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INVITED REVIEW

Molecular mimicry modulates plant host responses to pathogens

Pamela Ronald* and Anna Joe

Department of Plant Pathology and the Genome Center, University of California, Davis, CA 95616, USA.
**For correspondence. E-mail pcronald@ucdavis.edu*

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• **Background** Pathogens often secrete molecules that mimic those present in the plant host. Recent studies indicate that some of these molecules mimic plant hormones required for development and immunity.
• **Scope and Conclusion** This Viewpoint reviews the literature on microbial molecules produced by plant pathogens that functionally mimic molecules present in the plant host. This article includes examples from nematodes, bacteria and fungi with emphasis on RaxX, a microbial protein produced by the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae*. RaxX mimics a plant peptide hormone, PSY (*p*lant peptide containing *s*ulphated *t*yrosine). The rice immune receptor XA21 detects sulphated RaxX but not the endogenous peptide PSY. Studies of the RaxX/XA21 system have provided insight into both host and pathogen biology and offered a framework for future work directed at understanding how XA21 and the PSY receptor(s) can be differentially activated by RaxX and endogenous PSY peptides.

Key words: Molecular mimicry, plant pathogen, microbial mimic, RaxX, XA21, PSY, engineering receptor

INTRODUCTION: WHAT IS BIOLOGICAL MIMICRY?

Biological mimicry and molecular mimicry

In classical mimicry, species that are attractive to predators as food have evolved mechanisms that facilitate resemblance to inedible species that are dangerous or unpleasant to eat. A well-known example is Henry Bates's work on Amazonian butterflies. Bates theorized that palatable species occasionally produced mutant forms with visual characteristics in their wings similar to toxic or unpalatable species, making them less likely to be chosen by birds for food (Bates, 1862). Like this case described by Bates, early studies of biological mimicry focused on the visual resemblance between species. For example, some orchid species produce a flower that mimics bee females to attract males for pollination (Pasteur, 1982). Another example is an early barnyard grass, a weed that resembles rice, and thus farmers do not pull it out from rice fields (Barrett, 1983).

In 1964, researchers discovered examples of molecular mimicry: structural, functional or immunological similarities of molecules that are shared between infectious pathogens and the hosts that they infect. Molecular mimicry has been observed in diverse species of pathogens that infect both plants and animals. Because establishment and maintenance of pathogenesis depend on the passing and receiving of signals between host and pathogen, some of the molecules produced by pathogens mimic host components to gain evolutionary advantages (Mitchum *et al.*, 2012; Eves-Van Den Akker *et al.*, 2016). Such microbial molecules functionally or structurally resemble host factors that are required for host survival, such as ligands of host receptors, substrates of host enzymes, or host proteins themselves (Knodler *et al.*, 2001; Nesic *et al.*, 2010). Some of these microbially produced molecules mimic plant hormones that control growth, development, and regulation of innate immunity.

In this Viewpoint, we highlight recent examples of molecular mimics produced by bacteria, nematodes and fungal pathogens.

CORONATINE

A well-studied case of hormone mimicry in plants is the production of coronatine by the gram-negative biotrophic bacterium *Pseudomonas syringae* (Weiler *et al.*, 1994). Coronatine is a structural and functional mimic of jasmonoyl-L-isoleucine (JA-Ile), a bioactive form of the plant hormone jasmonic acid (JA) (Weiler *et al.*, 1994; Fonseca *et al.*, 2009). Thus, coronatine targets a JA receptor, COI-1 (CORONATINE INSENSITIVE1)/JAZ (JASMONATE ZIM DOMAIN) co-receptor, and activates JA signalling (Katsir *et al.*, 2008; Melotto *et al.*, 2008) (Fig. 1). JA signalling by coronatine suppresses salicylic acid (SA)-dependent defence through antagonistic cross-talk (Robert-Seilaniantz *et al.*, 2011). SA plays a central role in regulating plant defences against biotrophic and hemibiotrophic pathogens, including *P. syringae* (Robert-Seilaniantz *et al.*, 2011). These studies indicate that coronatine produced during *P. syringae* infection mimics JA action, suppressing the host defence response to facilitate infection (Geng *et al.*, 2012).

CLAVATA3/ENDOSPERM SURROUNDING REGION-RELATED PEPTIDES

Plant-parasitic nematodes also produce mimics of endogenous plant hormones. All sedentary plant-parasitic nematodes produce peptides similar to plant CLAVATA3/ENDOSPERM

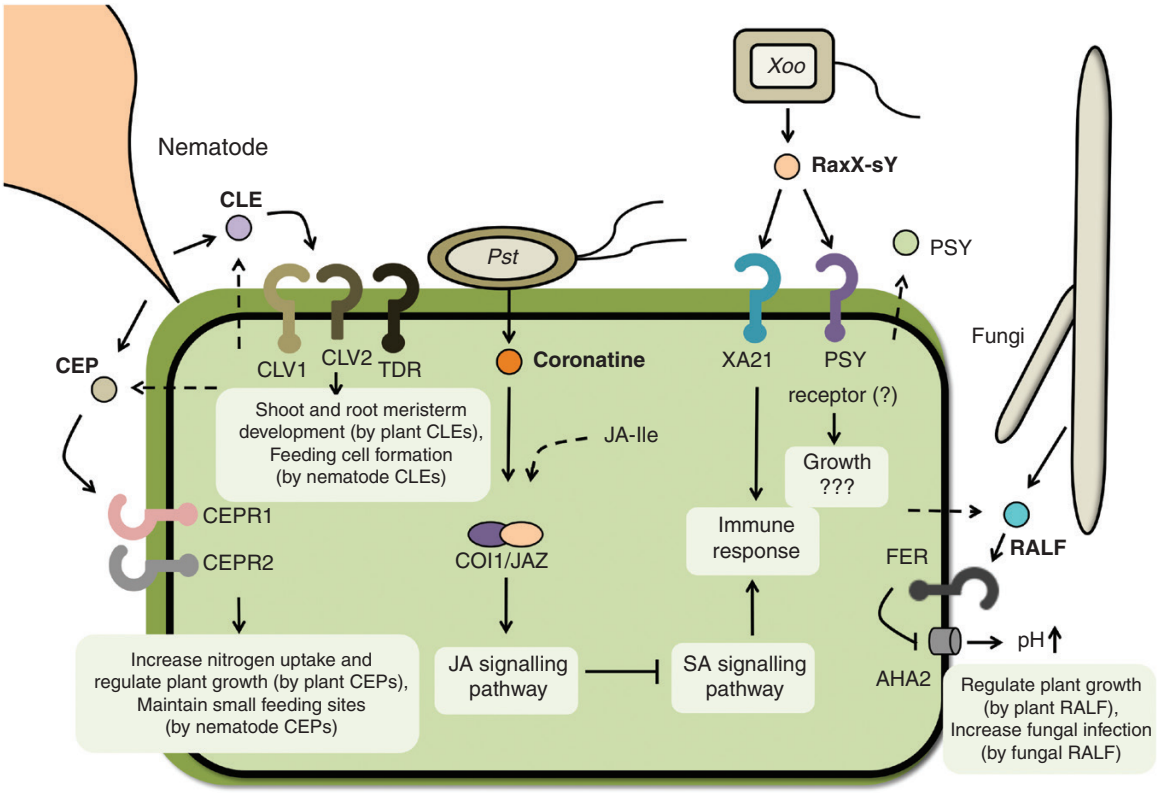

FIG. 1. Plant pathogens produce molecular mimics that modulate plant signalling pathways. *Pseudomonas syringae* pv. *tomato* (*Pst*) produces coronatine, a structural and functional mimic of jasmonoyl-L-isoleucine (JA-Ile). Coronatine binds the plant JA receptor, COI1/JAZ, to activate the JA signalling pathway, which suppresses salicylic acid (SA)-mediated signalling and inhibits the immune response. Nematodes secrete a mimic of a plant peptide called CLAVATA3/ENDOSPERM SURROUNDING REGION-related (CLE), which is perceived by the plant CLAVATA1 (CLV1)/CLV2 heterodimer or a tracheary element differentiation inhibitory factor (TDIF) receptor (TDR). It is hypothesized that nematode CLE peptides subvert plant CLE-mediated shoot and root meristem development to instead produce feeding cells for the nematode. Another class of nematode effectors mimics the plant C-TERMINALLY ENCODED PEPTIDEs (CEPs). Plant CEPs are produced in the roots under nitrogen starvation and then move through xylem vessels to shoots, where they are recognized by two receptors, CEPR1 and CEPR2. Activation of CEPRs induces nitrogen-demand signals, which increase expression of nitrogen transporters, inhibit primary root elongation and initiate lateral root development to take up nitrogen. The benefit to the nematode is that nematode CEPs induce more nitrogen uptake and keep the size of the feeding site small for biotrophic interaction with plants. Nematodes need to maintain small feeding sites to prevent excessive nutrient drainage and allow host plants to survive. The fungal pathogen *Fusarium oxysporum* secretes a mimic of the plant *r*apid *al*kalinization *f*actor (RALF) peptide. Plant RALF targets the FERONIA (FER) receptor to activate a plasma membrane H(+)-ATPase 2 (AHA2) and thus alkalinizes the extracellular space *in planta*. RALF-induced extracellular alkalinization regulates the plant cell expansion required for plant growth and development. Fungal RALF-induced alkalinization in the plant apoplast is beneficial to fungal infection and multiplication but the underlying mechanism remains unclear. The sulphated RaxX (RaxX-sY) peptide from *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) mimics the plant peptide hormone PSY (*p*lant peptide containing *s*ulphated *t*yrosine). RaxX-sY activates PSY signalling and promotes plant growth. Rice XA21 recognizes and responds specifically to microbial RaxX to activate the immune response. Straight lines indicate the secretion of pathogen molecules. Dashed lines indicate products of the endogenous factor from the plant. Question marks indicate pathways that have not yet been fully elucidated.

SURROUNDING REGION-related (CLE) peptides, which regulate shoot meristem differentiation, root growth and vascular development (Chen *et al.*, 2015; Guo *et al.*, 2017). A-type plant CLEs suppress shoot and root apical meristem activity and promote cell differentiation, while B-type plant CLEs function in the vascular meristem to suppress differentiation of tracheary elements and promote procambial cell division (Katsir *et al.*, 2011). Nematodes produce both A-type and B-type CLEs and secrete those precursor proteins into plant tissues, where they undergo post-translational modifications and proteolytic processing to become bioactive CLE peptides resembling plant CLEs (Chen *et al.*, 2015). The mature nematode CLEs, which are 12-amino-acid arabinosylated glycopeptide, strongly interact with plant CLE receptor complexes (Wang *et al.*, 2005; Mitchum *et al.*, 2008; Yamaguchi *et al.*, 2016; Guo *et al.*, 2017). A-type CLEs bind CLAVATA1 (CLV1)/CLV2 heterodimer, while B-type CLEs bind a tracheary element differentiation inhibitory factor (TDIF)-receptor (TDR) (Kucukoglu and Nilsson, 2015). The plant requires synergistic interaction between A- and B-type CLEs, spatial regulation and negative feedback for fine-tuning of vascular development (Whitford *et al.*, 2008; Guo *et al.*, 2017). Unlike plant CLE-mediated signalling, nematodes secrete A- and B-type CLEs directly into procambial cells in the roots, which induces massive cell proliferation and formation of feeding cells, where nematodes obtain nutrients (Fig. 1) (Guo *et al.*, 2017).

C-TERMINALLY ENCODED PEPTIDES

C-TERMINALLY ENCODED PEPTIDEs (CEPs) are a large and diverse family of effector peptides produced by some

sedentary plant-parasitic nematodes (Bobay *et al.*, 2013; Ogilvie *et al.*, 2014; Bird *et al.*, 2015; Eves-Van Den Akker *et al.*, 2016). CEPs are plant peptide hormones that are 15 amino acids in length with hydroxylation modification on prolines (Ohyama *et al.*, 2008). Plant *CEP* genes are highly upregulated in the portion of roots undergoing nitrogen starvation (Imin *et al.*, 2013). Root-derived plant CEP peptides move through xylem vessels to the shoots, where they interact with two leucine-rich repeat receptors, CEPR1 and CEPR2 (Tabata and Sawa, 2014). The CEP/CEPR interaction induces shoot-derived polypeptides named CEP downstream 1 (CEPD1) and CEPD2 (Ohkubo *et al.*, 2017). CEPD1 and CEPD2 in turn travel to the roots (specifically to those roots in nitrogen rich soils) and then upregulate *Arabidopsis* nitrogen transporter *NRT2.1* to take up nitrogen in the roots (Ohkubo *et al.*, 2017) (Fig. 1). Over-expression of CEP genes or exogenous application of CEPs suppresses root cell proliferation and thus reduces primary root elongation and accelerates lateral root development. Taken together, these observations show that root-derived CEPs under nitrogen starvation induce systemic N-demand signals for nitrate uptake from nitrogen-rich soil, while suppressing root growth where nitrogen is limiting. Nematode CEPs also upregulate *NRT2.1* expression and reduce primary root length. Nematode CEPs limit expansion of feeding sites, which they rely on for nutrient acquisition for several weeks. It is hypothesized that nematodes keep the size of feeding sites small, to their own benefit because over-sized feeding sites may drain excessive nutrient from plants and kill host plants (Eves-Van Den Akker *et al.*, 2016). Altogether, nematodes mimic plant CEPs to sustain their biotrophic interaction with plants (Eves-Van Den Akker *et al.*, 2016).

RAPID ALKALINIZATION FACTOR

The root-infecting fungus *Fusarium oxysporum* secretes a functional mimic of the plant regulatory peptide RALF (*r*apid *al*kalinization *f*actor) (Masachis *et al.*, 2016). Plant RALF targets the receptor FERONIA (FER), which inactivates plasma membrane H(+)-ATPase 2 (AHA2) and thus inhibits proton transport (Murphy and De Smet, 2014). This plant RALF/FER interaction induces extracellular alkalinization and impacts cell expansion during plant growth and development (Murphy and De Smet, 2014). The root-infecting fungus *F. oxysporum* secretes fungal RALF (F-RALF) to induce FER-mediated extracellular alkalinization in the host tissue in a similar manner to plant RALFs (Masachis *et al.*, 2016). Extracellular alkalinization has been reported to contribute to fungal infection and indeed F-RALF-induced alkalinization increases fungal pathogenicity (Murphy and De Smet, 2014; Masachis *et al.*, 2016) (Fig. 1). The underlying mechanisms remain to be elucidated. Stegmann *et al.* (2017) recently found that RALF peptides inhibit the FERONIA-mediated formation of active immune receptor complexes. Thus, fungal RALFs may suppress the plant immune response by inhibiting the assembly of receptor kinase complexes. Taken together, these findings show that fungal pathogens use F-RALFs to increase fungal infection and to suppress host immunity.

Together, these studies indicate that molecular mimics can suppress host immune responses, facilitate infection and/or enhance plant health, maintenance of which is critically important for propagation and survival of biotrophic pathogens.

THE XA21 IMMUNE RECEPTOR

Here, we describe the discoveries that led to the identification and characterization of a small sulphated protein produced by a plant pathogen that triggers an immune response in rice plants expressing the XA21 immune receptor and that induces root growth. These findings were presented at the Annals of Botany Lecture in Melbourne Australia, and are detailed in two publications (Pruitt *et al.*, 2015, 2017).

Discovery of XA21

In 1905, British geneticist and plant breeder Rowland Biffen demonstrated that it is possible to generate wheat varieties with resistance to a devastating disease by genetic manipulation. He cross-pollinated a resistant wheat variety with a susceptible wheat variety and showed that the resulting seed carried the resistance of the parent. Today, more than 100 years after Biffen's discovery, plant breeders have introduced 'resistance genes' into virtually every crop plant that we consume. However, for many years the identity of these genes and the functional basis of resistance remained elusive.

An explosion of research in the 1990s led to the isolation of several resistance genes from diverse plant species (Martin *et al.*, 1993; Bent *et al.*, 1994; Jones *et al.*, 1994; Whitham *et al.*, 1994; Lawrence *et al.*, 1995; Song *et al.*, 1995). Many of the resistance genes encoded intracellular proteins carrying a nucleotide binding site (NBS), leucine-rich repeats (LRRs), coiled coil and Toll /IL-1 receptor (TIR) domains that recognize specific races of a microbial species. Some of the resistance genes encoded cell surface receptors and were later named pattern recognition receptors (PRRs). PRRs recognize molecules that are conserved across a large class of microbes.

In 1995, we isolated the *Xa21* gene, which encodes a PRR carrying both an extracellular receptor domain and an intracellular kinase domain (Song *et al.*, 1995). Rice plants carrying *Xa21* confer broad-spectrum resistance to the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Wang *et al.*, 1996), which normally caused a devastating disease of rice in Asia and Africa (Ikeda *et al.*, 1990).

Discovery of rax *genes (required for activation of* xa21*-mediated immunity)*

Efforts to identify the microbial molecule that activates the XA21-mediated immune response led to the identification of a number of *Xoo* genes that are required for activation of XA21 (*rax* genes). These genes encode a tyrosine sulphotransferase, RaxST, and three components of a predicted type 1 secretion system (T1SS): a membrane fusion protein, RaxA; an adenosine triphosphate (ATP)-binding cassette (ABC) transporter, RaxB; and an outer membrane protein, RaxC. *raxST*, *raxA* and *raxB* are located in a single operon (*raxSTAB*) (da Silva *et al.*, 2004). RaxB falls into a clade of ABC transporters that carry a

proteolytic peptidase domain in their N-termini, termed C39, which recognizes and cleaves a conserved N-terminal leader sequence (Dirix *et al.*, 2004, Havarstein *et al.*, 1995). On the basis of these findings, we hypothesized that the activator of XA21-mediated immunity is tyrosine-sulphated by RaxST, a peptide processed and secreted via RaxABC T1SS (da Silva *et al.*, 2004).

Discovery of RaxX

In 2015, we reported the discovery of RaxX, a small protein with a tyrosine sulphation site and a predicted N-terminal leader sequence. The RaxX sequence is highly conserved in many *Xanthomonas* species. This conservation suggested that RaxX serves a key biological function. *Xoo* strains that lack RaxX, or carry mutations in the single RaxX tyrosine residue (Y41), are able to evade XA21-mediated immunity (Pruitt *et al.*, 2015). Y41 of RaxX is sulphated by the prokaryotic tyrosine sulphotransferase RaxST. Sulphated, but not non-sulphated, RaxX triggers hallmarks of the plant immune response in an XA21-dependent manner. A sulphated, 21-amino-acid synthetic RaxX peptide (RaxX21-sY) is sufficient for this activity (Pruitt *et al.*, 2015).

As a result of the explosion of studies on PRRs in both plant and animal systems, it has now become clear that the molecules recognized by PRRs can be conserved quite widely across genera (e.g. flagellin) or more narrowly within a genus (e.g. Pep13) (Brunner *et al.*, 2002) and, further, that sequence variation and posttranslational modifications can modulate PRR-dependent pathogen recognition (Takeuchi *et al.*, 2003; Sun *et al.*, 2006). Like microbial molecules recognized by other PRRs, the RaxX protein is conserved within a class of microbes (RaxX is conserved in most, but not all, *Xanthomonas* species) and is post-translationally modified (McDonald *et al.*, 2017).

These results indicate that the molecules recognized by NBS-LRR proteins and those recognized by PRR proteins share overlapping characteristics. Furthermore, the transcriptomic patterns triggered by different types of resistance proteins are overlapping (Tao *et al.*, 2003; Tsuda *et al.*, 2008). Although the many terms used to describe plant–pathogen interactions in the past [avirulence gene, effector-triggered immunity (ETI), pathogen associated molecular patterns (PAMPs)-triggered immunity (PTI) etc. (Jones and Dangl, 2006)] were very useful in advancing our understanding of plant–pathogen interactions, the discovery of diverse receptors and their ligands in the last 10 years has blurred the distinction between NBS-LRR and PRR proteins. Thus, broad generalizations that place these receptors and their corresponding microbial determinants into specific classes are less useful today than previously. For this reason, we avoid using the older terms.

RaxX shares similarity to a plant peptide hormone

Although RaxX shares no homology with microbial proteins of known function, sequence analysis revealed that RaxX residues 40–52 are similar to the plant peptide hormone PSY (*p*lant peptide-containing *s*ulphated *t*yrosine) (Amano *et al.*, 2007, Pruitt *et al.*, 2015, 2017). Alignment of PSY peptides from different plants revealed a highly conserved 13-amino-acid region, which corresponds precisely to the region of sequence similarity between RaxX and *Arabidopsis* PSY1 (AtPSY1) (Pruitt *et al.*, 2015, 2017). AtPSY1 is the best-characterized member of the plant PSY peptide family. AtPSY1 is an 18-amino-acid glycopeptide with a single sulphotyrosine residue that is secreted and processed and promotes root elongation primarily through regulation of cell size (Amano *et al.*, 2007). AtPSY1 is widely expressed in *Arabidopsis* tissues (Amano *et al.*, 2007). AtPSY1 promotes acidification of the apoplastic space through activation of membrane proton pumps (Fuglsang *et al.*, 2014). This acidification is thought to activate pH-dependent expansins and cell wall remodelling enzymes that loosen the cellulose network (Cosgrove, 2000; Hager, 2003). Concomitant water uptake by the cell leads to cellular expansion.

The sequence similarity suggests the possibility that RaxX functionally mimics PSY1. We recently demonstrated that RaxX peptides derived from diverse *Xanthomonas* species promote root growth, mimicking the growth-promoting activities of PSY peptides (Pruitt *et al.*, 2017). We also showed that, unlike RaxX, PSY peptides do not activate XA21-mediated immunity (Pruitt *et al.*, 2017). Thus, XA21 is a highly selective immune receptor capable of specifically recognizing the bacterial mimic (Fig. 1).

What is the biological function of RaxX?

In a classical evolutionary arms race, both the pathogen and host develop and deploy an arsenal of strategies to infect or resist their partner (Jones and Dangl, 2006). For example, many pathogens secrete an array of molecular factors designed to manipulate host biology and suppress the immune response. In turn, plants have developed a set of immune receptors that recognize these molecules or their activities and launch mechanisms to destroy the pathogen, which the pathogen then tries to counter. Our studies of the RaxX/XA21 interaction provide another example. Based on our findings, we propose a model whereby, in the absence of XA21, *Xoo* and other xanthomonads use sulphated RaxX to mimic PSY1-like peptides to suppress host defence responses, facilitate infection or enhance plant health, maintenance of which is critically important for propagation and survival of biotrophic pathogens (Amano *et al.*, 2007; Mosher *et al.*, 2013; Mosher and Kemmerling, 2013). We recently showed that a *Xoo raxX* mutant is less virulent than wild-type *Xoo* on rice leaves in the absence of XA21 (Pruitt *et al.*, 2017). This result supports the ideas that that RaxX is required for the full virulence of *Xoo* to infect rice leaves and that XA21 later evolved to recognize and respond specifically to RaxX. We have not yet identified the molecular mechanism utilized by RaxX to enhance virulence.

The robust immune response conferred by XA21 is likely to cause a strong selective pressure on the *raxX* gene in *Xoo*. Indeed, we have identified natural variants of RaxX21 that are able to evade the activation of XA21 immunity by altering one or two amino acids (e.g. P44, A46 and/or P48) (Pruitt *et al.*, 2015). Variation of RaxX P44 and P48 completely abolishes the immunogenic activity of RaxX in XA21 rice (Pruitt *et al.*, 2015). The amino acid change of A46 has a partial effect. However, these RaxX variants retain the ability to mimic the growth-stimulating properties of

PSY (Pruitt *et al.*, 2017). We do not know whether the amino acid changes in these RaxX variants arose in response to the presence of XA21 or were pre-existing in the *Xoo* population. Epidemiological studies with documentation of disease occurrence over time and space are needed to further investigate the evolution of *raxX*.

It is also possible that RaxX evolved to mimic specific host PSY peptides. Indeed, three residues in RaxX21 from *Xoo* (strain PXO99) are identical to those in OsPSY1a (Pruitt *et al.*, 2017). Similarly, the amino acids of RaxX21 from *X. oryzae* pv. *oryzicola* (strain BSL256) are similar to those in OsPSY2 (Pruitt *et al.*, 2017). If these two peptides evolved to mimic different PSY peptides, it would indicate that there are multiple PSY receptors in rice, which differentially recognize diverse PSY peptides. Unlike RaxXs from *Xoo* and *Xoc*, comparative genetic analysis of other sequenced RaxX and PSY peptides did not reveal strict correlation between the sequences of RaxX from the pathogen and PSYs from a corresponding compatible host (Pruitt *et al.*, 2017). However, alignment of the plant PSY sequences highlights variations at positions 5, 7 and 9 that correspond to RaxX amino acids 44, 46 and 48 (Pruitt *et al.*, 2017). The variation in both plant and pathogen RaxX shares several common amino acids, which suggests that the change is not random. These observations have led us to additional questions about this system: Do specific amino acids affect the ability of peptides to activate specific PSY receptor(s)? Is the PSY receptor(s) able to recognize diverse amino acid variants?

Tyrosine sulphation is critical for RaxX activity

Our results indicate that tyrosine sulphation is critical for RaxX activity. The presence or absence of sulphation and the residues near Y41 are decisive for the ability of RaxX to trigger XA21-mediated immunity and to activate PSY signalling. We have identified RaxX in at least nine *Xanthomonas* species that infect maize, cassava, sugar cane, tomatoes, peppers, wheat, alfalfa, onions, banana and citrus (Pruitt *et al.*, 2015, 2017). In all of these strains, *raxX* is encoded upstream of *raxST*, *raxA* and *raxB* and carries a conserved tyrosine residue (Pruitt *et al.*, 2015, 2017). The co-localization of the *rax* genes suggests that they function in a common biological process and that the RaxX proteins in these species are also substrates for RaxST-mediated tyrosine sulphation.

Although tyrosine sulphation has not been demonstrated in other prokaryotic species, it is a common posttranslational modification of eukaryotic proteins and plays important roles in regulating plant development and in mediating the interactions of plants and animals with microbes. For example, in humans, sulphation of the C-C chemokine receptor type 5 (CCR5) is critical for binding of the envelope glycoprotein gp120 by HIV (Farzan *et al.*, 1999). In addition to PSY, plants produce at least three other classes of tyrosine sulphated peptides: *p*hyto*s*ulpho*k*ine (PSK) (Matsubayashi and Sakagami, 1996), *r*oot meristem *g*rowth *f*actor (RGF) (Matsuzaki *et al.*, 2010) and Casparian strip integrity factor (CIF) (Doblas *et al.*, 2017; Nakayama *et al.*, 2017). PSK, RGF and CIF are also processed, secreted, and play a role in the regulation of growth and development in the root.

Further directions: anticipatory breeding and receptor engineering

The robust immunity conferred by XA21 on most *Xoo* strains is a highly valued agronomic trait. For this reason, XA21 has been introgressed into diverse rice varieties grown by rice farmers (Chen *et al.*, 2000; Toenniessen *et al.*, 2003; Sundaram *et al.*, 2008; Gopalakrishnan *et al.*, 2008; Huang *et al.*, 2012; Win *et al.*, 2012). The discovery of *raxX* allelic variants that can evade XA21-mediated recognition can be used to screen *Xoo* field populations for the presence of potentially virulent variants. Such an anticipatory breeding approach would alert farmers to the need to plant different varieties or mixtures before strains that overcome XA21-mediated immunity cause crop damage.

In some cases, knowledge of plant receptor–ligand interaction has been used to engineer resistance to abiotic and biotic stress. For example, Park *et al.* (2015) described engineering of the abscisic acid (ABA) receptor, PYR1 (Pyrabactin Resistance 1), to activate ABA-mediated drought tolerance. Park *et al.* (2015) established a set of PYR1 mutants that contains all possible single amino acid substitutions in the ABA binding pocket. The mutant library was then screened for receptor mutants that could respond to mandipropamid (MD). This is a fungicide that controls severely pathogenic oomycetes. The authors identified several mutated receptors that weakly responded to MD. Based on the screening results, the effective mutated residues were combined and extra mutations were added in the engineered PYR1 to optimize the MD response. These experiments led to the generation of a new receptor, $PYR1^{MANDI}$, which is activated by nanomolar levels of MD. $PYR1^{MANDI}$ can give a solution to a few problems associated with the natural response by ABA/PYR, which is moderate and is activated too late to protect crops from drought stress. Synthesized ABA is too expensive to spray on crop fields. Application of $PYR1^{MANDI}$ to crop fields will allow MD to efficiently activate ABA-mediated drought tolerance (Park *et al.*, 2015; Rodriguez and Lozano-Juste, 2015).

Over-expression of PRRs has been used to improve the resistance of plants to infection by bacterial pathogens. However, in some cases the engineered plants do not have sufficient resistance to suppress disease. For example, a chitin receptor conferred moderate resistance to *Magnaporthe oryzae*, the causal agent of rice blast disease (Kishimoto *et al.*, 2010). To create plants with enhanced resistance, Kishimoto *et al.* (2010) constructed a chimeric receptor that contains the extracellular domain of chitin elicitor binding protein (CEBiP) and the intracellular domain of XA21. The new receptor has high affinity to chitin, and rice plants expressing the chimeric receptor exhibited enhanced resistance to *M. oryzae* (Kishimoto *et al.*, 2010). These results indicate that engineering receptors can be an effective strategy for enhancing disease resistance.

These reports of plant receptors engineered for tolerance to biotic and abiotic stress suggest that knowledge of receptors that recognize microbial mimics, which often serve as virulence factors, may also lead to novel strategies for disease prevention (Park *et al.*, 2015; Rodriguez and Lozano-Juste, 2015). Recent studies of the JA receptor support this idea. The endogenous JA receptor is sensitive to both JA-Ile and the mimic coronatine. By making a structure-guided point mutation of a single amino acid, Zhang *et al.* (2015) generated a modified JA receptor that has reduced sensitivity to coronatine but retains

endogenous JA-Ile recognition. *Arabidopsis* with the modified JA receptor displayed enhanced resistance to coronatine-producing *Pseudomonas* strains, and has a normal phenotype in the absence of infection (Zhang *et al.*, 2015). The Zhang *et al.* study demonstrates how an understanding of bacterial mimicry of host factors can be used to engineer plants with enhanced resistance to bacterial pathogens.

CONCLUSIONS

Studies of the RaxX/XA21 system have provided insight into both host and pathogen biology and offered a framework for future work directed at understanding how XA21 and the PSY receptor(s) can be differentially activated by RaxX and endogenous PSY peptides. These studies also suggest mechanisms for engineering plants for enhanced resistance. For example, XA21 variants engineered for resistance to strains that can evade XA21-mediated immunity would be of use to farmers. Knowledge of the RaxX genetic variants in virulent *Xoo* strains makes this strategy theoretically possible. Future availability of a XA21/RaxX co-crystal structure would allow researchers to identify amino acids of XA21 that are required for recognition and response to RaxX. With this knowledge, substitutions in the identified key residues could be tested for their ability to respond to RaxX genetic variants. If successful, these results would set the stage for engineering rice plants with new receptors that recognize the RaxX variants. Furthermore, because *Xanthomonas* species affect virtually all crop plants, similar approaches could be used to engineer resistance to non-rice pathogens. For example, *X. axonopodis* pv. *citrumelo*, *X. euvesicatoria* and *X. oryzae* pv. *oryzicola*, which cause serious diseases on citrus, tomato and rice, carry alternative *raxX* alleles.

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