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The evolution of disease resistance genes

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Abstract

Several common themes have shaped the evolution of plant disease resistance genes. These include duplication events of progenitor resistance genes and further expansion to create clustered gene families. Variation can arise from both intragenic and intergenic recombination and gene conversion. Recombination has also been implicated in the generation of novel resistance specificities. Resistance gene clusters appear to evolve more rapidly than other regions of the genome. In addition, domains believed to be involved in recognitional specificity, such as the leucine-rich repeat (LRR), are subject to adaptive selection. Transposable elements have been associated with some resistance gene clusters, and may generate further variation at these complexes.

Introduction

The continued survival of most organisms depends on the presence of specific genetic systems to maintain diversity in the face of a changing environment. Classic examples include antigenic variation in trypanosomes and immunoglobulin gene formation in mammals. Plants are constantly being subjected to external stresses that require them to respond in an appropriate manner. Among these stresses, the plant's response to pathogen attack has been scrutinized intensely. The understanding of how the plant 'senses' or 'identifies' a foreign microbe as a pathogen and relays this information is beneficial not only to our basic understanding of how these processes work, but has practical applications for the improvement of agriculture.

Plants utilize a variety of strategies to defend against pathogen attack. One strategy is to strengthen the cell wall, thereby making a barrier between the plant cell and the pathogen. For example, enzymes involved in lignin and callose biosynthesis are induced upon pathogen attack. A second strategy the plant utilizes is the production of antimicrobial compounds, such as toxic secondary metabolites, and hydrolytic enzymes. The predominant strategy plants use to defend against pathogens, however, is the hypersensitive response (HR). Reminiscent of apoptosis in mammals, the HR is characterized by a rapid, localized cell death at the point of pathogen attack/recognition. The first insight into the genetics of plant disease resistance involving the HR was the pioneering work done by Flor [20]. Flor proposed a gene-for-gene model for the genetic interaction between plant and pathogen. This model states that a dominant gene from the host interacts with a corresponding dominant avirulence gene from the pathogen. The interaction between the two corresponding genes, the host resistance gene and the pathogen avirulence gene, illicits a HR, thus providing resistance.

The newly emerged tools of molecular biology helped refine this model and led to the hypothesis that a ligand produced by the pathogen interacts, presumably directly, with a corresponding plant receptor, which then trigger activation of a defense response. Under this model, pathogen and plant genes involved in this interaction are subject to different evolutionary forces. Since virulence is recessive, a simple loss-of-function mutation in the avirulence gene of the pathogen allows it to become virulent on the host. In

contrast, the plant must gain a new resistance function to counter new pathogen biotypes or species. A plant must possess a mechanism flexible enough to ensure response to a new pathogen. Most plant species contain a large number of highly polymorphic disease resistance genes, most of which share common structural domains [3]. It has long been speculated that DNA rearrangements play a key role in evolution of these genes, thus allowing plants to generate novel resistance to match the changing pattern of pathogen virulence [57, 58]. In support of this hypothesis, studies of the maize disease resistance locus *rp1* revealed that recombination of flanking markers was associated with the creation of novel resistance phenotypes [60]. This review focuses on the evolution of resistance genes using recent information gained from molecular genetic analysis of resistance genes.

Structure and classes of resistance genes

Numerous genes have been identified in plants that confer resistance to plant pathogens [11]. The first disease resistance gene to be cloned and characterized was *Hm1* [29]. *Hm1* provides resistance to the fungal pathogen *Cochliobolus carbonum* by inactivating the HC toxin produced by this fungus [29]. *Hm1* represents a class of resistance genes that encode detoxifying enzymes. In contrast, most of the resistance genes that have been cloned and characterized resemble components involved in signal transduction [3, 17]. These resistance genes can be classified into four general categories based on their predicted protein structures. Despite the differences between classes of resistance genes characterized to date, the finding that the majority of resistance genes encode signal transduction proteins suggests that different plants utilize similar strategies in the prevention of disease.

The first resistance gene cloned that resembled a signal transduction component was *Pto* [40]. *Pto* is a serine/threonine kinase that provides resistance to *Pseudomonas syringae* pv. *tomato* containing the avirulence gene *avrPto* (Figure 1A) [40]. Although *Pto* does not contain a readily identifiable ligand binding motif, studies have shown that *Pto* and the pathogen's corresponding avirulence gene product, *avrPto*, directly interact [65].

Several resistance genes show remarkable similarity to previously identified receptors from a diverse group of organisms. A common motif evident among these proteins is the leucine-rich repeat (LRR). LRRs are believed to mediate protein-protein interactions, or determine specific recognition of ligands by the receptor molecules [35]. LRR domains of resistance gene products show similarity to diverse proteins controlling cell-cell communication in development and signaling suggesting that these genes may have evolved through duplication and divergence of common ancestors [10, 38, 72]. This has been observed in other species such as the *Drosophila Toll* and *Dif* genes [55]. To date, genes involved in development have not been associated with resistance gene clusters.

One group of LRR containing resistance genes include the *Cf* class from tomato, and comprise *Cf2*, *Cf4*, *Cf5*, and *Cf9* [30, 54]. The *Cf* class of resistance genes provide resistance to the fungal pathogen *Cladosporium fulvum*. These genes encode putative extracellular receptors, containing LRR repeats at the N-terminal domain of the protein, plus a membrane anchor and a short intracellular tail at the C-terminal domain (Figure 1B) [30, 54].

Xa21 provides resistance to *Xanthomonas oryzae* pv. *oryzae* in rice. Analysis of the predicted amino acid sequence indicates that *Xa21* has three major domains that are characteristic of receptor kinases, a LRR extracellular ligand-binding domain, a transmembrane domain (TM), and a kinase domain (Figure 1C) [66]. It is interesting to note that the structure of *Xa21* contains elements of both the *Cf* class of resistance genes, and *Pto*. Several genes have been identified in plants that are structurally similar to *Xa21*, and govern a variety of functions including development [38, 72]. Closely related to the *Cf* genes, *Xa21*, the resistance gene *Xa21D*, encodes a presumed extracellular receptor lacking a membrane anchor (Figure 1D) [73].

The majority of cloned resistance genes resemble intracellular receptors. These genes contain a predicted nucleotide binding site (NBS) followed by a LRR domain at the C-terminal end of the protein [3, 17]. The NBS-LRR class of resistance genes can be further subdivided into two classes. One subclass contains a leucine zipper (LZ) motif at the N-terminal domain (Figure 1E). *Rps2* and *Rpm1* both provide resistance to strains of *Pseudomonas syringae* that contain the avirulence gene *avrRpt2* and belong to this class [6, 22, 49]. The other class contains homology to the cytoplasmic domains of the *Drosophila Toll* gene and the interleukin-1 receptors (Figure 1F). Examples of this class include the tobacco *N* gene, L_6 from flax, and *Rpp5* from *Arabidopsis* [36, 53, 75].

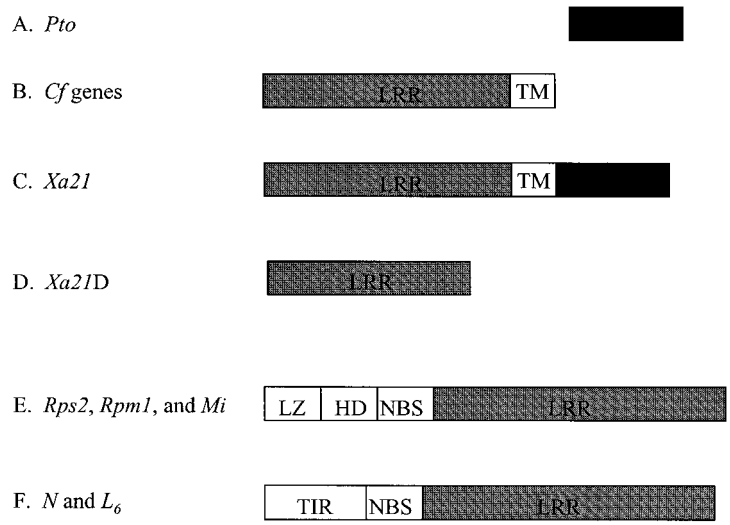

Figure 1. Representation of resistance gene classes. LRR, leucine-rich repeat; TM, transmembrane domain for *Xa21* or membrane anchor for the *Cf* class; LZ, leucine zipper; HD, hydrophobic domain; NBS, nucleotide binding site; TIR, Toll/Interleukin-1 signaling domain.

Genomic organization of resistance genes

Genes conferring race-specific resistance are commonly clustered in the genome [11, 27, 57]. In maize, a group of genes that confer resistance to the common rust fungus, *Puccinia sorghi*, cluster to a small region on the short arm of chromosome 10 [26, 77]. This region contains the *rp1* complex and is a classic example of a complex disease resistance gene locus. Fourteen dominant genes have been identified that map within the *rp1* complex and provide a biotype-specific resistance. In addition to the *rp1* complex, two other loci, *rp5* and *rpG*, map ca. 2 to 3 cM distal to the *rp1* complex [26, 70]. Genetic studies of the *rp1* complex and *rpG* indicate a high level of meiotic instability among these genes [5, 26]. Most of the genetic reassortment events at the *rp1* complex are associated with flanking marker exchange [26, 70]. Some *rp1* variants have been identified that do not show flanking marker exchange, presumably due to gene conversion events [23].

In addition to the genes in the *rp1* area, the *rp3* locus also confers resistance to *Puccinia sorghi*. Six alleles have been named at the *rp3* locus, *Rp3-A* through *F*, which maps close to the centromere on chromosome 3 [63, 64, 77]. The six *Rp3* alleles were identified in different cultivars and given separate allelic designations [63, 77]. However, these six alleles cannot be differentiated with the rust biotypes in the current collection, but can be differentiated with RFLPs (Richter and Hulbert, unpublished). The area that contains the *rp3* locus also contains other genes that condition resistance to pathogens, namely *WSMV* and *MV1* [44].

In flax, five loci have been identified that provide resistance to the rust fungus *Melampsora lini* [19, 27]. These loci have been named *K*, *L*, *M*, *N* and *P* [19, 27]. Both the *L* locus and the *M* locus have been extensively studied. Like *Rp1*, the *M* locus is a complex locus that is composed of an array of linked genes. In contrast, the *L* locus of flax consists of a single gene with 13 distinct alleles [2].

Members of a resistance gene family are often arranged as tandem direct repeats, which is consistent with their origin through gene duplication and their continued evolution through unequal exchange. One example is the *Xa21* gene family. *Xa21* belongs to a family of related sequences in rice with most of its members mapping to a 230 kb region on chromosome 11 [68]. Analysis of this region using cosmid clones and bacterial artificial chromosomes (BACs) has identified seven *Xa21* family members (A1, A2, B(Xa21), C, D, E, and F) residing on four contigs (Figure 2). The first contig spans a 40 kb interval and contains Xa21 and member C. The second contig spans an interval of 150 kb and contains members D, A1, and A2. The last two contigs each contain one family member (E and F) and span intervals of 130 kb and 40 kb respectively.

Comparative mapping studies have shown a remarkable synteny among the grass genomes [14]. Because of the observed synteny between cereal genomes, Bennetzen and Freeling proposed that the

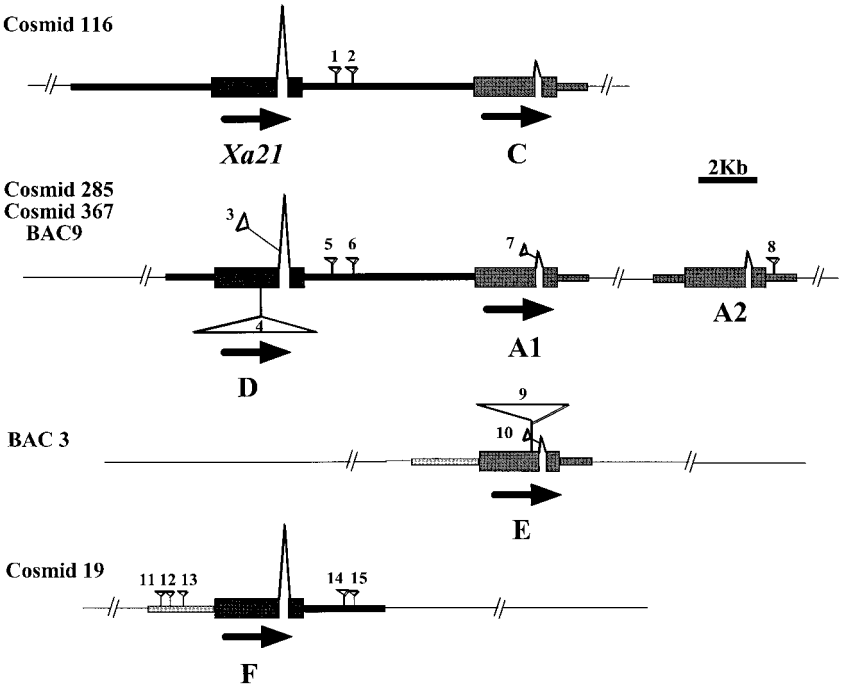

Figure 2. Cosmid and BAC contig of seven *Xa21* family members. The arrows represent the direction of the open reading frames. Letters beneath the arrows refer to the names of the *Xa21* family members. Wide bars represent coding regions, while narrow bars represent non-coding regions. Introns are represented by angled lines. Regions that have not been sequenced are represented by thin lines. Fifteen transposable elements were also identified and are numbered 1 through 15. Adapted from Song *et al.* [60]).

grasses could be used as a single genetic system [4]. Resistance genes and their analogues, however, do not appear to mark regions of synteny. The barley *mlo* and *Rpg1* genes are not found in the syntenic region in the rice genome although the order of flanking markers is conserved between barley and rice [33, 52]. In addition to disease resistance genes with known specificities, resistance-like gene sequences (RLGs), whose functions are unknown, map as clusters in rice, *Arabidopsis*, potato, tomato and soybean [7, 32, 37]. Comparative mapping studies of RLGs have shown that they do not mark regions of synteny. Based on a comparative mapping studies of monocot RLGs, Leister *et al.* [37] suggest that resistance genes diverge more rapidly than the rest of the genome through sequence divergence or ectopic recombination. For example, out of 6 rice and 7 barley RLGs tested only 10 map to syntenous regions on the foxtail genome.

Duplication and recombination

Duplication plays a central role in creating complex genetic systems [51]. Duplication can create new loci, alter gene family number through recombination, or generate repeated sequences within a gene. For example, studies of the major histocompatibility complex (MHC) showed that human and mouse genomes contain regions that emerged as result of chromosomal duplication [2]. Similarly, at least two clusters of *Cf-9* homologues have been found on the short arm of chromosome 1 of tomato [54], suggesting that *Cf* clusters are products of duplications.

Recombination can lead to amplification or reduction of the number of resistance gene family members. For example, the presence of two nearly identical functional *Cf-2* genes suggests that they arose through a recent gene duplication event [15]. Analysis of the *Cf-2*/*Cf-5* locus, where only a few sequences homologous to *Cf* genes reside, has revealed a rare susceptible recombinant that also arose via an unequal crossover event leading to a reduction of the *Cf* homologue numbers [15]. Molecular analysis of five *Cf-4*/*Cf-9* disease-sensitive recombinants demonstrated that each was generated by chromosomal mispairing of intergenic sequences and unequal crossing-over [54].

Several other resistance gene families show evidence of recombination and duplication. The *Xa21*

multigene family contains a large duplication of at least 17 kb; one of the duplicated genes confers the same race-specific resistance as *Xa21* [68, 69]. The presumed duplication and diversification of the *Pto* gene family led to the generation of alternative recognition capabilities of the encoded proteins [41]. Finally, it has been proposed that the flax *M* locus carrying tandemly arrayed specificities evolved from a rare duplication of an ancestral *M* gene [18]. Repeated DNA flanking of the locus may have enhanced subsequent duplication through unequal crossing-over events.

In some cases, recombination between diverged family members occurs at highly conserved stretches of nucleotides. For instance, a large proportion of recombination events at the *Xa21* locus were localized to a highly conserved domain in the 5′-coding domain resulting in new promoter/gene combinations [68]. Similarly, the recombination exchange site in mutants at the *M* locus can be localized to a 45 bp region that is invariant between LRR repeats [2].

In addition to swapping of large gene regions, recombination can lead to fine structural changes within a gene. The repeated structure of the LRR coding regions could facilitate intragenic (and intergenic) recombination leading to expansion and contraction of the LRR number as demonstrated in mutants of *M* and *Rpp5*. Whereas the wild-type *M* gene contains two DNA repeats encoding LRRs, spontaneous mutants have been identified that contain a single LRR region [2]. The mutant alleles with a single LRR repeat may have been generated by an unequal exchange between the first repeat in one *M* gene and the second repeat in its homologue [11]. A fast-neutron-generated *Rpp5* mutant contains an intragenic duplication of four complete LRRs. This duplication may have arisen from an unequal crossing-over event between two sequences of identity in the LRRs [53].

Rapid sequence exchange among tandemly repeated gene families generally leads to sequence homogenization between constituent members of the gene family [54]. How can variability be maintained in the presence of concerted evolution? To address this question, Parniske *et al.* sequenced three haplotypes at the *Cf-4*/*Cf-9* locus. Comparison of intergenic regions revealed a high degree of sequence rearrangements, whereas a patchwork of sequence similarities is observed in the coding regions [54]. The patches of similarity could result either from successive rounds of reciprocal recombination or from gene conversion events. In a homozygous background, the *Cf-9* gene was very stable. In contrast, the meiotic stability of *Cf-9* was dramatically reduced in a *Cf-4*/*Cf-9* transheterozygous background. Parniske *et al.* [54] proposed that the polymorphism of the intergenic regions suppresses unequal recombination in homozygotes and sister chromatids, thereby preventing sequence homogenization of the gene family. In this situation, recombination between regions of high homology within a coding region may actually contribute to the maintenance of a useful combination of resistance gene specificities. In a *Cf-4*/*Cf-9* transheterozygous background, homologous sequences aligned unequally are used as recombination templates. Such unequal recombination alters the number of gene family members as well as the composition of the clusters resulting in increased variation within the population.

Recombination provides a mechanism for generating new race specificities either by reshuffling existing genes or by creation of novel resistances to biotypes that neither parental allele was resistant to [60]. Thirteen variants identified at the *rp1* complex had a resistance spectrum that was different from the parental lines from which they were derived [60]. Eight of these variants retained a subset of the biotype specificities of one of their parents, suggesting that these variants were composed of two or more tightly linked genes, each separable by recombination. In addition, four of the 13 variants were identified as being resistant to a rust biotype neither parent was resistant to. Analysis of the flanking markers of these four novel genes indicated that they arose by crossing-over.

Although clustering and rapid evolution of resistance genes suggests that a gene conferring resistance to one pathogen species could evolve to recognize a different pathogen species, there is limited evidence supporting this hypothesis. Future cloning and sequencing of linked genes conferring resistance to different pathogens may eventually demonstrate such a common evolutionary origin.

One example of a resistance locus possessing dual specificities is the nematode resistance gene, *Mi-1*, in tomato. *Mi-1* has been cloned and represents the (leucine zipper/nucleotide binding site/leucine-rich repeat) class of resistance genes [48, 76]. At least seven homologues have been identified for *Mi*-1, clustering around a 650 kb introgressed region from *Lycopersicon peruvianum*. Two genes were identified at the *Mi*-1 locus, *Mi*-1.1 and *Mi*-1.2. The *Mi*-1.2 was identified as the copy that provided nematode resistance [76]. Resistance to the potato aphid, *Macrosiphum eu-*

phorbiae (Thomas), is encoded by the *Meu1* locus in tomato. Genetic studies of *Meu1* indicated that it was tightly linked to *Mi*-1 [30]. Resistance to the potato aphid was always associated with the presence of the nematode resistance gene, *Mi*. Subsequent studies showed that the *Mi*-1.2 copy also provides an isolate-specific resistance to the potato aphid [62]. The dual specificity of *Mi*-1 may reflect similar mechanisms of feeding by both pests [62].

Lesion mimic mutants

Recombination at resistance gene loci can also lead to the generation of lesion mimic mutants that display in the absence of a pathogen, a phenotype similar to the hypersensitive response normally controlled by resistance genes. This observation led to the hypothesis that similar types of genes are involved in both phenotypes and that some lesion mimic alleles may be derived from resistance gene loci [57]. One possible explanation is a decrease in ligand binding specificity of the resistance gene leading to promiscuous activation of HR signaling [24, 57]. The best evidence to date for this hypothesis comes from the *rp1* locus. Four *Rp1* variants that display lesion mimic phenotypes were identified at the *rp1* locus, two of which arose from crossover events, suggesting a role for recombination in the creation of these variants [24]. Three of the four *Rp1* lesion mimics illicit race non-specific necrosis when challenged by several rust biotypes [24]. It is also possible that alterations could occur in the signaling domain of a resistance gene [24, 57]. This class of lesion mimics may arise in resistance genes encoding kinases, like *Pto* or *Xa21*. Using site-directed mutagenesis to alter amino acids in the activation domain of *Pto*, Rathjen *et al.* constructed a constitutively active kinase that initiated an HR in the absence of its match avirulence gene [59].

The barley powdery mildew resistance gene *mlo* and the *lsd* (lesion-simulating disease response) and *acd2* (accelerated cell death) genes from *Arabidopsis* provide other examples of genes displaying a lesion mimic mutant phenotype together with defense responses associated with disease resistance [9,12, 21]. However, in these cases, no genes conferring race-specific resistance have yet been mapped to these loci. The two that have been cloned, *mlo* and *lsd1*, encode proteins with structures distinct from other cloned resistance genes indicating that not all lesion mimic mutants have a direct evolutionary link to resistance genes [9, 13]. In these cases, the signaling pathways that respond to resistance gene stimulation may be affected by mutations.

Adaptive selection of pathogen recognition domains

Characterization of nucleotide substitution patterns in resistance gene families have provided insight into the function and evolution of particular coding domains. For the investigation of function, the ratio of nucleotide substitutions that lead to amino acid replacements (nonsynonymous substitutions, dn) and nucleotide substitutions that do not alter amino acids (synonymous substitutions, ds) is particularly informative. In most protein-coding genes, the dn/ds ratio is less than unity; this observation is consistent with functional constraint against amino acid replacements [34]. Conversely, a dn/ds ratio significantly greater than unity indicates that adaptive selection events have fueled divergence between genes [25, 46]. Evidence of adaptive selection is rare but appears to be most common in gene regions that function in host and pathogen recognition [19]. It is expected that regions that bind ligand will be subject to stronger adaptive selection than regions that play a structural role. For example, the antigen recognition site of alleles at the class I MHC loci in human and mouse display a dn/ds ratio greater than unity indicating that the antigen recognition site is subject to strong adaptive selection events whereas structural regions of the protein are not [25].

Identifying domains that contain a dn/ds ratio greater than unity might indicate regions of functional importance. Analysis of 11 *Cf* gene family members revealed that the predicted solvent-exposed residues of the *b*-strand/*b*-turn region of the LRR domain exhibit increased dn/ds ratios relative to other residues in the LRR domain suggesting that solvent-exposed residues play a role in ligand binding [54]. Similarly, a comparison of nucleotide substitutions in the LRR coding regions of *Xa21* and the gene family member *Xa21D* revealed that although *Xa21* and *Xa21D* share 99.1% sequence identity, nonsynonymous substitutions occur significantly more frequently than do synonymous substitutions in the LRR [73]. This result indicates that this region has a functional importance and would be consistent with the LRR's putative role in ligand binding [73]. The greater than unity ratio of dn/ds indicates that the LRR domain, which governs race-specific pathogen recognition, is subject to adaptive evolution.

In addition, the high levels of sequence variation observed among resistance gene family members suggest that events such as gene conversion and/or unequal crossing-over is sufficiently low as to prevent homogenization of the family members [47]. Although most models predict that resistance genes possessing novel ligand-binding capabilities arise from recombination events, Meyers *et al.* predict that novel ligand specificities would arise from single amino acid changes in the solvent-exposed surface of the LRR [47].

Diversification of resistance gene family members by transposable-like elements

The human MHC class 2 region is among the most polymorphic part of the human genome. Multiple repetitive sequences representing more than 20 different families have been characterized in the MHC region [1]. Part of the inter- and intraspecific variation observed in the MHC is caused by different integration patterns of retroelements. Comparative studies of different human haplotypes and primate species revealed that retroelement insertions have contributed to genome plasticity of the MHC during primate evolution. Retroelements also contribute to recombination and genomic instability by serving as sites for recombination and translocation events [1].

In plants, it has long been hypothesized that transposable elements play a role in the reconstruction of genomes in response to environmental stresses such as tissue culture, irradiation or pathogen infection [43, 74]. In partial support of this hypothesis, Pouteau *et al.* [56] demonstrated that the transcription of the tobacco retrotransposon *Tnt1* is induced by a broad spectrum of microbial and fungal elicitors. Transposable element insertion into and excision from regulatory and coding regions can change the coding capacity and expression patterns of the gene [39, 45, 74].

There is no evidence for the generation of new specificity at resistance gene loci as a result of the insertion and excision of a transposable element. However, it has been shown that transposable element-induced gene alterations can cause resistance gene inactivation and diversification. For example, in the case of the maize fungal resistance gene, *Hm1*, which confers resistance to *Cochliobolus carbonum* race 1, a 315 bp insertion (designated *dHBr*) was found in a mutant allele of this gene [29]. Moreover, it was the insertion of a transposable element (a 256 bp element named *Drone*) that disrupted the *Hm1*-conferred resistance in an inbred of maize and, as a result, led to the genesis of the leaf spot and ear rot disease of maize in 1938 [50]. In flax, 2 mutants of *L6* carry small (300 bp) insertion elements that inactivated the gene [36].

In rice, transposable element-like sequences appear to be a major source of variability of the *Xa21*-gene family members. Seventeen transposable element-like sequences grouped into eleven families

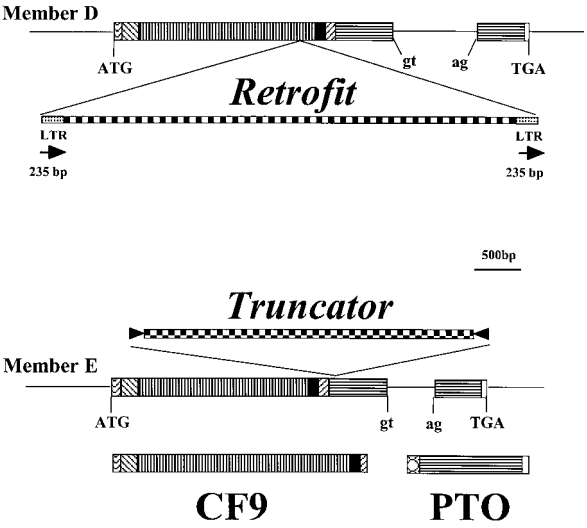

Figure 3. Position of the transposable element-like sequences *Retrofit* and *Truncator* in the coding region of the D and E members of the *Xa21* gene family. Representations of *Pto* and *Cf9* are shown below member E for comparison. Adapted from Song *et al.* [60].

Table 1. Seventeen *Xa21*-associated transposable-like elements.

Name	Class	Location	Reference
gaigin-Ol1	MITE	5′ F	[8]
gaigin-Ol2	MITE	5′ F	[8]
Tourist-Ol1	MITE	5′ F	[8]
Tourist-Ol2	MITE	3′ A2	[8]
Wanderer-Ol1	MITE	pTA8100	[8]
Ds-rice 1	Ac/Ds	3′ *Xa21*	[69]
Ds-rice 2	Ac/Ds	3′ D	[69]
Ds-rice 3	Ac/Ds	3′ F	[69]
Xa21-CACTA	CACTA	pTA818	[28, 61, 69]
Krispie	Novel	D Intron	[69]
Snap-Ol1	Novel	A1 Intron	[69]
Snap-Ol2	Novel	E Intron	[69]
Crackle	Novel	3′ F	[69]
Pop-Ol1	Novel	3′ *Xa21*	[69]
Pop-Ol2	Novel	3′ D	[69]
Retrofit	Retroelement	D	[69]
Truncator	Novel	E	[69]

(Table 1), including three families of miniature inverted repeat transposable elements (MITEs), five novel elements, *Ds*-like elements, a *CACTA*-like element and a retrotransposable element, are present at the *Xa21* locus [8, 68, 69]. Integration of two of these elements into coding sequences creates open reading frames that encode truncated proteins. The insertion of the transposable element, *Retrofit*, into member *Xa21*D generated a truncated protein that lacked a membrane-spanning domain (Figure 3) [68]. It is interesting that the truncated open-reading frame from this family member can confer an attenuated resistance with *Xa21* specificity [68]. Another transposable element insertion, member E of the *Xa21* family, created a truncated protein that now resembles one of the *Cf* class of resistance genes (Figure 3) [73]. No resistance, however, was associated with member E. Transposable element insertions into 5′- and 3′-flanking regulatory regions were also observed (Table 1). Many of the elements seem to have been active over the entire evolutionary period of the *Xa21* gene family [68].

It is tempting to speculate that transposable elements contribute to the evolution of resistance gene diversity. Movement of these elements in response to pathogen-induced stress would provide genetic plasticity with a possible selective advantage. However, because the insertion of transposable elements at the *Hm1*, *L6* and *Xa21* loci results in loss of function or impaired function, the question of whether transposable element movement can confer a selective advantage remains open. Transposable elements may, however, have an effect on recombination. The excision process of certain classes of transposable elements may involve double-stranded breaks and homology-dependent repair [66]. Using a transgenic tobacco assay to study the effects of ectopic recombination between two homologous, but non-allelic sequences, Shalev [66] found that excision of *Ac* increased the recombination frequencies between the two ectopic sites. The effect of transposable elements on recombination is less clear. The transposable element *Mutator* has been observed to increase the frequency of recombination at the *Knotted1* locus [42]. However, the frequency of meiotic recombination at the *bz* locus in maize was not affected by the presence of *Ac* in one of the heteroalleles [16].

Conclusions

Several evolutionary patterns can be discerned from the study of resistance genes. The first is duplication events which play a significant role in the creation of resistance gene families. The duplication of a progenitor resistance gene and subsequent divergence can create or amplify additional clusters of a gene family. In addition, unequal recombination and gene conversion at intergenic regions creates additional variability within the population. These events can reassort existing resistance genes in the array into new combinations. Intragenic recombination and gene conversion provides a mechanism to generate novel resistance specificities. Some of these recombination events occur at highly conserved regions. Diversity at the LRR domain provides an evolutionary advantage for recognizing, binding, and defending against a broad array of pathogens [25]. Finally, movement of transposable elements may result in further allelic diversity, either by disrupting genes, or by influencing recombination or other chromosomal rearrangements such as translocations. More sequence information is needed, however, to determine if transposable elements are more abundant or active at resistance gene loci than other regions of the genome.

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