pathogen interactions and, more generally, of protein–DNA interactions.

Distribution and structural features of TALEs

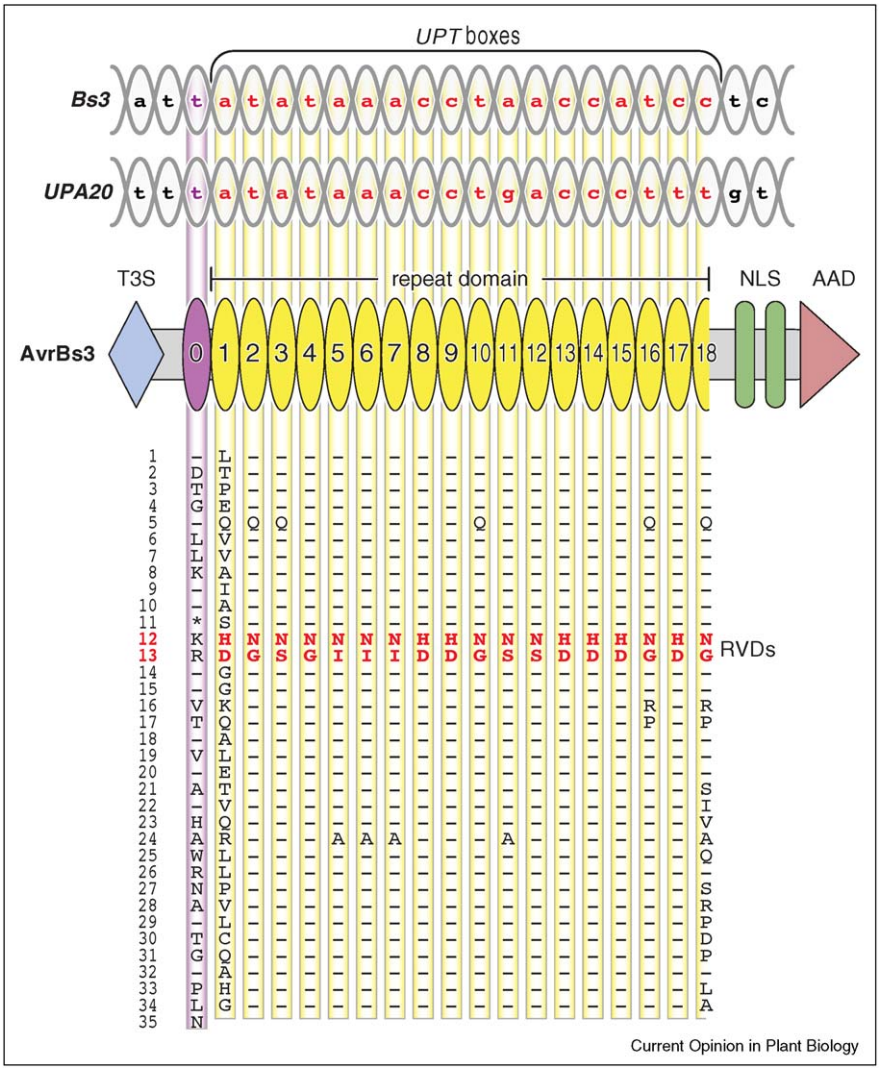
TALEs are found in several but not all pathogenic members of the genus *Xanthomonas*. Individual *Xanthomonas* strains may have as few as one or as many as several dozen TALEs [1,7]. Closely related proteins have been found in several biovars of *Ralstonia solanacearum*; some of these are delivered into host cells, but whether they act as transcriptional activators is not yet known [8–11]. The HsvB and HsvG host specificity determinants of the sugar beet and *Gypsophila* pathogen *Pantoea agglomerans* localize to plant cell nuclei, bind double stranded DNA, and activate transcription in yeast, but are structurally distinct from the TALEs of *Xanthomonas* [12]. These fascinating proteins have been reviewed elsewhere [13] and are not discussed in detail here.

TALEs have in common an N-terminus required for type III secretion and a C-terminus containing nuclear localization signals (NLS) and an acidic activation domain (AAD) typical of transcription factors (Figure 1). TALEs differ in the middle, a region of typically 33–34 amino acid (aa) long, near-perfect repeats that ends in a 20 aa long truncated repeat. 35 aa repeats also occur, and some members of the family, including all of the *Ralstonia* proteins, have almost exclusively 35 aa repeats. The number of repeats varies from protein to protein, and repeat polymorphism exists within and across TALE proteins. The polymorphism is concentrated at residues 12 and 13, referred to as the repeat-variable diresidue (RVD) [14••]. Note that the final, truncated repeat contains an RVD, which is in most cases NG (we describe RVDs herein by using single-letter codes for the two amino acids). Nearly 20 distinct RVDs occur among TALEs examined to date, but four, HD, NG, NI, and NN, account for 75% of the total [14••]. NMR analysis of 1.5 repeats from the *X. citri* TALE PthA recently revealed that the fold is the same as that of tetratricopeptide repeat (TPR) proteins, namely, two antiparallel α helices per repeat; this positions the RVDs on a solvent exposed surface, as predicted by Schornack *et al.* (C. Benedetti, personal communication; [2]).

TALEs as transcriptional activators—a historical view

The TALE AAD was first shown to be functional in the AvrXa10 protein of the rice pathogen *X. oryzae* pv. *oryzae*

Figure 1



Structural features of TALEs and matching binding sites (UPT boxes) exemplified by AvrBs3, the founder of the TALE family, and the corresponding pepper *Bs3* and *UPA20* promoters. AvrBs3 contains a N-terminal type III secretion and translocation signal (T3S), a repeat domain consisting of 17.5 repeat units (yellow ovals), two nuclear localization signals (NLS) and a C-terminal acidic activation domain (AAD). Repeat polymorphism occurs largely at residues 12 and 13, the repeat-variable diresidues (RVDs, uppercase boldface red letters). Note that the final, partial repeat contains an RVD. The repeat domain is preceded by a postulated 0th repeat (purple oval) that has only weak sequence similarity but a predicted structural similarity to the repeats. The *UPT* box of the pepper *Bs3* and *UPA20* promoters (lowercase boldface red letters) contain as all known *UPT* boxes an invariant 5′ T (purple). The AvrBs3 repeat residues are numbered and depicted in single-letter code below the structural representation. Residues identical to, or differing from repeat 1 are depicted by dashes or single-letter code, respectively. A gap that was introduced to optimize the alignment between the proposed 0th and the 1st repeat unit is denoted by an asterisk (*).

(*Xoo*), as a fusion to the yeast Gal4 DNA binding domain [15]. Shortly thereafter, a study of the TALE AvrXa7 from the same bacterium demonstrated binding of double stranded DNA, though specificity was not determined [16]. TALE-dependent expression of a resistance (*R*) gene, *Xa27* in rice, was reported in 2005 [17••]. This was a novel paradigm for effector triggered immunity, and the first example of what we refer to as an 'executor' *R* gene, the product of which is not involved in recognition of the effector, but only in carrying out defense when it is expressed. The following year, transcriptional activation of the rice gene *Os8N3* by the major virulence factor PthXo1 of *Xoo* was shown to be important for bacterial multiplication and disease development, giving rise to the notion of host susceptibility (*S*) genes as targets of TALEs [18••]. That activation is direct and depends on specific, repeat-mediated binding to the target gene promoter, that is, TALEs are indeed transcription factors, was then demonstrated with AvrBs3 and two of the genes it activates in pepper, the executor gene Bs3 and the putative S

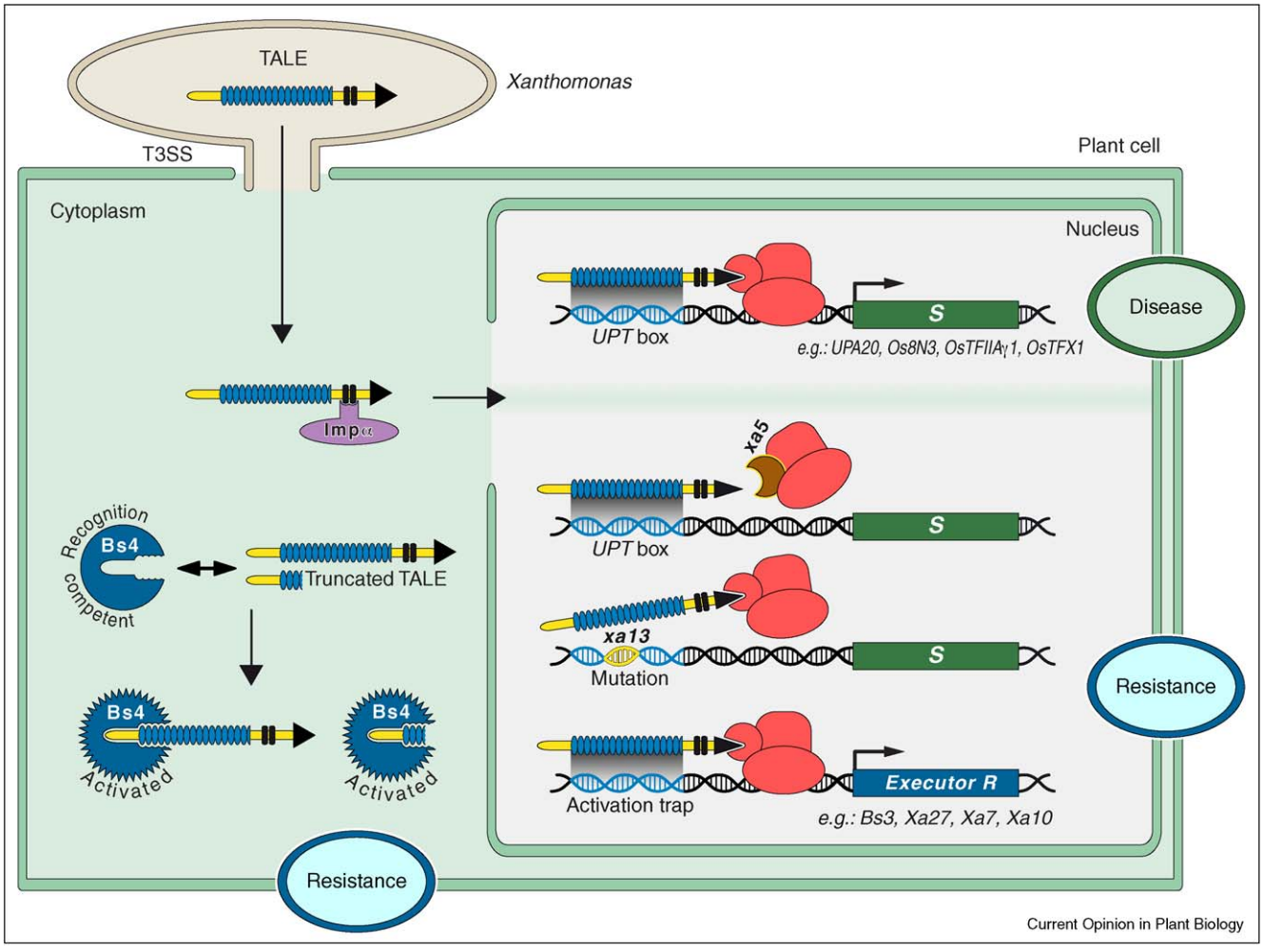
gene UPA20 [19••,20••]. More recently, the precise nucleotide sequences required for activation of these promoters by AvrBs3 were determined [21•,22•]. Highly conserved among genes *up*regulated by *A*vrBs3, this *UPA* box functioned from wherever it was placed in a promoter, directing initiation of transcription from 40 to 50 bp downstream of its 5′ end [22•].

Resistance mechanisms that target TALEs

Surprisingly diverse *R* genes have been isolated that mediate resistance to TALE containing xanthomonads (Figure 2). The pepper *Bs3* and rice *Xa27* genes recognize the TALEs AvrBs3 and AvrXa27, respectively [17••,19••,22•]. Both *R* genes contain distinct, effector-specific promoter motifs, designated as *UPT* (*up*regulated by *T*ALE) boxes, that direct physical interaction with and activation by the matching TALEs [19••,22•,23••].

The pepper *Bs3* gene mediates not only recognition of the *Xcv* AvrBs3 protein but also of the *X. gardneri* AvrHah1 protein, a TALE that has a repeat composition similar to AvrBs3 [24]. Recent studies revealed that AvrHah1 like AvrBs3 transcriptionally activates the *Bs3* promoter (T. Lahaye and S. Recht, unpublished). Thus TALE recognition in *Bs3* and *Xa27* immune pathways is at the *R* promoters, while the R proteins themselves are defense executors only. Transcriptional activation of *R* promoters may be the most common mechanism for recognition of TALEs since TALE mutant derivatives that lack either the NLS or ADD domains are generally

Figure 2



TALE roles in plant disease and resistance. Upon injection via the type III secretion system (T3SS) TALEs can be detected in the host cytoplasm as exemplified by the tomato NB-LRR type resistance (R) protein Bs4, which is capable of detecting the full-length TALE AvrBs4 as well as deletion derivatives that lack nuclear localization signals. Upon interaction with importin α (impα) TALEs translocate to the nucleus, bind to matching *UPT* boxes, and transcriptionally activate matching host susceptibility (*S*) genes such as *UPA20*, *Os8N3*, *OsTFIIAγ1*, or *OsTFX1*, resulting in disease. Activation of *S* genes can be suppressed either by a variation in the RNA II polymerase complex (red shapes) such as *xa5* (brown shape) or by mutations in the individual *UPT* boxes (*xa13* mutation). Plants have also evolved activation traps for resistance in which executor *R* genes are under transcriptional control of *UPT* boxes that would normally be present in *S* gene promoters.

incapable to trigger matching plant *R* genes [2]. One exception is the nucleotide binding site-leucine-rich repeat (NB-LRR) type R protein Bs4 from tomato that mediates recognition of but is not transcriptionally activated by the matching TALE AvrBs4 [25]. Since Bs4 also recognizes NLS or AAD deficient alleles [26] it seems likely that the recognition is of AvrBs4 structure rather than activity.

Given that the vast majority of plant *R* genes encode NB-LRR type proteins [27], it is surprising that Bs3 and Xa27 share no sequence homology with these or any other plant R proteins, and are distinct from each other. The rice *R* genes *Xa7* and *Xa10*, which confer resistance to strains expressing TALEs AvrXa7 and AvrXa10, respectively, map to regions that in the reference genome similarly contain no sequences homologous with known plant *R* genes [28–30]. Thus they may also be executor R proteins.

In addition to *R* genes that recognize TALE structure (*Bs4*) or subvert TALE function (*Bs3* and *Xa27*), which are genetically dominant, there are recessive *R* genes that interfere with TALE activity. For example, the rice *xa13* gene comprises alleles of the *S* gene *Os8N3* (synonym: *Xa13*) that are not activated by the corresponding TALE PthXo1. These are distinguished by differences in their promoters [18••,31•,32]. A recent study showed that the *Os8N3* promoter contains a functional UPT_{PthXo1} box, while all *xa13* genotypes do not [33•]. While mutations in the *UPT* box of an *S* gene like *Os8N3* may block the action of a single TALE, the rice *xa5* gene is postulated to interfere with the function of multiple TALEs. Located on chromosome 5, *xa5* encodes a V39E substitution variant of the γ subunit of the general transcription factor TFIIA [34]. Notably, transcriptional activation of the rice *Xa27* gene by the matching TALE AvrXa27 is severely attenuated in *xa5* genotypes [35•]. Reduced transcriptional activation in *xa5* genotypes was also reported for the rice *Os11N3* gene targeted by TALE AvrXa7 (G. Antony *et al.*, abstract in *Phytopathology* 2009, **99**:S5). Although complementation was not carried out to show conclusively that the effect was due to *xa5*, given that TFIIAγ is a component of the RNA II polymerase complex, it is tempting to speculate that TALEs recruit the complex via interaction with TFIIAγ to activate transcription. The *xa5*-encoded V39E substitution might reduce the affinity of this interaction, resulting in reduced TALE mediated activation of host target genes important for infection. Intriguingly, TALE PthXo7, from a strain virulent to *xa5* genotypes, transcriptionally activates a *TFIIAγ* paralog that is located on chromosome 1 and is normally expressed at very low levels [36•]. This strain might have evolved PthXo7 as a means of compensating for the reduced functionality of the *xa5*-encoded protein. In support of this hypothesis, a strain against which *xa5* is normally effective reached almost 10-fold higher titers in rice *xa5* genotypes when transformed with *PthXo7* [36•].

The variety of the resistance mechanisms plants have evolved to defend against pathogens expressing TALEs is remarkable. This is particularly true in light of the fact that effector triggered immunity in most studied cases is mediated by NB-LRR type R proteins, which function as sentinels [27]. Of special interest are recessively inherited *R* genes, which are likely to provide further insight into the virulence functions of TALEs.

TALE targeted genes in disease

Host whole genome expression analyses led to the identification of the rice *S* gene *Os8N3* activated by PthXo1 and of *UPA20* and several other pepper genes upregulated by AvrBs3 [18••,20••,21•]. *Os8N3* is related to *MtN3*, a *Medicago truncatula* gene differentially expressed during nodulation [37]. In rice, *Os8N3* is required for normal pollen development [31•]. Plants constitutively overexpressing *Os8N3* were as susceptible to a PthXo1 mutant strain of *Xoo* as to the wild type, and *Os8N3* silenced plants were resistant [18••]. In a strain naturally lacking PthXo1, an alternative major virulence factor TALE, AvrXa7, acts by turning on another member of the *Os8N3* family, *Os11N3* (G. Antony *et al.*, abstract in *Phytopathology* 2009, **99**:S5). How activation of rice *Os8N3* or *Os11N3* renders the plant susceptible to colonization is not yet understood.

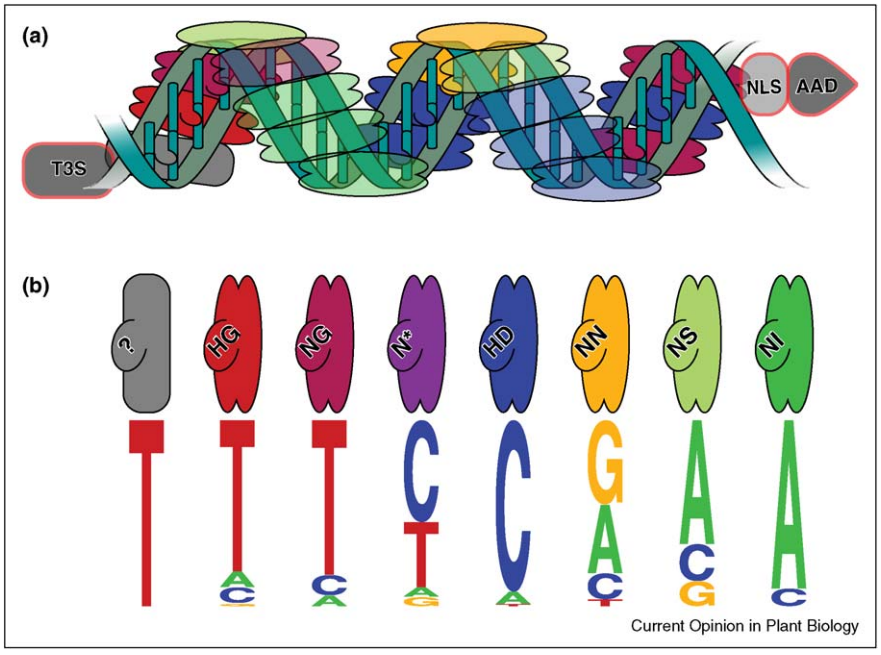
As noted, *Xoo* TALE PthXo7 activates TFIIAγ1, and some evidence suggests that this contributes to susceptibility in a *xa5* background [36•]. Another rice transcription factor gene, *TFX1*, a member of the basic region leucine zipper (bZIP) family, is activated by *Xoo* TALE PthXo6 [36•]. *TFX1* was activated by each of a diverse collection of *Xoo* isolates, suggesting that PthXo6 is broadly conserved and important. A knockout in the strain from which it was identified indeed caused a reduction in virulence that could be rescued by ectopic, constitutive expression of *TFX1* [36•]. Thus, *TFX1* is another *S* gene important for bacterial blight of rice.

The AvrBs3-induced pepper *UPA20* gene encodes a transcription factor of the basic helix-loop-helix type. Its transcriptional activation results in the hypertrophy that takes place at infection loci in bacterial spot of pepper [20••]. Hypertrophy may play a role in eruption of the bacteria onto the leaf surface [38]. Other *UPA* genes form a diverse group for which roles in susceptibility are still unclear, but intriguingly *UPA16* is a member of the *MtN3* family, like *Os8N3* and *Os11N3* [20••,21•]. Identification of the *UPA* genes led to the detection of the *UPA* box, which played a paramount role in the discovery of the mechanism for DNA recognition by TALEs, discussed below.

DNA recognition by TALEs

Moscou and Bogdanove [14••] and Boch *et al.* [39••] recently demonstrated that the sequence of RVDs in a

Figure 3



TALE–DNA recognition. **(a)** Tentative model for TALE–DNA association. The central TALE–DNA binding domain consists of 33–35 aa repeat elements that we predict form a solenoid superhelical fold that wraps around the DNA. Repeat-variable diresidues (RVDs, positions 12 and 13 in each repeat) define the DNA specificity of the TALE and are assumed to associate one-on-one with specific nucleotides or base pairs. Repeats on the viewer-proximal face of the DNA are semi-transparent for clarity. **(b)** The most common RVDs and the nucleotides they associate with. Letter height represents frequency relative to other nucleotides for that RVD. Nucleotides represent the plus strand of the binding site, but it is not known whether contacts are made on the plus strand, the minus strand, or both. Frequencies are according to [14••].

TALE corresponds directly to the sequence of nucleotides in the binding site, one to one in a linear and contiguous fashion (Figure 3). This correspondence suggests a mechanism for binding that explains TALE specificity and enables prediction of TALE targets.

Moscou and Bogdanove deciphered the mechanism computationally [14••]. For several TALEs with known activation targets they scanned the target promoter sequences for alignments that showed the least randomness in the associations of different RVDs with different nucleotides. Using the frequencies with which individual RVDs associated with C, A, T, or G in these alignments, the authors mined the rice genome and microarray data to find additional alignments to TALEs of *X. oryzae* pathovars. Across all alignments, comprising 382 RVD–nucleotide associations, they observed that HD was by far the most common RVD and associated strongly with C. Next most frequent were NG with T, and NI with A. NN was the fourth most common RVD and associated most frequently with G, but nearly as often with A, and sometimes with T or C. Other well-represented RVDs included NS, found with G, A, or C; N* (missing residue 13, for a 33 aa repeat), which associates with C and T; and HG, which resembles NG in its associations. Several other RVDs occurred only rarely. Thus, the RVD–nucleotide associations constituted a partially degenerate code. The authors found no effect of neighbors on association frequencies. Flanking the binding site there were no shared sequences except a strictly conserved T at position −1.

Boch *et al.* discovered the code by projecting the RVDs of AvrBs3 onto the *UPA* box [39••], which is roughly the same length in base pairs as the number of repeats in AvrBs3 [21•,22•], and then using the associations in that alignment to find matches for seven other TALEs in the promoters of their respective activation targets. They then constructed artificial promoter sequences predicted to match three TALEs from *X. campestris* pv. *armoraciae* (*Xca*) and showed that these were functional and specific. Using the *Xca* TALE Hax3 they also demonstrated that the conserved T at −1 was crucial for promoter activation. They tested the functional significance of the association frequencies for HD, NI, NG, and NS by permutating G, A, T, and C at all locations at which the RVD occurs in the 15 RVD long *Xca* Hax4 protein (the RVDs occur 3–5 times each). For NS, permutations had no detectable effect. For each of the others, permutation to any but the most frequently associated nucleotide, C for HD, A for NI, and T for NG, abolished detectable activity. In a similar set of experiments, the authors showed that arbitrary, artificial TALEs activated corresponding promoters

constructed using the code. With one of them, they tested nucleotide specificity for NN. Consistent with its association frequencies, for NN at four locations in a 13 RVD sequence, G at all positions gave the best activity, A reduced but did not abolish activity, and C and T eliminated detectable activity.

The results from both studies indicate that TALE–DNA recognition depends on preferential association of individual RVDs with specific nucleotides. It remains unclear if the mechanism is simple, depending for binding on a minimum number of perfect matches over a certain length alignment, or complex, involving differential contributions of different RVD–nucleotide associations that might depend on their position within the alignment.

Complexities in TALE–DNA binding

The complex model for TALE–DNA binding is supported by the observations of Kay *et al.* [21•] and Römer *et al.* [22•] that single base pair mutations in AvrBs3 binding sites result in both qualitative and quantitative effects. In a recent study, Römer *et al.* [33•] observed a drastic loss of activity when C, T, or A was substituted for G at just the first position in the binding site for the 24 RVD TALE PthXo1, which is an NN. This observation is unexpected given that the RVD NN should bind equally well to G and A nucleotides according to the observed association frequencies. Also, three NNs in a row at positions 4–6 of the 23 RVD PthXo6 each align with A, not G, in its binding site, and the much shorter AvrHah1 (14 RVDs) begins with an NN that aligns with A, and both of these TALEs are active [14••,24,33•]. Whether activity of PthXo6 and AvrHah1 would be augmented by substituting G at those positions is not known, but the seemingly conflicting observations suggest that TALE–DNA recognition is more complex than implied by the simplicity of the code.

Although Moscou and Bogdanove [14••] found on average no influence of immediate neighbors on RVD–nucleotide associations, neighbor effects might come into play at certain positions in a TALE–DNA alignment, or, individual affinities might be affected by distal RVDs. Length and overall composition of the sequence might also affect the stringency of RVD–nucleotide specificities at different positions: DNA contact point distances between repeats could vary slightly with different RVDs and introduce strain at certain sites along an alignment, affecting whether a mismatch there would be tolerated or would kill binding. It is relevant to note here that the TPR fold recently shown for 1.5 repeats in solution places repeats at too great a distance from one another to align with consecutive bases in B DNA, suggesting that TALEs undergo compacting conformational changes on binding (C. Benedetti, personal communication). These might relate to the apparent variability in the consequences of mismatches. In this context, regional variation in stability along the TALE–DNA interface seems particularly plausible as an explanation for the apparent variability in the consequences of mismatches at different positions. Another possibility is that polymorphism outside the RVD (e.g., positions 4, 16, 17, and 24 in Figure 1) affects RVD–nucleotide specificity.

Prospects

Despite the unknowns, discovery of the code has opened the door to several biological and biotechnological pursuits. *UPT* boxes can now easily be identified in TALE targeted promoters [33•]. This allows a molecular breeding approach to identify promoter variant alleles of major *S* genes as new sources of resistance. It is possible to identify new TALE targets by searching host genome sequences for matching binding sites. For TALEs that are major virulence factors, such as the PthA proteins of *X. citri* [40,41], *S* gene candidates could be isolated in this fashion. Searches could be narrowed by incorporating whole genome expression data to focus on significantly upregulated genes. Required confirmation could then be carried out by overexpression and RNA interference or mutant testing. Executor *R* genes could be identified similarly. In addition to bioinformatic approaches, which will be applicable to sequenced genomes, physical interaction of TALEs with host target promoters might be exploited in affinity based purification approaches to capture *S* and *R* genes.

Engineering executor *R* gene promoters to contain *UPT* boxes for multiple, important, or widely conserved TALEs could be an effective means of achieving durable and broad-spectrum resistance. Feasibility of this approach was recently demonstrated by Römer *et al.* [23••], who showed in a transient expression assay that *UPT* boxes for three distinct TALEs retained function and specificity when stacked together in a single promoter. Conversely, the *in vitro* generation of functional TALEs by Boch *et al.* [39••] suggests that TALEs can be engineered for arbitrary gene activation or as translational fusions for directing other proteins, such as negative regulators, methylases or nucleases, to specific DNA targets [42].

Important puzzles remain to be solved before we fully understand the function and potential utility of TALEs. Foremost among these is their structure bound to DNA, and the structure/function relationships that explain the complexities in the code. Another is how TALEs interact with the host transcriptional machinery, and whether their binding to DNA can affect the activity of endogenous transcriptional regulators. In microarray analysis of rice responses to *Xoo* (15 distinct TALEs) and *X. oryzae* pv. *oryzicola* (28 distinct TALEs), however, no significant reductions were observed for any transcript, suggesting that TALEs activate transcription in whatever context they find a *UPT* box, and do not interfere with oppositely

oriented endogenous activators (A.J. Bogdanove, unpublished). A third important line of inquiry is evolution: the origin of TALEs, their diversity, the effect of large numbers of these potentially highly recombinogenic genes on bacterial genomes, why TALEs are numerous in pathogens of some crops but not others, are important and still unanswered questions. Fortunately, the intriguing unknowns are drawing scientists from diverse disciplines to TALE research. The next several years should see a cascade of exciting advances.

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- • of special interest
- •• of outstanding interest

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By examining the effect on gene expression in rice of the *Xoo* TALE PthXo1, which is a major virulence factor, the authors discover *Os8N3* as an activation target. RNA silencing reveals *Os8N3* to be an *S* gene, required for susceptibility to this strain. The authors provide evidence that *Os8N3* is an allele of the recessive *R* gene *xa13*, establishing together with [31•] a new paradigm for plant disease resistance.

19. •• Römer P, Hahn S, Jordan T, Strauß T, Bonas U, Lahaye T: **Plant–pathogen recognition mediated by promoter activation of the pepper *Bs3* resistance gene**. *Science* 2007, **318**:645-648.
Positional cloning of the pepper *Bs3* gene that mediates recognition of AvrBs3 uncovers a structurally unique *R* gene that is transcriptionally activated by AvrBs3. Swapping experiments between *Bs3* and the allelic *Bs3-E* gene demonstrate that the promoter and not the coding sequence determines recognition specificity. Notably the promoter of the *Bs3* but not the *Bs3-E* gene contains a *UPA* box. Together with [20••] this study shows that identical TALE target boxes are present in *Xanthomonas R* and *S* gene promoters.

20. •• Kay S, Hahn S, Marois E, Hause G, Bonas U: **A bacterial effector acts as a plant transcription factor and induces a cell size regulator**. *Science* 2007, **318**:648-651.
The analysis of two pepper genes that are *up*regulated by *A*vrBs3 uncovers a shared promoter element, the *UPA* box. The authors show that AvrBs3 binds to the *UPA* box via the repeat domain in a sequence specific manner. Together with [19••] this manuscript shows for the first time evidence for sequence specific DNA binding by a TALE.

21. • Kay S, Hahn S, Marois E, Wieduwild R, Bonas U: **Detailed analysis of the DNA recognition motifs of the *Xanthomonas* type III effectors AvrBs3 and AvrBs3Δrep16**. *Plant J* 2009, **59**:859-871.
Sequence comparison of 11 AvrBs3-induced host genes allows refinement of the previously defined *UPA* box [20••]. DNase I footprint studies demonstrate that the *UPA* box corresponds to the center of the AvrBs3-protected DNA region.

22. • Römer P, Strauß T, Hahn S, Scholze H, Morbitzer R, Grau J, Bonas U, Lahaye T: **Recognition of AvrBs3-like proteins is mediated by specific binding to promoters of matching pepper *Bs3* alleles**. *Plant Physiol* 2009, **150**:1697-1712.
Permutation analysis of the *Bs3* promoter provides information on the exact boundaries of the *UPA* box and on the functional relevance of each individual box nucleotide. These mutational studies also provide the first experimental evidence that the conserved T at −1

is crucial to transcriptional activation. Together with [19••,20••,21•,24] this report provides the experimental fundament to decipher TALE–DNA recognition.

23. Römer P, Recht S, Lahaye T: **A single plant resistance gene** •• **promoter engineered to recognize multiple TAL effectors from disparate pathogens**. *Proc Natl Acad Sci U S A* 2009, **106**:20526-20531.
Using transient expression in *Nicotiana benthamiana* the authors demonstrate that three distinct *UPT* boxes can be functionally combined into one complex promoter. Given that many economically important xanthomonads deliver multiple TAL effectors into host cells, the engineering of *R* genes capable of recognizing multiple TAL effectors provides a potential approach for engineering broad spectrum and durable disease resistance.

24. Schornack S, Minsavage GV, Stall RE, Jones JB, Lahaye T: **Characterization of AvrHah1 a novel AvrBs3-like effector from *Xanthomonas gardneri* with virulence and avirulence activity**. *New Phytol* 2008, **179**:546-556.

25. Schornack S, Peter K, Bonas U, Lahaye T: **Expression levels of *avrBs3*-like genes affect recognition specificity in tomato *Bs4* but not in pepper *Bs3* mediated perception**. *Mol Plant Microbe Interact* 2005, **18**:1215-1225.

26. Schornack S, Ballvora A, Gürlebeck D, Peart J, Baulcombe D, Baker B, Ganal M, Bonas U, Lahaye T: **The tomato resistance protein Bs4 is a predicted non-nuclear TIR-NB-LRR protein that mediates defense responses to severely truncated derivatives of AvrBs4 and overexpressed AvrBs3**. *Plant J* 2004, **37**:46-60.

27. Takken FL, Tameling WI: **To nibble at plant resistance proteins**. *Science* 2009, **324**:744-746.

28. Gu K, Sangha JS, Li Y, Yin Z: **High-resolution genetic mapping of bacterial blight resistance gene *Xa10***. *Theor Appl Genet* 2008, **116**:155-163.

29. Chen S, Huang Z, Zeng L, Yang J, Liu Q, Zhu X: **High-resolution mapping and gene prediction of *Xanthomonas oryzae* pv. *oryzae* resistance gene *Xa7***. *Mol Breed* 2008, **22**:433-441.

30. Zhang Y, Wang J, Pan J, Gu Z, Chen X, Jin Y, Liu F, Zhang H, Ma B: **Identification and molecular mapping of the rice bacterial blight resistance gene allelic to *Xa7* from an elite restorer line Zhenhui 084**. *Eur J Plant Pathol* 2009, **125**:235-244.

31. Chu Z, Yuan M, Yao J, Ge X, Yuan B, Xu C, Li X, Fu B, Li Z, • Bennetzen JL *et al.*: **Promoter mutations of an essential gene for pollen development result in disease resistance in rice**. *Genes Dev* 2006, **20**:1250-1255.
The authors characterize the *xa13* gene for resistant to bacterial blight of rice. They show that it is involved in pollen formation, linking mechanisms in disease and development. They also show that *xa13* differs from *Xa13* only in its promoter sequence, and unlike *Xa13* it is not induced during infection. Together with [18••], this study helped to establish the concept of non-inducible *S* gene alleles as sources of resistance.

32. Yuan M, Chu Z, Li X, Xu C, Wang S: **Pathogen-induced expressional loss of function is the key factor in race-specific bacterial resistance conferred by a recessive *R* gene *xa13* in rice**. *Plant Cell Physiol* 2009, **50**:947-955.

33. Römer P, Recht S, Strauß T, Elsaesser J, Schornack S, Boch J, • Wang S, Lahaye T: **Promoter elements of rice susceptibility genes are bound and activated by specific TAL effectors from the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae***. *New Phytol* 2010, In Press, doi:10.1111/j.1469-8137.2010.03217.x.
Using the code, the authors successfully predict several *UPT* boxes in the promoters of rice genes and experimentally validate activation by specific TALE family members from *Xoo*. However, some observations suggest that TALE–DNA recognition is more complex than implied by the simplicity of the code (see text).

34. Iyer AS, McCouch SR: **The rice bacterial blight resistance gene *xa5* encodes a novel form of disease resistance**. *Mol Plant Microbe Interact* 2004, **17**:1348-1354.

35. Gu K, Tian D, Qiu C, Yin Z: **Transcription activator-like type III** • **effector AvrXa27 depends on OsTFIIAγ for the activation of *Xa27* transcription in rice that triggers disease resistance to *Xanthomonas oryzae* pv. *oryzae***. *Mol Plant Pathol* 2009, **10**:829-835.
The authors observe that *Xa27* activation by AvrXa27 is compromised in rice plants homozygous for *xa5*, the variant *TFIIAγ* gene that confers broad-spectrum resistance to *X. oryzae*. This report provides support for the hypothesis that TALEs depend on interaction with TFIIAγ to recruit the RNA II polymerase complex.

36. Sugio A, Yang B, Zhu T, White FF: **Two type III effector genes of** • ***Xanthomonas oryzae* pv. *oryzae* control the induction of the host genes *OsTFIIAγ1* and *OsTFX1* during bacterial blight of rice**. *Proc Natl Acad Sci U S A* 2007, **104**:10720-10725.
As an example of the power of whole genome microarray analysis to identify TALE targets, this report describes the isolation of two TALEs from *Xoo* that specifically induce a paralog of *xa5* and a bZIP transcription factor gene, respectively. The authors show that the latter is an *S* gene. Their results paint a picture of extensive TALE-driven transcriptional reprogramming of rice during infection.

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38. Brunings AM, Gabriel DW: ***Xanthomonas citri*: breaking the surface**. *Mol Plant Pathol* 2003, **4**:141-157.

39. Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, •• Lahaye T, Nickstadt A, Bonas U: **Breaking the code of DNA binding specificity of TAL-type III effectors**. *Science* 2009, **326**:1509-1512.
By projecting the RVD sequence of AvrBs3 onto the *UPA* box, the authors discern the TALE–DNA recognition code. Then using largely an experimental approach, they further define and test it. Importantly, they demonstrate that *UPT* boxes constructed using the code are functional, and that artificial TALEs with new specificities can be engineered.

40. Al-Saadi A, Reddy JD, Duan YP, Brunings AM, Yuan Q, Gabriel DW: **All five host-range variants of *Xanthomonas citri* carry one *pthA* homolog with 17.5 repeats that determines pathogenicity on *citrus*, but none determine host-range variation**. *Mol Plant Microbe Interact* 2007, **20**:934-943.

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42. Voytas DF, Joung JK: **DNA binding made easy**. *Science* 2009, **326**:1491-1492.