European Journal of Plant Pathology **106:** 699–713, 2000.

Mini review

Plant resistance genes: their structure, function and evolution

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Accepted 31 May 2000

Key words: gene-for-gene resistance, plant–pathogen interaction, avirulance genes, disease

Abstract

Plants have developed efficient mechanisms to avoid infection or to mount responses that render them resistant upon attack by a pathogen. One of the best-studied defence mechanisms is based on gene-for-gene resistance through which plants, harbouring specific resistance (*R*) genes, specifically recognise pathogens carrying matching avirulence (*Avr*) genes. Here a review of the *R* genes that have been cloned is given. Although in most cases it is not clear how *R* gene encoded proteins initiate pathways leading to disease resistance, we will show that there are clear parallels with disease prevention in animal systems. Furthermore, some evolutionary mechanisms acting on *R* genes to create novel recognitional specificities will be discussed.

Abbreviation: HR – hypersensitive response; LRR – leucine-rich repeat; NBS – nucleotide binding site; LZ – leucine zipper; TIR – Toll/interleukin I receptor; RDR – required for disease resistance; PCD – programmed cell death; ROS – reactive oxygen species.

Introduction

Although plants are under continuous attack by pathogens, most encounters result in plant resistance and disease being the exception. Passive defence lines such as cell walls, wax layers and chemical barriers confer broad resistance to a wide variety of pathogens (Knogge, 1996; Osbourn, 1996). If a pathogen overcomes this first line of defence, there is a second line, which is mounted by proteins encoded by specific resistance (*R*) genes. At this level at least four fundamentally different resistance mechanisms can be recognised:

i. The *R* gene product inactivates a toxin, which is produced by the pathogen, and which normally induces necrosis or inhibits the induction of active defence responses. An example is the *Hm1* gene from maize (Johal and Briggs, 1992), which encodes an NADPH-dependent reductase that inactivates the HC-toxin produced by race 1 of the fungus *Cochliobolus carbonum*. The toxin induces disease symptoms on susceptible plants by inhibiting histone deacetylase (Brosch et al., 1995).
ii. The *R* gene product encodes a pathogenicity target. Thus, absence of this target results in plant resistance. An example of such a gene is the *T-urf13* mitochondrial gene from maize which not only confers male sterility but also sensitivity to a pathotoxin (T-toxin) produced by *Bipolaris maydis* race T and hence susceptibility to this pathogenic fungus (Braun et al., 1989). Maize varieties that lack the *T-urf13* gene are resistant to the fungus.
iii. The *R* gene product primes the plant defence responses. To date the only example is resistance to *Erysiphe graminis* f.sp. *hordei* provided by the *mlo* gene of barley. Resistance,

obtained by mutagenesis of susceptible plants carrying *Mlo* alleles, is durable and active against a broad spectrum of fungal isolates (Büschges et al., 1997). The *Mlo* gene product might act as a negative regulator of plant cell death and other plant defences. Presence of the recessive allele only will, therefore, prime the plant for its responsiveness upon infection by a pathogen, resulting in low threshold levels for induction of defence responses. This model is consistent with the spontaneous cell wall appositions and local necrotic lesions on the leaves observed in *mlo* plants in the absence of the pathogen (Wolter et al., 1993). Strikingly, *mlo* plants are more susceptible to *Magnaporthe grisea* (Jarosch et al., 1999).

iv. The *R* gene product mediates specific recognition of a pathogen that expresses a matching avirulence (*Avr*) gene (i.e. gene-for-gene resistance), for a review on fungal *Avr*s, see Laugé and De Wit (1998) and for a review on bacterial *Avr*s see Bonas and Van den Ackerveken (1999). Specific recognition of a pathogen-derived *Avr* gene product, a so-called elicitor, by the host activates a signal transduction cascade that involves protein phosphorylation, ion fluxes, generation of reactive oxygen species (ROS) and other signals (Hammond-Kosack and Jones, 1996; Higgins et al., 1998; Somssich and Hahlbrock, 1998; Yang et al., 1997). These signals subsequently trigger transcription of plant defence genes encoding proteins such as glutathione S-transferases, peroxidases, cell wall proteins, proteinase inhibitors, hydrolytic enzymes (e.g. chitinases and 1,3-β-glucanases), pathogenesis-related (PR) proteins and enzymes involved in secondary metabolism (Zhu et al., 1996). In addition, plant cells that are in direct contact with the invading pathogen die. This phenomenon is called the hypersensitive response (HR) which is the hallmark of gene-for-gene-based resistance (Hammond-Kosack and Jones, 1996). HR is an active process that requires protein synthesis (reviewed by Greenberg, 1997) and shares many similarities with programmed cell death (PCD) as observed in animals (Dangl et al., 1996; Heath, 1998; Nürnberger et al., 1997; Ryerson and Heath, 1996). HR is often correlated with the induction of resistance in non-inoculated parts of the plant. This systemic acquired resistance (SAR) results in a significant reduction of disease symptoms caused by many pathogens (Delaney, 1997; Ryals et al., 1996).

Structure and function of proteins encoded by disease resistance genes

As shown in Table 1, *R* genes with a defined resistance specificity have been cloned from both monocotyledons and dicotyledons. These genes confer resistance against a wide range of pathogens, including viruses, bacteria, fungi, nematodes and even aphids. Although the life style and mechanism of infection by these organisms varies significantly, the *R* gene products are remarkably similar. All R proteins contain a leucine-rich repeat (LRR) domain. The intercellular (I) R proteins also contain a nucleotide binding site (NBS) and in some cases a leucine zipper (LZ) domain or a domain with homology to the receptor Toll and the interleukin-1 receptor (TIR). In the extracellular (E) proteins the LRR domain is accompanied by a membrane-spanning region and in one case by a cytoplasmic protein kinase domain.

Structure of the leucine-rich repeat domain

Based on the predicted localisation of the LRR domain, which can be either intracellular or extracellular, *R* gene products can be divided into two classes (Table 1). The LRR domain consists of a repeated sequence motif, comprising leucine residues and sometimes asparagine and proline residues at conserved positions (Kobe and Deisenhofer, 1994). The average repeat length of 24 amino acids is relatively constant between the products of the different *R* genes, whereas the number of repeats can vary from 14 in RPS2 to 37 in Cf2. The LRRs of NBS-containing R proteins have imperfect repeats resembling the cytoplasmic adenylate-cyclase of yeast (Kataoka et al., 1985). Non-NBS R proteins contain LRRs with a more tight consensus sequence including a conserved glycine residue (Bent, 1996). These proteins also contain a signal peptide for extracellular targeting and apparent transmembrane domain features.

The crystal structure of one LRR-containing protein, porcine RNAse inhibitor, has been resolved (Kobe and Deisenhofer, 1993). The tertiary structure of the LRR domain of this protein has unusual long repeats of 28–29 amino acids and resembles a horseshoe. This horseshoe structure probably differs from the three dimensional structure of the LRR domain encoded by

Table 1. Classes of resistance genes, classified by their structural domains, like transmembrane region (TM), nucleotide binding site (NBS), Toll/interleukin 1 receptor domain (TIR), leucine-rich repeat (LRR) and protein kinase (PK) domain. The predicted position of the protein (intracellular (I) or extracellular (E)) is indicated

Plant species	*R* gene	Localisation	Structure	Pathogen	Matching gene	Reference
Tomato	*Prf*	I	LZ–NBS–LRR	*Pseudomonas syringae* pv. *tomato*	*AvrPto*	(Salmeron et al., 1996)
Arabidopsis	*RPS2*	I	LZ–NBS–LRR	*Pseudomonas. syringae* pv. *tomato*	*AvrRpt2*	(Bent et al., 1994)
Arabidopsis	*RPM1*	I	LZ–NBS–LRR	*Pseudomonas syringae* pv. *maculicola*	*AvrRpm1, avrB*	(Grant et al., 1995)
Arabidopsis	*RPS5*	I	LZ–NBS–LRR	*Pseudomonas syringae* DC3000	*AvrPphB*	(Warren et al., 1999)
Arabidopsis	*RPP8*	I	LZ–NBS–LRR	*Peronospora parasitica*	*AvrRpp8*	(McDowell et al., 1998)
Tomato	*Mi*	I	LZ–NBS–LRR	*Meloidogyne incognita* and *Macrosiphum euphorbia*		(Milligan et al., 1998; Rossi et al., 1998; Vos et al., 1998)
Tomato	*I2c*	I	NBS–LRR	*Fusarium oxysporum*		(Ori et al., 1997)
Tomato	*I2*	I	NBS–LRR	*Fusarium oxysporum*		(Simons et al., 1998)
Rice	*Xa1*	I	NBS–LRR	*Xanthomonas oryzae* pv. *oryzae*		(Yoshimura et al., 1998)
Rice	*Pib*	I	NBS–LRR	*Magnaporthe grisea*		(Wang et al., 1999)
Potato	*Rx*	I	NBS–LRR	Potato virus X	Coat protein	(Bendahmane et al., 1999)
Potato	*Gpa2*	I	NBS–LRR	*Globodera rostochiensis*		(Stiekema et al. 1999)
Wheat	*Cre3*	I	NBS–LRR	*Heterodera avenae*		(Lagudah et al., 1997)
Pepper	*Bs2*	I	NBS–LRR	*Xanthomonas campestris*	*AvrBs2*	(Tai et al., 1999)
Corn	*Rp1-D*	I	NBS–LRR	*Puccinia sorghi*		(Collins et al., 1999)
Rice	*Pi-ta*	I	NBS–LRR	*Magnaporthe grisea*	*AvrPITA*	(Valent, 1999)
Barley	*Mla*	I	NBS–LRR	*Erysiphe graminis*		(Schulze-Lefert, 1999)
Tobacco	*N*	I	TIR–NBS–LRR	Tobacco mosaic virus	Replicase	(Whitham et al., 1994)
Arabidopsis	*RPP1, 10, 14*	I	TIR–NBS–LRR	*Peronospora parasitica*		(Botella et al., 1998)
Flax	L^6 L^{1-12}	I	TIR–NBS–LRR	*Melampsora lini*		(Lawrence et al., 1995)
Flax	*M*	I	TIR–NBS–LRR	*Melampsora lini*		(Anderson et al., 1997)
Arabidopsis	*RPP5*	I	TIR–NBS–LRR	*Peronospora parasitica*		(Parker et al., 1997)
Arabidopsis	*RPS4*	I	TIR–NBS–LRR	*Pseudomonas syringae* pv. *pisi*	*AvrRps4*	(Gassmann et al., 1999)
Rice	*Xa21*	E	LRR-TM-PK	*Xanthomonas oryzae* pv. *oryzae*		(Song et al., 1995)
Tomato	*Cf-2*	E	LRR–TM	*Cladosporium fulvum*	*Avr2*	(Dixon et al., 1996)
Tomato	*Cf-4*	E	LRR–TM	*Cladosporium fulvum*	*Avr4*	(Thomas et al., 1997)
Tomato	*Hcr9-4E*	E	LRR–TM	*Cladosporium fulvum*	*Avr4E*	(Takken et al., 1998)
Tomato	*Cf-5*	E	LRR–TM	*Cladosporium fulvum*	*Avr5*	(Dixon et al., 1998)
Tomato	*Cf-9*	E	LRR–TM	*Cladosporium fulvum*	*Avr9*	(Jones et al., 1994)
Sugar beet	$HS1^{pro-1}$	E	LRR–TM	*Heterodera schachtii*		(Cai et al., 1997)

the *R* gene products with shorter repeats (Jones and Jones, 1996), as shorter repeats probably lead to a linear, rod-like structure. Extracellular LRRs are predicted to have a conserved hydrophobic face which has a structural role, while the other side contains side chains of non-conserved, interstitial amino acid residues that might interact with a specific ligand (Parniske and Jones, 1999; Thomas et al., 1998)

Role of the LRR domain in recognitional specificity of R proteins

The LRR domain is an obvious candidate for receptor function. Although the LZ and TIR domains could also have this role, it is more likely that these are involved in downstream signalling (see below). Proteins containing LRR domains mediate interactions with other proteins (Kobe and Deisenhofer, 1994) playing a role in, for instance, signal transduction cascades or acting as receptors for peptide hormones (Moyle et al., 1995). Support for the assumption that specificity resides in the LRR domain comes from plants that have mutations in the LRR domain and show loss of resistance or altered resistance specificity. Minor alterations in the LRR domain of e.g. RPS2, RPM1 and N result in a lack of HR activation upon infection by an avirulent pathogen (Bent et al., 1994; Grant et al., 1995; Mindrinos et al., 1994). Furthermore, domain swap experiments between the various flax L proteins identified the LRR region as a major component providing pathogen recognition specificity (Ellis et al., 1997). Transgenic expression of *Xa21D*, a homologue of *Xa21*, results in the same, albeit partial resistance specificity shown as by *Xa21* itself. The *Xa21D* gene contains a transposon insertion, resulting in a truncated Xa21D protein that only carries the LRR and TM domain (Wang et al., 1998), which is apparently sufficient for race-specific recognition. As has been shown for the *Cf* genes (Parniske et al., 1997; Thomas et al., 1998), analysis of large numbers of *R* gene families has revealed that variation between the LRR domains directly correlates with recognitional specificity of the individual homologues.

Localisation of R proteins and their matching elicitors

It has been proposed that *Avr* products act directly as ligands for receptors encoded by host *R* genes (Jones, 1997). If this hypothesis is correct, the *R* gene product and matching elicitor are expected to occur in the same cellular compartment, a phenomenon which has been observed in most cases. The products of genes conferring resistance to obligate intracellular pathogens, such as *Rx* and *N*, conferring resistance to potato virus X (PVX) and tobacco mosaic virus (TMV), respectively, are indeed predicted to be localised cytoplasmically. Similarly, most products of resistance genes against bacteria are predicted to reside in the cytoplasm (Table 1). Indeed most bacterial *Avr* products only induce HR in resistant plants when present inside the plant cell (Bonas and Van den Ackerveken 1999). An exception is the Xa21 protein, mediating resistance against *X. oryzae* pv *oryzae*, which contains a large, extracellular LRR domain in addition to an intracellular kinase domain (Table 1). However, since the interacting elicitor has not been identified, it is not known whether avirulence depends on a functional Hrp-dependent type III secretion system that injects an elicitor in the cytoplasm of the host cells, followed by perception of the protein in this compartment.

Among *R* genes against fungal pathogens the *Cf* genes, which confer resistance to the tomato leaf mould fungus *Cladosporium fulvum*, are predicted to encode products that are for the major part extracellular. The corresponding elicitors of *C. fulvum* have been shown to be present in the apoplastic space of leaves of plants colonised by the fungus (Joosten and De Wit, 1999), indicating co-localisation of the elicitors and *Cf* gene products. The product of the *I2* gene, conferring resistance to *Fusarium oxysporum* f.sp. *lycopersici*, is also located in the cytoplasm. The matching elicitor, however, has not yet been isolated, so it is not known whether they co-localise. As *F. oxysporum* f.sp. *lycopersici* is able to penetrate living cells during infection, there is direct contact between the cytoplasm of the host cells and the fungus, suggesting that a direct interaction between *R* gene product and elicitor is possible (Olivain and Alabouvette, 1999).

Resistance genes *RPP5* and *RPP8* confer resistance of *Arabidopsis* to the oomycete *Peronospora parasitica* and their products are expected to be cytoplasmic (Table 1). The fungus is in intimate contact with the host via haustoria and it is tempting to speculate that the pathogen transfers *Avr* gene products directly into the host cell along the extrahaustorial matrix. Cloning of *Avr*s of this oomycete might give some clues on the fate of their products in the plant.

Thus, from studies carried out so far the localisation of *R* gene products has not been proven in many cases, but on those occasions were both *Avr* and *R* gene products have been characterised, the *R*-gene products and matching elicitors appear to occur in the same cellular compartment.

Direct or indirect interaction between R and matching AVR proteins

Although the R protein and its matching *Avr* gene product often co-localise, only in one case a direct interaction between the two has been demonstrated. The elicitor Avr-Pita from *Magnaporthe grisea*, an apparent zinc metalloprotease, interacted in the yeast-two-hybrid system (Fields and Song, 1989) with the LRR domain of the protein encoded by the *Pi-ta* gene. This direct interaction was confirmed when Avr-*Pita* was spotted onto a membrane and labelled *Pi-ta* (either the full protein or the LRR domain alone) was used as a probe. The product of a mutated *Pi-ta* allele, which does not confer resistance to the fungus, did not interact with Avr-*Pita* either in the two-hybrid system or in the western membrane binding assay, showing that binding is needed for biological function (Bryan et al., 1999).

The inability to demonstrate a direct interaction between elicitors and their cognate R proteins in other cases suggests that binding is not always direct, but possibly involves a co-receptor which could be the target for the elicitor in its function as virulence factor (see next section). Supportive evidence for an indirect interaction between R and AVR proteins originates from three observations.

Firstly, binding studies using labelled elicitors to identify a receptor often reveal the presence of a high affinity-binding site in plants that either contain or lack the matching *R* gene. For example, binding studies using ^{125}I-labelled AVR9 elicitor of *C. fulvum* revealed the presence of a high affinity-binding site on the plasma membrane of tomato both containing or lacking the *Cf-9* gene, but also in other solanaceous plants and even in a number of non-solanaceous plant species, such as cucumber, barley and oat (Kooman-Gersmann, 1998; Kooman-Gersmann et al., 1996). Solanaceous species all contain genes homologous to *Cf-9*, whereas species from other genera do not. Expression of *Cf-9* in *Arabidopsis*, a species that lacks *Cf-9* homologues, did not result in detectable AVR9 binding to membrane fractions. Kooman-Gersmann (1998) therefore concluded that neither the *Cf-9* homologues nor *Cf-9* itself are required for high affinity-binding of AVR9. Similar binding studies were performed using syringolides, which are acyl glucosides produced by gram-negative bacteria expressing *AvrD* and which induce a HR on soyabean cultivars that carry the *Rpg4* gene. It was found that both resistant and susceptible cultivars contain a 34-kDa protein that specifically binds the elicitor (Ji et al., 1998).

Secondly, by using the yeast-two-hybrid system, a direct interaction was found between Pto, a serine/threonine kinase without obvious binding domains, and AvrPto, originating from the bacterium *P. syringae* pv *tomato*, (Scofield et al., 1996; Tang et al., 1996). Domain swaps between Pto and the product of the closely related *Fen* gene, which does not interact with AvrPto, identified a region of 25 amino acids in Pto containing a conserved kinase domain essential for AvrPto binding. Using site-directed mutagenesis, one single amino acid (threonine 204) in this domain of Pto was shown to be essential for both interaction with AvrPto and AvrPto-specific induction of defence responses. The binding affinity of Pto for AvrPto mutants correlates well with the ability of the various AvrPto mutants to induce HR in *Pto*-containing plants (Tang et al., 1996), suggesting that these components also interact *in planta*.

Thirdly, immunoprecipitation studies revealed co-precipitation of a complex containing both an *R* gene product and its matching elicitor. RPS2, the product of resistance gene *Rps2* of *Arabidopsis*, was found to co-precipitate in a complex when antibodies raised against avirulence gene product AvrRpt2 of *P. syringae* were used (F. Katagiri, Novartis Agricultural Discovery Institute, San Diego, USA, personal communication). In conclusion, it appears that although direct binding between elicitors and *R*-gene products has rarely been observed, both components can co-localise in a complex.

R proteins and signal transduction

As discussed above, circumstantial evidence suggests that, although R proteins determine the specificity of the host for recognition of elicitors, in most cases they do not directly interact with the matching elicitor. A current model proposes that R proteins detect interaction of a virulence factor (= elicitor) of a pathogen

with a pathogenicity target present in its host. In this so-called 'guard' model, interaction of the R protein with the complex results in activation of plant defence responses including the HR (Van der Biezen and Jones, 1999). This model could explain why pathogens carry genes that allow its recognition by the host and enable the plant to resist attack by the pathogen. The model predicts that elicitors normally function as virulence factors, a hypothesis supported by the observation that some *Avr* genes are always maintained within a pathogen population (Vivian and Gibbon, 1997). For a number of bacterial (e.g. AvrBs2, AvrRpt2 and AvrRpm1) and fungal (e.g. ECP2 and NIP1) elicitors, it has indeed been proven that deletion or mutation of the encoding genes renders the pathogen less virulent on a susceptible host (Dangl, 1999; Kearney and Staskawicz, 1990; Laugé et al., 1997; Rohe et al., 1995).

Pathogenicity targets affected by the elicitor could, for example, be proteins involved in defence or in host metabolism. Evidence for an involvement of a pathogenicity target in defence comes from the identification of proteins that interact with Pto. By using the yeast-two-hybrid system a direct interaction between Pto and the transcription factors Pti4, Pti5 and Pti6 (*Pt*o *i*nteracting) was found, in the absence of AvrPto. Upon phosphorylation by Pto, these transcription factors bind to elements present in the promoter of ethylene-induced genes, including many basic PR proteins (Zhou et al., 1997). Pto also interacts with Pti1, a serine/threonine kinase that is also phosphorylated by Pto (Zhou et al., 1995). This suggests that Pto activates at least two distinct pathways. Overexpression of Pto results in increased basal defence responses against a variety of pathogens (Tang et al., 1999). Binding of AvrPto to Pto might affect Pto function and thereby lower the basal resistance level of the plant. Prf, an NBS–LRR R protein, might detect Pto–avrPto interaction and then induce a pathway leading to HR (Van der Biezen and Jones, 1999). In this model the pathogenicity target (Pto) is more or less guarded by the resistance gene product Prf. Prf has another function, as it also mediates induction of HR upon treatment with the insecticide fenthion, when the plant harbours the *Pto* homologue *Fen* (Salmeron et al., 1996). Therefore, Prf might safeguard two homologous complexes. Dual specificity of an R protein for distinct elicitors has been observed before, for instance for *Rpm1* which mediates recognition of both avrB and the unrelated AvrRPM1 elicitor (Grant et al., 1995).

Signal transduction by R proteins with a putative signalling domain

Upon pathogen recognition, many defence responses, such as the HR, PR protein accumulation and an oxidative burst are activated (for reviews see Baker et al., 1997; Hammond-Kosack and Jones, 1996; Somssich and Hahlbrock, 1998; Yang et al., 1997). How *R* gene products mediate the activation of these responses is, in most cases, not clear. It is likely that the LZ, NBS and TIR domains play a role in signalling. The LZ region could facilitate homo- or hetero-dimerisation (Landschulz et al., 1988), while the conformation of the complex might be determined by interaction with an elicitor. By employing the yeast-two-hybrid system, it should be possible to determine whether LZ regions in *R* gene products are able to interact with each other or with additional proteins.

The NBS domain can, in theory, activate kinases or G proteins by transfer of phosphate (Hammond-Kosack and Jones, 1997). Many proteins that contain a NBS are essential for processes like development, differentiation and vesicle transport (Bourne et al., 1991; Traut, 1994). The presence of such a highly conserved domain in many *R* genes suggests an important role for the NBS domain in R protein function. Mutations in the NBS domain of *RPS2* abolished the ability to induce HR in the presence of AvrRPT2. A similar effect was found in most mutations in the NBS domain of the tobacco *N* gene (Baker et al., 1997). Although NBS domains appear to be essential for R protein function, there is no biochemical evidence yet that these domains are functional via ATP- or GTP-binding.

Although the TIR domains in N, M, L6, RPP-1, −5, −10 and −14 and RPS4 (Table 1) have only limited homology to the cytoplasmic domain of the Toll receptor and the interleukin 1-receptor, it has been proposed that the proteins might function in a similar way (Figure 1). Toll is a receptor protein with an extracytoplasmic LRR domain that controls both polarity in Drosophila embryos (Hashimoto et al., 1988) and expression of the anti-fungal peptide drosomycin in flies infected by fungi (Lemaitre et al., 1996). Upon binding of Spätzle (a small ligand), the Toll protein activates the cytoplasmic protein Tube (Figure 1). Tube subsequently recruits Pelle, a serine/threonine kinase homologous to Pto, by transferring it to the plasma membrane. Pelle phosphorylates the Cactus–Dorsal protein complex, which leads to the degradation of Cactus and release of Dorsal. Dorsal, a transcription

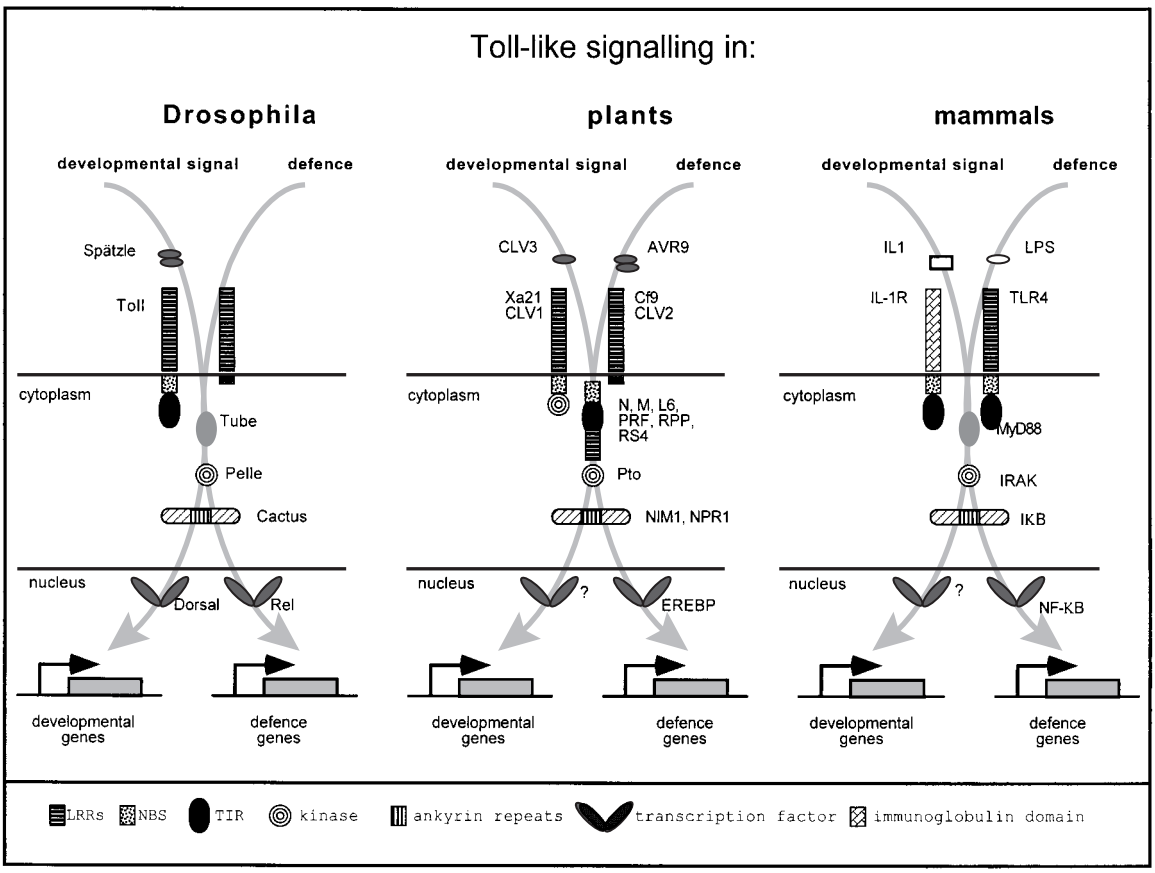

Figure 1. Schematic representation of Toll signalling pathways in Drosophila, plants and mammals responsible for induction of genes that play a role in development or defence. A small ligand, which can be either a small protein like Spätzle, AVR9 or CLAVATA3 (CLV3), or alternatively a lipopolysaccharide (LPS) or interleukin 1(IL1), binds to a Toll–IL like receptor. The transmembrane receptor contains either a leucine-rich repeat (LRR) domain or an immunoglobulin domain and can be linked to a nucleotide binding site (NBS) or a kinase domain. Binding to the Toll receptor or homologues, like Xa21, Cf, CLV, the interleukin-1 receptor or the Toll-like receptor 4 (TLR4), activates the signalling cascade. The TIR domain activates Tube (or a homologue like Myeloid differentiation factor (MyD88)), which recruits Pelle (or Pto or the IL 1 receptor-associated kinase (IRAK)) to the membrane. Pelle then phosphorylates Cactus (or NIM1, NPR1 or IκB) which is degraded, thereby releasing the transcription factor Dorsal (or EREBP or NF-κB). Dorsal moves as a dimer to the nucleus and regulates nuclear gene expression. See text for details.

factor of the Rel/NF-κB family, subsequently activates gene expression in the nucleus.

The human interleukin-1 system, which is involved in inflammatory and immune responses, is regulated in a similar manner. In macrophages, upon activation of the Toll-like interleukin-1 receptor (Sims et al., 1989), the protein kinase IRAK (homologous to Pelle) is activated. IRAK activates the IκB/NF-κB complex, which leads to the release of the inhibitor protein IκB from the complex, allowing the NF-κB transcription factor to activate gene expression.

The analogy between genes that control the activation of defence responses in animals and plants extends beyond the Toll-like domains found in some plant R proteins. From many proteins involved in the Toll signalling pathway, plant homologues have been identified that play a role in the induction of defence responses. Spätzle has structural homology with AVR9 from *C. fulvum.* Toll, the next protein in the signalling cascade, in addition to the Toll domain, contains extracellular LRRs, similar to the Cf proteins and Xa21. Pelle shares homology with Pto, but also with

the serine/threonine kinase domain of Xa21. Cactus, a protein with ankyrin domains, is homologous to the NPR1 protein that is involved in downstream signalling mediated by *Arabidopsis* resistance genes (see below).

Additional support for the hypothesis that plants activate defence gene expression in a way similar to animals, originates from the observation that ROS are generated rapidly upon *R*-mediated activation of defence responses (Doke et al., 1996). In macrophages the transcription factor NF-κB is activated by IRAK and regulated by ROS. The generation of ROS in plants and mammals depends on the assembly of a membrane-localised NADPH oxidase complex (Hammond-Kosack and Jones, 1997). Components of this complex, such as gp91phox homologues, have been identified in plants (Groom et al., 1996).

R gene products might activate downstream components using their NBS/TIR or LZ domains, but they could also initiate HR directly via an NB–ARC domain. This domain, recently recognised in many *R* gene products, is also present in CED-4 and APAF-1 proteins, which play an important role in apoptosis (Kumar and Colussi, 1999). CED-4/APAF-1-type activators can bind the to the BCL2 complex that is present at the mitochondrial membrane. By doing this, the complex binds to and inactivates free caspases, while the resulting protein complex (apoptosome) prevents cytochrome C from leaking out of the mitochondria. Upon activation of the death programme, the apoptosome dissociates, releasing the caspases that are subsequently activated by cytochrome C (Kumar and Colussi, 1999). *R* gene products could, by analogy with the CED-4/APAF-1-type activators be part of an apoptosome-like complex. Upon elicitor binding the protein complex might dissociate, resulting in the HR, which is considered a special form of PCD (Van der Biezen and Jones, 1998; Heath, 1998). Indeed, degradation of the *Arabidopsis* RPM1 disease resistance gene product coincides with the onset of HR (Boyes et al., 1998).

Signal transduction by R proteins without a putative signalling domain

R proteins like the tomato Cf proteins, which lack a LZ/TIR–NBS domain, also lack an obvious signalling domain. It is, therefore, likely that such proteins interact with membrane-bound proteins that possess the ability to transduce a signal into the cytoplasm. Such a complex could be analogous to the CLAVATA (CLV) complex that is involved in meristem development in *Arabidopsis*. An active CLV signalling complex is thought to consist of CLV1 (a Xa21 homologue), CLV3 (a small ligand) and CLV2 (a Cf homologue) (Jeong et al., 1999; for additional references see Joosten and De Wit, 1999). The fourth component in the complex is a kinase-associated protein phosphatase (KAPP) that binds to autophosphorylated CLV1 and seems to be a negative regulator of CLV1 function (Stone et al., 1998; Williams et al., 1997). In addition in this complex a Rho GTPase-related protein was found, which might be involved in signalling into a mitogen-activated protein kinase (MAPK) pathway. The analogy between the Cf and CLV systems, both responding to an extracellular proteinaceous ligand, suggests that signalling might be similar (Joosten and De Wit, 1999), as MAPK activation also plays a role in Cf-mediated resistance (Romeis et al., 1999).

A remarkable difference between CLV- and Cf-mediated signalling is the stability of the ligands. AVRs are cysteine-rich proteins, containing disulfide bridges (Van den Hooven et al., 1999), which are expected to confer sufficient stability to the protein allowing it to pass through the apoplast to reach the receptor present at the plasma membrane of the host cells (Joosten et al., 1997). Mature CLV3 does not possess any cysteine residues and is secreted by the meristem cells, which also encode the corresponding receptors (Fletcher et al., 1999), omitting the need for a protein to be stabilised by disulfide bonds allowing subtle signalling over a gradient. Interestingly, the recent cloning of the male determinant of self-incompatibility in *Brassica* reveals that signalling between pollen and stigma also occurs via small proteins secreted by the pollen. These act as ligands for the Stigma S-locus receptor, which consists of an S-locus glycoprotein (SLG) and a membrane-spanning receptor kinase (SRK). The proteins that are secreted by the pollen are rich in cysteine residues and are therefore designated S-locus cysteine-rich (SCR) proteins (Schopfer et al., 1999). The authors speculate that the SCRs contain disulfide bridges essential for a specific tertiary structure and resistance against proteolytic breakdown. In order to prove that Cf signalling requires partners similar to those present in the CLV complex, a biochemical approach should be followed to identify the interacting partners.

Signalling components downstream of R proteins

A genetic approach, based on mutagenesis, has been followed to find interacting partners of R proteins. Using EMS, four tomato genes, *Rcr1, Rcr2, Rcr3*

and *Rcr5* (*R*equired for *C. fulvum r*esistance), which map at distinct loci, have been identified. *Rcr3* is essential for full Cf2 function, whereas mutation of the other genes causes only partial loss of disease resistance (Hammond–Kosack et al., 1994; Hammond-Kosack and Jones, 1996). Proteins encoded by these genes appear to act in the same signalling pathway as Cf proteins and they might interact directly or indirectly with the Cf proteins. Cloning of the *Rcr* genes is in progress. *RDR* (*R*equired for *D*isease *R*esistance) loci have also been identified in barley, both for *Mlo* (*Ror1* and *Ror2*) (Freialdenhoven et al., 1996) and *Mla12* (*Rar1* and *Rar2*) gene function (Lahaye et al., 1998). *Rar1* has recently been isolated and shown to be member of a novel gene family encoding a zinc-binding protein, which is conserved in humans and *Caenorhabditis elegans*, but not in yeast (Shirasu et al., 1999). Silencing the gene resulted in loss of an oxydative burst in an incompatible interaction with powdery mildews in *Mla*-containing barley (Schulze-Lefert, 1999).

In *Arabidopsis*, two classes of *RDR* genes are specific for certain R proteins. *Eds1* (*E*nhanced *d*isease susceptibility) is required for R proteins with a TIR domain, like RPS4 and most RPPs (Aarts et al., 1998), whereas *Ndr1* (*N*o *d*isease *r*esistance) is mainly required for *R* gene products that contain an LZ domain, like RPM1, RPS2 and RPS5. The only exception so far is RPP8, which has an LZ domain but does not depend on *Ndr1* or on *Eds1* for function (Aarts et al., 1998). The observation that mutation of *Ndr1* or *Eds1* affected a subset of resistance genes of a similar type in their ability to mediate the activation of defence responses, indicates that in *Arabidopsis*, at least two different signalling pathways exist.

The *Ndr1* gene encodes a 219 amino acid protein that has two possible membrane-spanning regions (Century et al., 1997). Expression of *Ndr1* is induced in response to infection by various pathogens, however, neither its function nor the relative position in the signal transduction pathway is known. Interestingly, constitutive expression of *Ndr1* in *Arabidopsis* leads to plants with broad-spectrum resistance. Resistance responses in such transgenic plants are not constitutively activated when plants are grown under non-inducing conditions. However, when compared to wild-type plants, the responses are induced to a larger extent upon infection by pathogens (Cao et al., 1998).

Recently, the *Eds1* gene was cloned. Its product has homology to eukaryotic lipases (Falk et al., 1999), suggesting that EDS1 has a function in hydrolysis of lipids. Possibly, the protein is involved in processing intermediates of jasmonic acid, a lipid-derived signalling molecule involved in the induction of plant defences (Thomma et al., 1998). Alternatively, the protein could participate in a novel, unknown lipid-based signalling cascade. Similarly, the *Pad4* gene of *Arabidopsis*, which is required for the expression of multiple defence responses, including phytoalexin accumulation, upon infection by avirulent strains of *P. syringae* pv *maculicola*, also encodes a lipase-like protein (Jirage et al., 1999).

In a screen for *Arabidopsis* mutants defective in SAR induction, the *Nim1* (*N*on-*i*nducible *i*mmunity) mutation was identified and the responsible gene isolated (Ryals et al., 1997). The *nim1* mutant was found to contain a mutation in the same gene as two other *Arabidopsis* mutants: *npr1* (*N*o *PR* gene induction (Cao et al., 1997)) and *sai1* (*SA i*nsensitive (Shah et al., 1997)). The *Nim* gene encodes a protein with ankyrin motifs, a motif found in proteins with diverse functions that play a role in protein–protein interactions. Interestingly, the NIM1 protein also shows homology to the transcription factor inhibitor IκB, thus might fit in a similar pathway as described above for Pto and R proteins with a TIR domain. Ryals and coworkers (1997) therefore proposed that the protein functions as a transcriptional regulator that, upon activation, translocates a transcription factor to the nucleus, thereby inducing gene expression (Ryals et al., 1997). Recently, it was shown that NPR1 directly interacts with basic leucine zipper protein transcription factors that bind to sequences required for the induction of the *PR-1* gene (Zhang et al. 1999; for a review on genes controlling downstream signalling of NDR1 and EDS1, see Glazebrook 1999).

Evolution of resistance genes

Due to the high mutation rate of many plant pathogens, monogenic resistance is not durable in most cases. Mutants, which have changed from avirulent to virulent, will have a selective advantage as their host range has been broadened and they will therefore multiply more efficiently. Plants, however, have a wide range of recognitional specificities and susceptibility is the exception, suggesting that co-evolution between host and pathogen frequently occurs in nature. During evolution, new resistance specificities must have been generated to cope with the newly evolved virulent strains of pathogens.

A clue to the mechanisms by which sequence diversification in plant resistance genes is promoted, comes from their genomic organisation. Some *R* genes, such as *Hm1* and *RPM1* (Johal and Briggs 1992; Grant et al., 1995), are only present as a single copy gene, and are absent in susceptible plants. Most *R* genes, however, are organised in complex loci that contain an array of homologous genes. Examples of *R* genes that are present in clusters include *Rp1, Rpp5, Xa21, Pto, Dm3, I2, N, M* and the *Cf* genes. The tandem array organisation of homologous sequences probably facilitates inter- and intragenic recombination events, unequal crossing-over and gene duplications (Hammond-Kosack and Jones, 1996; Michelmore and Meyers, 1998). For example, the *Cf* gene clusters of tomato, which have been introgressed from wild species in cultivated tomato, resemble a patchwork of chimeric sequences. The variation between the individual members probably results from successive rounds of reciprocal recombination. Normally, such recombinations lead to sequence homogenisation. However, it has been suggested that the intergenic regions determine and control the recombinations, thereby preventing homogenisation (Parniske et al., 1997). In a homozygous plant, unequal recombination between sister chromosomes is repressed, resulting in meiotic stability. In a suitable heterozygous plant, unequal alignment of homologous genes and subsequent recombinations or gene-conversions are allowed, resulting in the observed unstable genotypes (Parniske et al., 1997). Analysis of recombinants obtained from test crosses between *Cf-4* and *Cf-9* plants (Thomas et al., 1997) revealed that three of the four recombinations occurred at a preferred, homologous region located between *Hcr9* (*H*omologues of *C. fulvum r*esistance gene *Cf-9)* genes. A more detailed analysis, including two *Hcr9* clusters (Northern light and Southern cross), present at the short arm of chromosome 1 but outside the *Cf-4/9* cluster, revealed that extensive sequence exchange has occurred between the individual homologues (Parniske et al., 1999).

These observations suggest that events such as unequal crossing-over and gene-conversion are sufficient to generate rearranged genes that encode a novel resistance specificity. Although these events may be important in the generation of novel recombinant genes (or gene-clusters), there appears to be a more important mechanism, which can be regarded as a process of adaptive evolution responsible for fine-tuning of novel recognition specificities. Sequence analysis of the *Cf-4/Cf-9* cluster of tomato suggested that adaptive selection plays an essential role in generation of sequence diversity (Parniske et al., 1997). The analysis was performed by comparing the ratio of non-synonymous (Ka) to synonymous (Ks) nucleotide substitutions in the various *Hcr9*s. A ratio of 1 indicates random mutagenesis, a ratio smaller than 1 is indicative of sequence conservation, while a ratio larger than 1 suggests sequence diversification. Comparing the Ka : Ks ratio of 11 *Hcr9*s revealed that the sequence encoding the N-terminal LRR region (LRRs 1–16) has a Ka : Ks ratio larger than 1. The highest ratio is found at the x position in the xxLxLxx motif, in which x is any amino acid and L is a conserved leucine residue or any aliphatic amino acid. This suggests an important role for the solvent-exposed residues of LRRs in specific binding of a ligand, or a ligand–receptor complex. The other domains of the *Hcr9*s have Ka : Ks ratios smaller than one, indicative of amino acid conservation.

Ratios larger than 1 are rare for most genes, but common in genes for which diversifying selection is important: for example genes of the major histocompatibility complex (MHC) of mammals (Hughes and Nei, 1988), and genes that express surface antigens of parasites and viruses (Endo et al., 1996). The MHC is involved in cellular and humoral immune responses and consists of a large cluster of homologous genes that by recombination and gene conversion generates new recognitional specificities. However, the most significant alterations that modify specificity, are specific substitutions of key residues in the antigen-binding domain (Trowsdale, 1993). A similar diversifying selection seems to act on most other *R* genes, as ratios larger than 1 have also been found for solvent-exposed LRR regions of products of the *R* genes *Dm3, I2, Mi, L6, Rx* and *Xa21* (Meyers et al., 1998). How the hypervariability in the solvent-exposed LRR region is accomplished is not known, but the parallels with the MHC are intriguing.

Novel specificities for recognition of both the MHC and *R* gene clusters are generated at the individual level. This results in large and diverse specificities at the population level. An example in plants is the diverse occurrence of *Cf* genes within the *Lycopersicon* genus (Haanstra, 2000; Laugé, 1999). Screening of various accessions of *L. pimpinellifolium*, using recombinant potato virus X (PVX) expressing extracellular proteins (AVRs and ECPs) of *C. fulvum* revealed that for every protein tested, plants occur that respond with a specific

HR (Laugé, 1999). Specific recognition of ECP2 was also found in some *Nicotiana* species, which are not a host for *C. fulvum*, suggesting that generation of new recognitional specificities occurs at random (Laugé, 1999). Random evolution of recognitional specificities might even result in alleles of *R* genes that mediate recognition of 'self-proteins'. Indeed, plants have been found that, at specific developmental stages or under certain environmental conditions, show HR-like systemic necrosis. These plants, which are referred to as 'disease lesion mimics', were found to contain mutations that often mapped to known *R* gene loci and are thought to contain *R* genes that have gone 'bad' (Jones, 1994). For the *N* gene of tobacco it has been shown that specific mutations do result in generation of disease lesion mimics (Baker et al., 1997), suggesting that this phenotype can be conferred by 'damaged' *R* genes.

In conclusion, since plants are not able to escape attack by pathogens, they have developed effective defence mechanisms, which either prevent infection or involve active responses that render the plant resistant. In addition to resistance specificity that allows recognition of particular races of a pathogen, plants have evolved mechanisms to generate novel recognitional specificities to cope with new races. The rate by which these new specificities are generated will, in the long run, determine the outcome of the continuous struggle between host and pathogen.

Acknowledgements

Prof. Dr. Pierre J.G.M. de Wit and Ir. Rob Weide are gratefully acknowledged for critical reading of the manuscript. F.T. is financially supported by an EC-biotech grant (BIO4-CT96-0515). M.J. is appointed on a research project granted by the biotech company Zeneca-Mogen.

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