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Resistance Genes (*R* Genes) in Plants

Kim E Hammond-Kosack, *Centre for Sustainable Pest and Disease Management, Hertfordshire, UK*

Kostya Kanyuka, *Centre for Sustainable Pest and Disease Management, Hertfordshire, UK*

The activation of plant defence to restrict pathogen invasion is often conferred by resistance (*R*) proteins. The most prevalent class of *R* proteins contain leucine-rich repeats (LRRs), a central nucleotide binding site and a variable amino terminal domain. Other classes possess an extracellular LRR domain, a transmembrane domain and sometimes an intracellular serine/threonine kinase domain. *R* proteins function in pathogen perception and/or the activation of conserved defence signalling networks. Upon infection, specific effectors produced by pathogens and presumed to promote growth in host tissue, are either directly recognized by different *R* proteins or are recognized by a targeted plant protein which is itself guarded by *R* proteins. Subsequently, various defence signalling networks are activated via *R* protein phosphorylation, oligomerization, degradation, conformational changes and by the shuttling of *R* proteins between the plant cell cytoplasm and the nucleus. The overall outcome is dramatic cellular reprogramming and the activation of coordinated defence responses both locally at the site of infection as well as systemically throughout the plant. Many *R* gene loci appear to be under positive genetic selection, which rapidly diversifies paralogous sequences. Some *R* genes are present in plant genomes at single loci as either a single sequence or an allelic series whilst others reside within tight or loose clusters of related *R* sequences. For a century, plant breeders have genetically characterized and used *R* genes to reduce the impact of pathogens on crop production. More recently, various transgenic approaches have been tested to provide broader spectrum control and improved durability.

Innate Immunity and Resistance in Plants

In natural ecosystems plant disease epidemics are rare. This is because most plant species can successfully defend themselves against the various infection strategies deployed by different types of plant attackers, for example, fungi, oomycetes, bacteria, viruses, insect pests and nematodes. These interactions are generally evolutionarily stable and the nonadopted plant species is referred to as a 'nonhost'. In these encounters, pathogens frequently reveal their presence to the plant's 'innate immune system' through microbe-associated molecular patterns (MAMPs) formerly termed pathogen-associated molecular patterns (PAMPs). MAMPs are evolutionarily conserved molecules and are often indispensable for the pathogen's life. Examples of MAMPs include structural elements from within the fungal cell wall and the bacterial flagellum. MAMPs act as general elicitors recognized by the plant via specialized MAMP receptors, which in turn leads to the induction of defence responses (Table 1). MAMPs are not present in the plant and therefore signal 'nonself'.

Pathogenic species have evolved the ability to overcome preformed defences, to evade MAMPs detection and/or to suppress actively plant defence responses and thus infect one to many plant species and cause visible disease. Often the affected host tissues are restricted to either a specific organ or cellular location, for example roots, leaves, flowers, the epidermis or vascular tissue. In these so called 'compatible interactions', the plant activates various 'basal defence' strategies to restrict the colonization of 'virulent' pathogens. This minimizes the pathogen's ability to damage the plant's normal growth, development and dispersal (Figure 1). Only a few oomycete and virus pathogens frequently cause a high incidence of plant death. In intensive agricultural systems, where often only a restricted number of crop species are continually grown, some pathogen species can become prevalent. Without due care devastating 'disease epidemics/pandemics' can occur which limit the yield, quality and even the safety of the harvested crop.

At the beginning of the twentieth century, plant geneticists began to investigate interspecies compatibility. These studies revealed that individuals in a pathogen population could cause disease only on some plants in a given population; the rest were resistant, i.e. no disease occurred.

Table 1 Plant microbe-associated molecular patterns (MAMPs) receptors

Class	Pattern recognition receptor (PRR)	Plant species	Predicted features of MAMPs protein	Molecule/protein recognized	Pathogen species	Reference
1	LeEix1 LeEix2	Tomato	LZ-eLRR-TM-ECS	EIX – an ethylene induced xylanase	<i>Trichoderma viride</i>	^a
2	FLS2	<i>Arabidopsis</i>	eLRR-TM-kinase	flg22 – a 22 amino acid peptide derived from the N-terminal fragment of the flagellin protein	Multiple bacteria species	^a
3	EFR	<i>Arabidopsis</i>	eLRR-TM-kinase	EF-Tu – acetylated N terminus of the elongation factor Tu	Bacteria	Zipfel <i>et al.</i> (2006)
4	GBP – 75-kDa b-glucan binding protein	Soybean and <i>Fabaceae</i> species	Soluble, cell wall located protein with intrinsic endo- β -glucanase activity	HG – hepta β -glucoside	<i>Phytophthora</i> cell-wall derived	^a
5	N-glycoproteins of 162 and 50 kDa	Tobacco, <i>Arabidopsis</i> and <i>Acer pseudoplatanus</i>	Plasma membrane localized	Lipid-transfer proteins–elicitors which bind sterols	<i>Oomycetes</i> (<i>Phytophthora</i> species and <i>Pythium</i> species)	^a
6	CEBiP	Rice	Plasma membrane localized glycoprotein with two extracellular LysM motifs	Chitin oligomers (chitooligosaccharide)	Fungi	Kaku <i>et al.</i> (2006)
7	100-kDa Pep-13 binding protein	Parsley	Plasma membrane localized	Pep13 – a surface exposed 13 amino acid sequence present within a cell wall transglutaminase	<i>Phytophthora sojae</i> and other <i>Phytophthora</i> species	^a
8	Not known	Tobacco	Plasma membrane localized	RNP-1 cold shock inducible RNA-binding protein	Gram-negative and Gram-positive bacteria	^a
9	Not known	<i>Arabidopsis</i> and rice	Not known	LPS- lipopolysaccharides	Gram-negative bacteria	^a
10	HrBP1	<i>Arabidopsis</i>	Not stated	Harpin	<i>Erwinia amylovora</i>	Eden

Note: For further details see the following articles and their references: Zipfel *et al.* (2006); Kaku *et al.* (2006); EDEN Biosciences, http://www.edenbio.com/usa/technology/download/wp_8.pdf
^aReviewed by Nürnberger *et al.* (2006).

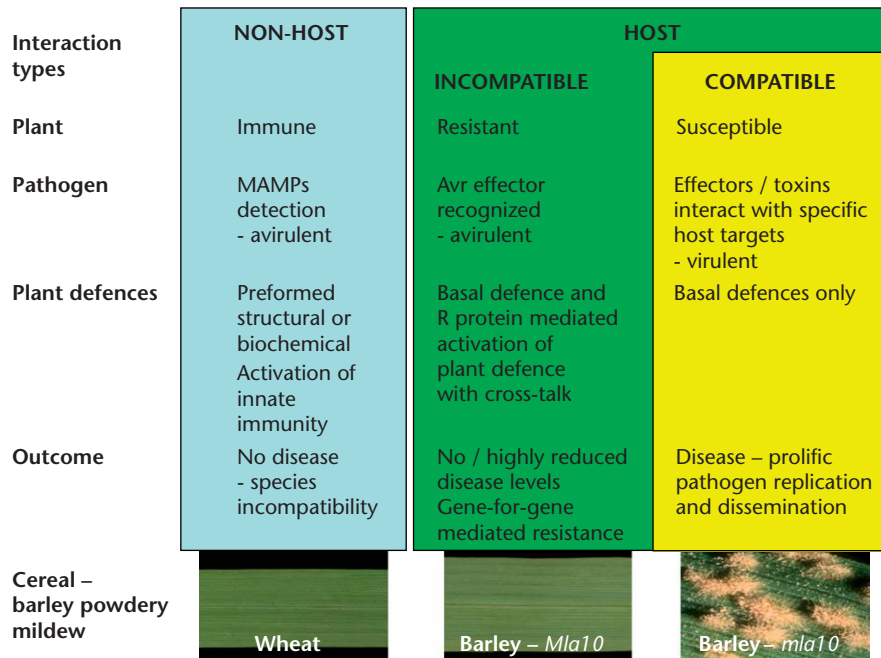


Figure 1 The different interaction types and layers of plant resistance.

In the early 1940s, Harold Flor formally investigated the genetics of the interaction between flax and the fungal flax rust pathogen and proposed the ‘gene-for-gene model’. This model predicts that plant resistance will occur only when a plant possesses a dominant ‘resistance gene’ (*R*) and the pathogen expresses the complementary dominant ‘avirulence gene’ (*Avr*). Incompatibility is therefore determined by complementary pairs of dominant genes. An alteration or loss of the plant resistance gene (*R* changing to *r*) or of the pathogen avirulence gene (*Avr* changing to *avr*) leads to disease (compatibility). Although the gene-for-gene model is not applicable to all host–pathogen interactions, it holds true when the invading pathogen species colonizes primarily through living plant tissue and the host induces a rapid and highly localized cell death response at the site of infection called the ‘hypersensitive response’ (HR). Sometimes, however, resistance is inherited as a recessive trait (see below). Once twentieth century plant breeders realized that plant resistance was frequently inherited as a dominant trait, plant breeding programmes were developed to identify resistant germplasm in wild relatives of crop plants and then introgress the corresponding resistance *R* genes for agricultural benefits. Occasionally the outcome was stable disease control. More frequently a ‘boom and bust cycle’ was observed (see additional figures). In these latter situations, a few years after the *R* gene was introduced into commercial production, the gene ceased to protect the crop and severe disease epidemics occurred.

Pathogen Effectors and Avirulence Gene Products

Why does a virulent pathogen make *Avr* gene products? The outcome is clearly undesirable, a restricted host range in plant populations where individuals possess the corresponding *R* gene. This dilemma puzzled students and researchers alike for almost 60 years. However, in the late 1990s, a relatively straightforward answer emerged from studying plant–bacterial interactions. Phytopathogenic bacteria were found to produce a suite of molecules termed ‘effectors’ which are delivered directly into the plant cells during initial infection by a special type of secretion system, termed ‘type III secretion’. These effectors either alter the plant’s physiological state to benefit pathogen colonization or are used to suppress the activation of host plant defences. In response, the plant has evolved specific countermeasures to protect against these pathogen-induced cellular reprogramming events. The protective countermeasures included the evolution of *R* genes whose products lead to the direct or indirect recognition of a specific sub-set of pathogen effector gene products and the activation of defence responses in the attacked tissues. Only this recognized sub-set of effectors is identified as *Avr* genes by plant genotypes possessing the corresponding *R* gene (Figure 1).

Resistance Gene Types, Mode of Inheritance, Mode of Action and Patterns of Expression

Dominant resistance genes

Since 1992 many disease resistance genes have been cloned from experimentally tractable species such as maize, *Arabidopsis*, tomato and tobacco and subsequently from experimentally more challenging species, for example, barley, flax, potato, pepper, rice and even more recently from hexaploid wheat. The majority of known R proteins group into just a few main classes based primarily upon their combination of a limited number of structural motifs (Table 2 and Figure 2). Most R proteins control only pathogen races, which express the corresponding effector protein(s) and are therefore called race-specific R proteins. Occasionally, effective resistance is conferred against multiple races and even different pathogen species. These R proteins are called

race-non-specific. Interestingly, monocotyledonous and dicotyledonous species possess strikingly similar R protein structures implying that fundamental modes of pathogen recognition and defence signalling have been preserved through plant evolution and diversification. The most prevalent class of functionally defined R genes encode intracellular nucleotide-binding/leucine-rich repeat (NB-LRR) proteins, with either an *N*-terminal putative leucine-zipper (LZ) or other coiled coil (CC) sequence. Alternatively at the *N*-terminus of these NB-LRR R proteins there is a region with high similarity to the Toll and Interleukin 1 receptor protein, which are involved in innate immunity in drosophila and mammals. This region is referred to as the TIR region. In recent articles these two classes are referred to as 'CNL' and 'TNL' R proteins, respectively (McHale *et al.*, 2006; Meyers *et al.*, 2005). The only other intracellularly located R class encode serine/threonine protein kinases, for example, tomato Pto. The tomato plasma membrane-bound (Cf) proteins which confer resistance to the fungal pathogen *Cladosporium fulvum* represent

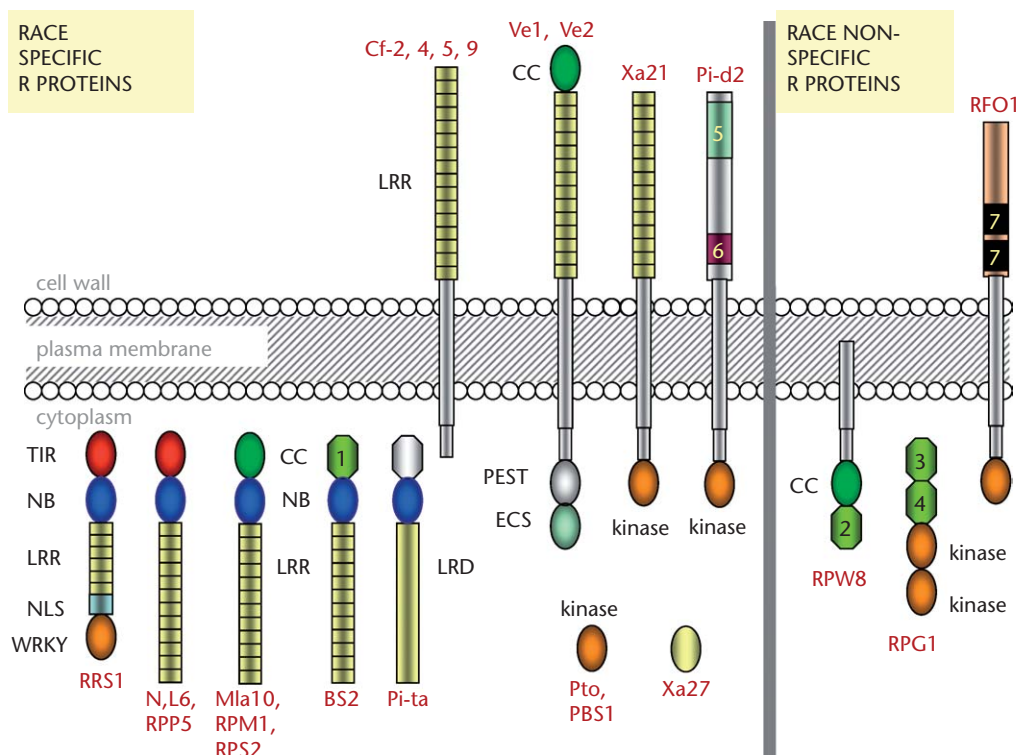


Figure 2 R protein classes and their cellular location. The predicted domains of R proteins which confer either race-specific or race nonspecific resistance are presented schematically: CC, coiled-coil domain; TIR, Toll and Interleukin 1 receptor-like motif; NB, nucleotide binding site; LRR, leucine-rich repeat; NLS, nuclear localization signal; ECS, endocytosis signal; PEST, Pro-Glu-Ser-Ther-like sequence; WRKY, motif characteristic of some plant transcription factors; 1, 2, 3, 4 – novel domains that lack significant homology to known proteins; 5, domain with homology to a B-lectin; 6, structure with a weak similarity to a PAN domain; 7, structure with homology to epidermal growth factor (EGF)-like domain; Cf-2, Cf-4 and Cf-5 confer resistance to *Cladosporium fulvum* races expressing, respectively, Avr2, Avr4 and Avr5; L6 flax rust resistance 6; Mla10, resistance to *Blumeria graminis* f.sp. *hordei* expressing AvrA10; RPM1, resistance to *P. syringae* pv. *maculicola* expressing AvrRpm1 or AvrB; RPP5, resistance to *Hyaloperonospora parasitica* expressing ATR5. Further details of all other named R protein are given in Table 2. The two Ve proteins differ slightly in protein structure. Ve1 contains a putative CC domain but no PEST sequence in the C terminus, whereas Ve2 lacks the CC domain at the *N* terminus but contains a C-terminal PEST sequence. The MLO protein has not been included in this figure because the absence/reduced levels of this protein is thought to confer resistance. Adapted from Hammond-Kosack KE and Parker JE (2003) Deciphering plant-pathogen communication: Fresh perspectives for molecular resistance breeding. *Current Opinions in Biotechnology* 14: 177–193. Reproduced by permission of Elsevier.

Table 2 The major classes of cloned plant disease resistance genes^a

Class	Gene	Plant	Pathogen	Infection type/organ attacked	Predicted features of R protein	Race specific	Year isolated	Reference
1A	<i>Pto</i>	Tomato	<i>Pseudomonas syringae</i> pv. <i>tomato</i> (<i>avrPto</i>)	Extracellular bacteria/leaf	Intracellular serine/threonine protein kinase	Yes	1993	^b
1B	<i>PBS1</i>	<i>Arabidopsis</i>	<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i> (<i>avrPphB</i>)	Extracellular bacteria/leaf	Different sub-family	Yes	2001	^c
2A	<i>RPS2</i>	<i>Arabidopsis</i>	<i>Pseudomonas syringae</i> pv. <i>maculicola</i> (<i>avrRpt2</i>)	Extracellular bacteria/leaf	CC-NB-LRR Intracellular protein CNL protein	Yes	1994	^b
2B	<i>N</i>	Tobacco	Mosaic virus	Intracellular virus/leaf and phloem	TIR-NB-LRR Intracellular protein TNL protein	Yes	1994	^b
2C	<i>Bs2</i>	Pepper	<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> (<i>avrBs2</i>)	Extracellular bacteria/leaf	NB-LRR Intracellular protein	Yes	1999	^b
2D	<i>RRS-1</i>	<i>Arabidopsis</i>	<i>Ralstonia solanacearum</i>	Extracellular bacteria/leaf	TIR-NB-LRR-NLS-WRKY	Yes	2002	^c
2E	<i>Pi-ta</i>	Rice	<i>Magnaporthe grisea</i> (<i>avrPita</i>)	Hemibiotrophic intracellular fungus without haustoria/leaf	NB-leucine rich domain (LRD)	Yes	2000	^b
3A	<i>Cf-9</i>	Tomato	<i>Cladosporium fulvum</i> (<i>Avr9</i>)	Biotrophic extracellular fungus without haustoria/leaf	eLRR-TM-sCT Extracellular protein with single membrane spanning region and short cytoplasmic carboxyl terminus RLP protein	Yes	1994	^b
3B	<i>Ve1</i> <i>Ve2</i>	Tomato	<i>Verticillium albo-atrum</i>	Extracellular vascular wilt fungus without haustoria/root and stem	CC-eLRR-TM-ECS eLRR-TM-PEST-ECS	Yes	2001	^c
4	<i>Xa-21</i>	Rice	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> (all races)	Extracellular bacteria/leaf	eLRR-TM-kinase RLK protein	Yes	1995	^b
5	<i>RPW8</i>	<i>Arabidopsis</i>	Multiple powdery mildew species	Biotrophic intracellular fungus with haustoria/leaf	Small, probable membrane protein with CC domain	No	2001	^c
6	<i>Rpg1</i>	Barley	<i>Puccinia graminis</i> f.sp. <i>tritici</i>	Biotrophic intracellular fungus with haustoria/stem	Receptor kinase-like protein with 2 tandem kinase domains	No	2002	^c

(Continued)

Table 2 Continued

Class	Gene	Plant	Pathogen	Infection type/organ attacked	Predicted features of R protein	Race specific	Year isolated	Reference
7	<i>RFO1</i>	<i>Arabidopsis</i>	<i>Fusarium oxysporum</i> f.sp. <i>matthiola</i> <i>Fusarium oxysporum</i> f.sp. <i>raphani</i>	Extracellular vascular wilt fungus without haustoria/ root and stem	Wall-associated kinase-like kinase, WAK-TM-kinase	No	2005	Diener and Ausubel (2005)
8	<i>Xa27</i>	Rice	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> (<i>avrXa27</i>)	Extracellular bacteria/leaf	113 amino acid protein with two predicted α -helix domains	Yes	2006	Gu <i>et al.</i> (2005)
9	<i>Pi-d2</i>	Rice	<i>Magnaporthe grisea</i>	Hemibiotrophic intracellular fungus without haustoria/leaf	B-lectin receptor – TM - kinase	Yes	2006	Chen <i>et al.</i> (2006)

Note: For further details see the following articles and their references: Diener and Ausubel (2005); Gu *et al.* (2005); Chen *et al.* (2006).

^aThe table gives the details of the first *R* gene isolated in each class.

^bThe references for *R* genes isolated prior to 2001 are summarized in Martin *et al.* (2003) and Hammond-Kosack and Jones (1997).

^cThe references for *R* genes isolated between 2001 and 2003 are summarized in Hammond-Kosack and Parker (2003).

another class of R proteins. Cf proteins consist of an extracellularly located LRR (eLRR) domain, a single transmembrane (TM) spanning domain and a small putatively cytoplasmic tail lacking any obvious functional motif. This class of R proteins is referred to as receptor-like proteins (RLPs). A structurally related class to the RLPs possesses an eLRR, a single TM and a cytoplasmic serine/threonine kinase domain. This class of R proteins is known as receptor-like protein kinase (RLKs). A few dominantly inherited R proteins do not fit these five major classes (Table 2, Figure 2). These include maize Hm1, *Arabidopsis* RPW8, sugar beet Hs1^{PRO1}, tomato Ve proteins, barley Prg1, *Arabidopsis* RFO1, rice Xa21 and rice Pi-d2.

Resistance proteins domains

Each resistance protein provides the plant with up to two unique capabilities; firstly recognition of specific pathogen(s) and secondly activation of defence responses. For a few R proteins both functions have been formally demonstrated to reside within a single protein sequence, for example tomato Pto. However, for the majority only a single role is known or suspected. For example, domain swap experiments between the flax rust L and P resistance proteins have so far identified the LRR region to have a role in recognizing specific pathogen races. The motifs and domains now known in R proteins to contribute specific functions are described in Table 3.

Direct or indirect recognition of pathogen effectors

Physical interaction between R proteins and pathogen effectors has only rarely been demonstrated. For example, the tomato Pto protein kinase, which is tethered to the plasma membrane via a *N*-terminal myristoylation site interacts directly with its cognate bacterial effector AvrPto within the serine/threonine kinase activation domain at residue threonine 204. Tomato Pto also interacts directly with a second bacterial effector AvrPtoB, which has intrinsic E3 ubiquitin ligase activity (Figure 3a). For three NB-LRR, R proteins direct interaction with effectors has also been demonstrated. The *Arabidopsis* RRS1-R protein interacts with bacterial type III effector Pop2 (see below), rice Pi-ta interacts with AVR-Pita (a predicted secreted metalloprotease) from the Ascomycete rice blast fungus *Magnaporthe grisea*, whilst the flax L5, L6 and L7 proteins interact in yeast with the corresponding AvrL567 protein variants from the Basidiomycete flax rust fungus *Melampsora lini*. However, failure by the research community to detect direct R-Avr interactions in plants or *in vitro*, prompted in the late 1990s speculation over the existence of alternative recognition targets and even multi-protein recognition complexes.

Therefore, in 2002 the research community proposed the 'Guard Hypothesis'. This predicts that an effector protein interacts with a host target, which is itself recognized by

more than one R protein. The *Arabidopsis* RIN4 protein is an example of a host target for type III bacterial effectors, which is recognized by at least two CNL R proteins (Figure 3b). In these instances RIN4 is a 211-amino acid acetylated protein, which is plasma membrane associated. RIN4 is the target for the structurally unrelated bacterial effectors AvrRpm1 and AvrB. Both effectors induce phosphorylation of RIN4, which is predicted to activate the R protein RPM1. AvrRpt2 is a cysteine protease and is a third effector recognized by RIN4 inside the plant cell. AvrRpt2 cleaves RIN4 at two sites. Cleavage of RIN4 activates the NB-LRR R protein, RPS2. Similar downstream defence signalling pathways are subsequently activated irrespective of the R protein involved.

Very recently barley epidermal cells engineered to express a fluorescently labelled version of powdery mildew resistance protein Mla10 (CNL R protein) identified the protein's presence at low levels in the plant cell nucleus. The cognate powdery mildew avirulence protein Avra10 labelled with a different fluorescent protein tag was also found in the plant cell nucleus (Figure 3c). These are important new observations because neither the R nor the avirulence protein sequences possess known motifs for nuclear targeting. In the presence of Avra10, Mla10 was found to interact directly with a WRKY DNA binding protein. This new evidence suggests that a direct route to activate plant defences is a formal possibility. The *Arabidopsis* R protein RRS-1 also possesses a WRKY binding motif (see below).

Direct transcriptional activation of defence by NB-LRR R proteins may be required to occur ahead of the defence signalling activated in the cytoplasm. In plants as diverse as *Arabidopsis*, tobacco, barley and wheat both basal and *R*-gene-mediated resistance are regulated by the cytoplasmically localized signalling complex SGT1/RAR1/HSP90 [SGT1 (suppressor of G2 allele of SKP1), RAR1 (required for Mla-dependent resistance 1) and HSP90 (heat shock protein 90)]. The proteins in this complex work together to stabilize various NB-LRR R proteins. The SGT1 component is also essential for the function of the E3 ubiquitin ligase complex, which targets proteins for degradation by the 26S proteasome. Alternatively, transcriptional activation may occur in tandem with cytoplasmic signalling events activated by the R protein, thereby providing an overarching synchrony to the cellular reprogramming which ensures the activation of a successfully coordinated defence response.

The tomato RLPs Cf-2, Cf-4, Cf-5 and Cf-9 each direct resistance responses to a different extracellular cysteine-rich peptide produced by *C. fulvum*. Fungal Avr2 is recognized in the extracellular space by the cysteine protease Rcr3. The Cf-2 protein is thought to guard the Rcr3 and by sensing Avr2-Rcr3 interaction to activate plant defence responses (see additional figures). How Cf-4 possessing tomato plants perceive Avr4 is not known. However, recently an NB-LRR protein called NRC1 has been shown to act downstream of Cf-4 and is required for defence activation (Figure 3d). This is the first demonstration of a formal

Table 3 The known and predicted domains and motifs in R proteins

	Protein domain/motif /signatures	Distinctive structural features	Proven/postulated function in plant defence	Reference
1	LRR domain	Concatenated repeats of 23 or 24 amino acids in length Inner hydrophobic surface and solvent exposed outer surface Extracellular type (24 aa) LxxLxxLxxLxLxx(N)xLxGxIPxx Intracellular type (23 aa) LxxLxxLxxLxLxx(N/C/T)xLxxIPxx	Protein–protein interactions Controls direct or indirect recognition of specific pathogen races Many interact with other eLRR transmembrane spanning proteins which provide intercellular signalling capacity Controls direct or indirect recognition of specific pathogen races Interactions between LRR and NB domain may contribute to recognition specificity Dominant negative interaction with a shared downstream defence signalling component(s) proposed	<i>a, b</i>
1a	C-terminal nonLRR (CT) region		Barley MLA1/MLA6 recognition specificity is determined by different but overlapping LRRs as well as the CT region	Shen <i>et al.</i> (2003)
2	N-terminus of CNL and TNL NB-LRR proteins		Downstream signalling and defence activation	<i>b</i>
2a	CC domain	Coiled-coil structure –a repeated heptad sequence with interspersed hydrophobic amino acid residues Location of myristoylation sequence	Protein–protein interactions and protein dimerization; CC domain of barley MLA10 interacts with WRKY1/2 transcription factors Tethering of R protein to plasma membrane	(<i>b</i>) and Shen <i>et al.</i> (2006)
2b	TIR-domain	Sequence similarity to the cytoplasmic domain of the TOLL and IL-1R proteins	Avr dependent TIR-TIR interactions required for tobacco N protein oligomerization	Mestre and Baulcombe (2006)
2c	NB-ARC -domain	Nucleotide binding domain shared by Apaf-1, certain R proteins and CED-4	Binds and hydrolyses ATP in the tomato I-2 protein Proposed function – a NTP hydrolysing molecular switch which performs reversible intra- and intermolecular protein phosphorylation and autophosphorylation	<i>c</i>
2d	Prf-specific domain	Unique N-terminal amino acids 1–546 outside the NB-ARC-domain	Direct interaction with Pto <i>in vivo</i>	<i>d</i>
3	Effector target interaction domain	~ 150 amino acids in length located between the CC and NB domain in <i>Arabidopsis</i> RPM1	Required for bacterial pathogen effectors to interact with plant target proteins	<i>e</i>

4	Serine-threonine kinase domain	Intracellular Eleven conserved sub-domains	Protein phosphorylation and autophosphorylation Direct binding of different bacterial effectors	^b
5	Myristoylation motif	The consensus sequence Met-Gly X-X-X-Ser/Thr – is required for the covalent attachment of myristate to the Gly residue	Tethering of intracellular protein to the plasma membrane	^b
6	WRKY motif	Plant specific zinc-finger transcription factor A domain of 60 amino acids, which contains the amino acid sequence WRKY at its N-terminal end and a putative zinc finger motif at its C-terminal end	Frequently induced during plant defence and binds W-box cis-acting elements found in many defence inducible promoters WRKY proteins function as transcriptional activators and repressors	Chen <i>et al.</i> (2006)
7	PEST motif	Pro-Glu-Ser-Thr sequence	Often involved in protein ubiquitination, internalization and degradation Presence suggests a short R protein half-life once cytoplasmic localization occurs	^f
8	ECS- endocytosis signature in the C-terminal cytoplasmic tail	YXX ϕ where ϕ represents an amino acid with a hydrophobic side chain and X represents any amino acid	Protein endocytosis (LeEix only)	^f
9	WAK	Wall-associated kinase domain with two EFG domains	WAK proteins are tightly bound to the plant cell wall. May bind pectin	Diener and Ausubel (2005)
10	B-lectin domain	A bulb-type mannose specific binding lectin (B-lectin) domain	Found only in rice Pi-d2, thought to provide a hydrophobic pocket with the potential to bind a hydrophobic ligand	Chen <i>et al.</i> (2006)
11	PAN domain	Contains a conserved core of three disulphide bridges. In some members of the family there is an additional fourth disulfide bridge that links the N and C termini of the domain	Found only in rice Pi-d2, thought to mediate protein – protein or protein – carbohydrate interactions	Chen <i>et al.</i> (2006)

Note: For further details see the following articles and their references: Shen *et al.* (2003); Mestre and Baulcombe *et al.* (2006); Diener and Ausubel (2005); Chen *et al.* (2006); Shen *et al.* (2006).

^aHammond-Kosack and Jones (2000);

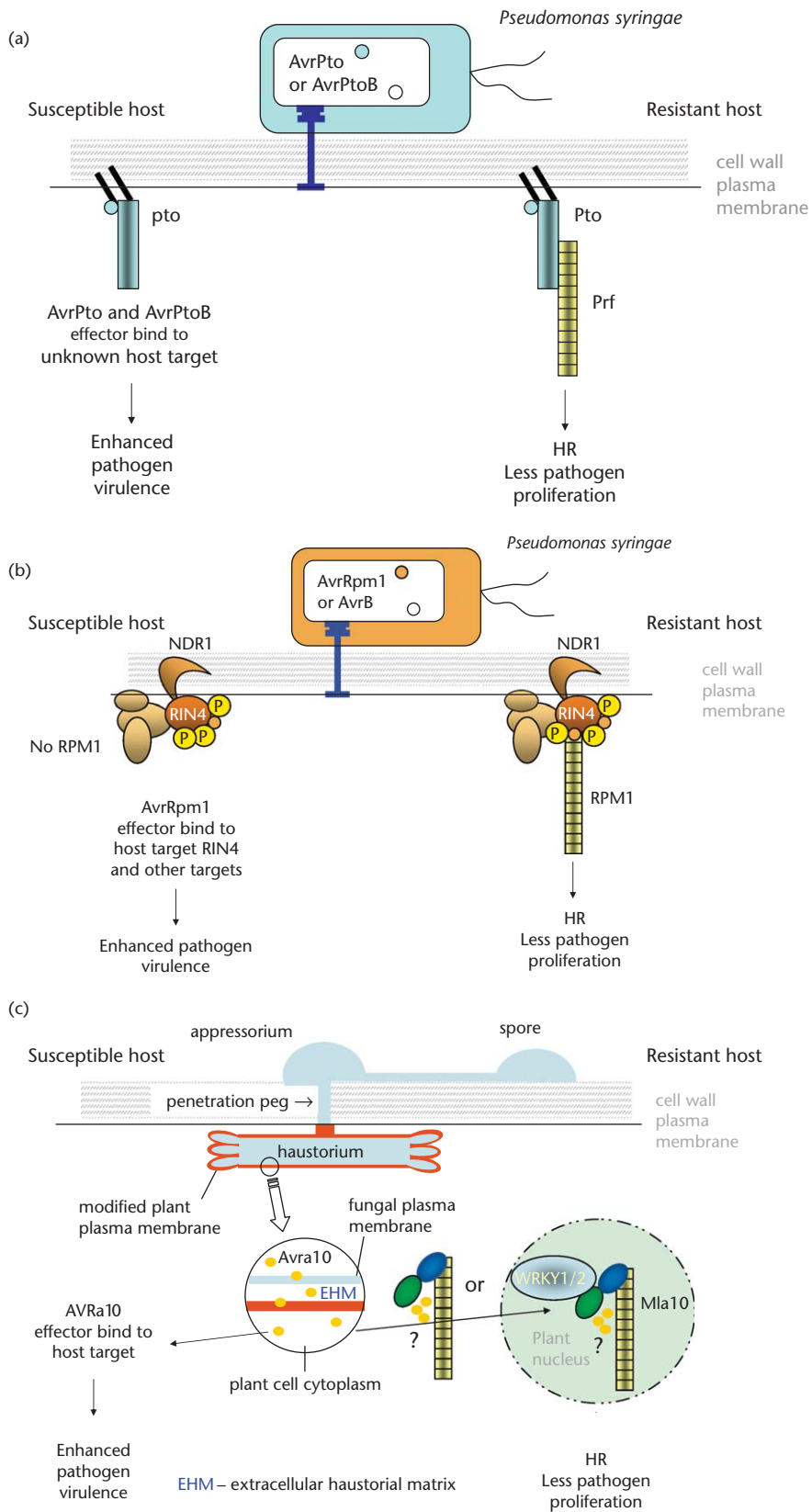
^bMartin *et al.* (2003);

^cTakken *et al.* (2006);

^dMucyn *et al.* (2006);

^eJones and Dangl (2006);

^fHammond-Kosack and Parker (2003).



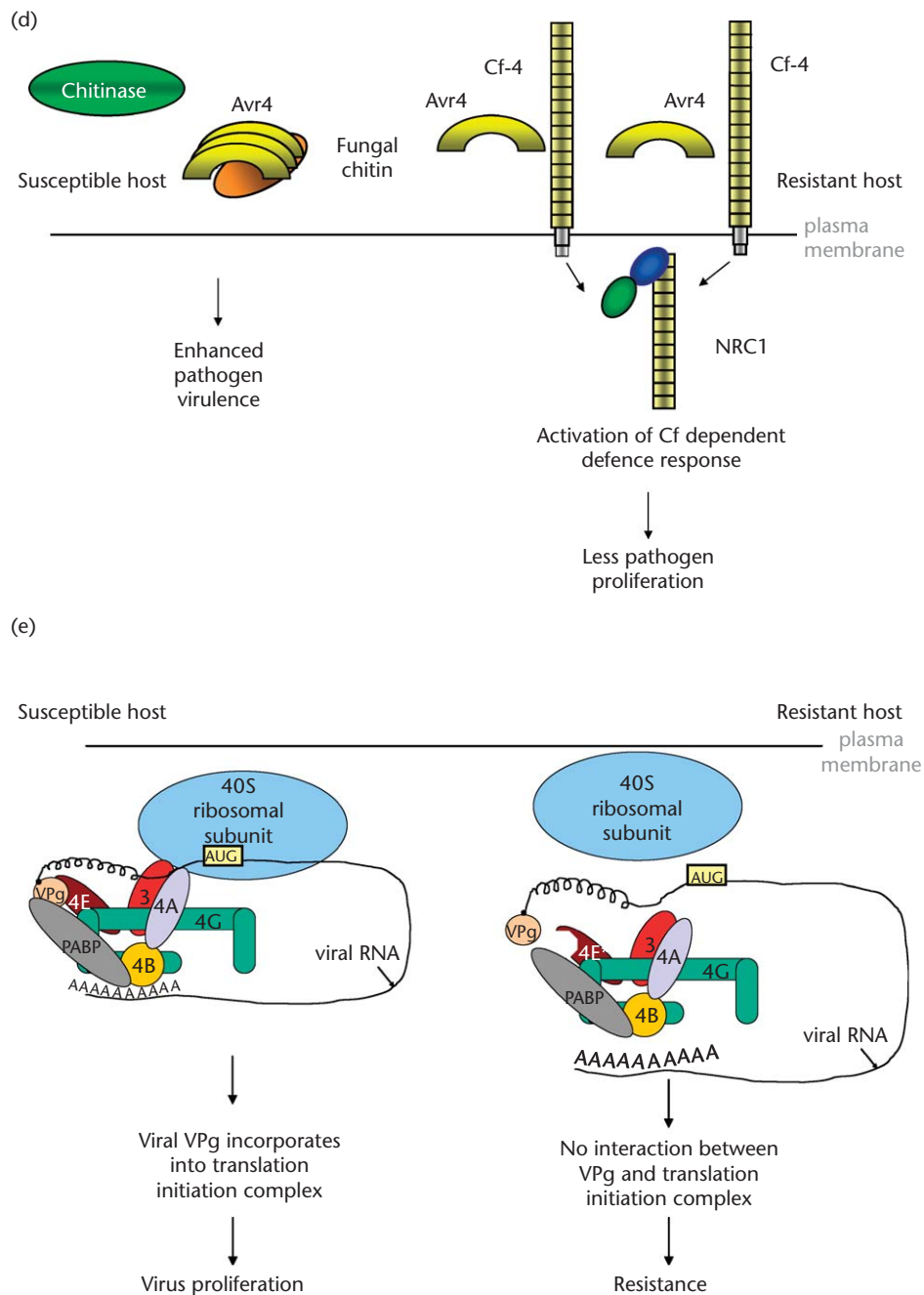


Figure 3 (Continued)

link between NB-LRR- and RLP-mediated defence activation.

The rice Xa-21 protein, an RLK, confers resistance to *Xanthomonas oryzae* pv. *oryzae* bacteria. Although it is not known how the recognition of different races occurs, the kinase domain interacts with the protein XB3 via its ankyrin repeat domain and transphosphorylation occurs. The protein XB3 possesses a RING finger motif and is an E3 ubiquitin ligase. The physical interaction with XB3 is

thought to stabilize the Xa-21 protein, thereby maintaining R protein levels and hence the capacity to activate fully the defence response.

Some R proteins confer resistance to different pathogens. For example, tomato Mi-1, a CNL R protein, confers resistance to three species of root feeding nematode as well as an aphid and a whitefly pest. Possibly, recognition of a common signal type or the guarding of a common host susceptibility target is involved in defence activation. Other

R loci show dual specificity because of tightly linked genes with nearly identical sequences. An example is the neighbouring genes in the potato genome, *Gpa2* and *Rx1* both of which encode CNL proteins and confer resistance to a nematode and a virus, respectively (see additional figures). *Gpa2/Rx1* protein domain swapping experiments have recently demonstrated that the recognition specificity is conferred by the C-terminal half of the LRR domain.

Recessive resistance genes

From approximately 40 cloned resistance genes, which confer resistance to fungal and bacterial pathogens only four are recessive: barley *mlo*, *Arabidopsis edr1*, *Arabidopsis rrs1-r* and rice *xa5*. Whilst the functions of dominantly inherited *R* gene products are beginning to emerge, the molecular mechanisms involved in recessive resistance are less well understood.

Conserved recessive resistance

Naturally occurring or chemically induced mutations in the barley and *Arabidopsis mlo* gene confer broad-spectrum resistance to all races of powdery mildew fungi. Wild type *Mlo* encodes a novel class of plant-specific seven transmembrane domains proteins. It is located in the plasma membrane and acts as a negative regulator of defence and/or programmed cell death. MLO protein colocalizes in the cell and physically interacts with ROR2/PEN1 which belongs to the syntaxin class of proteins known as mediators of the fusion of vesicles with target membranes. Vesicle mediated exocytosis at the site of attempted fungal infection leads to localized 'papilla' formation which inhibits penetration. Therefore, it has been proposed that MLO is involved in some vesicle-associated processes at the plant cell plasma membrane that the fungus has evolved to utilize for its own growth advantage.

Recessive cereal resistance genes

The rice gene *xa5* confers broad-spectrum resistance to most races of the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*. Wild type *Xa5* encodes a subunit of the general transcription factor TFIIA required by RNA polymerase II. It has been suggested that the mutant *xa5* protein possesses an enhanced ability to interact with the acidic transcription activation domain of the bacterial avirulence protein Avr x a5. This in turn leads to retardation of host cell transcription and ultimately to rapid cell death and resistance.

Recessive noncereal resistance genes

The *Arabidopsis* gene *rrs1-r* confers broad-spectrum resistance to multiple strains of the causal agent of bacterial wilt, *Ralstonia solanacearum*. It encodes a novel protein that structurally resembles the prevalent TNL sub-class of R proteins. However in contrast to 'classical' R proteins RRS1 also contains at its C-terminus a WRKY motif characteristic of some plant transcriptional factors. The RRS1 protein moves from the cytoplasm to the plant nucleus upon physical interaction with Pop2, a bacterial type III effector protein. When in the nucleus *rrs1-r* activates transcription of defence genes via its WRKY motif (Table 3).

The *Arabidopsis edr1* mutation provides good resistance to a range of bacterial and fungal pathogens. The wild type *EDR1* gene encodes a Raf-like mitogen-activated protein kinase kinase kinase (MAPKKK). The *edr1* mutation causes a premature stop codon, which eliminates the kinase domain, suggesting that, like MLO in barley, EDR1 is a negative regulator of disease resistance in *Arabidopsis*. The *edr1*-mediated resistance at least to powdery mildew requires an intact salicylic acid (SA) signalling pathway and suggests this process is negatively regulated by EDR1.

Figure 3 R proteins and defence activation (a) Tomato Pto is a polymorphic serine-threonine kinase. Pto directly binds the unrelated bacterial effector proteins AvrPto and AvrB. Both effectors are delivered to the interior of the plant cell by the type III secretion system (TTSS) (blue syringe). Defence activation by Pto requires the NB-LRR protein Prf. The N-terminus of the monomorphic Prf protein binds to Pto to form a molecular complex (right). Both the AvrPto and AvrB effector proteins contribute to virulence in *pto* mutant genotypes (left). Adapted from Jones and Dangl (2006) The plant immune system. *Nature* 444: 323–329. Reproduced by permission of Nature Publishing Group. (b) *Arabidopsis* RPM1 is a plasma membrane tethered NB-LRR protein. The bacterial effectors AvrB and AvrRpm1 are delivered to the plant cell cytoplasm by the TTSS, then modified by eukaryotic-specific acetylation and as a consequence targeted to the plasma membrane. The biochemical functions of AvrB and AvrRpm1 are unknown. Both target RIN4, which interacts with the cytoplasmically localized N-terminal portion of NDR1. RIN4 becomes phosphorylated (+P) and activates RPM1 (right). In the absence of RPM1, both effectors act on RIN4 and other host targets, which contribute to virulence (left). The brown ovoids in this panel represent as yet unknown proteins. Adapted from Jones and Dangl (2006) The plant immune system. *Nature* 444: 323–329. Reproduced by permission of Nature Publishing Group. (c) Barley Mla10 is an NB-LRR protein, which resides in both the host cell cytoplasm and the nucleus. The powdery mildew avirulence protein AVRa10 is delivered into the inside of the plant cell either via the haustorium and the extracellular haustoria matrix (ECM) or directly from fungal hyphae. In the presence of AVRa10, Mla10 protein in the nucleus interacts with specific transcription factors, WRKY1/2. The identified WRKY proteins act as repressors of MAMP-triggered basal defence. Neither AVRa10 nor Mla10 possess known nuclear localization signals. It is unknown whether recognition of the presence of AVRa10 first occurs in the plant cell cytoplasm or the nucleus (right). How AVRa10 contributes to mildew virulence is also unknown (left). This figure is drawn from data published by Shen *et al.* (2007) Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. *Science* 315: 1098–1103, as well as from unpublished data. (d) The receptor-like R protein Cf-4 in tomato activates defences to *C. fulvum* races producing the extracellular effector Avr4. Avr4 binds fungal chitin and protects *C. fulvum* hyphae from degradation by extracellular plant chitinases. Tomato Cf-4 either through indirect or direct recognition of Avr4 activates plant defences. Defence activation by Cf-4 and some other Cf proteins requires the NB-LRR protein NRC1. (e) Recessive resistance to potyviruses controlled by mutant alleles of an eukaryotic translation initiation factor 4e (*elF4E*). Initiation of translation in plants (and other eukaryotes) uses a multi-protein complex comprising of initiation factors 3, 4A, 4B, 4E, 4G, poly(A)-binding proteins (PABPs), 40S ribosomal subunit and several other minor components. An interaction between the mRNA cap structure (m⁷GpppG) and eIF4E is required for efficient translation. Potyviruses produce a small protein called VPg, which is covalently attached to the 5'-end of their RNA genomes and is likely to play a role similar to the mRNA cap structure during translation initiation (left). Some naturally occurring structural variants of eIF4E confer resistance to potyviruses. This is thought to be caused by their inability to bind potyviral VPg and recruit potyviral RNA into the translation initiation complex (right).

Recessive resistance to viruses in cereal and noncereal species

Recessively inherited resistance is quite common for viral diseases and often confers 'immunity' as opposed to the HR associated defence. Viruses are the smallest known organisms encoding only a few functional proteins. To complete their life cycle they often hijack components of the host plant's biochemical machinery. Therefore, the recessive antiviral resistance genes are most likely to correspond to mutations in plant host factors required at specific steps in the virus life cycle.

Recently it has been demonstrated that mutations in the eukaryotic translation initiation factor 4E (eIF4E) or its isologue eIF(iso)4E in a number of plant species confer resistance primarily against viruses belonging to the *Potyviridae* family (Figure 3e). These viruses encode a small protein called VPg that is attached to the 5' end of the viral RNA genome. VPg is a likely functional equivalent of the eukaryotic mRNA cap structure which is important for translation initiation and recruitment of ribosomes via its interaction with eIF4E and/or eIF4(iso)E. Both, potyviral VPg and plant eIF4E/eIF(iso)4E are multifunctional proteins with roles in processes other than initiation of protein synthesis. The exact purpose or outcome of the VPg-eIF4E interaction has not yet been deciphered. Mutations in eIF4E are likely to result in abolition of VPg-eIF4E interaction leading to failure of the potyvirus to complete their life cycle (i.e. translation, replication or cell-to-cell trafficking), which is manifested as resistance.

So far, two mechanistic scenarios have emerged to explain recessively inherited resistance. Resistance can occur via the loss of function of the wild type plant protein product, which actively contributes to the disease causing ability of the pathogen. Alternatively, the plant protein changes through sequence modifications and is therefore difficult for the pathogen to use.

Resistance to toxin producing pathogens – susceptibility factors

Proteinaceous and secondary metabolite toxins are produced by specific taxonomic lineages of pathogenic fungi and bacteria, for example, Pleosporales fungi and Pseudomonad bacteria. These toxins are produced either constitutively or during the infection process and cause the debilitation of important host cellular functions, including histone deacetylation and protein translation. As a consequence, visible tissue chlorosis and/or cell death occurs. Some toxins are absolutely required for a pathogen's disease causing ability whereas others just alter the severity of the associated symptoms in different plant species. In susceptible plant genotypes specific toxin targets are present. In contrast, resistant genotypes either possess an insensitive form of the toxin target site or have evolved *R* loci coding for toxin modification/degradation/elimination processes (Table 4).

Expression of resistance genes and prevalence of R proteins

During normal plant growth and development most *R* genes conferring race-specific resistance are constitutively expressed at low levels. Similarly *R* protein levels, detected by antibodies or protein tags, are low in abundance in healthy plants and only rarely accumulate either locally or systemically in plants undergoing a defence response. An exception is the recently isolated rice *Xa27* gene, which is differentially expressed in the presence of the AvrXa27 bacterial effector. For some *R* genes, for example, the tobacco *N* gene, transcript splice variants have been detected and shown to be important for *R* function.

The expression of single plant *R* proteins is not required for life even in environments where the pathogen thrives. However, for one *Arabidopsis* *R* gene, *RPM1*, a 9% decrease in total seed production has been recorded relative to isogenic *rpm1* plants. When *R* genes are expressed from their native promoter in taxonomically unrelated species or over-expressed in their native plant species this frequently leads to precocious defence activation and plant cell death.

R Genes and their Evolution

Sequencing of plant and pathogen genomes

Complete sequencing of genomes of a model dicotyledonous plant *Arabidopsis thaliana* (thale cress), a model monocotyledonous plant *Oryza sativa* (rice) and a model tree species *Populus trichocarpa* (black cottonwood) has revealed the genome organization of different classes of plant *R* genes and permitted the evolutionary analyses of the major *R* gene classes. These studies have identified that the majority of *R* genes in plants encode NB-LRR class of *R* proteins. The *Arabidopsis*, rice and *Populus* genomes are predicted to contain approximately 150, 400 and 235 NB-LRR genes, respectively. It is not known, whether all NB-LRR-encoding genes play a role in disease resistance. Almost two-thirds of these genes in *Arabidopsis* encode TNLs and another third encodes CNLs, whereas *Populus* contains more CNLs (~60%) than TNLs (~40%). Interestingly, the rice genome completely lacks the TNL-encoding genes. Evolutionary studies suggest CNLs are likely to be the more ancient subclass of *R* genes. Thus, for example, (1) in phylogenetic analyses rice and *Arabidopsis* CNLs cluster together suggesting their common ancestral origin, (2) in the phylogeny tree CNLs have longer branch lengths compared with the TNL tree and (3) positions of introns in CNL-encoding genes are less conserved than in TNL-encoding genes. Global EST sequencing projects and PCR-based surveys confirmed that all plants maintain large and diverse NB-LRR families involved in pathogen surveillance or other unknown functions. These studies also confirm that lineages within the NB-LRR superfamily are not equally represented among all plant taxa. The presence of TNLs in pine and moss indicates that this subfamily of

Table 4 R loci conferring resistance to host-selective and host non-selective toxins produced by fungal and bacterial pathogens

Toxin type	Plant R Locus	Plant Species	Resistance mechanism	Pathogen	Infection type/ Organ attacked	Toxin name	Toxin type	Toxin function	Race specific	Reference
Host-selective										
1	<i>Hm1</i>	Maize	Detoxifying enzyme HC-toxin reductase	<i>Cochliobolus carbonum</i> (race 1)	Fungal necrotroph/ leaf	HC	Cyclic tetrapeptide	Inhibition of histone deacetylase enzyme activity which is thought to interfere with the transcriptional activation of plant defence genes	Yes	Johal and Briggs (1992)
2	<i>Asc-1</i>	Tomato	TM helix-LAG1 motif facilitates a sphingolipid-dependent transport mechanism	<i>Alternaria alternata</i> f.sp. <i>lycopersici</i>	Fungal necrotroph/ leaf	AAL	Sphinganine analogue mycotoxins – (SAMs) – aminopentol esters	Triggers programmed cell death which likely involves ceramide signalling and cell cycle disruption	No	Brandwagt <i>et al.</i> (2000)
3	Minus <i>T-urf13</i> gene in mitochondrial genome	Maize	Lack of pore formation in mitochondrial membrane	<i>Cochliobolus heterostrophus</i> (race T)	Fungal necrotroph/ leaf	T-toxin	Polyketide – linear polyketols	Direct binding to the tetrameric URF13 protein in the mitochondrial membrane, causes pore formation and leakage of mitochondrial contents	Yes	^a
4	vb/pc-2 (not cloned)	Oats	Not known	<i>Cochliobolus victoriae</i>	Fungal necrotroph/ leaf	Victorin	Cyclized chlorinated peptide	Binds to the P protein of the glycine decarboxylase complex in mitochondria	Yes	^a
5	ToxA insensitivity locus located to chromosome 5BL (not cloned)	Wheat	Lack of entry (internalization) from the extracellular space into the plant mesophyll cells of the resistant genotype	<i>Pyrenophora tritici-repentis</i>	Fungal necrotroph/ leaf	Ptr Tox A and Ptr Tox B	13.2 and 6.6 kDa proteins	Induction of host cell death in the absence of the pathogen. Binding to the chloroplast protein ToxABP1	Yes	^(a) and Manning <i>et al.</i> (2007)

Host nonselective

6	<i>Fhb1</i> (Chr3B)	Wheat	Masking of toxins by the formation of conjugated toxin-glycosides	<i>Gibberella zeae</i> and <i>Fusarium culmorum</i>	Fungal, nonbiotroph/floral, stem base and roots	Deoxynivalenol	B-type sesquiterpenoid trichothecenes	Binds to the peptidyl transferase protein in the ribosome and inhibits protein translation	No	Lemmens <i>et al.</i> (2005)
7	None	Tobacco	Toxin efflux or toxin reduction (yeast)	<i>Cercospora nicotianae</i>	Fungus	Cercosporin	Superoxide generating perylenequinone photosensitizer	Singlet oxygen generation leading to peroxidation of membranes	No	Daub and Ehrenshaft (2000)
8	Tabtoxin resistance gene (<i>ttr</i>) from <i>Pst</i> overexpressed in plants	Beans, soybean	Acetyltransferase activity	<i>Pseudomonas syringae</i> pv. <i>tabaci</i>	Bacteria	Tabtoxin	Dipeptide	Hydrolysed by plant cells to release tabtoxinine, the active toxin which inactivates glutamine synthetase and leads to ammonia accumulation	No	Anzai <i>et al.</i> (1989)

Note: For further details see the following articles and their references: Johal and Briggs (1992); Brandwagt *et al.* (2000); Manning *et al.* (2007); Lemmens *et al.* (2005); Daub and Ehrenshaft (2000); Anzai *et al.* (1989).
^aWolpert *et al.* (2002).

NB-LRRs had evolved prior to the angiosperm–gymnosperm split and was lost during evolution of monocotyledonous plants.

Plant receptor-like proteins (RLPs) are known to play key roles in development as well as defence against pathogens. Whole genome sequencing projects revealed that *Arabidopsis* and rice encode 56 and 90 RLPs, respectively. In phylogenetic analyses, the majority of RLPs cluster into four distinct superclades, three of which include RLPs known to be involved in plant defence. Sequence comparisons reveal diagnostic amino acid residues that may specify different molecular functions in the various RLP subtypes. These analyses of rice RLPs identified at least 73 candidate resistance genes and four genes potentially involved in development.

In 2003, the draft genomic sequence of the first plant pathogenic fungus *Magnaporthe grisea* became available. In just four years due to their small genome size and improved sequencing techniques the genomes or more than 20 plant pathogenic fungal and oomycete species have emerged. Through comparative bioinformatics analyses, species specific loci have been identified and the full complement of secreted effector proteins predicted. These new pathogen studies should rapidly accelerate our understanding of R protein function and evolution.

How are new resistance specificities generated?

The majority of NB-LRR-encoding *R* genes are clustered in plant genomes (Meyers *et al.*, 2005). Over 60% of all the predicted NB-LRR genes in the *Arabidopsis* genome reside in 40 clusters ranging in size from two to ten tightly linked genes. Cluster size varies between 5.95 Kbp and 79.03 Kbp. The remaining 40 genes exist as singletons. The rice genome contains an approximately equivalent proportion of clustered NB-LRR genes. This physical clustering suggests that tandem duplication is an important source of new NB-LRR genes. Gene copy number can vary widely among ‘haplotypes’ (genetically distinct lines) within a species. One extreme example is the maize *Rp1* rust resistance locus, which consists of a family of closely related NB-LRR genes. The number of *Rp1* paralogues in different haplotypes varied from a single gene to >50 genes. Such variation in gene copy number has probably arisen due to mispairing and unequal crossing over between clustered NB-LRR genes, or between repetitive sequences such as retrotransposons that are frequently present in intergenic regions of *R* gene clusters. Thus, clustering of *R* genes permits the generation of novel resistance specificities via genetic recombination (DNA crossing over) or gene conversion (unequal crossing over) (Figure 4). Bioinformatic analyses demonstrate that genes in clusters are under diversifying selection, a positive selection that rapidly diversifies paralogous sequences, resulting in a higher rate of nonsynonymous substitutions (nucleotide substitutions that result in amino acid replacements) relative to synonymous substitutions (nucleotide substitutions that do

not cause an amino acid replacement) (McDowell and Simon, 2006). The greatest variation so far identified is in the LRR-encoding domain, a structural feature of the *R* genes that is thought to be involved in recognition of pathogen effectors.

The distribution of *Arabidopsis* and rice RLPs in superclades is similar to NB-LRR genes with large genomic clusters containing 2 or more members, supporting a hypothesis of rapid evolution by tandem duplication and gene shuffling events. Some clusters contain several highly homologous genes conferring resistance to the same or different races of the same pathogen (e.g. *Cladosporium fulvum* resistance *Cf* genes).

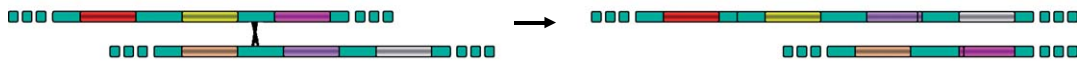
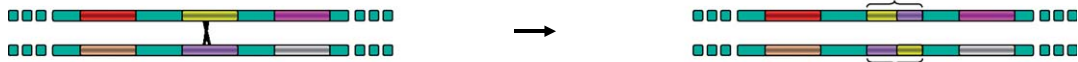
Some *R* genes are present as single-copy loci. Good examples are *Arabidopsis* *RPM1* and *RPS5* genes controlling resistance to different strains of *Pseudomonas syringae*. There is no allelic variation at either the *RPM1* or *RPS5* locus; resistant genotypes contain a functional copy whilst in susceptible genotypes the gene is deleted. It has been proposed that these single copy *R* loci have evolved a very long time ago and now are subject to purifying selection (i.e. a negative natural selection against mutations that result in changes to functionally important amino acid residues) and therefore a reduction in gene diversity. A second type of single gene evolution occurred at the flax *L* locus specifying resistance to different races of the flax rust pathogen *Melampsora lini*. Strong diversifying selection has given rise to at least a dozen alleles at this locus in global flax germplasm. Novel alleles at the *L* locus are known to be generated by frequent interallelic recombination events (Figure 4).

The evolution of recessive resistance genes has rarely been investigated. These genes are generally present as single-copy loci distributed at different locations in plant genomes. At certain loci (e.g. barley *rym4* which corresponds to the eukaryotic translation initiation factor *4E* gene, *eIF4E*) a moderate allelic variation exists with at least four naturally occurring alleles documented. Although no formal proof of recessive resistance gene clustering has yet been obtained, the mapping to the same genetic interval of several resistance specificities to the same pathogen has been documented. For example, barley *rym8*, *rym9*, *rym12* and *rym13* conferring resistance to bymoviruses (*Potyviridae* family) map to a narrow genetic interval in the sub-telomeric region on the long arm of the chromosome 4H.

Parallels between Plant and Animal Defence

Remarkable similarities have been uncovered in the molecular mode of MAMP perception in animals and plants (Nürnberg *et al.*, 2004). This includes the discovery that the *Arabidopsis* LRR-receptor-like kinase FLS2 is very similar to the mammalian Toll-like receptor TLR5, which lacks a kinase domain. Both FLS2 and TLR5 recognize

A. Recombination events during meiosis following cross-pollination

A1. Re-assortment of *R* genes within a clusterA2. Expansion and reduction of an *R* gene clusterA3. Evolution of novel *R* genes

B. Recombination events during meiosis following self-pollination

B1. Expansion and reduction of an *R* gene cluster plus evolution of novel *R* genesB2. Evolution of novel *R* genes (with shorter or longer LRR regions)II. Recombination events at simple *R* locus

C1. Intragenic recombination in a heterozygote (domain swap)



C2. Misalignment and intragenic recombination in a homozygote (shortening and extension of an LRR region)



Figure 4 Some possible mechanisms of *R* gene evolution. Novel *R* alleles can be generated by several different mechanisms. However, the frequency of their occurrence depends upon whether the plant species is maintained as an outbreeding (cross-pollinating) population (A) or an inbreeding (self-pollinating) population (B and C). The evolution of resistance in plants appears to occur primarily at the single-gene level so that novel specificities arising by way of intergenic recombination between similar genes (B1) are rare. The variant *R* alleles eventually selected encode proteins with increased effectiveness or confer a novel recognition capacity. The intergenic regions are shown in light blue. The genes highlighted by either a bracket or a double headed arrow represent different recombination products.

flagellin as a MAMP. Moreover, defence signal transduction activated following pathogen recognition in both animal and plant defence involves changes in cytoplasmic calcium concentrations, the production of signalling molecules such as nitric oxide (NO), reactive oxygen species (ROS) and lipid-based hormones (lipoxins in animals and oxylipins in plants), the activation of mitogen-activated protein kinase (MAPK) cascades and the activation of transcription factors.

Deployment of R Proteins and New Ways to Widen the Utility of *R* Genes in Crop Protection

Nontransgenic approaches

Conventional plant breeding has significantly improved the resistance of crops to important diseases. However the

time consuming and often challenging phenotypic selection of the desired resistant progeny at multiple stages during the breeding programme creates difficulties when trying to respond effectively and stop the spread of new virulent pathogen strains (see additional figures).

By employing DNA marker technologies many breeding limitations can be overcome. Molecular markers closely linked to *R* genes are now routinely used by leading breeding companies in marker-assisted selection (MAS) programmes for the rapid and efficient transfer of *R* genes and other useful traits into elite crop breeding lines. However, the best DNA markers are those derived from the *R* gene sequence. Markers allow each *R* gene to be traced accurately through each breeding generation without the need to carry out difficult and laborious phenotypic screens. As a result more plant generations per year can be completed. However, MAS is expensive which currently restricts its use. MAS can simplify the simultaneous introduction (i.e. 'pyramiding') of several *R* genes targeting different races of the same pathogen into one elite breeding line. The pyramiding approach (Figure 5) should provide broader spectrum and more durable disease resistance. For example, in rice various *Xa* genes confer resistance to the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Several years of field experimentation in the Philippines have consistently demonstrated that rice cultivars containing the resistance gene *Xa7* exhibit the lowest disease severity. Although *Xoo* races virulent on *Xa7* genotypes were recovered from the fields these were all reduced in aggressiveness (disease causing ability). *Xa7* is therefore predicted to be a durable *R* gene and is desirable to include in a *R* gene pyramiding scheme.

The usefulness of cultivar multilines and mixtures for disease control has been tested as an alternative to *R* gene pyramiding (see additional figures). Multilines are mixtures of genotypes bred for phenotypic uniformity of agronomic traits, whilst cultivar mixtures are mixtures of agronomically compatible cultivars with no additional breeding for phenotype uniformity. The choice of *R* genes to be included within a multiline or mixture needs to be relevant to the pathogen population in question. Their effectiveness has been well demonstrated for the control of rusts and powdery mildews of small grain cereals.

In the recent past, techniques for routine accurate detection of single nucleotide polymorphisms have emerged. These have permitted the development of a novel method, called TILLING (Targeting Induced Local Lesions IN Genomes) for the discovery of allelic series of point mutations at any gene of interest in populations of chemically induced mutants. EcoTILLING is used for assessing allelic variation at targeted genetic loci in natural plant germplasm (i.e. ecotypes, landraces and other exotic genotypes). In these methods gene regions of interest are first amplified by PCR (polymerase chain reaction) with fluorescent labelled primers from pooled samples of DNA from mutant lines or ecotypes. Then the PCR products are heated and slowly cooled, resulting in a proportion of heteroduplex DNA formation between wild type and mutant samples. Subsequently, the DNA is digested with an endonuclease, CEL1, which cleaves mismatch positions in heteroduplex molecules. Following cleavage the location of the mismatch nucleotide positions can be identified by various separation methods including DHPLC (denaturing high-pressure liquid chromatography) or polyacrylamide gel

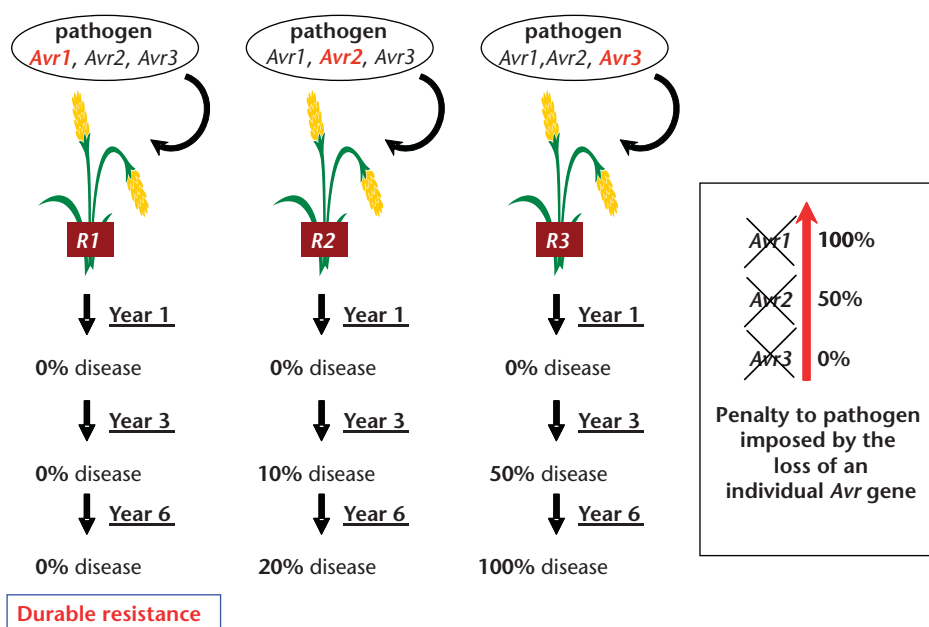


Figure 5 Plant disease control using *R* genes sequences. Designing plant genotypes based on information from pathogen avirulence (effector) genes. *R* genes imposing a high penalty to the pathogen for adaptation are likely to be durable.

electrophoretic analysis. By TILLING/EcoTILLING genotypes bearing different variant alleles of a *R* gene, effector target, defence signalling gene or defence response gene can be identified. These genotypes can then be evaluated for improved resistance to specific pathogens, followed by rapid introgression of the best alleles either singly or in combination into elite breeding materials.

Transgenic approaches

Many transgenic ways to control disease have been suggested using the isolated plant *R* genes, pathogen *Avr* genes, other components of the defence signalling network and the plant specific target sites required for pathogenesis. Four examples are given to illustrate the breadth of possibilities under investigation.

(a) *Transforming susceptible plants with cloned R genes* – By ‘plant transformation’ several different *R* gene alleles, each effective against a single pathogen species or race, can be simultaneously introduced into semi-elite and elite germplasm. In theory, this should slow down the process of pathogen evolution, because for a virulent pathogen to emerge this would require all the corresponding *Avr* genes to mutate simultaneously within a single pathogen isolate. Also by taking the plant transformation route the interspecies infertility barrier is removed, therefore a wider source of useful resistance gene sequences is available. For example, the potato CNL *R* genes *Rpi-blb1* and *Rpi-blb2* both originate from the diploid species *Solanum bulbocastanum*. Through time-consuming breeding schemes involving ploidy manipulations and a series of bridge crosses, various interspecific hybrids (the ABPT lines) were generated. Field testing of these lines at multiple sites in the Netherlands over 20 years revealed either no lesion or only late season development of the late blight pathogen (*Phytophthora infestans*). Recently, through a transgenic approach several commercial tetraploid potato cultivars have been transformed with both *Rpi-blb1* and *Rpi-blb2* genes. The effectiveness of the transgenic material in controlling this devastating oomycete pathogen is currently under farm-scale evaluation.

(b) *Over-expression of pre-activated resistance gene sequences* – Most *R* genes exhibit exquisite recognition specificity. To overcome this deficit, new *R* genes have been created in the laboratory through single point mutations, which are autoactivating (*R** genes). For several NB-LRR, i.e. tomato I2, potato Rx and flax L6, specific amino acid changes of either the NB or ARC2 domain, results in autoactivation and this leads to *Avr* independent defence signalling. The disadvantage of *R** alleles is that their presence typically leads to a fitness penalty and hence expressions levels must be maintained at a low level.

(c) *Cloned R and Avr genes can be used in combination to promote acquired resistance* – The rapid activation of localized defence responses at the site of pathogen infection, often associated with a HR, is the most

prevalent and effective mechanism used by plants to minimize pathogen attack. By combining *R* and *Avr* gene expression in a single plant genotype, it is possible to engineer a ‘trigger’ for HR. However, if both are expressed constitutively in the same plant, a devastating whole plant induced HR results in plant death. Therefore, the expression of this two-component system must be tightly regulated. The desired phenotype can be obtained by using synthetic promoters designed only to be pathogen inducible (Rushton *et al.*, 2002). This ensures defence activation is precisely localized. This engineering strategy will probably work best against biotrophs because localized plant cell death would lead to pathogen starvation. This approach has been well tested using the *R-Avr* gene combination of tomato *Cf-9* and *C. fulvum Avr9* and shows considerable promise in controlling multiple pathogens.

(d) *Artificial evolution of R gene sequences to broaden the spectrum of pathogens recognized* – An alternative approach to method b (described above) is to undertake a random *in vitro* mutagenesis of the domains in the *R* gene sequence known to contribute to pathogen recognition and to select gene variants, which confer novel recognition specificities. The LRR domain of the potato *Rx* gene has recently been exposed to error-prone recombinatorial PCR. A subset of the mutant *Rx* sequences, which do not affect the conserved nonpolar residues in the repeat, were shown to confer resistance against the original viral strain as well as additional potato virus X strains and a second distantly related virus species. This short-term evolution of the LRR domain, although currently done in the laboratory in a random manner, holds promise for enhancing resistance against multiple strains of the same pathogen. It may even be possible to retrieve useful resistance from *R* genes, which have already been overcome in the field.

Concluding Remarks

In the 15 years since the isolation and confirmation of the first *R* gene sequence, our understanding of how *R* proteins function and how *R* loci have evolved to recognize nonself and coordinately activate plant defence responses has dramatically improved. However, many questions remain stubbornly unanswered. For example, what is the molecular basis of quantitative trait locus (QTL) mediated resistance? What features are possessed by effector proteins from obligate biotrophic fungi? How and where in the plant cell do the effectors from biotrophs interact with host targets and for what purpose? What is the 3 dimensional crystal structure of the extracellular and intracellular LRR domains present in many classes of *R* proteins? How do plants perceive and execute resistance mechanisms towards necrotrophic pathogens, which feed on dead tissue, because these interactions rarely conform to the

gene-for-gene hypothesis? Which signals control the inter-relationships between plant development, hormone signaling and the activation of basal and R protein mediated defence responses? And in polyploid plant species, how does a locus residing in only one of the genomes evolve to confer resistance?

In the foreseeable future, the sequencing of new plant and pathogen genomes as well as additional isolates, strains and races of already sequenced species will accelerate greatly our abilities to predict and monitor the dynamics of host–pathogen interactions through comparative bioinformatics analyses and diagnostics. By using high throughput techniques to assign gene functions such as virus induced gene silencing (VIGS) for plant sequences, targeted gene disruption for pathogens and RNA interference for both, our mechanistic understanding of host–pathogen compatibility and incompatibility events will steadily increase. This new knowledge and new techniques should be particularly invaluable to the study of plant resistance to nonbiotrophic pathogens. Although of high agricultural importance, the mechanisms underlying resistance to necrotrophs are not well understood.

The elimination of all diseases from agricultural crops is unlikely. Most pathogen populations continue to evolve in the face of new constraints imposed by changing agricultural practices, the application of pesticides and the deployment of resistant germplasm. A new unknown is the effect of climate change. Modelling of host–pathogen interactions from single molecule interactions, to the events occurring within different cellular compartments, between cells, within a whole plant, between plants in a crop and within the wider landscape will reveal where our knowledge is most sparse. This approach should also reveal the potential points of pathogen weakness as well as the strengths in host resistance, which can be exploited to provide new options for sustainable disease control.

Additional Figures

To accompany the text, figures are available online at: <http://www.rothamsted.bbsrc.ac.uk/ppi/staff/khk.html>

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References

- Anzai H, Yoneyama K and Yamaguchi I (1989) Transgenic tobacco resistant to a bacterial disease by the detoxification of a pathogenic toxin. *Molecular General Genetics* **219**: 492–494.
- Brandwagt BF, Mesbah LA, Takken FLW *et al.* (2000) A longevity assurance gene homolog of tomato mediates resistance to *Alternaria alternata* f. sp. *lycopersici* toxins and fumonisin B1. *Proceedings of the National Academy of Sciences of the USA* **97**: 4961–4966.
- Chen X, Shang J, Chen D *et al.* (2006) A B-lectin receptor kinase gene conferring rice blast resistance. *Plant Journal* **46**: 794–804.
- Daub ME and Ehrenschaft M (2000) The photoactivated *Cercospora* toxin cercosporin: contributions to plant disease and fundamental biology. *Annual Review of Phytopathology* **38**: 461–490.
- Diener AC and Ausubel FM (2005) *RESISTANCE TO FUSARIUM OXYSPORUM 1*, a dominant *Arabidopsis* disease resistance gene, is not race specific. *Genetics* **171**: 305–321.
- EDEN Biosciences, http://www.edenbio.com/usa/technology/download/wp_8.pdf
- Gu K, Yang B, Tian D *et al.* (2005) *R* gene expression induced by a type-III effector triggers disease resistance in rice. *Nature* **435**: 1122–1125.
- Hammond-Kosack KE and Jones JDG (1997) Plant disease resistance genes. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 573–605.
- Hammond-Kosack KE and Jones JDG (2000) Responses to plant pathogens. In: Buchanan BB, Gruissem W and Jones RL (eds) *Biochemistry and Molecular Biology of Plants*, pp. 1102–1156. Rockville, MD: American Society of Plant Physiology.
- Hammond-Kosack KE and Parker JE (2003) Deciphering plant–pathogen communication: fresh perspectives for molecular resistance breeding. *Current Opinions in Biotechnology* **14**: 177–193.
- Johal GS and Briggs SP (1992) Reductase activity encoded by the *Hm1* disease resistance gene in maize. *Science* **258**: 985–987.
- Jones JDG and Dangl JL (2006) The plant immune system. *Nature* **444**: 323–329.
- Kaku H, Nishizawa Y, Ishii-Minami N *et al.* (2006) Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proceedings of the National Academy of Sciences of the USA* **103**: 11086–11091.
- Lemmens M, Scholz U, Berthiller F *et al.* (2005) The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for fusarium head blight resistance in wheat. *Molecular Plant – Microbe Interaction* **18**: 1318–1324.
- Manning VA, Hardison LK, Ciuffetti LM (2007) Ptr ToxA interacts with a chloroplast-localized protein. *Molecular Plant – Microbe Interaction* **20**: 168–177.
- Martin GB, Bogdanove AJ and Sessa G (2003) Understanding the functions of plant disease resistance proteins. *Annual Review of Plant Biology* **54**: 23–61.
- McDowell JM and Simon SA (2006) Recent insights into *R* gene evolution. *Molecular Plant Pathology* **7**: 437–448.
- McHale L, Tan X, Koehl P and Michelmore RW (2006) Plant NBS-LRR proteins: adaptable guards. *Genome Biology* **7**: 212 (doi: 10.1186/gb-2006-7-4-212).
- Mestre P and Baulcombe D (2006) Elicitor-mediated oligomerization of the tobacco N disease resistance protein. *Plant Cell* **18**: 491–501.

- Meyers BC, Kaushik S and Nandety RS (2005) Evolving disease resistance genes. *Current Opinion in Plant Biology* **8**: 129–134.
- Mucyn TS, Clemente A, Andriotis VME *et al.* (2006) The tomato NBARC-LRR protein Prf interacts with Pto kinase *in vivo* to regulate specific plant immunity. *The Plant Cell* **18**: 2792–2806.
- Nürnberg T, Brunner F, Kemmerling B and Piater L (2004) Innate immunity in plants and animals: striking similarities and obvious differences. *Immunological Reviews* **198**: 249–266.
- Nürnberg T and Kemmerling B (2006) Receptor protein kinases-pattern recognition receptors in plant immunity. *Trends in Plant Sciences* **11**: 519–522.
- Rushton PJ, Reinstädler A, Lipka V, Lippok B and Somssich IE (2002) Synthetic plant promoters containing defined regulatory elements provide novel insights into pathogen- and wound-induced signalling. *The Plant Cell* **14**: 479–762.
- Shen QH, Saijo Y, Mauch S *et al.* (2007) Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. *Science* **315**: 1098–1103.
- Shen Q-H, Zhou F, Bieri S *et al.* (2003) Recognition specificity and RAR1/SGT1 dependence in barley *Mla* disease resistance genes to the powdery mildew fungus. *Plant Cell* **15**: 732–744.
- Takken FLW, Albrecht M and Tameling WIL (2006) Resistance proteins: molecular switches of plant defence. *Current Opinion in Plant Biology* **9**: 383–390.
- Wolpert TJ, Dunkle LD and Ciuffetti LM (2002) Host-selective toxins and avirulence determinants: what's in a name? *Annual Review of Phytopathology* **40**: 251–285.
- Zipfel C, Kunze G, Chinchilla D, *et al.* (2006) Perception of the bacterial PAMP EF-Tu by the receptor *EFR* restricts *Agrobacterium*-mediated transformation. *Cell* **125**: 749–760.

Further Reading

- Agrios GN (1997) *Plant Pathology*, 4th edn. San Diego, CA: Academic Press.
- Consonni C, Humphry ME, Hartmann HA *et al.* (2006) Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nature Genetics* **38**: 716–720.
- Day B, Dahlbeck D and Staskawicz BJ (2006) NDR1 interaction with RIN4 mediates the differential activation of multiple disease resistance pathways in *Arabidopsis*. *The Plant Cell* **18**: 2782–2791.
- Farnham G and Baulcombe DC (2006) Artificial evolution extends the spectrum of viruses that are targeted by a disease-resistance gene from potato. *Proceedings of the National Academy of Sciences of the USA* **103**: 18828–18833.
- Fritz-Laylin LK, Krishnamurthy N, Tör M *et al.* (2005) Phylogenomic analysis of the receptor-like proteins of rice and *Arabidopsis*. *Plant Physiology* **138**: 611–623.
- Kanyuka K, Ward E and Adams MJ (2003) *Polymyxa graminis* and the cereal viruses it transmits: a research challenge. *Molecular Plant Pathology* **4**: 393–406.
- Tian D, Traw MB, Chen JQ, Kreltman M and Bergelson J (2003) Fitness costs of *R*-gene-mediated resistance in *Arabidopsis thaliana*. *Nature* **423**: 74–77.