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REVIEW

Defended to the Nines: 25 Years of Resistance Gene Cloning Identifies Nine Mechanisms for R Protein Function OPEN

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Plants have many, highly variable resistance (*R*) gene loci, which provide resistance to a variety of pathogens. The first *R* gene to be cloned, maize (*Zea mays*) *Hm1*, was published over 25 years ago, and since then, many different *R* genes have been identified and isolated. The encoded proteins have provided clues to the diverse molecular mechanisms underlying immunity. Here, we present a meta-analysis of 314 cloned *R* genes. The majority of *R* genes encode cell surface or intracellular receptors, and we distinguish nine molecular mechanisms by which R proteins can elevate or trigger disease resistance: direct (1) or indirect (2) perception of pathogen-derived molecules on the cell surface by receptor-like proteins and receptor-like kinases; direct (3) or indirect (4) intracellular detection of pathogen-derived molecules by nucleotide binding, leucine-rich repeat receptors, or detection through integrated domains (5); perception of transcription activator-like effectors through activation of executor genes (6); and active (7), passive (8), or host reprogramming-mediated (9) loss of susceptibility. Although the molecular mechanisms underlying the functions of *R* genes are only understood for a small proportion of known *R* genes, a clearer understanding of mechanisms is emerging and will be crucial for rational engineering and deployment of novel *R* genes.

INTRODUCTION

Plants can trigger an effective immune response to a wide variety of fungal, bacterial, and viral pathogens. The robustness of the plant immune system is remarkable, given that plants are sessile and unremittingly exposed to potential pathogens while their immunity does not rely on mobile immune cells and adaptive somatic variation, both of which are so effective in vertebrates.

In plants, resistance (*R*) genes play a key role in their remarkable immune responses. *R* genes are usually dominant (but sometimes recessive) genes that provide full or partial resistance to one or more pathogens. We include receptors of pathogen-associated molecular patterns (PAMPs) as *R* genes because they provide partial and sometimes even full resistance (Lacombe et al., 2010). *R* genes exist in natural plant populations and have been used by humankind since early crop domestication. Selection during domestication favored dominant *R* genes providing full resistance, but recessive *R* genes and *R* genes that provide partial resistance may provide more durable resistance. Most identified *R* genes are polymorphic in plant populations, which led to their initial characterization and use in plant breeding programs. However, individual plants have up to a few hundred *R* gene analogs that make no identified contribution to resistance. Many of these *R* gene analogs are also fixed in plant species and are thought to contribute to non-host resistance (Schulze-Lefert and Panstruga, 2011).

The cloning of the first *R* gene was published in 1992 and the number of cloned *R* genes has steadily increased over the subsequent 25 years (Figure 1A). The first cloned *R* gene, *Hm1* from maize (*Zea mays*), encodes an enzyme that detoxifies *Helminthosporium carbonum* (HC) toxin from the fungal pathogen *Cochliobolus carbonum* (Johal and Briggs, 1992). The cloning of *Hm1* was followed by the cloning of *Pto* from tomato (*Solanum lycopersicum*) in 1993 (Martin et al., 1993), and then, in 1994, *Cf-9* from tomato (Jones et al., 1994), *N* from tobacco (*Nicotiana tabacum*; Whitham et al., 1994), and *RPS2* from *Arabidopsis thaliana* (Bent et al., 1994; Mindrinos et al., 1994). Since then, hundreds of examples of cloned *R* genes have been published.

Now, after 25 years of *R* gene cloning, we look back and determine mechanistic trends in the function of *R* gene products. Through a comprehensive review, we identified 314 cloned functional *R* genes (Supplemental Table 1). A mechanism has been suggested for 128 of the 314 identified *R* gene products (41%; Table 1); among these, we distinguish nine distinct mechanisms (Table 1, Figure 1A), explained further in this review, and supported by examples.

Many *R* genes confer recognition of pathogen-derived effectors and initiate effector-triggered immunity (ETI), which often involves the hypersensitive response (HR), a type of programmed cell death (Jones and Dangl, 2006). ETI is considered distinct from the other layer of induced immunity, PAMP-triggered immunity (PTI) (Jones and Dangl, 2006). In contrast to ETI, PTI involves recognition of conserved PAMPs and does not involve HR. In this review, we do not distinguish between PTI and ETI, as the PTI and ETI responses are very similar and the division has become hazy with the description of conserved effectors and polymorphic PAMPs (Thomma et al., 2011). We do, however, distinguish between extracellular and intracellular pathogen recognition mechanisms.

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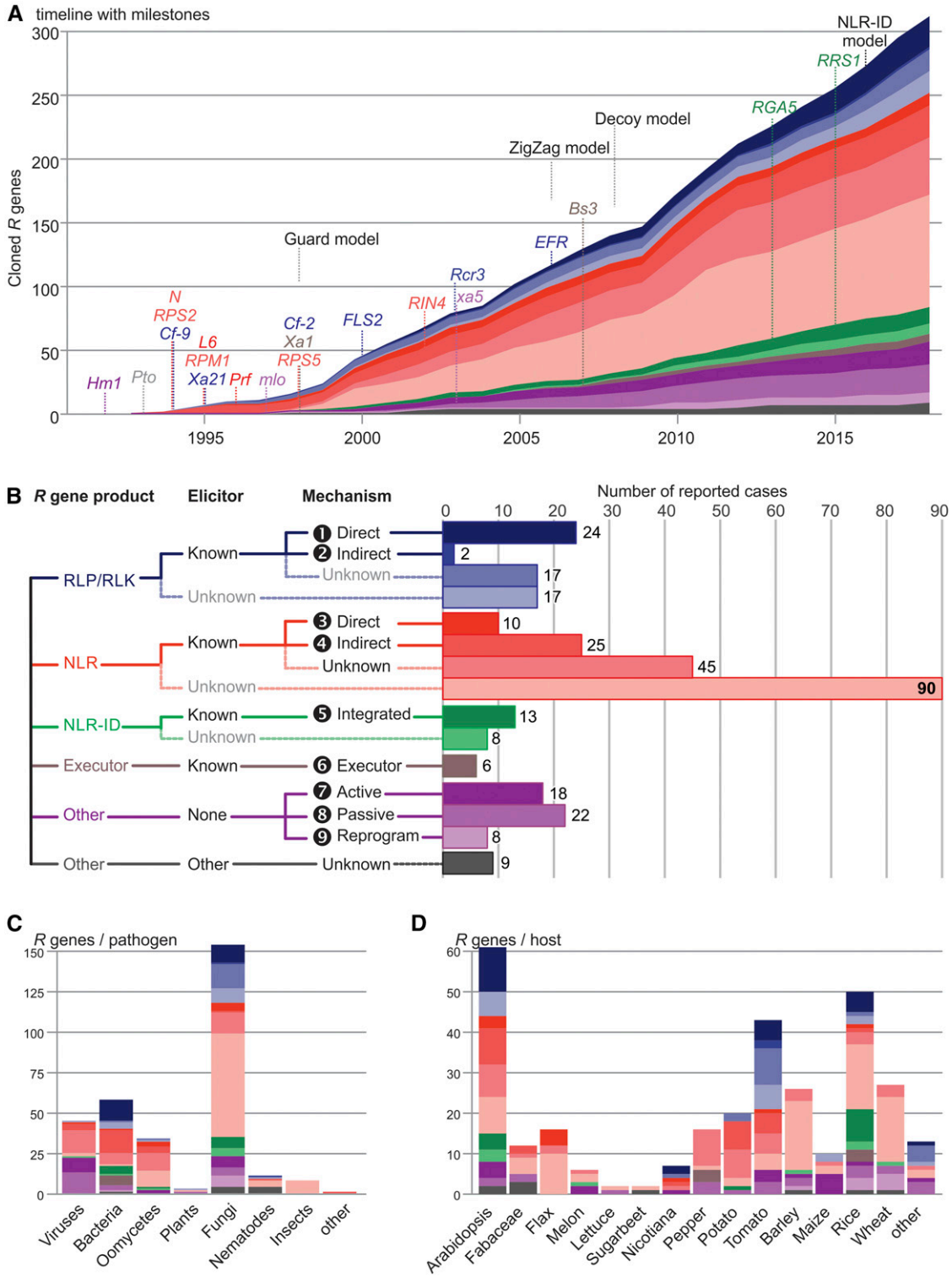

Figure 1. Analysis of Molecular Mechanisms Underpinning *R* Genes.

A near-comprehensive literature search resulted in a database of *R* genes conferring immunity to a wide range of plant pathogens (Supplemental Data Set 1). This list was filtered to remove duplicates: *R* genes that code for receptors that perceive pathogen components were counted only once if this component is conserved between pathogens. By contrast, if an *R* gene codes for a receptor that perceives structurally or sequence unrelated pathogen-derived components, these were counted multiple times. Furthermore, alleles of *R* genes with slightly different recognition spectra were counted as multiple *R*

OVERVIEW AND OCCURRENCE OF THE NINE MECHANISMS

Although many cloned *R* genes have no proposed mechanism in the literature, they most likely fit within these nine mechanisms (Figure 1B). These mechanisms can broadly be divided into two clades: perception (I) and loss of susceptibility (II). Perception-based mechanisms can be further subdivided into three subclades: perception involving receptor-like proteins/kinases (RLPs/RLKs) (Ia); Nod-like receptors (NLRs) (Ib); and Executor genes (Ic). RLPs/RLKs in the apoplast can perceive PAMPs and effectors directly (subclade Ia, mechanism 1) or indirectly (subclade Ia, mechanism 2). Likewise, NLRs can perceive effectors directly (subclade Ib, mechanism 3) or indirectly (subclade Ib, mechanism 4), or through integrated domains (IDs) (subclade Ib, mechanism 5). Perception of transcription activator-like (TAL) effectors occurs by triggering expression of executor genes (subclade Ic, mechanism 6).

We identify three loss-of-susceptibility mechanisms (clade II): active loss of susceptibility by the expression of immune-related components or specific enzymes involved in detoxification/degradation of pathogen and pathogen-associated components (clade II, mechanism 7); passive loss of susceptibility through loss of interaction with key pathogen targets (clade II, mechanism 8); and loss of susceptibility by host-reprogramming leading to reduced pathogen growth (clade II, mechanism 9).

Of the cloned *R* genes, 61% encode NLRs (191/313), but for most NLRs the perception mechanism is either unclear (45/191) or the perceived component is unknown (98/191) (Figure 1B). The other main class of cloned *R* genes (19%, 60/314), encode RLPs/RLKs, which can recognize pathogen-derived components directly (24/60) or indirectly (2/60). Finally, several cloned *R* genes are executor genes (6/314) or lead to loss of susceptibility (48/314) (Figure 1B). Interestingly, with the exception of mechanism 6 (executor genes), the mechanisms are not specific for immunity against certain types of pathogens (Figure 1C) or only used by particular plant species or taxonomic groups (Figure 1D). Instead, these mechanisms are deployed against different kinds of pathogens and appear to be universally used in the plant kingdom. However, the distribution of these mechanisms may not reflect the natural frequencies of mechanisms because of a strong research bias toward model systems.

Subclade Ia: Extracellular Perception

Plants perceive various PAMPs and effectors by *R* genes encoding for cell surface-localized RLPs or RLKs (Figure 2). This perception can be direct (mechanism 1) or indirect (mechanism 2).

Mechanism 1: Direct Perception at the Cell Surface

Many PAMPs are perceived directly at the cell surface by RLPs and RLKs. The best studied PAMP in plants is bacterial flagellin (Felix et al., 1999). Flagellin fragment flg22 is perceived by the RLK FLAGELLIN-SENSITIVE2 (FLS2) in Arabidopsis (Gómez-Gómez and Boller, 2000). The flg22 peptide binds directly to FLS2 triggering the recruitment of BRI1-ASSOCIATED RECEPTOR KINASE (BAK1), a RLK (Chinchilla et al., 2007). The crystal structure of FLS2 in complex with BAK1 and flg22 has been elucidated, revealing that flg22 acts as a "molecular glue" inducing complex formation of FLS2 with BAK1 (Sun et al., 2013). FLS2 orthologs from different plant species perceive different parts of bacterial flagellin. For instance, tomato SlFLS2 recognizes a 15-amino acid epitope that overlaps with flg22 (Robatzek et al., 2007), whereas different flagellin epitopes (flgII-28 and CD2-1) are recognized by the RLK FLS3 in tomato and an uncharacterized receptor in rice (*Oryza sativa*), respectively (Hind et al., 2016; Katsuragi et al., 2015). Furthermore, FLS2 receptors from different plant species display different affinities for the conserved part of flagellin from different pathogens, possibly reflecting coevolution with specific bacterial pathogens (Trdá et al., 2014).

Many other microbial PAMPs are recognized directly similar to flagellin (reviewed in Zipfel, 2014). These include EF-Tu, chitin, lipopolysaccharide, peptidoglycan, and other components of the bacterial cell wall (Zipfel, 2014), as well as bacterial RNA (Lee et al., 2016), possibly by direct interaction with RLP/RLKs. Although often not considered as being *R* genes, PAMP-recognizing RLKs provide partial resistance and can even confer full resistance when transferred to other plant species (Lacombe et al., 2010).

Perception of extracellular effector proteins may also occur via direct interactions with RLPs or RLKs. For example, nlp20, a fragment of NECROSIS AND ETHYLENE-INDUCING PEPTIDE1 (NEP1), has been coimmunoprecipitated with RLP23 (Albert et al., 2015). NEP-LIKE PROTEINs (NLPs) are secreted by bacterial, oomycete, and fungal plant pathogens (Böhm et al., 2014; Oome et al., 2014). Many NLPs function in the necrotrophic phase of these pathogens and have a cytotoxic role. Noncytotoxic NLPs, on the other hand, are expressed early during infection and serve an unknown role during the initial stages of host colonization (Oome et al., 2014). Both cytotoxic (Böhm et al., 2014) and noncytotoxic NLPs (Oome et al., 2014) trigger immune responses via RLP23 through the conserved nlp20 fragment (Albert et al., 2015). RLP23 requires the RLKs SUPPRESSOR OF BIR1 1 and BAK1 for signaling, triggering a weak immune response (Albert et al., 2015).

Figure 1. (continued).

genes, as were paralogs within species and orthologs in different species which are shown to be involved in immunity. Loss-of-susceptibility *R* genes that can act against several pathogens through an identical mechanism were counted only once. In addition, *R* genes that were shown to induce enhanced disease resistance when taken outside of their native context were removed, as were engineered *R* genes. Finally, some genes that are involved in autoimmunity resemble *R* genes, but these were not included unless these were proven to be involved in immunity against pathogens.

(A) A timeline summarizing the increased knowledge regarding *R* genes. The identified mechanisms were plotted cumulatively over time, with some of the milestones added. The date of the first cloned *R* gene was taken in case multiple host components are involved or the underlying molecular mechanism was elucidated later. These *R* genes were grouped according to the proposed mechanism of function as we understand it today.

(B) All identified *R* genes were grouped according to their proposed mechanism.

(C) The identified *R* genes were grouped by pathogen which they act against and colored by the molecular mechanism by which they function.

(D) The identified *R* genes were grouped by host species carrying them and colored by the molecular mechanism by which they function. Colors are explained in **(B)**.

Table 1. *R* Genes Classified by Nine Mechanisms

Mechanism	Description	*R* Genes (Plant Species)
1: RLP/RLK, direct	Recognition triggered by direct interaction of a pathogen-derived effector and a cell surface RLK/RLP receptor.	*EFR*, ***FLS2***, *LORE*, *LYK3*, *LYK4*, *LYK5*, *LYM1*/*LYM3*, *LYM2*, *RBGP1*, *RLP23* (Arabidopsis), *VvFLS2* (grapevine), *NbCORE*, *NbFLS2* (*N. benthamiana*), *CEBiP*, *LYP4*/*LYP6*, *OsFLS2* (rice), *CORE*, *FLS3*, *LeEIX2*, *SlFLS2* (tomato)
2: RLP/RLK, indirect	Recognition triggered either by effector binding to a host component or by effector-mediated modification of a host component, perceived by a cell surface RLK/RLP receptor.	***Cf-2*** (tomato) Guardee/decoy: *Rcr3*
RLP/RLK, unknown mechanism		*Ve1* (tomato), *StoVe1* (eggplant), *HLVe1-2A* (hop), *NgVe1* (*Nicotiana glutinosa*), *LepR3, RLM2* (oilseed rape), *StuVe1*, *ELR* (potato), *XA21* (rice), *9DC1, 9DC2, 9DC3, Cf-4, Cf-5, Cf-9, Hcr9-4E*, *I*, *I-3* (tomato)
3: NLR, direct	Recognition triggered by direct interaction of a pathogen-derived component and an NLR.	***RPP1****-{EstA/Nda/ZdrA}* (Arabidopsis), ***L5/L6/L7***, *M* (flax), *Roq1* (*N. benthamiana*), *Pi-ta* (rice), *Sw-5b* (tomato)
4: NLR, indirect	Recognition triggered either by effector binding to a host component or by effector-mediated modification of a host component, perceived by an NLR.	*HRT1,* ***RPM1***, ***RPS2***, ***RPS5***, ***SUMM2***, ***ZAR1*** (Arabidopsis), *Gpa2*, *R2*, *R2-like*, *Rpi-abpt*, *Rpi-blb3*, *Rx1*, *Rx2* (potato), *Rpg1-b*, *Rpg1r* (soybean), ***N*** (tobacco), ***Prf*** (tomato) Guardees/decoys: *TIP*, *RIN4*, *PBS1*, *CRCK3*, *ZED1*, *ZRK3*, *RKS1*, *PBL2* (Arabidopsis), *RanGAP2*, *BSL1* (soybean), *GmRIN4* (tobacco), *LescPth5*, *Fen*, *Pto* (tomato)
NLR, unknown mechanism		*RBA1*, *RCY1*, *RPP13-Nd-1*, *RPP13-UKID37*, *RPP13-UKID5*, *RPP5*, *RPS6*, *TAO1* (Arabidopsis), *Mla1*, *Mla10*, *Mla13* (barley), *Bs2* (black pepper), *P*, *P2* (flax), *Rxo1* (maize), *Fom-2* (melon), L^{1}, L^{1a}, L^{1c}, L^{2}, L^{2b}, L^{3}, L^{4}, *Pvr4*, *Tsw* (pepper), *R3a*, *R3b*, *R8*, *Rpi-blb2*, *Rpi-vnt1.1*, *Rpi-vnt1.2*, *Rpi-vnt1.3* (potato), *Pi9*, *Pib*, *Piz-t* (rice), *3gG2* (soybean), *N'* (tobacco), *Bs4*, *I2*, *Tm-2*, $Tm2^{2}$ (tomato), *Pm2*, *Pm3a*, *Pm3f* (wheat)
5: NLR-ID	Recognition is triggered either by effector binding to a domain or by effector-mediated modification of a domain that is integrated in a host NLR.	*RRS1B*, ***RRS1-R***, *RRS1-S* (Arabidopsis), *R1* (potato), *Pii-2*, ***Pik****-{1/h/p1/s}*, ***RGA5-A***, *Xa1* (rice)
6: Executor	Recognition triggered by transcriptional activation of the executor gene by a pathogen TAL effector.	***Bs3***, *Bs3-E*, *Bs4C-R* (pepper), *Xa10*, *Xa23*, *Xa7* (rice)
7: Other, active	Loss of susceptibility by directly disarming the pathogen by actively interrupting a key pathogenicity process.	*JAX1*, *RTM1*, *RTM2*, *RTM3* (Arabidopsis), *HvHm1* (barley), ***Hm1***, *Hm2*, *qMdr9.02*, *qRfg1*, *ZmTrxh* (maize), *At1*, *At2* (melon), *IVR* (*N. benthamiana*), *PaLAR3* (Norway spruce), *STV11-R* (rice), *Tm-1*, *Ty-1*, *Ty-3* (tomato)
8: Other, passive	Loss of susceptibility by mutation in a host component, leading to the inability to manipulate the host.	*rwm1*, *lov1* (Arabidopsis), *Rym-4*, *Rym-5* (barley), *retr01* (cabbage), *bc-3* (French bean), *Mo-1* (lettuce), *sbm1* (pea), $Pvr2^{1}$, $Pvr2^{2}$, *pvr6* (pepper), *Eva1* (potato), *xa13*, *xa25,* ***xa5*** (rice), *LGS1*, *Pc* (sorghum), *Asc-1*, *Ty-5*, *Pot-1* (tomato), *Tsn1*, *Snn1* (wheat)
9: Reprogramming	Loss of susceptibility by a deregulated host.	***mlo*** (barley), *GH3-2*, *GH3-8*, *pi21* (rice), *Lr34*, *Lr67*, *YrL693*, *Yr36* (wheat)

Several *R* genes have multiple synonyms. This table summarizes only one name per cloned *R* gene. Only the name of the sensor NLR is mentioned in case of NLR-IDs. *R* genes that can perceive multiple sequence-unrelated effectors have been counted multiple times. Bold indicates key examples discussed in this work.

A direct interaction has been demonstrated for the perception of the fungal ethylene-inducing xylanase (EIX) effector by the tomato RLP LeEIX2 (Ron and Avni, 2004; Rotblat et al., 2002). Expression of LeEIX2 in mammalian cells enables binding of EIX (Ron and Avni, 2004). Furthermore, several fungal endopolygalacturonases are perceived by the Arabidopsis RLP RBPG1 (Zhang et al., 2014), and these enzymes can be coimmunoprecipitated with RBPG1 (Zhang et al., 2014). In addition, perception of EIX and fungal endopolygalacturonases by LeEIX2 and RBPG1, respectively, is independent of their enzymatic functions (Rotblat et al., 2002; Zhang et al., 2014). A direct interaction is thought to also occur for the RaxX effector from *Xanthomonas oryzae*, which is perceived by the RLK encoded by the rice *R* gene *Xa21* (Pruitt et al., 2015). Likewise, the *Verticillium*-derived effector AVE1 is perceived by

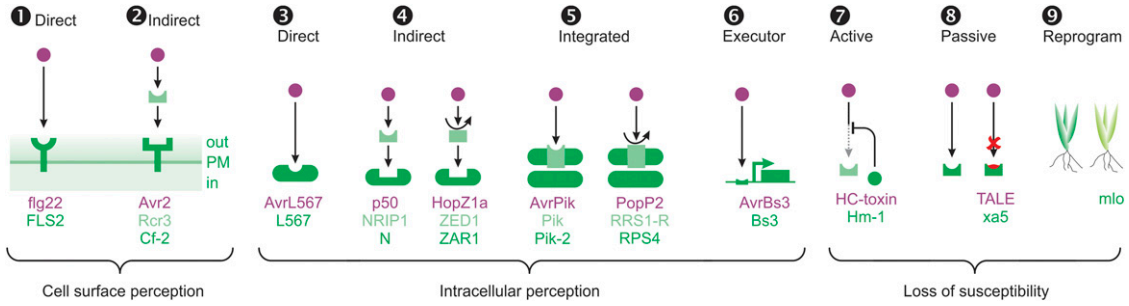

Figure 2. Nine Molecular Mechanisms Underpinning *R* Gene Functions.

Illustration of direct (1) and indirect (2) recognition at the cell surface; four different intracellular perception mechanisms (3–6); and three loss-of-susceptibility mechanisms (7–9). PAMPs and effectors are colored in purple, indirect receptors in light green, and direct receptors in dark green.

the tomato *R* gene *Ve1*, which encodes an RLP (de Jonge et al., 2012). However, no direct interaction has been shown for these effectors and their cognate Xa21 and Ve1 receptors.

Taken together, these examples indicate that direct perception of PAMPs and effectors at the cell surface is a common strategy underlying the partial and full disease resistance provided by *R* genes.

Mechanism 2: Indirect Extracellular Effector Perception

Pathogen perception at the cell surface can also occur indirectly, by recognition of modified host factors. The key example of this mechanism is the recognition of the fungal pathogen *Cladosporium fulvum* (syn. *Passalora fulva*) by the tomato *R* gene product Cf-2, a RLP which has been introgressed into cultivated tomato from *Solanum pimpinellifolium* (Dixon et al., 1996). *Cf-2* confers recognition of the fungal effector Avr2 from *C. fulvum* (Luderer et al., 2002), as well as the unrelated nematode effector GrVap1 from *Globodera rostochiensis* (Lozano-Torres et al., 2012). Avr2/GrVap1 perception requires *Rcr3*, which encodes a secreted papain-like Cys protease (Dixon et al., 2000). Both Avr2 and GrVAP1 function as protease inhibitors and directly interact with Rcr3 (Lozano-Torres et al., 2012; Rooney et al., 2005). Avr2 and GrVap1 act as virulence factors (van Esse et al., 2008; Lozano-Torres et al., 2014), likely by inhibition of various host proteases (Kaschani et al., 2010; Lozano-Torres et al., 2012, 2014; Shabab et al., 2008), including the Rcr3 paralog Pip1 (Shabab et al., 2008). These data suggest that Rcr3 might act as a decoy for the perception of Avr2 and GrVap1 (van der Hoorn and Kamoun, 2008). However, tomato lines lacking a functional *Rcr3* allele are more susceptible to *Phytophthora infestans*, but do not display an altered susceptibility toward *C. fulvum* or *Pseudomonas syringae* (Ilyas et al., 2015). This indicates a role for Rcr3 beyond effector recognition. A second argument against Rcr3 being a decoy is that close *Rcr3* orthologs are present in pepper (*Capsicum annuum*), potato (*Solanum tuberosum*), and tomato (Ilyas et al., 2015), whereas *Cf-2* is probably an evolutionarily much younger gene present only in tomato (Dixon et al., 1996; Seear and Dixon, 2003).

So far, Avr2/GrVap1 perception by Cf-2 and Rcr3 is the only example of indirect perception of effectors in the apoplast. However, perception of other effectors in the apoplast might also be indirect. For example, a direct interaction of tomato RLP Cf-9 with the *C. fulvum* effector Avr9 could not be detected (Luderer et al., 2001), and Avr9 perception seems to involve an additional Solanaceae-specific factor known as the high-affinity binding site for Avr9 (Kooman-Gersmann et al., 1996, 1998). Likewise, perception of the *Fusarium oxysporum* Avr3 effector by the tomato RLK I-3 may require an additional host component present in tomato, but absent in tobacco and *Nicotiana benthamiana* (Catanzariti et al., 2015).

Subclade Ib: Intracellular Perception

In addition to extracellular perception of pathogens, plants are able to recognize effectors inside the cell (Figure 2). Most cloned *R* genes encode NLRs, which can perceive effectors either directly (mechanism 3) or indirectly (mechanism 4). However, some *R* genes encode NLRs containing additional IDs (NLR-IDs) required for effector recognition (mechanism 5) (Kroj et al., 2016; Sarris et al., 2016). Finally, some effectors activate executor genes (mechanism 6).

Mechanism 3: Direct Intracellular Effector Recognition—Trigger-Happy NLRs

Direct perception of effectors is not restricted to the cell surface, as several effectors have been convincingly demonstrated to directly interact with NLRs to trigger immune responses. ARABIDOPSIS THALIANA RECOGNIZED1 (ATR1), for example, is an effector from the oomycete pathogen *Hyaloperonospora arabidopsidis*, and ATR1 directly interacts with the Arabidopsis NLR RECOGNITION OF PERONOSPORA PARASITICA1 (RPP1), leading to its recognition (Krasileva et al., 2010). Various *RPP1* alleles have distinct recognition specificities for different *ATR1* alleles, and this specificity is mediated by the LRRs of RPP1 (Steinbrenner et al., 2015). The AvrL567 effector from the fungal pathogen *Melampsora lini* is another effector that directly interacts with its cognate receptor, the flax L5, L6, and L7 NLRs, which are encoded by allelic genes (Dodds et al., 2004, 2006). Different variants of AvrL567 are perceived differentially by these receptors (Dodds et al., 2006; Wang et al., 2007), and the specificity for these AvrL567 variants resides in the LRR domain of the NLR (Ravensdale et al., 2012; Wang et al., 2007).

Interestingly, effector perception is also affected by cooperative polymorphisms in other domains of the NLR besides the LRR

(Ravensdale et al., 2012). This suggests a mechanism of perception where effector binding competes with intramolecular interactions (Ravensdale et al., 2012). The L5, L6, and L7 receptors exist in an equilibrium between "on" and "off" states, and effector binding stabilizes the "on" form, thereby initiating immune signaling (Bernoux et al., 2016). This equilibrium model may be more widely applicable to NLR signaling. Indeed, the NLR Sw-5b directly recognizes a small 21-amino acid PAMP-like peptide region from the viral movement protein NSm (NSm^{21}), and it has been argued that binding of NSm^{21} destabilizes the interaction between the LRR and NB-ARC domain triggering recognition (Zhu et al., 2017).

In the case of RPP1 and L5/L6/L7, allelic NLR-encoding genes perceive allelic effector variants. However, it is not always the case that homologous and/or allelic NLRs perceive homologous effector proteins. For example, rice and barley (*Hordeum vulgare*) have homologous NLRs residing in the same *R* gene cluster; these NLRs perceive sequence-unrelated effectors from *Magnaporthe oryzae* and *Blumeria graminis*, respectively (Lu et al., 2016; Wu et al., 2015). In addition, potato orthologs of two different tomato NLR-encoding genes, tomato *Sw-5b*, which perceives a viral effector, or tomato *I2*, which perceives a fungal effector, can perceive oomycete effectors (Giannakopoulou et al., 2015; Vossen et al., 2016). The exact molecular mechanism by which sequence-unrelated effectors from different pathogens can be perceived directly by highly similar NLRs is unknown. In many cases, the specificity-determining region in the NLR is in the C-terminal LRR region (Shen et al., 2003), while in other cases mutations in regions other than the NLR are sufficient to alter specificity (Giannakopoulou et al., 2015). Therefore, it seems that small changes in several parts of the NLR can sensitize the NLR to destabilization by sequence-unrelated effectors, triggering recognition. In addition, evolving a different specificity to sequence-unrelated effectors may be facilitated by the fact that these effectors might adopt a similar fold. Indeed, several sequence-unrelated effectors from *M. oryzae* have been shown to adopt a similar fold (de Guillen et al., 2015; Maqbool et al., 2015). A structural understanding of effector binding by these NLRs would provide clues to the exact molecular mechanism of direct perception, but these experiments are notoriously challenging.

Mechanism 4: Indirect Intracellular Recognition—Decoys and Guardees

Many effectors are perceived indirectly by NLRs, through their interaction with—or enzymatic modification of—additional host components. These additional host proteins have also been called guardees or decoys to suggest that they are effector targets or mimics of effector targets, respectively.

An example of indirect interaction is the recognition of the 50-kD helicase (p50) domain of *Tobacco mosaic virus* by the tobacco NLR N, which requires NRIP1, a chloroplast-localized rhodanese sulfurtransferase (Caplan et al., 2008). NRIP1 is recruited to the cytoplasm by the p50 effector, and NRIP1 only interacts with N in the presence of the p50 effector. It is thought that N activation requires a prerecognition complex of NRIP1 and the p50 effector (Caplan et al., 2008).

An example of indirect detection via enzymatic host modification is the perception of the *P. syringae* Type-III effectors HopZ1a (Lewis et al., 2010) and HopF2a (Seto et al., 2017), and the *Xanthomonas campestris* Type-III effector AvrAC (syn. XopAc) (Wang et al., 2015b; Xu et al., 2008) by the functionally conserved NLR ZAR1 (Baudin et al., 2017; Lewis et al., 2010; Peele et al., 2014). HopZ1a is perceived through its acetyltransferase activity on the PM-localized pseudokinase HOPZ-ETI-DEFICIENT1 (ZED1), a receptor-like cytoplasmic kinase (RLCK) XII family member (Lewis et al., 2013). AvrAC is perceived through its activity on the RLCK VII family member PBL2 (Wang et al., 2015b). When PBL2 is uridylylated by AvrAC, it interacts with the ZED1-like RKS1, which in turn forms a stable complex with HOPZ-ACTIVATED RESISTANCE1 (ZAR1), leading to a weak HR (Wang et al., 2015b). HopF2a is an ADP-ribosyltransferase and its recognition requires ZAR1 and the ZED1-like ZRK3, which may act as an adaptor for effector recognition similar to RKS1 (Seto et al., 2017). ZED1 and PBL2 are thought to act as decoys for the perception of HopZ1a or AvrAC, respectively. Indeed, *ZED1* does not contribute to immunity or susceptibility to *P. syringae* in the absence of ZAR1, indicating that ZED1 acts as a decoy (Lewis et al., 2013). By contrast, PBL2 is not a strict decoy, as it appears to be required for perception of certain PAMPs, including flg22 and elf18 (Zhang et al., 2010). It is yet undetermined whether *PBL2* contributes to resistance or susceptibility to *X. campestris* in the absence of *ZAR1*. The exact molecular mechanism by which ZAR1 perceives modulation of various RLCKs is unknown. ZAR1 is a classic example of how one *R* gene product can perceive the action of multiple effectors from multiple pathogens through interactions with several host components, all of which may be able to recruit additional components.

Another functionally conserved NLR that indirectly perceives multiple effectors is tomato Prf (Bombarely et al., 2012; Salmeron et al., 1996; reviewed in Ntoukakis et al., 2014). Prf interacts with several RLCK VII family members, including Pto and Fen (Salmeron et al., 1996). Interaction with Pto leads to perception of the *P. syringae* Type-III effectors AvrPto and AvrPtoB, which bind Pto (Kim et al., 2002). AvrPto acts as a kinase inhibitor (Xing et al., 2007), while AvrPtoB acts as a weak kinase inhibitor and has an additional E3 ligase domain to target its interactors for destruction by the host proteasome (Cheng et al., 2011; Rosebrock et al., 2007). Prf also interacts with the Pto-homolog Fen, leading to immune signaling in response to the insecticide Fenthion (Martin et al., 1994). It is thought that Prf oligomerizes with several molecules of Pto and/or Pto homologs and that effector binding to one of these kinases in this complex would induce a series of trans-phosphorylation events resulting in an immune response (Ntoukakis et al., 2014). The oligomerization with different Pto homologs within one receptor complex could expand the recognition spectrum of Prf (Ntoukakis et al., 2014).

The NLRs ZAR1 and Prf each interact with multiple host proteins; by contrast, RPM1 INTERACTING PROTEIN4 (RIN4) is a host protein that interacts with multiple NLRs. RIN4 forms complexes with the NLRs RPS2 and RPM1 (Axtell and Staskawicz, 2003; Mackey et al., 2002). The *P. syringae* Type-III effectors AvrB and AvrRpm1 induce phosphorylation of RIN4 by recruiting the Arabidopsis RIPK protein kinase (Liu et al., 2011a; Mackey et al., 2002). The phosphorylation of RIN4 by RIPK at a conserved threonine residue (T166) reduces RIN4 interaction with the prolyl-peptidyl isomerase ROTAMASE CYP1 (ROC1) leading to an

altered conformation of RIN4, which triggers RPM1-mediated immune signaling (Li et al., 2014). In the absence of RPM1, the phosphorylation of RIN4 enhances its role as a negative regulator of immunity (Lee et al., 2015; Liu et al., 2009; Wilton et al., 2010), indicating RIN4 is not a decoy. Cleavage of RIN4 by the *P. syringae* Type-III effector AvrRpt2 inhibits RPM1-mediated immune signaling (Kim et al., 2005). This cleavage requires AvrRpt2 maturation by eukaryotic prolyl-peptidyl isomerases such as ROC1 (Coaker et al., 2005), which is required for RPM1-mediated immune signaling. In response, the NLR RPS2 perceives RIN4 cleavage, triggering immune signaling (Axtell and Staskawicz, 2003). RIN4 binding to RPS2 keeps RPS2 in an "off" state, and cleavage releases the inhibition of RPS2 triggering immune signaling (Day et al., 2005). In addition, RPS2 weakly perceives AvrRpm1 (Kim et al., 2009). In the absence of *RPM1*, AvrRpt2-dependent cleavage of RIN4 does not contribute to virulence (Lim and Kunkel, 2004), indicating that in the absence of AvrB or AvrRpm1, RIN4 acts as a decoy for AvrRpt2. Soybean (*Glycine max*) has independently converged on a similar perception mechanism, where the soybean NLRs RPG1-b and RPG1r perceive the manipulation of the soybean RIN4 ortholog by AvrB and AvrRpm1, respectively (Ashfield et al., 2004; Kessens et al., 2014; Selote and Kachroo, 2010).

Other NLRs also perceive effectors indirectly using additional host proteins. The NLR RPS5, for example, perceives the *P. syringae* Type-III effector AvrPphB (Warren et al., 1998), a family C58 cysteine protease (Zhu et al., 2004). AvrPphB contributes to virulence by cleaving several RLCKs (Zhang et al., 2010), including RIPK, thereby inhibiting RPM1-mediated recognition of AvrB (Russell et al., 2015). The perception of AvrPphB by RPS5 requires *PBS1*, which encodes a RLCK VII family member (Ade et al., 2007; Shao et al., 2003; Swiderski and Innes, 2001). PBS1 binds to RPS5 to form a prerecognition complex (DeYoung et al., 2012), and subsequent cleavage of PBS1 by AvrPphB triggers a subtle conformational change that allows an exposed loop in PBS1 to trigger activation of RPS5 (DeYoung et al., 2012; Qi et al., 2014). It remains to be demonstrated whether PBS1 cleavage by AvrPphB contributes to the virulence of *P. syringae* in the absence of *RPS5* and therefore whether PBS1 is a decoy. However, PBS1 may not be a decoy, as *PBS1* contributes to PAMP-triggered immune responses (Zhang et al., 2010) and is under strong purifying selection, even though not all Arabidopsis accessions carry *RPS5* (Caldwell and Michelmore, 2009), indicating a physiological function for PBS1 in the absence of *RPS5*.

Finally, sometimes NLRs monitor cellular homeostasis. The Arabidopsis NLR SUMM2, for example, perceives the phosphorylation status of the calmodulin binding RLCK CRCK3 (Zhang et al., 2017). CRCK3 is phosphorylated by a MAP kinase signaling cascade involving the MAP kinases MEKK1, MKK1, and MKK2, as well as MPK4 (Zhang et al., 2017). The CRCK3 phosphorylation status in the absence of MPK4, MKK1/MKK2, or MEKK1 kinase activity triggers an immune response that is dependent on SUMM2 (Zhang et al., 2012). The *P. syringae* Type-III effector HopAI1 inactivates MPK4, thereby triggering an immune response that is dependent on SUPPRESSOR OF MKK1 MKK2 2 (SUMM2; Zhang et al., 2012). SUMM2 is therefore an example of an NLR monitoring cellular homeostasis. Perception of an alteration of homeostasis, rather than the actual effector target itself, may allow for the perception of many effectors whose effects ultimately converge on the same host components. CRCK3 has no known function beyond its signaling role through SUMM2, suggesting it might act as a decoy (Zhang et al., 2012).

In conclusion, NLRs often trigger immune signaling by monitoring either (1) the interaction of effectors with host proteins, (2) the enzymatic modification of host proteins, or (3) cellular homeostasis. More bacterial effectors have been shown to be perceived indirectly by NLRs than effectors of other classes of pathogens (Figure 1C). This likely reflects a better understanding of bacterial effectors as well as the fact that bacteria are easier to manipulate, rather than a biological difference in how these different types of pathogens are perceived by plants. Although there are several clear candidates acting as guardees (RIN4 and PBL2) or decoys (ZED1 and Pto), it is unclear in other cases (PBS1 and CRCK3). Furthermore, several of these proposed decoys seem more conserved than the corresponding NLR.

Mechanism 5: NLR-IDs—Sensors Embedded within Receptors

Some NLRs carry integrated domains (NLR-IDs) (Kroj et al., 2016; Sarris et al., 2016) thought to be required for effector recognition. As these additional domains seem to have integrated repeatedly and recently, it is not unlikely they still possess their original activity in the absence of the recognized effector, unlike a decoy (Sarris et al., 2016). Furthermore, it remains to be demonstrated on a case-by-case basis whether the ID is required for effector perception. Three NLR-IDs have been studied in detail: Arabidopsis RRS1 and rice RGA5 and Pik. Remarkably, these NLR-IDs are coexpressed from the same promoter in opposing orientation with a NLR lacking the ID, and heterodimerization of these paired, coexpressed NLR proteins is required for signal transduction.

Arabidopsis RRS1 is a NLR containing an integrated C-terminal WRKY transcription factor domain and cooperates with the NLR RPS4 to trigger effector recognition. The WRKY domain of RRS1 interacts with the *P. syringae* Type-III effector AvrRps4, triggering RPS4-dependent immune responses (Sarris et al., 2015). PopP2 is a *Ralstonia solanacearum* Type-III effector of the YopJ family that acts as an acetyltransferase, acetylating key lysines in the RRS1 WRKY domain. In the Col-0 allele of *RRS1* (*RRS1-S*), this acetylation blocks AvrRps4 recognition (Sarris et al., 2015), while the Nd-1 and Ws-2 alleles of *RRS1* (*RRS1-R*) signal in response to this acetylation, in addition to recognizing AvrRps4 (Le Roux et al., 2015; Sarris et al., 2015). Furthermore, the *RRS1-R* allele recognizes a yet unidentified effector from the fungus *Colletotrichum higginsianum* (Sarris et al., 2015), and the *RPS4-RRS1* locus also underlies a quantitative trait locus for resistance against *X. campestris* (Debieu et al., 2016). Therefore, *RRS1* can recognize effectors from various bacterial and fungal pathogens. Furthermore, *RRS1-R* can perceive the direct interaction of AvrRps4 with its integrated WRKY domain, as well as perceive the enzymatic modification of this domain by PopP2. The biological significance of the RRS1 WRKY domain binding to W-box DNA sequences (Sarris et al., 2015) outside of the context of perceiving pathogen effectors is not known. Remarkably, AvrRps4 is also perceived in a similar way in Arabidopsis by a homologous pair of NLRs, designated RRS1B (containing a WRKY ID), and RPS4B (Saucet et al., 2015).

The rice RGA5 and Pik NLRs both contain an integrated RATX1 or HMA domain, which is related to the *Saccharomyces cerevisiae* copper binding protein ATX1 (Cesari et al., 2013). The HMA domain is located at the C terminus of RGA5 and between the CC and NB-ARC domain in Pik, representing independent integration events. RGA5 interacts with the *M. oryzae* effectors Avr-Pia and Avr-CO39 using its HMA-domain, and this triggers an RGA4-dependent immune response (Cesari et al., 2013). Alleles of the *M. oryzae* effector Avr-Pik bind to the HMA domain of Pik alleles (Maqbool et al., 2015), which together with Pik-2 triggers immune signaling (Ashikawa et al., 2008). Substitutions in the HMA-domain of different *Pik* alleles revealed a correlation between the recognition specificity of these different Pik alleles and the different Avr-Pik variants (Maqbool et al., 2015).

In conclusion, perception by NLR-IDs can be the result of direct binding of the effector or due to enzymatic activity of the effector on the integrated domain. In addition, studying NLR-IDs can provide valuable insight into the molecular targets of effectors, as these integrated domains likely represent domains targeted by these effectors (Kroj et al., 2016). All studied NLR-IDs require a genetically linked NLR to trigger signaling. However, genetically unpaired NLRs can also cooperate. In Arabidopsis, tobacco, and tomato, unlinked "helper" NLRs have been shown to act downstream of several canonical "sensor" NLRs (Bonardi et al., 2011; Peart et al., 2005; Wu et al., 2017). In addition, helper-NLRs are required for effector perception by RLPs (Fradin et al., 2009; Gabriëls et al., 2007), thereby linking effector perception in the apoplast to intracellular signaling by NLRs.

Mechanism 6: Executor Genes—Promoter Traps for TAL Effectors

Executor genes are *R* genes that are transcriptionally activated by transcription activator-like effectors (TALEs) produced by *Xanthomonas* species and confer immunity to the *Xanthomonas* strains carrying these TALEs. TALEs function by binding to specific DNA sequences, altering host transcription of key susceptibility factors. Executor genes function as a promoter trap for these TALEs, forcing them to promote transcription of genes involved in immunity. Promoters of executor genes therefore act as decoys, mimicking the promoter regions of these susceptibility factors, leading to the initiation of immune responses (van der Hoorn and Kamoun, 2008). So far, six executor genes have been cloned: rice *Xa27* (Gu et al., 2005), *Xa10* (Tian et al., 2014), and *Xa23* (Wang et al., 2015a), and pepper *Bs3*/*Bs3-E* (Römer et al., 2007), and *Bs4C-R* (Strauss et al., 2012). Identified executor genes either encode for proteins with a catalytic function (*Bs3* and *Bs3-E* encode for a putative flavin monooxygenase; Römer et al., 2007) or for proteins with multiple putative transmembrane domains (*Xa27*, *Xa10*, *Xa23*, and *Bs4C-R*) (Zhang et al., 2015). Importantly, increased understanding of the specificity of DNA binding by TALEs has enabled the development of synthetic executor genes mediating immunity against multiple *Xanthomonas* strains (Hummel et al., 2012; Römer et al., 2009; Zeng et al., 2015). This strategy could be used to engineer resistance against the pathogen *R. solanacearum*, which also expresses TALEs, known as RipTALs (de Lange et al., 2013).

Clade II: Loss of Susceptibility

Not all *R* genes encode proteins involved in mechanisms relying on perception. Several *R* genes rely on loss of susceptibility, sometimes also called susceptibility (*S*) genes (van Schie and Takken, 2014). Loss of susceptibility can occur in three ways (Figure 2): active (mechanism **7**), passive (loss of interaction; mechanism 8), or by host reprogramming (by mutations in components of cellular pathways; mechanism 9). Many loss-of-susceptibility mechanisms of resistance are very durable, but can carry a cost for the host and therefore can lead to a yield penalty. When active and passive loss-of-susceptibility mechanisms become fixed in populations, they lead to Type-I non-host resistance, typically characterized by the absence of a strong immune response (Mysore and Ryu, 2004).

Mechanism 7: Active Loss of Susceptibility—Blocking Key Pathogen Strategies

R genes underlying active loss-of-susceptibility mechanisms encode host proteins that are directly involved in disarming the pathogen by actively interrupting a key pathogen process. Active loss-of-susceptibility mechanisms are diverse and can act broadly against many pathogens, or be specific to some. These active mechanisms are often constitutively expressed, but some can be upregulated upon immune signaling. In addition, active loss-of-susceptibility mechanism may also lead to the production of PAMPs and danger-associated molecular patterns (DAMPs), which are then perceived and amplify the original response. For example, *Hm1*, the first *R* gene cloned (Johal and Briggs, 1992), encodes a NADPH-dependent reductase that is specifically involved in detoxifying HC toxin (Johal and Briggs, 1992). HC toxin is produced by *C. carbonum* race 1 (CCR1), the cause of leaf blight and ear mold in maize. Orthologs of *Hm1* are present in the grass family, and in barley such orthologs contribute to non-host resistance to CCR1 (Sindhu et al., 2008). Many different active loss-of-susceptibility mechanisms of resistance are employed against viruses. For example, the tomato *Tm-1* gene product, which confers resistance to *Tomato mosaic virus* inhibits the replication of *Tomato mosaic virus* RNA by binding to replication proteins (Ishibashi et al., 2014). In addition, the allelic tomato *Ty-1*/*Ty-3* resistance genes encode for γ-clade RNA-directed RNA polymerases, which induce RNA-directed DNA methylation to provide immunity against single strand DNA geminiviruses (Butterbach et al., 2014; Verlaan et al., 2013).

Mechanism 8: Passive Loss of Susceptibility—Losing Interaction with Key Host Targets

Loss of interaction of a key host susceptibility factor with a pathogen effector is a common mechanism underpinning recessive *R* genes. Indeed, half of the known *R* genes against viruses involve such a loss of interaction (Truniger and Aranda, 2009). The majority of the so far identified recessive *R* genes act against potyviruses (Kang et al., 2005) and encode mutant translation initiation factors of the 4E or 4G family, which are unable to interact with the cap structure on viral transcripts, thereby conferring resistance to potyviruses (Truniger and Aranda, 2009). In addition,

one-third of the *R* genes against *X. oryzae* pv *oryzae*, the causal agent of bacterial blight, are inherited recessively (Liu et al., 2011b), and the underlying mechanisms seems often to involve loss of interaction with key host targets. A recessive mutation in the promoter of the rice *xa13* allele does no longer facilitate its manipulation by the TALE AvrXa13, leading to loss of susceptibility (Chu et al., 2006). Likewise, a single amino acid mutation in the rice gamma subunit of transcription factor IIa (OsTFIIAγ5), encoded by the recessive *xa5* resistance gene, confers resistance to various *X. oryzae* strains in adult plants (Iyer and McCouch, 2004) by restricting bacterial movement (Iyer-Pascuzzi et al., 2008). OsTFIIAγ5 directly interacts with TALEs and is required for their activity (Yuan et al., 2016). The *xa5* mutation likely affects interaction with TALEs to varying degrees, thereby conferring varying degrees of resistance to different *X. oryzae* pathovars (Gu et al., 2009; Huang et al., 2016; Yuan et al., 2016).

Mechanism 9: Passive Loss of Susceptibility by Host Reprogramming

Host reprogramming by mutations in components of cellular pathways is a common strategy leading to durable resistance against a broad range of pathogens. This form of loss of susceptibility is often a recessive trait, but may also involve dominant-negative alleles. The genes involved in this category are often called *Adult Plant Resistance* (*APR*) genes, as the resistance they confer is often only effective in later stages of plant life (Ellis et al., 2014). Furthermore, *APR* genes often confer partial resistance against a broad range of pathogens (Ellis et al., 2014). It is important to note that the mechanisms underlying this type of resistance do not necessarily act only in the adult plant; therefore, the term *APR* genes does not fully cover this category. Furthermore, perception-based mechanisms as well as loss-of-interaction mechanisms may only function in the adult plant.

The prime example of a senescence-associated loss-of-susceptibility mechanism is mediated by recessive loss-of-function *mlo* (*Mildew Locus O*) alleles in both monocots and dicots (Hückelhoven and Panstruga, 2011). *MLO* encodes a plant-specific integral membrane protein with an unknown molecular function that acts as a negative regulator of cell death in response to both biotic and abiotic stress (Piffanelli et al., 2002). *MLO* loss-of-function alleles are associated with spontaneous cell death (Büschges et al., 1997). In both barley and Arabidopsis, *MLO* is coexpressed with *PEN1*, *PEN2*, and *PEN3* (or its barley orthologs), which are required for an active response against powdery mildews (Humphry et al., 2010). *MLO* acts as a negative regulator of the *PEN1*/*PEN2*/*PEN3* pathways (Humphry et al., 2010), and in both barley and Arabidopsis, these genes are required for *mlo*-dependent immunity (Humphry et al., 2006). Therefore, loss of a general suppressor of cell death can confer specific resistance to powdery mildews in both monocots and dicots by deregulation of the PEN1 and PEN2/PEN3 pathways. This principle has been used to engineer a transgene-free tomato *mlo* mutant resistant to powdery mildews (Nekrasov et al., 2017).

Analogous to the *mlo* mechanism, the recessive resistance toward rice blast is conferred by a loss-of-function mutation in *Pi21*, a gene encoding a HMA domain protein that acts to suppress the plant defense response (Fukuoka et al., 2009). It is interesting to note that HMA domain proteins are crucial virulence targets for different pathogenic fungi and that several sequence-unrelated effectors adopt a similar fold that converge on HMA domain proteins (de Guillen et al., 2015; Maqbool et al., 2015). Therefore, engineering loss-of-function alleles of HMA domain-encoding genes analogous to *pi21* may confer broad resistance to various pathogenic fungi in unrelated plant species. Other genes conferring disease resistance through senescence-associated mechanisms include wheat (*Triticum aestivum*) *Lr34*, which encodes an ABC transporter and confers dominant adult resistance to leaf rust, stripe rust, and powdery mildew (Krattinger et al., 2009). In addition, wheat *WKS1* (syn. *Yr36*) encodes a kinase and START domain-containing protein that confers dominant partial resistance to a variety of stripe rusts (Fu et al., 2009). WKS1 functions by reducing the ability of chloroplast thylakoid ascorbate peroxidases (tAPXs) to detoxify peroxides, which contributes to cell death (Gou et al., 2015). Finally, wheat *Lr67* is a dominant *R* gene that confers partial adult resistance against all races of leaf rust, stripe rust, stem rust, and powdery mildew and is caused by a mutation in a predicted hexose transporter that differs by two amino acids from the susceptible allele (Moore et al., 2015). Lr67 displays a dominant-negative effect through heterodimerization with the susceptible allele product, reducing glucose uptake (Moore et al., 2015), ultimately leading to leaf tip necrosis (Herrera-Foessel et al., 2014). The resistance these genes confer is seemingly dependent on a deregulated initial immune response, resulting in a quicker and stronger immune response that is able to partially suppress the pathogen. However, it is possible that some of these genes in fact act through a different molecular mechanism.

DISCUSSION

Since the cloning and molecular characterization of the first *R* genes in the early 1990s, a steady amount of *R* genes and interactors has been identified (Figure 1A). Even still, while Phytozome lists ~14 000 NLR-encoding genes, only 191 have been shown to act as *R* genes (Goodstein et al., 2012). With the advance of novel techniques such as SMRT RenSeq and other next-generation sequencing-based techniques (Steuernagel et al., 2016; Witek et al., 2016), the number of annotated *R* genes is expected to increase at a faster pace than before. Understanding the molecular mechanisms underlying these *R* genes, however, will still require careful case-by-case examination. Indeed, of the examined 314 *R* genes, only 128 have a proposed mechanism, based on an even lower number of detailed studies into molecular mechanisms. The underlying mechanisms of the remaining 186 *R* genes is yet unknown. Understanding these molecular mechanisms in addition to discovering more *R* genes is vital, as it allows us to transfer these traits to other species as well as rationally engineer disease resistance, thereby extending the recognition spectra of *R* genes to outside of what is found in nature. For instance, engineering of the AvrPphB cleavage site in PBS1 to that of other pathogen-derived proteases leads to their recognition in an RPS5-dependent manner (Kim et al., 2016). Likewise, Executor *R* genes have been engineered to increase their recognition spectra (Hummel et al., 2012; Römer et al., 2009; Zeng et al., 2015).

In addition to engineering novel *R* genes, high-throughput screens with effectors on non-host plants can identify *R* genes conferring non-host resistance, which may be very durable when deployed in susceptible hosts.

Critically, the distinction between direct and indirect interactions between the pathogen-derived elicitor and the *R* gene product is not as easily defined as it seems, both experimentally and conceptually. Direct interactions are not necessarily proven by bimolecular fluorescence complementation, fluorescence resonance energy transfer, or coimmunoprecipitations as the detected complex may contain additional host components required for the interaction. Likewise, even interactions in yeast two-hybrid assays do not exclude conserved eukaryotic factors facilitating the interaction. Direct interactions are best proven with purified proteins and by including noninteracting mutant proteins as controls. A direct interaction is confirmed in vivo when these mutations also disrupt the interaction in vivo. These assays are challenging and have often not been performed. Second, the requirement of an additional host protein for the interaction does not exclude that there is a physical interaction between the *R* gene product and the pathogen-derived effector, for instance, when a host protein acts as a molecular glue to enhance the direct interaction. Therefore, caution must be taken in the interpretation of results.

We have frequently discussed the compliance with guard and decoy models in mechanisms 3, 5, and 6. The molecular mechanistic interpretation of the difference between the guard and decoy model boils down to the question of whether a putative decoy has a function in the absence of the *R* gene. However, even when a guard/decoy is shown to have a role in the absence of the *R* gene, this may result from a decoy function, e.g., when several *R* gene products converge on guarding the same decoy. Similarly, a decoy may still have a function in a pathogen interaction in the absence of a perceived effector if multiple independent effectors from that pathogen converge on the same decoy triggering recognition. Therefore, mechanistically it may be better to refer to a guarded protein acting as a decoy when there is no clear virulence role in targeting the guard/decoy in the absence of an *R* gene in a given pathogen interaction. In other words, decoys only function in specific interactions where they act as coreceptors by mimicking the elicitor target.

It is also interesting to point out genes that have not been found among cloned *R* genes. First, signaling components of defense pathways, such as EDS1, NPR1, etc., are not among cloned *R* genes. It might be that these genes are not polymorphic in nature as they are central hubs in interconnected signaling networks, and alterations in these signaling components could disrupt multiple pathways. *R* genes therefore seem to be at the edges of signaling pathways. Second, no pathogenesis-related (*PR*) genes have been found to underlie *R* genes. This could be because most *PR* genes more likely function in immunity in an additive and quantitative manner. Finally, no *R* gene encoding for DAMPs or their receptors have been identified. DAMPs are endogenous "danger" signals that are produced or released upon cellular stress, cellular damage, or nonphysiological cell death and can be involved in either triggering or reinforcing immune signaling (Yamada et al., 2016). Because pathogens can cause DAMP release, DAMPs can theoretically facilitate indirect perception of pathogens. It is possible that transfer of DAMPs and their recognition machinery to other species may confer enhanced resistance to specific pathogens, although this remains to be demonstrated.

In conclusion, the past 25 years has revealed the molecular identity of many *R* genes and uncovered the underlying molecular mechanisms governing their functioning. However, much remains to be discovered. Understanding the molecular and structural mechanisms involved in *R* gene mediated disease resistance will allow us to engineer resistance for the crops of the future.

Supplemental Data

Supplemental Data Set 1. List of *R* genes.

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AUTHOR CONTRIBUTIONS

Both authors contributed to writing the article.

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