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CURRENT REVIEW

RxLR Effectors: Master Modulators, Modifiers and Manipulators

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Cytoplasmic effectors with an Arg-any amino acid-Arg-Leu (RxLR) motif are encoded by hundreds of genes within the genomes of oomycete *Phytophthora* spp. and downy mildew pathogens. There has been a dramatic increase in our understanding of the evolution, function, and recognition of these effectors. Host proteins with a wide range of subcellular localizations and functions are targeted by RxLR effectors. Many processes are manipulated, including transcription, post-translational modifications, such as phosphorylation and ubiquitination, secretion, and intracellular trafficking. This involves an array of RxLR effector modes-of-action, including stabilization or destabilization of protein targets, altering or disrupting protein complexes, inhibition or utility of target enzyme activities, and changing the location of protein targets. Interestingly, approximately 50% of identified host proteins targeted by RxLR effectors are negative regulators of immunity. Avirulence RxLR effectors may be directly or indirectly detected by nucleotide-binding leucine-rich repeat resistance (NLR) proteins. Direct recognition by a single NLR of RxLR effector orthologues conserved across multiple *Phytophthora* pathogens may provide wide protection of diverse crops. Failure of RxLR effectors to interact with or appropriately manipulate target proteins in nonhost plants has been shown to restrict host range. This knowledge can potentially be exploited to alter host targets to prevent effector interaction, providing a barrier to host infection. Finally, recent evidence suggests that RxLR effectors, like cytoplasmic effectors from fungal pathogen *Magnaporthe oryzae*, may enter host cells via clathrin-mediated endocytosis.

Keywords: effector targets, effector-triggered susceptibility, oomycete, RxLR and EER motifs, susceptibility factor

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Among the most exciting questions in plant research is how do pathogens manipulate, modify or modulate processes in plant cells to create a susceptible environment? In turn, how do plants detect these perturbations and defend themselves against diseases? Among biotrophic and hemibiotrophic oomycete pathogens, cytoplasmic effectors with an Arg-any amino acid-Arg-Leu (RxLR) motif downstream of a secretion signal peptide are encoded by hundreds of genes within the genomes of *Phytophthora* spp. and downy mildew pathogens (Kronmiller et al. 2023; Mandal et al. 2022; Tör et al. 2023). RxLR effectors are delivered into living plant cells from biotrophic structures called haustoria to suppress immunity (Boevink et al. 2020; Bozkurt and Kamoun 2020; Wang et al. 2017, 2018).

An explosion of information about the evolution, function, and recognition of RxLR effectors is entering the literature, with a search for the term 'RxLR effector' at the National Center for Biotechnology Information returning 106 papers since the turn of 2020, at the time of writing (April 2023). This is a fast-moving field of research. It is not possible to cover all the literature in a short, topical review, for which we apologize, but we will cover some of the exciting advances in three topics: i) How are RxLR effectors evolving in oomycete genomes? ii) What do RxLR effectors target and what are their modes of action (MOA)? iii) How do RxLR effectors contribute to host resistance and host range?

Genomics and Evolution of RxLR Effectors

The accessibility and reduced costs of DNA sequencing have stimulated a dramatic increase in oomycete genome sequences. Technologies such as PacBio have led to improved, chromosome-level assemblies of genomes, such as the tree pathogen *Phytophthora agathidicida* (Cox et al. 2022), the downy mildew *Peronospora effusa* (Fletcher et al. 2022), and the obligate biotrophic white rust pathogen *Albugo candida* (Furzer et al. 2022). The genomes of biotrophic downy mildews (Tör et al. 2023) and hemibiotrophic *Phytophthora* (Kronmiller et al. 2023; Mandal et al. 2022) plant pathogens contain potentially hundreds of RxLR-encoding genes, based on standard predictions involving string searches and hidden Markov models (HMMs) (Chepsergon et al. 2021; Whisson et al. 2007; Wood et al. 2020). The exception, within the oomycete plant pathogens that have a clear biotrophic association with hosts, is *Albugo*, which apparently lacks RxLR effectors but, instead, has predicted cytoplasmic effectors containing the motif CHxC, since

renamed CCG effectors based on motif analyses (Furzer et al. 2022).

The increase in *Phytophthora* genomes that are available has deepened the capabilities for genome comparison. A study of 128 genomes passing a quality threshold, representing 33 *Phytophthora* species, confirmed that RxLR effector genes are among the most diverse and rapidly evolving sets of genes in the genus. No core RxLR effector (CRE) grouping, representing effectors that are conserved at the sequence level across all *Phytophthora* species, could be identified (Mandal et al. 2022). Analyses of the flanking intergenic regions provided widespread support for the two-speed genome hypothesis proposed from studies of the *Phytophthora infestans* genome (Haas et al. 2009), suggesting that rapidly evolving genes, such as those encoding RxLR effectors, reside in gene-sparse, repeat-rich genomic regions, whereas housekeeping functions are sequestered in repeat-poor regions (Mandal et al. 2022). An independent study of the genomes of 31 *Phytophthora* species confirmed the preponderance of repeat-rich regions containing RxLR-encoding genes, in line with the two-speed genome (Kronmiller et al. 2023). They indicated that the numbers of apoplastic and cytoplasmic effectors increased from narrow to broad host range, but the latter levelled off in species with 'enormous' host ranges (Kronmiller et al. 2023). RxLR effectors were evident in the genomes of *Phytophthora* species colonizing all plant tissues, from leaves to roots, and infecting a wide variety of plants, including trees, shrubs, berries, vegetables, and perennials (Mandal et al. 2022). The inference is that RxLR effectors can evolve to manipulate diverse regulatory networks controlling immunity in different plant types and tissues.

The immune-targeting activities of many functionally validated RxLR effectors are consistent among oomycete phytopathogens with diverse hosts and lifestyles, but little is known about the roles of RxLRs in tree-pathogenic *Phytophthora* species. Two studies analyzed the genomes of tree pathogens to identify highly in planta–induced RxLR genes and exploited the power of transient expression in the model system *Nicotiana benthamiana* to confirm functions consistent with effector activities. *Phytophthora kernoviae* was transformed to express green fluorescent protein, which revealed that haustoria were observed during the early stages of infection of *N. benthamiana* and the tree hosts *Rhododendron ponticum* and European beech. Transient expression of *P. kernoviae* RxLR effectors in *N. benthamiana* enhanced colonization by the pathogen (S. Wang et al. 2021). Infection-induced RxLRs from *P. agathidicida*, which infects the iconic and ancient kauri trees of New Zealand, were again expressed transiently in *N. benthamiana* and were shown to either activate or suppress immunity (Guo et al. 2020). These studies infer the importance of both an intimate biotrophic association with the host and the involvement of RxLR effectors in promoting susceptibility.

Given that RxLR gene prediction models based on HMM or string searches are biased towards the genomes from which they are developed and may thus not be attuned to evolutionary adaptations in other genomes, Wood et al. (2020) took a different approach to identify candidate cytoplasmic effectors in the *Bremia lactucae* genome. They screened the genome for genes encoding proteins with the conserved WY structural fold, present in approximately 50% of predicted *Phytophthora* RxLRs. Their findings were instructive; 55 WY-containing effector candidates with signal peptides and infection-associated transcriptional accumulation were identified, but few of these had canonical RxLR motifs. In contrast, 33 had the EER motif. Of 11 WY-containing effectors that elicited hypersensitive responses (HRs) on wild or domesticated lettuce plants known to contain resistance genes, only two had RxLR motifs. A broader study of oomycete genomes revealed that 9 to 21% of WY effector candidates lacked the RxLR motif, most of which had the EER (Wood et al. 2020).

As many as 71 CRE were identified as conserved across 11 *Phytophthora parasitica* genomes. Intrinsically disordered regions were predicted in many of these. Potential short linear motifs (SLiMs) were identified in the C-terminal regions of 21 CREs that may represent sites of interaction with host target proteins (Chepsergon et al. 2021). As an example, a predicted SLiM in PpRxLR1 is a ubiquitin-associated motif (Chepsergon et al. 2021). Future work is needed to functionally validate such SLiMs, but this could be a powerful approach to predict interacting host proteins.

What Do RxLR Effectors Target and What Are Their MOA?

Despite their small size and general lack of enzymatic activity, RxLR effectors can modulate, modify, and manipulate many different processes in plant cells. Acting as small interfering proteins, they can display many different MOA (Fig. 1), including i) stabilization or ii) destabilization of protein targets, iii) inhibition or iv) use of host protein enzyme activity, v) disruption of host protein complexes, and vi) changing the localization of targets. It must be noted that some effectors have more than one host target and, accordingly, may display more than one MOA (He et al. 2020). Here, we summarize RxLR effector-target research over the last few years and add to the previous body of knowledge (summarized in He et al. [2020] and Fabro [2022]) of how these proteins use these MOA to manipulate immunity.

Transcriptional modulation

As many plant defense responses are controlled through regulation of transcription, transcription factors (TFs) and other transcriptional regulators are common RxLR targets. The effector HaRxLL470 from *Hyloperonospora arabidopsidis* interacts with the transcriptional activator Hy5, which regulates both light and immunity signaling and acts by preventing its binding to DNA, thus inhibiting the transcription of defense genes (S. Chen et al. 2021). Inhibition of positive transcriptional regulators of defense is a common strategy but is accomplished in various ways; for example, PsAvh113 from *Phytophthora sojae* promotes 26S proteasome–mediated degradation of its target TF, GmDPB (Zhu et al. 2023). By contrast, PsAvh110 binds to the heterochromatin-associated transcriptional activator GmLHP1-2 and competes with co-activator GmPHD6, disrupting the formation of a transcriptional complex and lowering the expression of defense genes (Qiu et al. 2023). Endoplasmic reticulum (ER) tail-anchored *N*-acetyl-L-cysteine (NAC) TFs have been demonstrated to be common targets of tail-anchored RxLRs from different pathogens and the effector MOA is to inhibit the cleavage of the tail anchor, which prevents the re-localization of NAC TFs to the nucleus (Breeze et al. 2023; McLellan et al. 2013; Meisrimler et al. 2019) (Fig. 1). *Plasmopora viticola* PvRxLR111 effector targets a negative regulator, VvWRKY40, but here, the effector stabilizes the TF, presumably to promote its defense-suppression activity (Ma et al. 2021). Similarly, Pi22798 from *P. infestans* uses the activity of a negative regulator of immunity by promoting the homodimerization of TF StKNOX3, rather than its heterodimerization with StKNOX7, to enhance pathogenicity (Zhou et al. 2022) (Fig. 1).

In addition to modulation of transcription, RxLRs manipulate transcript processing. For example, PsPSR1 can disrupt alternative splicing and small RNA (sRNA) production by inhibiting the RNA binding and helicase activities of splicing factor PINP1

(Gui et al. 2022). In another example, Pi04089 interacts with the RNA binding protein StKRBP1, which it stabilizes (Wang et al. 2015), the consequence of which is to alter defense gene expression and enhance susceptibility to *P. infestans* (Luo et al. 2021). Moreover, Pi23226 was found to bind 25S ribosomal RNA, preventing ribosome assembly leading to inhibition of translation-triggering cell death during the switch to necrotrophy in *P. infestans* infection (Lee et al. 2023). This demonstrates that RxLRs are also able to target non-protein components of the host, such as RNA, to interfere with defense and promote susceptibility.

Modifying phosphorylation

Kinase signaling is an important component of immune signaling, particularly following pathogen-associated molecular pattern (PAMP) perception, and many RxLRs target it. Recently, Liang et al. (2021) showed that receptor-like cytoplasmic kinase (RLCK) subfamily VII members, such as BIK1, that relay signaling from pattern recognition receptors (PRRs) on perception of *Phytophthora* PAMPs, are interactors of *Phytophthora capsici* RxLR25. In this case, protein stability and complex formation were unaffected, but RxLR25 prevented the PAMP-induced phosphorylation of RLCKs, ensuring they remain in an inactive

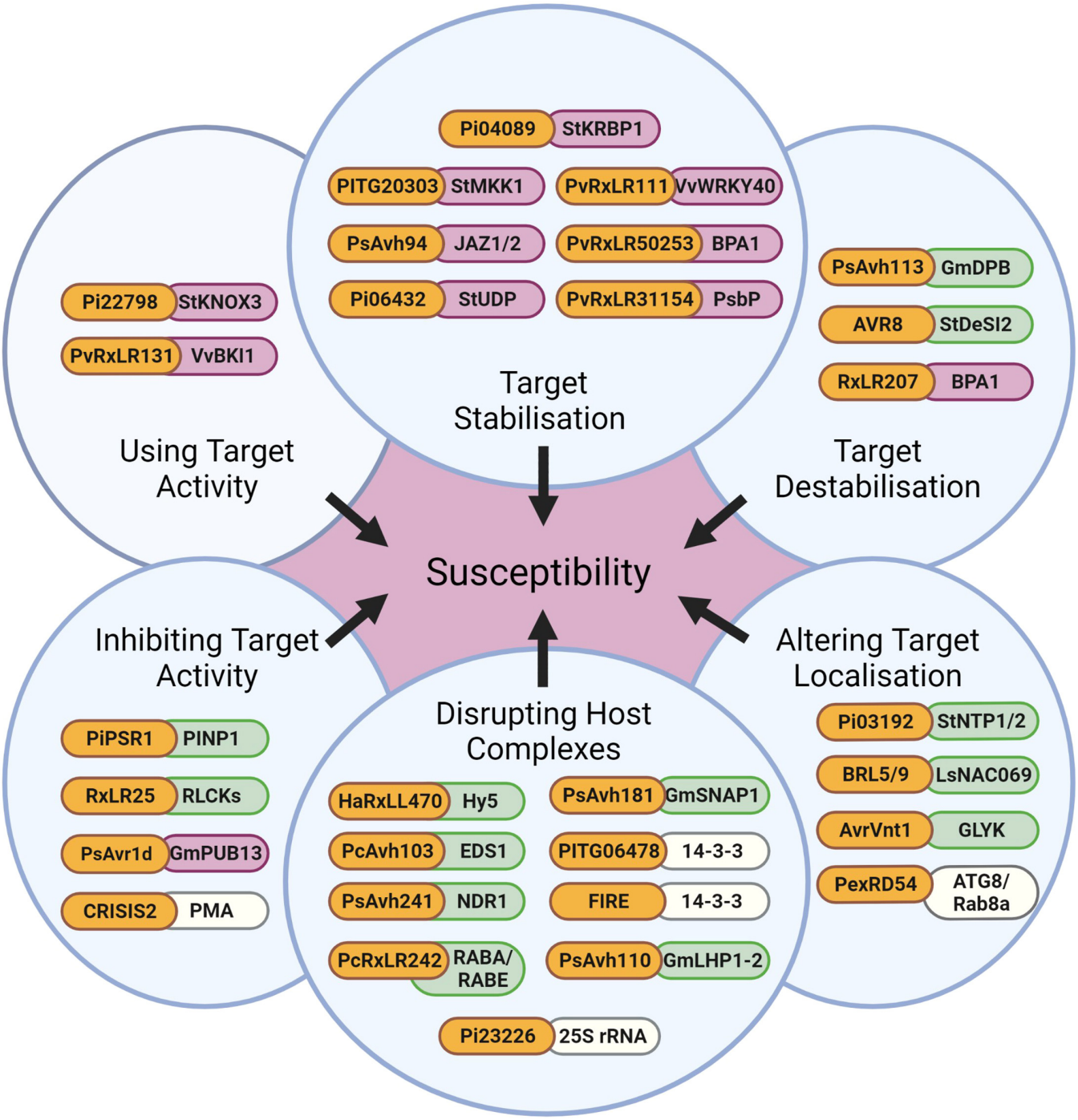

Fig. 1. Modes of action of RxLR effectors. The RxLR effectors (orange ovals) mentioned in this review are grouped according to their published modes of action (large circles) on the targets shown, which all lead to susceptibility in the host. The effector targets are colored to indicate positive (green ovals) and negative (magenta ovals) regulators of defense or unknown or multiple roles (white ovals). PMA = plasma membrane H^+-ATPase. Created with BioRender.

state and thus suppressing pattern-triggered immunity (PTI) (Liang et al. 2021). PTI is also suppressed by PITG20303 from *P. infestans*, which interacts with potato mitogen-activated protein kinase StMKK1. Active StMKK1 is a negative regulator of defense and PITG20303 stabilizes it (Fig. 1), leading to downregulation of PTI (Du et al. 2021). SnRK1 is a kinase associated with the allocation of resources at the point of crosstalk between growth and defense. The RxLR PBZF1 from *Plasmodiophora brassicae* interacts with SnRK1 and disrupts its downstream signaling, leading to enhanced pathogen colonization (W. Chen et al. 2021). Another *P. capcisi* RxLR, PcAvh1, interacts with a subunit of the phosphatase PP2A, which is responsible for dephosphorylating proteins. PcAvh1 MOA is unknown, but PP2A seems to act as a positive regulator of defense (Chen et al. 2019). Another kinase-related RxLR target is the BRI1 kinase inhibitor 1 (VvBKI1), which interacts with *Plasmopora viticola* PvRxLR131. Again, the effector MOA is unknown, but Lan et al. (2019) demonstrated that VvBKI1 activity is required for PvRxLR131-mediated susceptibility.

Manipulating central immune regulators

Plant proteins that act as central immune regulators, identified through historical mutant screens, have been known for decades. However, only in recent years have RxLR effectors been shown to target some of these proteins. EDS1 acts with partners such as PAD4 to facilitate salicylic acid (SA)-mediated transcriptional programming during both PTI and effector-triggered immunity (ETI). PcAvh103 from *P. capsici* interacts with the lipase domain of EDS1 and prevents it from forming a complex with PAD4 (Li et al. 2020) (Fig. 1). NDR1 is another important positive regulator of plant defense required for cell death signaling from coiled-coil type nucleotide-binding leucine-rich repeat (NLR) resistance proteins. *P. sojae* RxLR Avh241 interacts with NDR1 and prevents its homodimerization to suppress its activity (Yang et al. 2021). JAZ proteins are repressors of jasmonic acid (JA) signaling in plants and are normally turned over by the 26S proteasome following JA perception. The *P. sojae* RxLR Avh94 interacts with JAZ1 and JAZ2, stabilizing the proteins and preventing activation of JA signaling (Zhao et al. 2022) (Fig. 1). These examples show that RxLRs can inhibit a range of central immune regulators.

Modulating cell trafficking

Cellular trafficking is a process that RxLRs from multiple pathogens target. Recent large-scale target identification screens reveal many host proteins with connections to trafficking (McLellan et al. 2022; Pelgrom et al. 2020; Petre et al. 2021). Trafficking regulates several processes in plant cells, including secretion of antimicrobials to the site of infection and the recycling and signaling of receptors. RAB GTPases are trafficking switches that control vesicle fusion to and release from membranes. *P. capsici* RxLR242 interacts with a range of RABs from different subfamilies and inhibits secretion of PR1 by binding to active RABE1-7 and preventing its associations with vesicle-related proteins SNARE13, VAMP727, TRAPPC2, and TRAPPC4 (Li et al. 2022). RxLR242 mislocalizes PRRs such as FLS2 from the plasma membrane (PM) to the ER by competitively preventing RABA4-3 from binding the above vesicle-related proteins (Li et al. 2022) (Fig. 1). *P. sojae* effector PsAvh181 also interferes with secretion of defense-associated proteins such as PR1. It prevents the association between its target GmSNAP-1 and GmNSF, disrupting proper formation of the SNARE complex, which is required for vesicles to fuse to the PM (H. Wang et al. 2021a). In contrast, the RxLR PexRD54 from *P. infestans* that has previously been shown to suppress certain autophagy pathways by binding ATG8 and preventing it from forming a complex with Joka2/NBR1 (Dagdas et al. 2016, 2018) can also activate autophagy. Recent work has shown that PexRD54 does this by encouraging the association of Rab8a with ATG8 vesicles and directing them towards *P. infestans* haustoria (Pandey et al. 2021) (Fig. 1). Moreover, the critical AIM motif required for PexRD54 interaction with ATG8 has been lost in the PexRD54 orthologue from *Phytopthora mirabilis* (Maqbool et al. 2016; Zess et al. 2022), perhaps due to currently unknown selection pressures. It appears that blocking or encouraging complex formation may be a major MOA that modulates cellular trafficking (Fig. 1).

Modification of ubiquitination

Ubiquitination is a regulatory system involving a cascade of enzymes that add ubiquitin modifiers onto target proteins. This can change their activity, localization, or stability via degradation by the 26S proteasome (Langin et al. 2023) and is a major regulatory process controlling immunity. As regulation of protein stability is one of the MOAs of RxLR effectors (Fig. 1), it makes sense that these proteins would also target ubiquitination. Indeed, PsAvr1d interacts with the E3 ligase GmPUB13 and inhibits its self-ubiquitination and subsequent turnover, leading to its increased stability. PsAvr1d does this by mimicking an E2 ligase enzyme, binding at the E2 interaction site with a much higher affinity than the endogenous E2 enzyme (Lin et al. 2021). *P. infestans* effector Pi06432 was demonstrated to interact with and stabilize the ubiquitin-like domain containing protein StUDP. In turn, StUDP interacts with and alters the stability of several 19S proteasome regulatory subunits, leading to inhibition of the 26S proteasome, ultimately lowering accumulation of SA and SA signaling (Z. Wang et al. 2023).

SUMOylation is a regulatory system like ubiquitination and involves the attachment and removal of a small ubiquitin-like modifier (SUMO). The RxLR Avr8 from *P. infestans* interacts with deSUMOylating enzyme StDeSI2, which acts as a positive regulator of immunity. Avr8 destabilizes StDeSI2 in a 26S proteasome-dependent manner, thus suppressing defense (Jiang et al. 2023) (Fig. 1). There are many examples in the literature showing that various RxLRs from multiple pathogens modify ubiquitination to either stabilize or destabilize their targets. For example, *Plasmopora viticola* RxLR50253 caused the stabilization of its target BPA1, leading to suppression of cell death and resistance (Yin et al. 2022). In sharp contrast, *P. capsici* RXLR207 leads to degradation of BPA1, activating cell death while still promoting pathogen virulence (Li et al. 2019) (Fig. 1). These perturbations must be quite finely tuned to benefit the differing lifestyles of the pathogens, with *Plasmopora viticola*, an obligate biotroph of narrow host range in which cell death suppression is likely critical, versus *P. capsici*, a hemibiotroph with a short biotrophic phase and broad host range, in which cell death during the necrotrophic phase may be beneficial.

Manipulating chloroplast immunity

Effectors from non-oomycete pathogens have long been known to target chloroplast-mediated immunity. However, several RxLR effectors were recently also shown to target this process (Breen et al. 2023; Littlejohn et al. 2021). RxLR AVRvnt1 from *P. infestans* does this indirectly, by preventing the positive defense regulator glycerate 3 kinase (GLYK) from localizing to the chloroplast (Gao et al. 2020). Encoded by a chloroplast nuclear gene, GLYK has two isoforms with alternate start codons; in the light, the longer form with a chloroplast transit peptide (cTP) is produced and bound by AVRvnt1, triggering cell death in host genotypes with Resistance to *P. infestans* (Rpi)-vnt1. In darkness, the short form lacking the cTP is produced but not bound by AVRvnt1 and, thus, is unrecognized by the resistance protein (Gao et al. 2020). In this way, light regulates this chloroplast-related immune response. In addition, RxLR31154

from *Plasmopora viticola* localizes to the chloroplast and interacts with and stabilizes the susceptibility factor PsbP (Fig. 1), which is required for the stability and formation of photosystem II (Liu et al. 2021). Although these are the first examples of RxLR effectors targeting chloroplast proteins to alter defense, more are likely to emerge as a small number of RxLRs have been predicted to localize to this organelle (Liu et al. 2018; Pecrix et al. 2019).

Modulation of post-translational modification-interacting proteins

Plant proteins undergo a variety of post-translational modifications such as ubiquitination and phosphorylation, two processes highlighted above as targeted by RxLRs. The 14-3-3 proteins are regulatory proteins that specifically bind to phosphorylated sites on target proteins to control protein stability, localization, or activity. Several recent examples show that 14-3-3 proteins are utilized by RxLRs to modulate the targets of the 14-3-3 proteins. Seo et al. (2023a) demonstrated PITG06478 from *P. infestans* interacts with Nb14-3-3a/b proteins to inhibit the activity of PM H^+-ATPase (PMAs) leading to cell death. Normally 14-3-3 proteins would bind the phosphorylated C terminus of PMA to maintain it in an active state. PITG06478 is thought to sequester the 14-3-3 proteins to keep PMA in an inactive state (Seo et al. 2023a) (Fig. 1). Interestingly, the RxLR effector CRISIS2 from *P. capsici* also targets PMA to inhibit its activity leading to cell death. However, instead of indirectly inhibiting activity by removing 14-3-3 proteins like PITG06478, CRISIS2 associates directly with the PMA C terminus (Seo et al. 2023b). It is interesting to speculate that the CRISIS2 MOA may be to prevent 14-3-3 binding to activate PMA. The RxLR effectors Pi02860 from *P. infestans* (McLellan et al. 2022) and FIRE from *Phytophthora palmivora* (Evangelisti et al. 2023) have also recently been demonstrated to interact with 14-3-3 proteins. In the case of FIRE, it possesses a 14-3-3-binding motif, making it appear to be a 14-3-3 substrate, and was shown to be phosphorylated inside plant cells. How this aids pathogen infection is not yet known, but FIRE localizes around haustoria with 14-3-3 proteins and promotes colonization (Evangelisti et al. 2023). Perhaps by mimicking a substrate, it may prevent 14-3-3 proteins binding with genuine defense-associated substrates.

How Do RxLR Effectors Contribute to Host Resistance and Host Range?

Identifying oomycete avirulence (Avr) effectors that are recognized by the host and understanding how they are recognized by corresponding NLR resistance proteins (R proteins) are important steps to develop effective strategies for disease control. Studying the naturally occurring sequence variation in them is crucial for understanding the molecular basis of plant-pathogen co-evolution and for informing the potential durability of host resistance. Considerable efforts have been made by researchers to discover oomycete *Avr* genes, revealing that they tend to encode RxLR effectors, e.g., as reported by Hou et al. (2023). A commonly used and traditional method to identify *Avr* genes from oomycetes is map-based cloning. It remains relevant today with the recent isolation of Avr8 from *P. sojae* (Arsenault-Labrecque et al. 2022). Verification involved CRISPR-cas9 knockout and revealed that *PsAvr8* is the same gene as *PsAvr3a*. A second commonly used route to identify new oomycete *Avr* genes involves a combination of association genetics and effectoromics, a high-throughput approach that expresses effectors transiently in planta to select resistant germplasm. A recent example involves expression-based screening of RxLR genes from the lettuce pathogen *Bremia lactucae* in a range of 150 lettuce accessions containing different resistances (Pelgrom et al. 2019). Three RxLR effectors, BLN06, BLR38, and BLR40, triggered a HR in specific lettuce lines, with BLR38 recognition being associated with resistance to multiple *B. lactucae* races. Recently, Lin et al. (2020) demonstrated the utility of pathogen-enrichment sequencing (PenSeq) for identifying a novel *Avr* gene (*Avramr1*) in *P. infestans*. Briefly, the authors combined two sequencing approaches, PacBio long-read sequencing and complementary DNA pathogen-enrichment sequencing, to generate high-quality, full-length transcripts of genes expressed by the pathogen during infection (Thilliez et al. 2019). A total of 47 highly differentially expressed candidate genes were cloned and were individually co-expressed with Rpi-amr1 in *N. benthamiana* to identify the specific Avr protein that was recognized by it.

In addition to the recently described examples above, there are more than 10 *Avr* genes identified encoding proteins that are recognized specifically cognate *Rpi* genes in potato (Elnahal et al. 2020), and at least 12 *Avr* genes have been identified corresponding to 27 major *Resistance to P. sojae* (*Rps*) genes in soybean (Arsenault-Labrecque et al. 2018). The depth of this information is helping us to understand how Avr effectors evolve rapidly to evade detection by R proteins, leading to an ongoing "arms race" between plant hosts and pathogens. This has driven the evolution of diverse NLR receptors in plants and has also led to the evolution of diverse effector molecules in pathogens. To select the most durable *R* genes for deployment in agriculture, we need to understand the mechanism of effector recognition and the way the pathogen evades recognition.

The modes of recognition of RxLR Avr effectors by host NLRs are either i) direct recognition by physical interaction between NLR and Avr or ii) monitoring of the Avr target by the NLR to detect effector activity. An example of the first scenario is the direct physical interaction between Avramr3 from *P. infestans* identified by Rpi-amr3 from *Solanum Americanum* (Lin et al. 2022). Avramr3 has orthologues in many *Phytophthora* species and recognition is also conserved in other economically important pathogens, such as *P. parasitica* and *P. palmivora* (Fig. 2A).

The second scenario involves indirect recognition through monitoring for perturbations to the Avr target indicative of effector activity (the guard hypothesis). Direct recognition is predicted to overcome more readily by mutations in effectors that abolish R-Avr interaction, whereas indirect recognition is predicted to be more sustainable (De Wit 2007). Several recent examples of involvement of the guard hypothesis have been described. Li et al. (2021) revealed that GmPUB1-1 acts as a guardee and maintains the R proteins encoded by genes within the *Rps1-b* and *Rps1-k* loci in an inactive state. When PsAvr1b binds to GmPUB1-1, it disrupts the interaction between GmPUB1-1 and the R protein Rps-1b, releasing it to transition into an active state and trigger ETI. However, silencing *GmPUB1* in the background of Rps1-b causes full susceptibility of host plants. A further example is the recognition of *P. infestans* RxLR PiAvr2 by two evolutionarily distinct resistances, R2, from Mexican germplasm, or Rpi-mcq1, from South American potato genotypes. Each recognition event is mediated by host BSL phosphatases, which are virulence targets of PiAvr2 (Turnbull et al. 2017, 2019). The BSL phosphatases can thus be regarded as guardees. However, PiAvr2 targeting of BSL1 is monitored by R2, whereas targeting of BSL2 and BSL3 is required for Rpi-Mcq1 activation (H. Wang et al. 2021b) (Fig. 2B). The recognition of *P. infestans* PiAvrvnt1 by potato Rpi-vnt1 is light-dependent, takes place in the chloroplast, and is mediated by the host protein GLYK. GLYK acts as a guardee that is targeted by AVRvnt1, and the recognition event is sensed by Rpi-vnt1 to trigger a resistance response (Gao et al. 2020; Mondal 2021).

Recently, host potato plants have been shown to evolve multiple allelic variants of Rpi-chc1, with polymorphisms in the leucine-rich repeat domain of these variants that confer specific recognition of the superfamily of PexRD12/31 RxLR effectors (Monino-Lopez et al. 2021), implying that host-encoded receptors are continuously evolving to effectively provide resistance against *P. infestans*. Moreover, in addition to the example above of PiAvr2 from *P. infestans* that is recognized by R2 and Rpi-mcq1 (H. Wang et al. 2021b), PsAvr3a from *P. sojae* is recognized by both Rps3a and Rps8 in soybean (Arsenault-Labrecque et al. 2022; Dong et al. 2011). Thus, more than one *R* gene can independently evolve to detect a single RxLR Avr effector.

NLR immune receptors can recognize specific pathogen effectors either as singletons or as part of a resistosome complex. In a recent study by Ahn et al. (2023), effector AVRamr3 was shown to be recognized by NLR sensors Rpi-amr3 and Rpi-amr1, inducing oligomerization of the helper NLR required for cell death (NRC2) to form a resistosome conferring *P. infestans* resistance. In addition, a new study found that NRC4 helper NLR accumulates at haustoria formed at the site of pathogen invasion, where it interacts with other proteins to activate plant immunity to *P. infestans*. This dynamic localization and activation of the helper NLR allows for efficient and specific recognition of pathogens, leading to a faster, more effective immune response (Duggan et al. 2021).

To achieve successful infection, pathogens must escape, suppress, or alter NLR recognition events in ways that allow the pathogen to grow and reproduce. This can be accomplished by numerous approaches. Differential expression, gene deletion, and sequence polymorphisms are strategies employed by oomycete pathogens to avoid host recognition. *P. infestans* Avr2 is such an example, in which evading R2 involved either silencing of the gene or sequence polymorphism, to create PiAvr2-like, which avoids recognition (Gilroy et al. 2011). A recent study also found that *P. infestans* PiAvr3b protein sequences were only conserved in certain *Phytophthora* species, and not all the orthologs were capable of triggering cell death in the presence of the potato StR3b protein (Gu et al. 2023). These variants demonstrate the ability of other *Phytophthora* species to evade recognition or of potato to evolve StR3b to specifically detect the ortholog of *P. infestans* with which it will be co-evolving. In a recent overview, more examples and details are given of how *P. sojae* Avr effectors have evolved to avoid host recognition (Hou et al. 2023).

Some oomycete Avr effectors can evade the surveillance and defense of host plants through acquisition or evolution of an additional, epistatic effector that suppresses the immune-triggering event caused by the Avr effector. An example is the RxLR effector IPI-O4 in *P. infestans*, which can block the direct recognition of IPI-O1 (PiAvrBlb1), leading to suppression of RB/Rpi-Blb1-mediated HR, resulting in disease (Chen and Halterman 2017; Chen et al. 2012; Zhao and Song 2021) (Fig. 3A). Additional oomycete RxLR effectors can suppress NLR network-mediated immune responses by directly targeting NLR signaling components. An example is the *P. capsici* RxLR effector PcAvh103, which specifically targets the host lipase domain of Enhanced Disease Susceptibility 1 (EDS1) to disrupt its association with Phytoalexin Deficient 4 (PAD4) to suppress a range of ETI responses (Li et al. 2020) (Fig. 3B). A further example is AVRcap1b from *P. infestans*, which associates with *N. benthamiana* Target of Myb 1-like protein (NbTOL) NbTOL9a protein (Derevnina et al. 2021). This host protein acts as a negative regulator of NRC2- and NRC3-mediated HR in planta, and AVRcap1b requires NbTOL9a to fully suppress NRC3 activity (Fig. 3C). By contrast, RxLR PITG_15278 acts upstream of another component of the NLR network, helper NRC4, to suppress cell death mediated by RpiBlb2 (Derevnina et al. 2021) (Fig. 3D).

In addition to recognition of RxLR Avr effectors leading to ETI, host range and, indeed, nonhost resistance can also be determined by how well or whether effectors interact with and efficiently manipulate their targets. Recent work has shown that RxLR effectors from *P. infestans* that target proteins in the host plant potato often fail to interact with the candidate orthologues of their targets in the nonhost *Arabidopsis* (McLellan et al. 2022). Moreover, transgenic expression of the *P. infestans* RxLRs in *Arabidopsis* did not enhance colonization by the adapted oomycete pathogen *H. arabidopsidis*. By contrast, *H. arabidopsidis* RxLR effectors expressed in *Arabidopsis* did enhance colonization by this pathogen. In addition, there was an enrichment of *H. arabidopsidis* RxLR effectors interacting with the *Arabidopsis* orthologues (McLellan et al. 2022). The lack of interactions between *P. infestans* RxLRs and target orthologues in *Arabidopsis* is consistent with the hypothesis that there may be structural diversity in these target proteins in nonhost plants, at least at the sites of effector interaction. As an example, *P. infestans* effector Pi06087/PiSFI3, which targets the potato protein

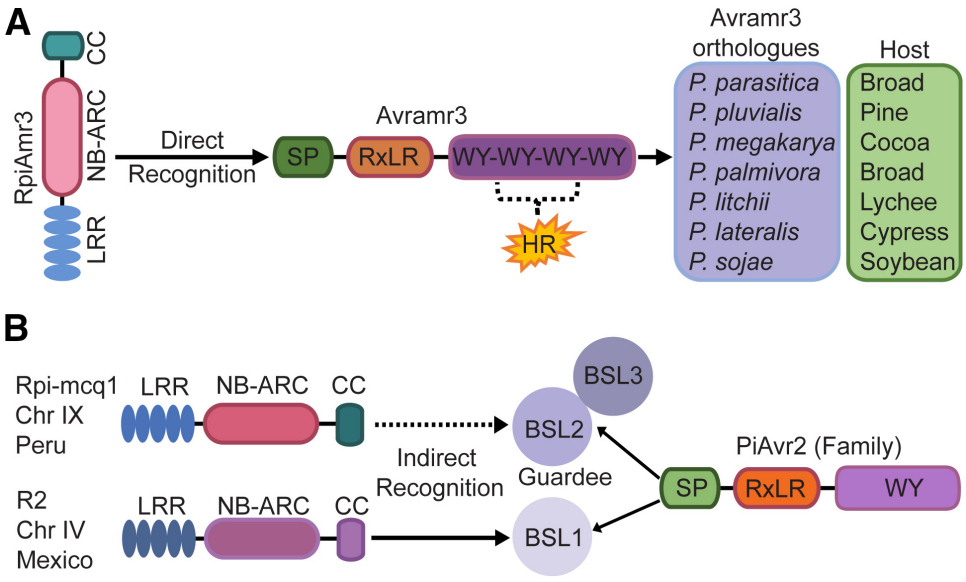

Fig. 2. Modes of RxLR effector recognition. **A,** Direct recognition, e.g., the RxLR effector Avramr3 is directly recognized by resistance protein RpiAmr3 to trigger a hypersensitive response (HR). The region of the effector recognized is indicated by a dotted bracket. Orthologues of AvrAmr3 that are present in a range of *Phytophthora* species that infect different hosts, as indicated, are recognized by Rpi-Amr3. **B,** Indirect recognition, e.g., the RxLR effector PiAvr2 is recognized by the independently evolved R2 and Rpi-mcq1 resistance proteins (present on chromosomes IV and IX as shown), and this is mediated by monitoring BSL1 or BSL2 and BSL3 (guardees), respectively, to trigger the HR. R2 interacts with BSL1 in the presence of Avr2 (solid arrow), whereas no such interaction was detected between Rpi-mcq1 and BSL2 or BSL3.

StUBK/StPUB33, fails to interact with the *Arabidopsis* orthologue AtPUB33. Excitingly, transgenic expression of AtPUB33 in host plants potato and *N. benthamiana* resulted in a small but significant reduction in *P. infestans* infection (McLellan et al. 2022). These observations suggest that breakdown in effector-target interactions can be exploited to provide disease resistance. If we know the precise sites of effector-target interaction, we may modify targets to undermine effector activity and reduce disease. How many such modifications would be needed to convert a host into a nonhost plant (McLellan et al. 2023)?

Future Directions and Prospects

As we identify more RxLR effectors from oomycete plant pathogens, we have the opportunity to use them as probes to identify corresponding target proteins in the host. Each effector-target interaction reveals a node within the network of modulation, modification, and manipulation that takes place to provide a susceptible environment by undermining plant immunity and, potentially, by providing sustenance to the colonizing pathogen. Recent years have revealed multiple MOAs of effectors upon their host targets as a wide range of plant mechanisms and processes are manipulated. A greater understanding of the overall immune system, the key points of vulnerability that are manipulated by effectors in concert, and especially, where effector activity is either redundant, conserved, or both across pathosystems will help to make the immune system more resilient to disease.

As we try to provide barriers to infection, we understand that some of these targeted processes are guarded by host NLRs (Gao et al. 2020; Li et al. 2021; H. Wang et al. 2021b). By contrast, some NLRs may directly protect against diverse *Phytophthora* pathogens with broad host ranges by recognition of conserved effectors (Lin et al. 2022). We are learning more about the structures of resistosomes (Ahn et al. 2023) and the network of NLRs that needs to be defeated during pathogen attack (Derevnina et al. 2021). We can see that modifying the structures of host targets to resemble their counterparts in nonhost plants has the potential to provide resistance (McLellan et al. 2022, 2023). In addition, despite there being potentially hundreds of RxLR effectors expressed and delivered to host cells by oomycete pathogens, some are essential. Host- or spray-induced gene silencing (HIGS or SIGS) are providing promising new solutions to defeat oomycete infections (Hou and Ma 2020), albeit double-stranded RNA uptake into *Phytophthora* needs to be improved (Qiao et al. 2021). Recently, HIGS and SIGS targeted towards essential RxLR effector genes have effectively reduced *P. capsici* infection (Cheng et al. 2022).

The processes by which RxLR effectors are secreted from oomycetes and the means by which they are delivered to host cells, taken up, or translocated, and ultimately reach their varied subcellular destinations remain a focus of study and a source of conjecture. Studies by Wang et al. (2017, 2018) demonstrated that RxLRs could be unconventionally secreted from haustoria and were observed to enter plant cells only in the presence of the pathogen. Exciting new findings show that RxLR effectors from *P. infestans* can enter the host cell via clathrin-mediated endocytosis (CME) (H. Wang et al. 2023). This discovery parallels the demonstration that cytoplasmic effectors from the fungal pathogen of rice, *Magnaporthe oryzae*, also are translocated into host cells via CME (Oliveira-Garcia et al. 2023). How, in each case, effectors transit from pathogen to plant cell, what triggers promote endocytosis, and how the effectors are released from endosomes to travel to their ultimate sites of activity are pressing questions to address in coming years.

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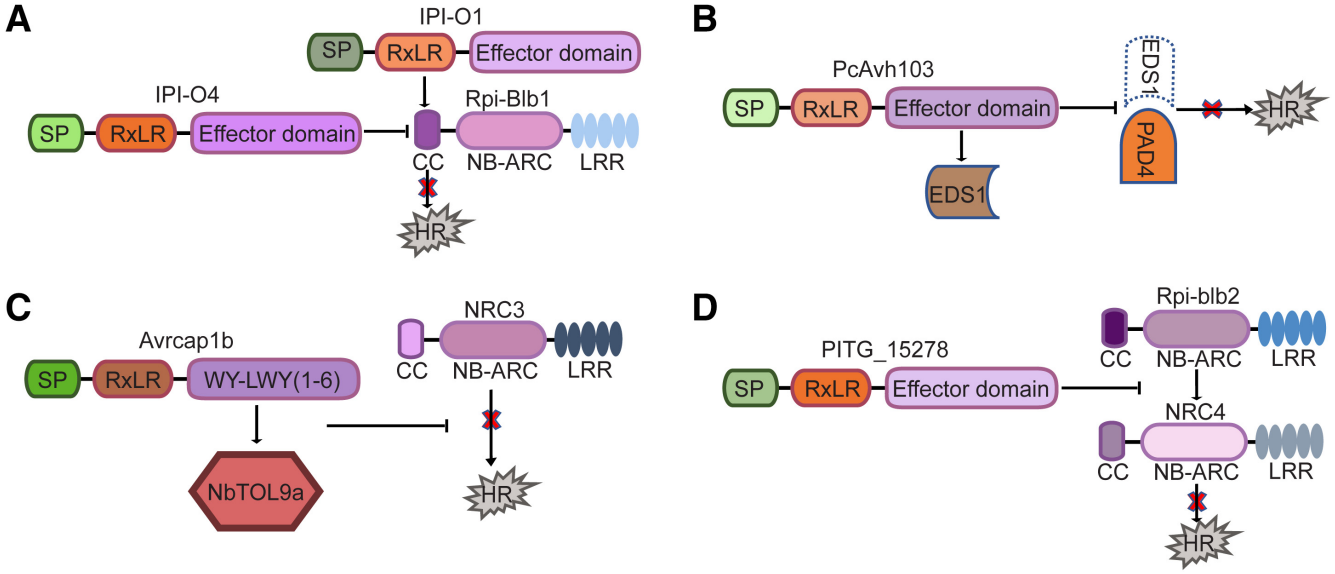

Fig. 3. Modes of effector-triggered immunity (ETI) suppression by RxLR effectors. **A**, The RxLR effector IPIO4 suppresses the nucleotide-binding leucine-rich repeat resistance (NLR)-triggered hypersensitive response (HR) caused by direct interaction between the coiled-coil (CC) domain of RpiBlb1 and IPIO1. SP = signal peptide. **B**, The effector PcAvh103 suppresses ETI by directly targeting NLR signaling component EDS1, preventing its interaction with PAD4. **C**, The effector Avrcap1b interacts with plant negative regulator NbTol9a to suppress NLR network helper NRC3-mediated HR. **D**, The *Phytophthora infestans* effector PITG_15278 suppresses the association between Rpi-blb1 and NLR network helper NRC4 to inhibit the downstream triggered HR (red cross).

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