Microbial Pathogen Effectors in Plant Disease

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Microbial plant pathogens use secreted effector proteins for successful infection of their host. This evolved state is rather exceptional as most microbes do not cause disease in the vast majority of plant species. An important primary activity of effectors is to interfere with a range of plant immune processes to evade and suppress pathogen detection, or to block immune signalling and downstream responses. Furthermore, effectors can enhance disease susceptibility by altering cellular processes and modulating host transcription. For most of these activities, effectors specifically target plant proteins that are central in these processes. An advanced virulence strategy is the post-translational modification by effectors of plant targets to change their activity or stability. The knowledge gathered on the molecular mechanisms underlying effector-triggered susceptibility of plants provides great potential for novel approaches of resistance breeding.

Introduction

Plant pathogenic bacteria, fungi, and oomycetes have diverse lifestyles and infection strategies, but have in common that they attempt to colonise and live at the expense of their host. Essential in infection by these microbial pathogens is the evasion or suppression of the host immune system and the modulation of host processes. To achieve this, pathogens secrete effector proteins that are collectively required to promote disease. On the other hand, effectors can also be potent triggers of the plant immune system and work against the pathogen producing them. In the molecular arms race between plants and their pathogens, effector genes are thus under constant evolutionary pressure.

In this article we discuss how bacteria, fungi and oomycetes use protein effectors to manipulate plant processes to their benefit. The immense and expanding number of identified effectors, different host targets, and mechanisms of action make it impossible to be comprehensive in this article. We have, therefore, chosen to provide general concepts with prime examples, highlighting the diversity and power of effector activities.

The Plant Immune System

During infection, conserved microbe- or pathogen-associated molecular patterns (M/PAMPs), e.g. flagellin, lipopolysaccharides (LPS), chitin or other pathogen-derived molecules, can be recognised extracellularly by pattern recognition receptors (PRRs) at the plant plasma membrane. Most plants resist infection through detection of PAMPs by PRRs resulting in PAMP-triggered immunity (PTI), which is associated with calcium influx, an oxidative burst, callose deposition and activation of a mitogen-activated protein kinase (MAPK) cascade to induce defence gene expression (Nicaise et al., 2009). Adapted pathogens evade detection or suppress PTI by translocating effector proteins into host cells to cause disease, known as effector-triggered susceptibility (ETS). In turn, within the host species, specialised polymorphic intracellular receptors (NLRs) containing a nucleotide-binding and leucine-rich repeat domain that can recognise pathogen effectors and induce effector-triggered immunity (ETI) have evolved. ETI is an amplified PTI response, which is often observed as a hypersensitive response (HR) that is associated with localised programmed cell death at the site of attempted infection (Jones and Dangl, 2006).
These domains are therefore referred to as integrated decoys for effector detection. R protein activation. Interestingly, a subset of NLRs contains a variable integrated domain that is required for effector proteins and may drive convergent evolution of multiple, unrelated effectors to target the same plant protein. Indeed, extensive effector–target protein–protein interaction assays have shown that a number of Arabidopsis proteins are targeted by multiple, unrelated effectors to target the same plant protein. Indeed, extensive effector–target protein–protein interaction assays have shown that a number of Arabidopsis proteins are targeted by diverse, sequence-unrelated effectors from pathogens of three kingdoms of life (Mukhtar et al., 2011; Weßling et al., 2014). Targeting these highly connected hubs with central regulatory roles allows pathogens to effectively suppress immune responses and rewire signalling.

Pathogens have evolved many different methods to effectively deal with the plant immune system (detailed later in Figure 2), allowing them to cause disease. In the following sections we present generalised concepts of effector activities that have allowed microbes to become pathogenic on plants.

### Effector Secretion and Translocation

Many microbial effectors exert their function inside the plant cell, while others function in the apoplast, the free diffusional space outside of the plant cell membrane (Figure 1). Gram-negative bacterial pathogens often translocate effectors directly into the host cytoplasm via their type III secretion system (T3SS) (Galán and Wolf-Watz, 2006). A syringe-like structure traverses the bacterial inner and outer membranes and the plant cell wall, resulting in a channel between the pathogen and host cytoplasm. Machine learning algorithms, developed on N-termini of reported type III effectors, have been used to predict T3SS signal sequences in candidate effectors (Arnold et al., 2009; Samudrala et al., 2009).

Fungi and oomycetes do not possess injection systems but translocate effectors in two steps that each use distinct amino acid motifs for targeting. First, secretion from the pathogen is ensured by a signal peptide or alternative motif on effector proteins. Secondly, the secreted effector requires a motif to facilitate translocation across the plant cell membrane. Once inside the host cell, dedicated cell sorting motifs, for example, nuclear localisation signals (NLSs), can mediate further transport of effectors to their final cellular destination.

Although a signal peptide is sufficient for secretion of apoplastic effectors, an additional host-translocation motif located directly downstream of the signal sequence is generally considered to be required to cross the plant cell membrane. In oomycete effectors, two major classes of host translocation motifs have been identified: the bipartite RXLR-dEER motif in RXLR-like effectors and LxFLAK motif in Crinklers (CRNs). It was shown that secretion of Avr3a by the oomycete Phytophthora infestans does not require the bipartite RXLR-dEER motif, but its mutation abolishes uptake into host plant cells (Whisson et al., 2007). Also, the LxFLAK containing N-termini of CRN 2, 8 and 16 mediate translocation (Schornack et al., 2010).

Fungal effector prediction is complicated by the absence of conserved sequence motifs or structural folds within and between species. An exception is the identification of an N-terminal [YFW]xC motif in effector candidates of the barley powdery mildew fungus. [YFW]xC effector candidate gene expression was upregulated in haustoria-rich epidermal tissue compared to spores and hyphae. Nevertheless, it is unclear whether these proteins are translocated into host cells or remain apoplastic (Godfrey et al., 2010).

A machine learning method, EffectorP, was recently developed to improve effector prediction. Training sets of known effector and non-effector sequences are used for pattern learning based on protein features; for example, many apoplastic fungal effectors are relatively small and cysteine-rich proteins. The authors confirmed the relevance of low-molecular weight and high-cysteine content as prediction criteria, but found that protein net charge and serine and tryptophan abundance are additional important discriminative features. However, EffectorP cannot distinguish apoplastic from translocated effectors (Sperschneider et al., 2015).

### Evading and Suppressing Detection in the Apoplast

Oomycetes and fungi secrete a range of molecules into the apoplast, for example, toxins, cell wall–degrading enzymes and effectors. In this section, we focus on apoplastic effectors that evade or suppress pathogen detection and defence by the plant (see example in Figure 2a). Plants produce papain-like cysteine proteases (PLCPs) that degrade non-self-proteins as part of their defence. To circumvent breakdown of their secreted proteins, pathogens secrete an array of protease inhibitors into the apoplast that target PLCPs, for example, the Avr2 effector of the fungus Cladosporium fulvum that inhibits the tomato PLCP Rcr3. Interestingly, in resistant Cf-2 tomato lines the Rcr3 protein is guarded, enabling Avr2 detection and subsequent activation of ETI. Similarly, P. infestans produces a family of cystatin-like protease inhibitors. These EpiC effectors inhibit a variety of PLCPs including not only tomato Rcr3 and Pip1 but also the Arabidopsis C14 protease, that locally accumulates around haustoria during infection. Another P. infestans effector, AvrBlb2, acts in the plant cytoplasm to prevent C14 secretion (Krüger et al., 2002; Bozkurt et al., 2011).
Molecular patterns of pathogens that are exposed during infection form a source of PAMPs. Chitin and peptidoglycan (PGN) are indispensable components of the fungal and bacterial cell wall, respectively. However, the release of chitin oligomers and PGN fragments, aided by plant hydrolases, effectively activates PTI. To evade detection of these fragments *C. fulvum* secretes the Avr4 and Ecp6 effectors that have complementary activities. Avr4 binds to chitin on the fungal cell wall, thereby preventing secreted plant chitinases access to their substrate (Van den Burg *et al.*, 2006). Chitin oligosaccharides, which nevertheless are released, are sequestered by LysM domains in Ecp6 proteins that occur in many fungal pathogens. This lowers the amount of free chitin oligomers that act as ligands for plant PAMP receptors (De Jonge *et al.*, 2010).

Figure 1  **Plant invasion and effector delivery strategies of bacteria and filamentous pathogens.** The delivery mechanisms and sites of action of effector proteins is largely determined by the pathogen lifestyle, as shown in this schematic drawing of a cross-section through an infected plant leaf. (a) Filamentous fungal and oomycete pathogens may directly penetrate the cuticle and cell wall. In order to gain access to the host cytoplasm, extracellular pathogens may form haustoria: specialised feeding structures that remain separated from the host by the host-derived extrahaustorial membrane (EHM). Effectors are secreted from the haustorium, remain extracellularly (orange), or may be translocated into the host (purple). (b) Other filamentous pathogens enter via stomata. Hyphae can grow between the cells in the apoplast or invade the plant cells and spread intracellularly whilst remaining separated from the plant cytoplasm by the plant-derived extra-invasive hyphal matrix. (c) Bacteria frequently gain access through stomata or wounds. Many Gram-negative species use a type III Secretion System (T3SS) to translocate effectors directly into the host cytoplasm.
Figure 2  Examples of subcellular processes in a plant cell that are targets of pathogen effectors. (a) Plants secrete a range of papain-like cysteine proteases into the apoplast to degrade non-self proteins. (b) Recognition of effectors by resistance proteins leads to effector-triggered immunity. (c) The secretory pathway delivers proteins and cell wall components to the apoplast, constituting a first line of defence. (d) Detection of PAMPs, for example, bacterial flagellin, by PRRs in the plant cell membrane activates an MAPK cascade that results in a PTI response. (e) The hormone salicylic acid (SA) accumulates in response to biotrophic pathogens, and acts together with NPR1 monomers that travel into the nucleus. There, activated NPR1 interacts with TGA transcription factors to promote transcription of SA-responsive genes. Another important defence hormone, jasmonic acid (JA), mediates the COI-dependent degradation of JAZ proteins. MYC2 is released in the process and drives the transcription of JA responsive genes.

Interference with Immune Signalling

Many translocated effectors suppress immune responses (Figure 2d). Interference of effectors with signalling, for example, that initiated by perception of bacterial flagellin by the Arabidopsis receptor Flagellin-Sensing 2 (FLS2), is observed at different levels. Firstly, pathogens can deploy effectors to degrade the PAMP. Monomeric flagellin, but not filamentous flagellin, can be degraded by the Pseudomonas syringae alkaline protease AprA. AprA cleaves within the 22 amino acid epitope of flagellin (flg22) that is recognised extracellularly by FLS2 (Pel et al., 2014).

Secondly, effectors can influence the accumulation of receptor complexes at the membrane. P. syringae effector HopU1 transfers an ADP (adenosine diphosphate)-ribose moiety to the
RNA (ribonucleic acid)-binding protein GRP7. This blocks the interaction of GRP7 with FLS2 mRNA and reduces accumulation of FLS2 protein levels during pathogen infection (Nicaise et al., 2013).

The membrane-localised receptor complex is the third level at which effectors can block signal transduction, for example, of the FLS2-BAK1 complex, which activates PTI in response to flagellar. BAK1 functions as a co-receptor in complexes with different receptors that have diverse roles in immunity or plant growth, by activating downstream components through its cytoplasmic kinase domain. In addition, the cytoplasmic kinase BIK1 associates with FLS2 and BAK1 in the cytoplasm. BIK1 undergoes sequential BAK1-mediated transphosphorylation and autophosphorylation within the activated complex and acts as a positive regulator of immunity (Lin et al., 2014). The bacterial effector AvrPtoB also associates with the FLS2-BAK1 complex and exerts its E3 ubiquitin ligase activity on FLS2 to target it for degradation. BAK1 was also found to interact with AvrPtoB but is not a major target for ubiquitination (Göhre et al., 2008). Another P. syringae effector AvrPphb, a cysteine protease, proteolytically cleaves BIK1 to further block PTI signalling (Zhang et al., 2010).

Activated PRR complexes undergo endocytosis that is associated with attenuation of signalling due to degradation or post-translational modifications. However, research in mammals demonstrated that signal transduction can continue in endosomal vesicles. Also, in plants, effectors could affect immune signalling from endocytic compartments. In plant cells expressing the P. infestans effector Avr3a, the amount of endosomal vesicles with activated FLS2 was reduced by almost half. At the same time, flg22-triggered defence gene activation and reactive oxygen species (ROS) production were strongly reduced. ROS act as signalling molecules to promote defence gene activation and HR. Avr3a interacts in vitro with the dynamin-related protein 2 that is required for FLS2 endosomal vesicle formation. The authors suggest that Avr3a interferes with vesicular trafficking to block FLS2 signalling (Chaparro-Garcia et al., 2015).

Activation of PAMP receptors, such as FLS2, triggers MAPK cascades. MAPK-mediated phosphorylation of plant proteins, including transcription factors and enzymes, initiates a wide range of defence responses. In the MEKK1, MKK4/MKK5 and MKP3/MKP6 cascade, at least two P. syringae effectors are active. Hopf2 prevents MKK5-mediated activation of MKP6. The ADP-ribosyltransferase activity of Hopf2 targets the region of MKK5 that is required for defence gene activation, suggesting that ADP-ribosylation blocks MKK5 activity (Wang et al., 2010). Just downstream, effector HopA1 inactivates MKP3 and MKP6 through dephosphorylation and thereby quenches flg22-induced immune responses (Zhang et al., 2007).

As a countermeasure, to detect effectors that interfere with PTI, the plant NLR SUMM2 guards MKP4. Inactivation of MKP4 by HopA1 in Arabidopsis is detected by SUMM2 resulting in an ETI response (Zhang et al., 2012).

In contrast to the staining of effector-mediated interference observed in the FLS2 signalling cascade, RPM1-interacting protein 4 (RIN4) is single-handedly targeted by at least five P. syringae effectors (AvrB, AvrRpm1, AvrRpt2, AvrPto and HopF2). RIN4 is a central player in the PTI response. It regulates stomatal aperture in a complex with plasma membrane H+\textsuperscript{+}-ATPases. Plants close their stomata in response to PAMP detection to limit bacterial entry. Interestingly, rin4 mutants no longer reopen stomata in response to coronatine (COR), a toxin produced by virulent strains of P. syringae. Moreover, rin4 mutants display enhanced resistance to a T3SS–deficient P. syringae mutant and increased callose deposition upon PAMP perception. Thus, RIN4 acts as a negative regulator of PTI.

AvrB promotes RPM1-Induced protein kinase (RIPK)-mediated RIN4 phosphorylation at threonine 21 and 166, and serine 160. Triple phosphorylated RIN4 promotes H+\textsuperscript{+}-ATPase activity, leading to increased stomatal aperture and thus bacterial growth (Lee et al., 2015). flg22 recognition induces phosphorylation at serine 141 to relieve RIN4-mediated suppression of PTI, for example, visible as increased callose deposition, which also occurs in a RIN4 S141 phosphomimic. However, AvrB-induced phosphorylation overrules flg22-induced ps141, returning the plant to a state of PTI repression, for example, leading to reduced callose deposition (Chung et al., 2014).

RIN4 is also an important player in ETI, where it acts as a guardee. In resistant Arabidopsis accessions, both induction of phosphorylation by P. syringae effector AvrRpm1 or AvrB, and proteolytic cleavage by AvrRpt2, trigger a RPM1- or RPS2-dependent ETI response, respectively. This response can be suppressed by another effector, AvrPphb, that cleaves the kinase RIPK, thereby preventing AvrB-induced phosphorylation of RIN4 and subsequent recognition by RPM1 (Russell et al., 2015).

**Suppression of Cell Death**

The HR that follows effector recognition restricts the growth of biotrophic pathogens and is often associated with programmed cell death (Figure 2b). Suppression of cell death and other ETI responses can render resistance genes ineffective. Initiation of effector-triggered cell death by several resistance proteins is partially mediated by MAPKs. Recognition of P. syringae AvrPto by tomato Pto activates the MAPKKKε and MAPKKKε proteins that are proposed to converge on MEK2. The P. infestans effector PexRD2 is able to suppress MAPKKKε-mediated cell death, but not cell death mediated by overexpression of MAPKKKa or constitutively active MEK2 (King et al., 2014). On the other hand, Xanthomonas axonopodis effector XopQ effectively suppresses MAPKKKa or constitutively active MEK2-mediated cell death, but not MAPKKKε-mediated cell death (Teper et al., 2015). These data suggest that activation of both MAPKKK pathways is required for AvrPto/Pto-triggered HR since expression of either effector is sufficient for impeding cell death. (King et al., 2014; Teper et al., 2015).

The P. infestans effector Avr3a suppresses cell death induced by the elicitor infestin1 (INF1) and several R protein/effector pairs such as Cf-9/Avr9, Cf-4/Avr4 and Pto/AvrPto. The host ubiquitin E3 ligase CMPG1 is critical in this process. Activation of CMPG1 leads to proteosomal degradation of itself and its substrates. Furthermore, the E3 ligase activity of CMPG1 is required for INF1-triggered cell death. Interaction with effector Avr3a stabilises the host E3 ligase and thereby suppresses cell death.
Altering Hormonal Signalling

Salicylic acid (SA) confers local and systemic resistance against (hemi-)biotrophic pathogens, whereas jasmonic acid (JA) and ethylene (ET) induce resistance to necrotrophs. Cross-talk between the SA and JA branch of the immune system allows for optimisation and prioritisation of immune responses. Several plant hormones such as auxin, abscisic acid (ABA), gibberellins and cytokinins further fine-tune responses. For example, auxin plant hormones such as auxin, abscisic acid (ABA), gibberellins and cytokinins further fine-tune responses. For example, auxin and ABA antagonise SA signalling, and vice versa (Pieterse et al., 2012). The flexibility in this extensive signalling network provides an opportunity for pathogens to shift the balance in their favour.

Biotic microbial attackers deploy effectors to attenuate or subvert SA metabolism and responses (Figure 2e). The corn smut fungus Ustilago maydis secretes an effector, Cmu1, that acts as a chorismate mutase. Chorismate is a precursor of SA metabolism and its conversion to prephenate by Cmu1 reduces its availability for SA production (Djamei et al., 2011). Also, in planta expression of the Xanthomonas campestris effector, XopJ, leads to a reduction in SA levels and SA marker gene expression. The bacterial XopJ proteolytically degrades the proteasomal subunit RPT6. In the absence of RPT6, proteasome activity is impaired, leading to accumulation of the ubiquitinated form of NPR1, a positive regulator of SA signalling. Ubiquitinated NPR1 could interfere with activation of NPR1 target genes and reduce immune responses (Ustün and Börnke, 2015).

Instead of decreasing SA levels, effector HaRxL44 of the downy mildew Hyaloperonospora arabidopsis (Hpa) shifts the balance from an SA response to a JA- and ET-based response, which is tailored to defend against necrotrophic pathogens and has limited effectiveness against biotrophic pathogens. HaRxL44 interacts with mediator subunit 19 (MED19a) and causes its proteasomal degradation. MED19a is part of a larger mediator complex that bridges the gap between transcriptional regulators and the transcription machinery. Expression of HaRxL44 or loss of MED19a lead to an increase in JA/ET marker gene expression and reduced resistance to Hpa and other biotrophs (Caillaud et al., 2013).

JA signalling is also induced by the P. syringae effectors HopX1 and HopZ1a. Both effectors promote degradation of JAZ proteins, which are negative regulators of the JA pathway. The subsequent activation of JA/ET defence responses antagonises the SA pathway through molecular cross-talk (Jiang et al., 2013; Gimenez-Ibanez et al., 2014). Several P. syringae strains take it a step further by producing COR, a phytoalexin that structurally mimics the bioactive form of JA. COR binding to the F-box protein coronatine-insensitive 1 (COI1) promotes association and subsequent degradation of JAZ proteins, thereby promoting JA signalling (Tanaka et al., 2015).

The necrotrophic fungus Botrytis cinerea benefits from induction of SA to antagonise JA. B. cinerea produces an exopolysaccharide (EPS) that promotes SA accumulation and NPR1-mediated suppression of JA-induced defence genes (El Oirdi et al., 2011). See also: Jasmonic Acid Signalling: Systemic Signalling in Plant Defence

Modifying Host Vesicular Transport

Transport by vesicle trafficking between different intracellular compartments and the extracellular environment is important for plant immunity. The plant secretory or exocytic pathway is required for delivery of plant proteins mediating the first line of defence at the plasma membrane or in the apoplast (Figure 2c). The P. syringae effector HopE1 targets the microtubule network to contribute to virulence. HopE1 interacts with microtubule-associated protein 65 (MAP65) in a calmodulin-dependent manner, which becomes redistributed from the microtubule network to the cytoplasm. Both map65-1 mutants and HopE1 overexpressing plants display reduced secretion of pathogenesis-related protein PR-1 and diminished callose deposition (Guo et al., 2016).

Secretion is also impaired by the P. infestans effector Avr1 that interacts with Sec5, a subunit of the exocyst complex. Sec5 is required for tethering of vesicles to their target membrane. Silencing of Sec5 enhances susceptibility to P. infestans and abolishes callose deposition upon P. syringae infiltration. In planta, expression of Avr1 mimics the loss of Sec5 as callose deposition is impaired, suggesting that Avr1 manipulates plant immunity through interaction with Sec5 (Du et al., 2015). See also: Callose and Related Glucans

Other cellular traffic is affected by the P. infestans effector, PexRD54, that interacts with the autophagy-related protein ATG8CL, and co-localises to autophagosomes. During autophagy, cellular components are degraded to recycle building blocks and energy. PexRD54 stimulates ATG8CL-mediated autophagy as expression of PexRD54 increases the number of ATG8CL-labelled autophagosomes. However, PexRD54 outcompetes the endogenous selective autophagy cargo receptor Joka2 in this process, suggesting it may steer autophagy towards defence related components (Dagdas et al., 2016).

Transcriptional Reprogramming of the Cell

Activation of immune pathways induces transcriptional reprogramming to activate defence responses. Pathogens therefore deploy nuclear-localised effectors that can modulate nuclear processes. The term ‘nucleomodulin’ has emerged to describe effectors that modify chromatin structure, affect epigenetic regulation or alter transcription (Bierne and Cossette, 2012).

Many Xanthomonas species encode transcription activator-like effectors (TALEs) that are injected into plant cells via the T3SS.
The overall structure of TALEs is conserved; at the N-terminus, a type III translocation signal required for transport into the plant cell, and at the C-terminus NLSs and an acidic transcriptional activation domain for import into the nucleus and activation of plant gene transcription, respectively. The middle region consists of a variable number of near-perfect repeats of 33–35 amino acids long that mediate DNA binding. The identity and specificity of TALEs is largely determined by residues 12–13 within each repeat. These are referred to as the repeat-variable diresidues (RVD) and almost 20 distinct combinations of amino acids occur at this position. The RVD is the only part of the repeat that directly interacts with the DNA helix, with the remainder of the repeat fanning out behind the RVD. Each RVD associates with a different affinity with adenine, cytosine, guanine and thymine. Some RVDs interact almost exclusively with a single nucleotide such as N1 with adenine or HD with cytosine. Others have similar binding affinities for several nucleotides as NN can bind both guanine and adenine, and NS binds all four nucleotides. The combination of highly specific and more promiscuous RVDs within a repeat region results in both binding specificity and flexibility (Bogdanove et al., 2010).

Although the TALE DNA-binding cipher has been broken, relatively little is known about the target genes of TALEs. AvrBs3 was the first TALE for which targets were identified on the basis of induced expression. These UPA (upregulated by AvrBs3) genes included putative auxin–induced genes and α-expansins. Of these, UPA20, a basic helix-loop-helix transcription factor, is postulated to be a direct target as its induction does not require de novo protein synthesis and interaction of AvrBs3 with a UPA20 promoter fragment was found using electrophoretic mobility shift assays. Overexpression of UPA20 is sufficient to induce the hypertrophy (cell enlargement) phenotype characteristic for plant tissue transiently overexpressing AvrBs3 (Kay et al., 2007).

Besides activating plant genes that increase plant susceptibility to disease, TALEs can also be responsible for their own demise. Transcription of the pepper Bs3 and rice Xa27 R genes is specifically induced by the TAL effectors AvrBs3 and AvrXa27, respectively, due to compatible binding elements in the R gene promoter regions. This type of R gene is also referred to as executor gene, because the product does not recognise an effector or guardee, but only functions to execute an immune response or cell death. In line with this function, these executor genes do not share sequence resemblance to NLRs (Bogdanove et al., 2010).

Also, the oomycete-specific CRN effectors predominantly reside in the nucleus upon translocation into host cells (Schornack et al., 2010). The nuclear-localised P. sojae effector PsCRN108 contains a helix-hairpin-helix motif that is associated with DNA binding. Arabidopsis lines overexpressing PsCRN108 displayed reduced accumulation of almost half of the heat shock proteins (HSP) transcripts. HSPs aid in proper folding of proteins and prevent the formation of protein aggregates. In line with their function, HSP expression is upregulated in response to various abiotic and biotic stresses. The authors propose that PsCRN108 suppresses basal defences by binding to heat shock elements in HSP promoters, thereby interfering with the binding of endogenous transcription factors. Markedly, PsCRN108 overexpression lines of Nicotiana benthamiana and Arabidopsis display enhanced disease susceptibility (Song et al., 2015).

While the effectors described above reprogram host cells themselves, another effective strategy is to interfere with host transcription factors. The potato-membrane-bound NAC transcription factors NPT1 and NPT2 are associated with the endoplasmic reticulum (ER) in their dormant state, but are activated upon perception of Phytophthora. Subsequently, re-localisation of the TFs to the nucleus occurs, presumably to induce defence-associated transcriptional reprogramming. To negate these effects, the P. infestans RXLR effector Pi03192 associates with the NPTs on the ER, where they maintain dormant so that translocation to the nucleus does not occur (McLellan et al., 2013).

Sweet Rewards

To acquire nutrients from the host, pathogens modify expression of the evolutionary conserved family of plant SWEET genes that encode for sugar transporters. The X. oryzae TAL effector PhXo1 was shown to directly interact with the rice SWEET11 (OsSWEET11) promoter and activate transcription. Loss of PhXo1 expression or mutations in the OsSWEET11 binding element lead to reduced pathogen growth. Pathogenicity can be regained by expression of effector AvrXa7 that activates OsSWEET14 (Chen et al., 2010).

Exploitation of host sugar transporters is not limited to Xanthomonas as P. syringae infection induced expression of AtSWEET4, AtSWEET5, AtSWEET7, AtSWEET8, AtSWEET10, AtSWEET12 and AtSWEET15. Also, the fungal pathogen Golovinomyces cichoracearum induced a distinct, but partially overlapping AtSWEET subset (Chen et al., 2010). As these latter pathogens do not have TALEs, the mechanism of SWEET gene induction is unknown.

Future Perspectives and Applications

As more pathogen effectors are discovered and their modes of action in supporting disease susceptibility in plants are unravelled, the obtained knowledge can be used to develop new methods for durable disease resistance. Here, three strategies are presented to illustrate the potential of effector-assisted breeding for resistance.

Knowledge of the effector repertoire of a pathogen can assist breeders in the identification of new R genes and advance the characterisation of previously found ones. Firstly, R gene identification can be accelerated by screening germplasm with effectors using transient expression systems such as Agrobacterium tumefaciens. A similar approach can identify R genes with extended effector specificity when different allelic variants of an effector are tested. Secondly, new cultivars or wild progenitors can be classified on the basis of effector responses. Natural R gene stacks can be broken down to a collection of effector recognition specificities (to Mendelize R genes). In addition, when R gene resistance is quantitative and only a partial resistance effect is observed in the field, breeding is simplified when it can be
linked to an easier quantifiable trait such as effector recognition. For example, field resistance mediated by Rpi-Smira2 in potato cultivar ‘Sarpo Mira’ is associated with recognition of RXLR effector AvrSmira2 (Rietman et al., 2012). Lastly, the identification of effectors essential for pathogen virulence by spatiotemporal monitoring of effector repertoires allows for rationalised gene deployment and may provide more durable resistance (Vleeshouwers and Oliver, 2014).

A second recently emerged strategy focuses on the plant targets of effectors; in particular, plant genes required for pathogen infection. Loss of these so-called Susceptibility (S) genes confers resistance to pathogen infection. In a screen for interactors of the HopZ family of P. syringae effectors, the Arabidopsis MLO2 protein was identified as a target. Interestingly, mlo resistance has been successfully used in barley against powdery mildew infection for over 70 years. Similar resistance is observed in Arabidopsis mlo2 mutants. S genes will be deployed only if the benefit gained by increased pathogen resistance outweighs the negative effect of gene loss. Alternatively, a germplasm set can be screened for alleles of the S gene in which the protein function is maintained but effector interaction is lost (Dangl, 2013).

Similar to the concept of R gene stacking, TAL effector–binding elements in executor gene promoter regions can be stacked. The promoter of Xa27 in rice conferring resistance to AvrXa27-containing X. oryzae strains was expanded with six additional TALE binding elements. The rice plants gained resistance to a variety of strains that are virulent on wild-type Xa27 plants. The difficulty in expanding promoter regions is the risk of unintentionally introducing endogenous regulatory elements that may be activated in response to environmental or developmental cues (Hummel et al., 2012).

The technological progress made in the past decade, especially the availability of low-cost large-scale sequencing techniques, has enabled researchers to determine the effector repertoire of many pathogens. The identification of plant targets for pathogen effectors has subsequently taken flight. With interest we await how these fascinating scientific discoveries will be translated to disease-resistant crops in the future.

References


**Further Reading**


