Effector Biology of Plant-Associated Organisms: Concepts and Perspectives

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Every plant is closely associated with a variety of living organisms. Therefore, deciphering how plants interact with mutualistic and parasitic organisms is essential for a comprehensive understanding of the biology of plants. The field of plant–biotic interactions has recently coalesced around an integrated model. Major classes of molecular players both from plants and their associated organisms have been revealed. These include cell surface and intracellular immune receptors of plants as well as apoplastic and host-cell-translocated (cytoplasmic) effectors of the invading organism. This article focuses on effectors, molecules secreted by plant-associated organisms that alter plant processes. Effectors have emerged as a central class of molecules in our integrated view of plant–microbe interactions. Their study has significantly contributed to advancing our knowledge of plant hormones, plant development, plant receptors, and epigenetics. Many pathogen effectors are extraordinary examples of biological innovation; they include some of the most remarkable proteins known to function inside plant cells. Here, we review some of the key concepts that have emerged from the study of the effectors of plant-associated organisms. In particular, we focus on how effectors function in plant tissues and discuss future perspectives in the field of effector biology.

Interactions with the biota are critical to plant life. In nature, every single plant is closely associated with a variety of living organisms, particularly microbes (Hines and Zahn 2009; Rodriguez et al. 2009; Redford et al. 2010). Plants are repeatedly attacked by a multitude of pathogens and pests, including viruses, bacteria, fungi, oomycetes, nematodes, and insects (Agrios 2005). Some plants can also establish parasitic relationships with other plants (Westwood et al. 2010). Given the ubiquity of plant-associated organisms in nature, deciphering how plants interact with mutualistic and parasitic organisms is essential for a comprehensive understanding of the biology of plants.

Despite the diversity and multitude of organisms that interact with plants, a series of common notions define our understanding of plant–biotic interactions. The field of plant–biotic interactions has recently coalesced around an integrated model that applies to all types of pathogens and mutualists (Dodds and Rathjen 2010). Both plants and associated organisms contribute molecular players that dictate the outcome of the plant–biotic interaction. For example, plants contribute cell surface and intracellular immune receptors, and the colonizing organisms produce a repertoire of effectors that modulate plant processes, including the induction and suppression of plant defenses (Fig. 1). This article focuses on effectors, molecules secreted by plant-associated organisms that alter host-cell structure and function (Hogenhout et al. 2009).

Effectors have generally evolved to enable parasitism, for example, by suppressing plant immunity or by modifying plant physiology to support growth and spread of the parasite. These responses are collectively known as effector-triggered susceptibility (ETS) (Fig. 1A) and are achieved through the perturbation of a set of host processes that we define as effector-targeted pathways (ETPs) (Table 1). However, effectors can also "trip the wire" and activate plant immune receptors, a response known as effector-triggered immunity (ETI), when it is mediated by intracellular immunoreceptors of the nucleotide-binding leucine-rich repeat (NB-LRR) class (Fig. 1B). In contrast, plant cell surface receptors recognize apoplastic effectors as well as conserved pathogen molecules known as pathogen-associated molecular patterns (PAMPs) (Fig. 1B) (Boller and Felix 2009; Thomma et al. 2011). These receptors, which are typically receptor kinases and receptor-like proteins, are collectively known as pattern recognition receptors (PRRs). The resulting immune response is known as PRR-triggered immunity or PAMP-triggered immunity (PTI).

Many pathogen effectors have evolved to suppress PTI, providing additional twists to the arms race coevolution between pathogens and plants (Chisholm et al. 2006; Jones and Dangl 2006; Dodds and Rathjen 2010). Effectors are therefore targeted by dynamic and sometimes opposite evolutionary forces because they can have either positive or negative fitness effects on the pathogen or pest, depending on the host plant genotype. As a consequence, effector genes are among the most rapidly evolving genes in the genome of plant-associated organisms.

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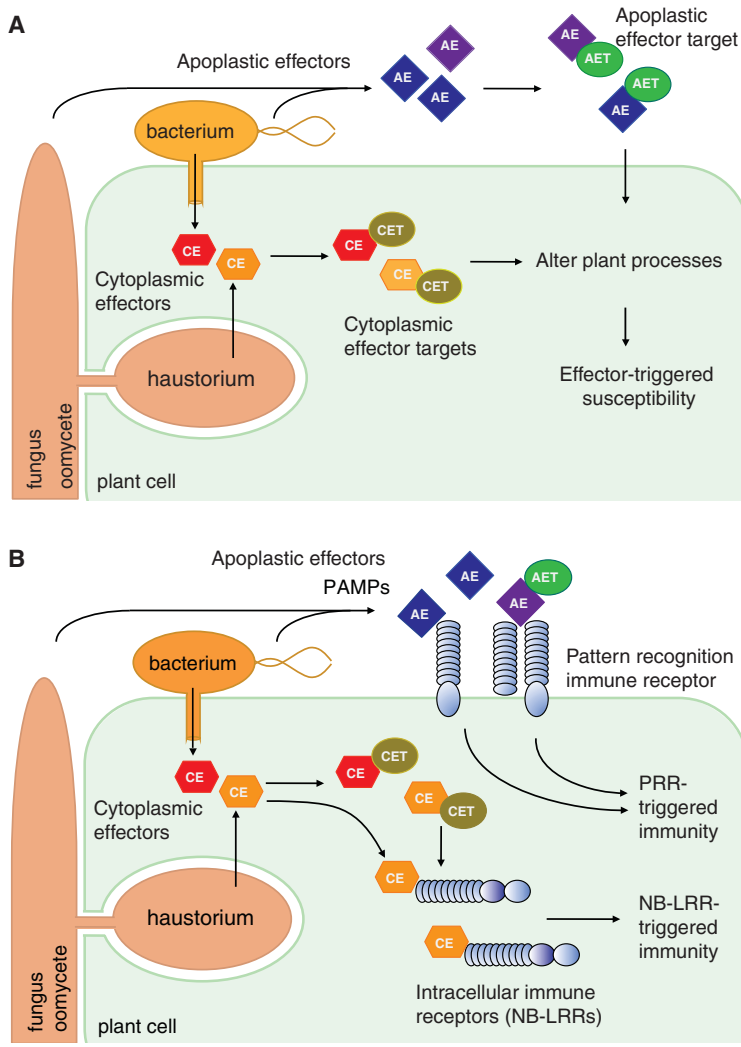

Figure 1. The concept of effectors in plant immunity. Infectious pathogens such as bacteria, fungi, oomycete, and nematodes deliver effectors at the interface of the host plant (apoplastic effectors, AE) or inside the cell (cytoplasmic effectors, CE). Host-translocated (cytoplasmic) effectors are delivered into the host cytoplasm through a type-III secretion pilus or specialized infectious structures called haustoria that form within the cell. Pathogen effectors traffic to various compartments, bind, and manipulate different host proteins called targets. Depending on their localization in the cells, these targets are designated as apoplastic effector target (AET) and cytoplasmic effector target (CET). Effector–target interactions impact the outcome of the interaction between the pathogen and its host. In susceptible genotypes (*A*), these molecular interactions can alter plant cell processes and suppress immune responses, leading to effector-triggered susceptibility (ETS) and host colonization. In resistant genotypes (*B*), these interactions are perceived by key sensing receptors of the immune system that, in turn, stop pathogen growth. Cell surface pattern recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs), apoplastic effectors, and/or apoplastic effector–target interactions to initiate PRR-triggered immunity (PTI). Intracellular nucleotide-binding receptors (NB-LRR) induce NB-LRR-triggered immunity (ETI) on recognition of cytoplasmic effectors and/or cytoplasmic effectors–target interactions. (Modified from Dodds and Rathjen 2010.)

Effectors have emerged as a central class of molecules in our integrated view of plant–microbe interactions. Understanding the molecular functions of effectors is widely accepted as essential for a mechanistic understanding of the processes underlying plant colonization by invading organisms. Effectors can also serve as molecular probes for unraveling key plant processes. Their study has significantly contributed to advancing our knowledge of plant hormones, plant development, plant receptors, signal transduction pathways, and epigenetics. Many pathogen effectors are extraordinary examples of biological innovation; they include some of the most remarkable proteins known to function inside plant cells. Among such remarkable effectors, we highlight the transcription activator-like (TAL) effectors of *Xanthomonas* bacteria, modular proteins that bind specific plant promoter elements to activate plant gene transcription (Boch et al. 2009); the bacterial effectors AvrPtoB and coronatine, which mimic plant E3 ubiquitin ligases (Janjusevic et al. 2006; Rosebrock et al. 2007) and the hormone

Table 1. Definition of terms used in this manuscript

Term	Definition
Apoplastic effector	Effectors that are secreted to the host extracellular space. They interact with host extracellular proteins and can also be recognized by PRRs.
Cytoplasmic (host-translocated) effector	Effectors that are translocated inside the host cell through specialized microbial structures such as the type-III secretion system or infection vesicles and haustoria. Inside the host cell, they traffic to different subcellular compartments where they exert their functions.
Effector	Microbial and pest secreted molecules that alter host-cell processes or structure generally promoting the microbe lifestyle. Effector functions are as diverse as suppressing immune responses or enhancing access to nutrients.
Effector helper	Host molecules genetically upstream of the effector-mediated target modulation that are required by the effector to exert its function but do not necessarily have a direct impact on the cellular process that the effector is targeting.
Effector target	Host molecules that the effector modifies to manipulate host processes, thus enhancing the microbe fitness and ability to colonize and spread to other hosts in susceptible plants.
Effector-targeted pathway (ETP)	Natural cellular physiological processes such as RNA silencing, innate immunity, intracellular trafficking, cell signaling, and transcription that are manipulated by effectors for the pathogen benefit.
Effector-triggered susceptibility (ETS)	The outcome of the deployment of effector molecules that favors pathogen virulence. ETS can be the result of suppressing innate immunity or enhancing the pathogen nutrient uptake.
Host susceptibility factor	Host molecules that are hijacked by the invading microbe to support effector functions. Both host targets and host helpers are susceptibility factors; they operate in conjunction to the advantage of the pathogen.
Nucleotide-binding leucine-rich repeat (NB-LRR) proteins	Intracellular (cytoplasmic) receptor proteins specific of plants that have evolved to recognize pathogen effectors or their impact on cellular processes. They contain a nucleotide-binding site (NB) and leucine-rich repeat (LRR) domains coupled to either coiled-coil (CC) or Toll/interleukin-1 receptor (TIR) motifs in the amino-terminal region. NB-LRR receptor activation usually leads to a cell death response known as the hypersensitive response (HR).
Pathogen-associated molecular patterns (PAMPs)	Microbial-derived signatures that are conserved across an entire class of microbes and that contribute to organism fitness. These molecules are often present in nonpathogenic microorganisms; therefore, they are also known as microbe-associated molecular patterns (MAMPs)
Pattern recognition receptors (PRRs)	Cell surface localized receptors that recognize conserved microbial patterns (PAMPs) or apoplastic effectors. PRRs allow the plant to discriminate between self and nonself molecules given their ability to sense microbial molecules. There are two classes of PRRs: receptor-like kinases (RLKs) and receptor-like proteins (RLPs). The extracellular domains that mediate recognition comprise LRRs or LysM motifs.

jasmonoyl-isoleucine (JA-Ile) (Weiler et al. 1994; Bender et al. 1999), respectively; SAP11 of phytoplasma bacteria that binds host TCP transcription factors and destabilizes CIN-TCPs to alter plant development and inhibit jasmonate (JA) synthesis (Sugio et al. 2011); and the viral protein P19 that binds double-stranded RNA with high affinity in order to suppress RNA interference (RNAi) in host plant cells (Lakatos et al. 2004).

This article summarizes and discusses some of the key concepts that have emerged from the study of the effectors of plant-associated organisms. In particular, we focus on how effectors function in plant tissues and discuss future perspectives in the field of effector biology.

EFFECTORS: USAGE AND DEFINITION

The term "effector" has become widely used in the field of plant–biotic interactions, begining ~12 years ago (for review, see Hogenhout et al. 2009). This rise in usage was in part because the neutral term "effector" addresses the conceptual limitations of teleological terminology such as "avirulence" and "elicitor." These outdated terms misrepresented the fact that the same molecules can have both positive and negative effects on the pathogen depending on the host genotype (see discussions in Hogenhout et al. 2009). Plant bacteriologists initially equated effectors to the proteins delivered inside plant cells via the type-III secretion system (T3SS). However, the usage broadened once it became evident that pathogens use a multitude of mechanisms to translocate proteins inside plant cells. More recently, the term effector has also been extended to secreted proteins of mutualistic microbes that contribute to the establishment of a symbiotic relationship with plants (Kloppholz et al. 2011; Plett et al. 2011).

As previously discussed, we favor a broad, inclusive definition of effectors as "molecules secreted by plant-associated organisms that alter host-cell structure and function" (Hogenhout et al. 2009). This broader definition is particularly suitable when only limited information about the function of the "effector" molecules is known, for instance, in the case of effectors with avirulence or elicitor activities, that is, that trigger plant defense responses. Once more information about the functions of an "effector" is revealed, it is preferable to use descriptors that reflect these specific activities (for instance, protease inhibitor).

Because of the prominence of immunity in the study of plant–microbe interactions, there is a perceptible tendency in the literature to assume that effectors always function as immunosuppressors. This view is too narrow; there is more to effector function than suppression of immunity. Although it appears that the majority of *Pseudomonas syringae* T3SS effectors function as immunosuppressors, many other distinct functions have been assigned to pathogen effectors. The TAL effectors of xanthomonads activate host genes involved in processes as diverse as cell size, sugar transport, and epigenetics (Duan et al. 1999; Chen et al. 2010; Domingues et al. 2010; de Souza et al. 2012). Aster yellows phytoplasmas secrete SAP proteins that directly perturb plant development to increase the mass of host green tissue and the likelihood of transmission by their insect vector (MacLean et al. 2011; Sugio et al. 2011). Surely, many more diverse functions of effectors remain to be discovered.

Another issue worth highlighting is the case of necrotrophs, pathogens that colonize dead plant tissues. Many necrotrophs secrete toxin effectors that actively cause tissue necrosis thus contributing to pathogen virulence (Mengiste 2012). Paradoxically, some necrotrophic fungi kill plants by activating immune receptors via secreted toxin effectors. In one well-characterized example, the *Arabidopsis LOV1* gene, a member of the NB-LRR resistance gene family, was shown to determine susceptibility to the fungus *Cochliobolus victoriae* and sensitivity to the toxin victorin (Lorang et al. 2007). In this case, the tables are turned—the immunoreceptor becomes a liability for the plant, contributing to susceptibility to a fungal disease.

EFFECTORS TRAFFIC TO DIFFERENT CELLULAR COMPARTMENTS IN PLANT CELLS

Microbes and other plant-associated organisms secrete effectors to different sites in the host plant. Several pathogens, including fungi and oomycetes, colonize plant intercellular spaces and secrete effectors that target defenses in the plant apoplast (Fig. 1A). Classic examples include the effectors of the dothidiomycete leaf mold fungus *Cladosporium fulvum* Avr2, Avr4, and ECP6, which target various extracellular processes of the host plant tomato (van den Burg et al. 2006; Bolton et al. 2008; van Esse et al. 2008). Whereas Avr2 is an inhibitor of tomato apoplastic cysteine proteases, ECP6 interferes with the perception of *C. fulvum* cell wall chitin by tomato cell surface immune receptors (de Jonge et al. 2010). Other microbes deliver their effectors inside plant cells, typically through specialized structures (Fig. 1A). Several fungi and oomycetes use hyphal extensions called haustoria to push inside plant cells, where they remain enveloped with a plant-derived membrane, resulting in a close interface through which effectors are thought to traffic inside host cells (Dodds et al. 2009; de Jonge et al. 2011). Effectors of the rice blast fungus *Magnaporthe oryzae* accumulate and translocate into host cells at a membranous cap known as the biotrophic interfacial complex (BIC) (Valent and Khang 2010). Bacteria rely on specialized secretion pili, such as pseudomonad and xanthomonad T3SSs (Buttner and He 2009). The crown gall bacterial pathogen *Agrobacterium tumefaciens* and several related species use a type-IV secretion apparatus to transfer a DNA fragment (T-DNA) inside plant cells in complex with bacterial virulence proteins such as VirD2 and VirE2 (Christie 2004). The T-DNA stably inserts into the plant genome to express biosynthetic enzymes for plant hormones and amino acids, resulting in the formation of tumors and production of bacterial nutrients. Phytoplasmas, plant pathogenic bacteria that, similarly to animal parasitic mycoplasmas diverged from Gram-positive bacteria through loss of outer cell wall and dramatic genome reductions, are intracellular obligate pathogens of plants (Hogenhout and Loria 2008). They directly secrete effector proteins inside host plant cytoplasm using the general Sec-dependent (type II) secretory pathway, and, unlike extracellular bacterial phytopathogens, do not appear to require specialized secretory systems for pathogenesis (Kakizawa et al. 2004).

Once translocated into the plant cytoplasm, effectors can traffic to different subcellular compartments, including organelles and various membrane compartments. A large number of effectors accumulate in the plant nucleus (see also Caillaud et al. 2012, this volume). Cytoplasmic effectors, such as xanthomonad TAL effectors, phytoplasma SAP11, and *Phytophthora* Crinklers (CRNs), carry nuclear localization signals (NLSs) enabling them to subvert the plant protein importin-α to mediate import into the plant nucleus (Van den Ackerveken et al. 1996; Bai et al. 2009; Schornack et al. 2010). The *P. syringae* type-III effector HopG1 targets plant mitochondria (Block et al. 2010), whereas HopI1 localizes to plant chloroplasts, where it suppresses salicylic acid synthesis and disrupts the thylakoid stack structure (Jelenska et al. 2007). Another *P. syringae* type-III effector, the acetyltransferase HopZ1a, targets the plant cytoskeleton (Lee et al. 2012a). HopZ1a disrupts microtubule networks, subsequently interfering with the plant secretory pathway and suppressing cell-wall-mediated defense (Lee et al. 2012a). Recently, some oomycete effectors, notably *Phytophthora infestans* AVRblb2 and AVR2, were shown to relocalize from the plasma membrane to accumulate around haustoria inside infected plant cells (Bozkurt et al. 2011; Saunders et al. 2012). This suggests that these effectors associate with dynamic focal processes that take place at pathogen penetration sites (Underwood and Somerville 2008). T3SS effectors of the plant pathogenic bacteria *P. syringae* and *Xanthomonas campestris* have been reported to target secretory pathways, suggesting that interference with polarized vesicle trafficking is a common pathogenesis process (Bartetzko et al. 2009; Kaffarnik et al. 2009; Nomura et al. 2011). One example is the *P. syringae* effector HopM1 that localizes to the *trans*-Golgi network/early endosome to destabilize the host protein AtMIN7, also known as BEN1 regulating

endosomal recycling (Tanaka et al. 2009; Nomura et al. 2011).

TEMPORAL ASPECTS OF PATHOGEN DEPLOYMENT OF EFFECTORS

Effectors not only need to be at the right location during infection but they also require deployment at the right time for successful colonization. Indeed, pathogens secrete different sets of effectors in sequential waves at different phases of parasitism. Pathogens have convergently evolved different ways to achieve this temporal regulation. At this point, these processes are better understood for animal parasites. During infection of vertebrate cells, the protozoan pathogen *Toxoplasma gondii* secretes effectors by sequential triggering of three distinct parasite organelles known as micronemes, rhoptries, and dense granules, at different phases during its intracellular growth (Carruthers and Sibley 1997). Bacterial enteropathogen *Salmonella enterica* uses a sorting platform involving chaperone proteins to sort and load the effectors to be secreted in a defined order via T3SS (Lara-Tejero et al. 2011). In bacterial plant pathogens, T3SS is also used to deliver effectors into plant cells in a regulated manner. T3SS effectors released early in the epiphytic growth of *P. syringae Psy*B728a are important for pathogen survival during this phase and T3SS effectors such as HopAA1 and HopZ3 may play specific roles during epiphytic growth of this pathogen on specific hosts (Lee et al. 2012b). Phytoplasmas, which require insect vectors for transmission to plants, differentially regulate effector genes for insect and plant colonization (Toruno et al. 2010; MacLean et al. 2011). Fungal pathogens *Colletotrichum higginsianum* and *Colletotrichum graminicola*, infecting *Arabidopsis thaliana* and maize, respectively, use transcriptional regulation to synthesize and secrete different sets of effectors and enzymes important for different phases of infection: The biotrophic phase is augmented by effectors and secondary metabolism enzymes whereas the necrotrophic phase brings in hydrolases and transporters (O'Connell et al. 2012). In *C. higginsianum*, the regulation is further refined to the prepenetration stage where ChCEs (*C. higginsianum* candidate effectors) are up-regulated presumably in preparation for host-cell ingress (Kleemann et al. 2012). In the oomycete *Phytophthora*, several RXLR-type effectors are induced at the cyst germination stage or at early infection stage, whereas several necrosis-inducing Nep1-like proteins (NLPs) are expressed at late infection stages, suggesting that they contribute to the necrotrophic phase (Qutob et al. 2002; Haas et al. 2009). In *P. infestans*, an effector known as SNE1 is transcriptionally regulated and expressed during the biotrophic phase, presumably to maintain biotrophy and to suppress the actions of a cell-death-inducing NLP that is expressed during the necrotrophic phase of infection (Kelley et al. 2010). Once the effectors reach the right location in host cells at the right time, they can interact with host proteins to exert their functions for successful invasion.

HOST-CELL TARGETS OF EFFECTORS

Effectors have evolved to bind and modify "target" host molecules to perturb plant processes (Figs. 1A and 2A). Essentially, any effector activity that would increase the fitness of the microbe, its ability to colonize the host plant, and spread to other hosts could potentially evolve. Some effectors, particularly bacterial T3SS effectors, are enzymes that biochemically modify host molecules, typically impeding their function or eliminating them (Cunnac et al. 2009; Deslandes and Rivas 2012). The enzymatic activities of effectors are diverse and include protease, hydrolase, phosphatase, kinase, transferase, and ubiquitin ligase activities (Shao et al. 2003; Abramovitch et al. 2006b; Janjusevic et al. 2006; Fu et al. 2007; Lee et al. 2012a; Rodriguez-Herva et al. 2012; van Damme et al. 2012). Other effectors do not carry enzymatic activities and act by binding host proteins to modulate their

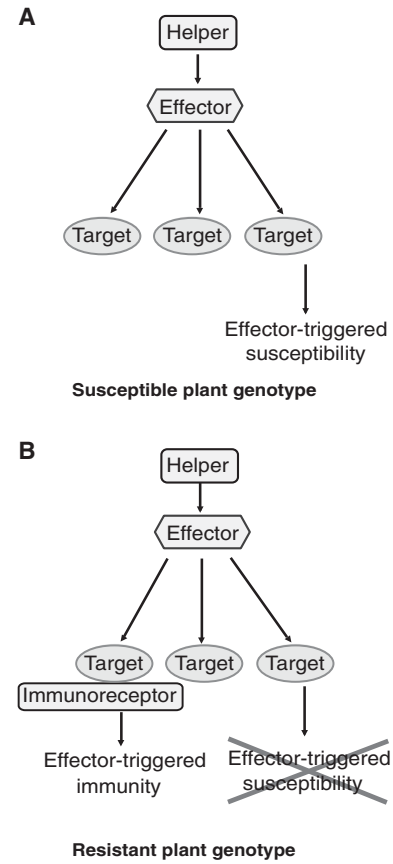

Figure 2. Effectors and their host-cell helpers and targets. This genetic model describes the position of pathogen effectors and their respective host-cell helpers and targets in the signaling pathways leading to susceptibility (*A*) and resistance (*B*). Effector targets and effector helpers are distinct plant susceptibility factors. Pathogen effectors recruit host helper proteins and interact with them for proper function. Activated effectors bind cognate targets, manipulate them, and form active effector–target complexes. In a susceptible interaction, the effector–target complex is not recognized and results in an altered cellular state of effector-triggered susceptibility (ETS). In a resistant interaction, this complex triggers host recognition by cognate immune receptors leading to effector-triggered immunity (ETI).

functions. Many such effectors inhibit plant enzymes such as kinases, proteases, glucanases, and peroxidases (Tian et al. 2004, 2007; Rooney et al. 2005; Damasceno et al. 2008; Xiang et al. 2008; Song et al. 2009; Hemetsberger et al. 2012). Another group of effectors have evolved to bind nucleic acids acting as modulators of gene expression. *Xanthomonas* TAL effectors directly bind elements in plant gene promoters to activate gene expression of host genes that benefit the pathogen (Duan et al. 1999; Boch et al. 2009; Domingues et al. 2010; de Souza et al. 2012). Among the host susceptibility genes that are induced by TAL effectors are the SWEET sugar transporters that are thought to release sugar to contribute to the nutrition of the invading bacteria (Chen et al. 2010).

Our knowledge of the identity of the host targets of effectors remains incomplete given the large number of uncharacterized effectors from a multitude of pathogens and symbionts. The full range of biochemical mechanisms by which effectors manipulate their targets is also poorly known. Initially, effectors were thought to be quite specific, targeting and modifying individual plant proteins. It now appears that effectors can be promiscuous; a single effector can associate with multiple plant proteins and may even affect distinct processes in the host plant (Fig. 2A). Classic examples include the *P. syringae* type-III effectors AvrPto and AvrPtoB that directly bind and interfere with several immune receptor kinases in tomato and *Arabidopsis* thereby interfering with multiple PTI signaling pathways (Abramovitch et al. 2006a; Göhre et al. 2008; Shan et al. 2008; Xiang et al. 2008; Gimenez-Ibanez et al. 2009; Zeng et al. 2012) and HopM1, which was shown by Nomura et al. (2006) to degrade a number of plant proteins in addition to AtMin7. Similarly, phytoplasma protein effector SAP11 binds to both plant class I and II TCP transcription factors but only destabilizes the latter (Sugio et al. 2011).

HOST-CELL HELPERS OF EFFECTORS

Not all effector-associated plant proteins are host targets that are modified by the effectors. Some plant proteins biochemically modify the effectors, contribute to effector maturation inside the plant cytoplasm, or serve as cofactors that form biochemically active complexes with the effector. Other host proteins enable the effectors to traffic to their final subcellular destination. Such proteins are best viewed as "effector helpers" rather than genuine "effector targets." These effector-associated helper proteins can be distinguished from effector targets because they genetically function upstream of the effector-mediated target modulation (Fig. 2B). Loss-of-function mutations in effector helpers typically interfere with the ability of the effector to perform its activity but do not necessarily affect the biological process targeted by the effector. In contrast, mutations in effector targets usually phenocopy the phenotype induced by the effector, such as suppression of host immunity or altered plant development (Block et al. 2008; Block and Alfano 2011; Canonne and Rivas 2012; Deslandes and Rivas 2012).

There are several vivid examples of host effector helpers. The type-III effector AvrRpt2 is a cysteine protease that is delivered inside plant cells by *P. syringae* as an inactive enzyme. Once inside the host cell, AvrRpt2 requires cyclophilin, a chaperone that catalyzes and accelerates protein folding (Coaker et al. 2005, 2006). Cyclophilin acts as a cofactor, activating the self-processing of AvrRpt2 and leading to the cleavage of AvrRpt2 target protein RIN4 (Coaker et al. 2005). Similarly, another *P. syringae* type-III effector, HopZ1a, is activated inside the host cell by phytic acid to become a functional acetyltransferase that acetylates tubulin, a plant target of HopZ1a (Lee et al. 2012a). Other type-III effectors require host-mediated biochemical modifications, such as myristoylation and phosphorylation, to become functional. For example, myristoylation of AvrRpm1 and AvrPto is required for both their avirulence and virulence activities, suggesting that plant N-myristoyltransferases are involved as host-cell helpers (Nimchuk et al. 2000; Anderson et al. 2006).

Another example of a host-cell helper is the plant protein importin-α that mediates nuclear trafficking and is recruited by several pathogen effectors including TAL effectors, SAP11, and Crinklers (Szurek et al. 2001; Bai et al. 2009; Schornack et al. 2010). TAL effector AvrBs3 in association with importin-α enables its accumulation in the host-cell nucleus where it directly binds to plant DNA harboring a conserved promoter element, which is its "real" target, to activate plant gene expression (Szurek et al. 2001; Kay et al. 2007, 2009). TAL effectors also likely associate with components of the plant transcriptional machinery. One candidate is the general transcription factor subunit TFIIAγ, which is encoded by the rice recessive *Xanthomonas* resistance gene *xa5* and may serve as a cofactor that enables TAL effectors to induce host gene expression (Iyer and McCouch 2004).

Effector helpers and targets are host proteins that are usurped or manipulated by the invading organism to enable the effectors to function and establish a state of effector-triggered host susceptibility. Hence, collectively they are both host susceptibility factors. However, the distinction between helpers and targets is conceptually useful for a mechanistic dissection of effector function, particularly because mutations in these two classes of host susceptibility factors yield different phenotypes.

EFFECTOR-TARGETED PATHWAYS: FUNCTIONAL REDUNDANCY AMONG PATHOGEN EFFECTORS

Pathogens must perturb key plant processes to establish a state of susceptibility in their host cells. Interestingly, a given pathogen taxon tends to converge on particular ETPs (Fig. 3). RNA silencing (RNAi) is an efficient antiviral defense system in plants. Consequently, successful plant viruses evolved effectors that suppress RNA silencing (Burgyan and Havelda 2011). Bacterial plant pathogens need to combat plant immunity elicited by conserved patterns such as flagellin and the elongation

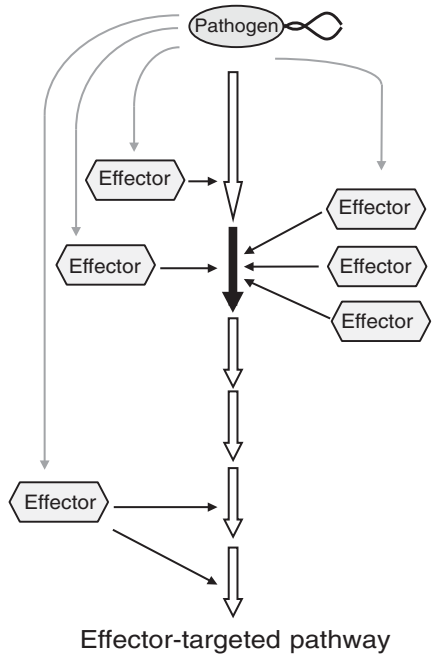

Figure 3. Functional redundancy among effectors. This scheme illustrates how effectors target different steps in an ETP or converge on a single target within an ETP. Several unrelated effectors can also converge onto the same host protein or hub (boldface arrow) in the ETP. This reinforces the strategy of invasive pathogens to interfere and circumvent generated host signals and defense.

factor thermo unstable (EF-Tu). Therefore, many of the bacterial type-III effectors suppress plant immunity, particularly the signaling pathways triggered by flagellin and EF-Tu (Block and Alfano 2011). Similarly, fungal plant pathogens have evolved effectors that disable the immune pathway triggered by the cell wall molecule chitin (de Jonge et al. 2011). Because the signaling pathways of the immune receptors detecting flagellin, EF-Tu, and chitin overlap, it is possible that fungal effectors can target the pathways activated by bacterial flagellin and EF-Tu and vice versa. Diverse phytoplasma species elicit developmental malformations in their host plants (Sugio and Hogenhout 2012). They secrete effectors that modulate plant development and plant–insect interactions, therefore ensuring the successful transmission of these obligate and insect-vectored bacterial pathogens.

Because a given pathogen taxon must perturb specific host pathways to be effective, it is perhaps not surprising that many effectors appear to be functionally redundant. As schematically depicted in Figure 3, functionally redundant effectors may affect different steps in an ETP or may converge on a single target within the pathway. For example, three different *P. syringae* type-III effectors bind and perturb the same *Arabidopsis* target protein RIN4 (Grant et al. 2006). Two of these effectors, AvrRpm1 and AvrB, induce phosphorylation of RIN4, whereas the protease AvrRpt2 cleaves RIN4. Two additional *P. syringae* type-III effectors, AvrPto and AvrPtoB, physically interact with the kinase domains of the immunoreceptors FLS2, EFR, and CERK1, thereby interfering with multiple PTI signaling pathways (Nicaise et al. 2009). In contrast, the effectors HopAI1 and HopF2 inhibit downstream steps of the same PTI signaling pathways targeted by AvrPto and AvrPtoB. HopAI1 and HopF2 target MPKs and MKKs, respectively, which are kinases that mediate PTI signaling following perception of microbial patterns (Wang et al. 2010; Wu et al. 2011). This example also illustrates how the same effector could target two or more different steps within an ETP (Fig. 3).

The effectors of filamentous pathogens also display functional redundancy. Both of the effectors Avr4 and Ecp6 of the leaf mold fungus *C. fulvum* interfere with chitin perception by the host plant tomato (de Jonge et al. 2011). Likewise, the RXLR-type effectors of oomycetes are thought to exhibit a high degree of functional redundancy. Genome and transcriptome analyses of different strains of the potato late blight pathogen *Phytophthora infestans* revealed a high degree of genetic and expression polymorphism (Haas et al. 2009; Raffaele et al. 2010; Cooke et al. 2012). Many effector genes are strain specific: Only 45 of the ~550 predicted RXLR effector genes have intact coding sequences and are induced in planta in all examined strains (Cooke et al. 2012). In the soybean pathogen *Phytophthora sojae*, 10%–15% of RXLR effectors are highly expressed during infection (Jiang and Tyler 2012). This suggests that the majority of the RXLR effectors of *P. infestans* and *P. sojae* are dispensable and possibly encode redundant functions.

Effector functional redundancy may also be a consequence of arms race coevolution between pathogens and hosts. The pathogen population could benefit from carrying a set of functionally redundant effectors to counteract plant immunity. Genetic and epigenetic modifications of gene function, including nonsynonymous mutations, deletion, and gene silencing, are usually deleterious, being removed from the pathogen population by purifying selection. On the other hand, purifying selection is unlikely to effectively operate on redundant effector genes because they functionally complement one another. This results in the maintenance of genes with relatively low fitness under the present environmental conditions and facilitates the emergence of strain-specific repertories of effector genes in the population. When the environment changes, for instance, through the emergence of disease resistance in the host plant, preexisting isolates harboring a set of effectors advantageous for the new conditions could be positively selected. This evolutionary "bet-hedging" strategy, in which the pathogen population benefits from extensive heterogeneity in the effector complement, could be one of the tactics that enable pathogens to avoid extinction in the arms race (Toruno et al. 2010; Simons 2011). For example, a functionally redundant heterogeneous effector repertoire would enable the pathogen population to include strains that can disable key ETPs while evading perception by plant immunoreceptors (Boller and Felix 2009). The extent to which "bet hedging" explains functional redundancy of plant pathogen effectors has not been explored

in depth and deserves to be more rigorously studied in the future.

EFFECTORS FROM UNRELATED PATHOGENS CONVERGE ON THE SAME HOST TARGETS

We discussed above how different effectors from the same pathogen have evolved to disable the same host target within an ETP (Fig. 3). In addition, effectors from phylogenetically unrelated pathogens can also converge on the same host targets. One example is the tomato defense cysteine protease RCR3, which is inhibited by effectors from three unrelated plant pathogens: *C. fulvum* AVR2, *P. infestans* EPIC1 and EPIC2B, and VAP1 from the root parasitic nematode *Globodera rostochiensis* (Song et al. 2009; Lozano-Torres et al. 2012). These findings highlight RCR3 as a "hub" component of apoplastic defenses of tomato that needs to be disabled for successful parasitism by fungal, oomycete, and nematode pathogens. Remarkably, plants have evolved to detect pathogen-induced perturbations of hub virulence targets such as RCR3. The tomato cell surface receptor-like protein Cf-2 confers resistance to *C. fulvum* and *G. rostochiensis* in an RCR3-dependent manner (see more on this topic in the following section) (Rooney et al. 2005; Lozano-Torres et al. 2012). More recently, Mukhtar et al. (2011) identified a set of 18 candidate hub target proteins following a large-scale yeast two-hybrid (Y2H) screen using effectors of both the oomycete *Hyaloperonospora arabidopsidis* and the bacterium *P. syringae*.

Although a number of effectors from unrelated pathogens have evolved to disable identical targets, in many cases any step in an ETP would be an effective target. Is it truly more beneficial for a pathogen to converge on a few "hub" host targets given that nodes would then be under strong selection to evade effectors? It seems that in many cases, pathogens have evolved effectors that disable multiple steps within a targeted pathway. For instance, plant viruses have evolved a multitude of mechanisms to target the RNA silencing machinery but have not evolved to focus on a single hub target (Burgyan and Havelda 2011).

Perhaps a more pertinent issue is to determine the degree to which unrelated pathogen taxa have converged on the same ETPs. Some common themes are starting to emerge. Effectors from diverse pathogenic species of bacteria, oomycetes, fungi, and insects have been reported to suppress an overlapping set of PTI signaling pathways (Gimenez-Ibanez et al. 2009; Bos et al. 2010; de Jonge et al. 2010; Wang et al. 2011). Both bacterial and oomycete plant pathogens target vesicle trafficking pathways to interfere with focal immunity, although the exact mechanisms are yet to emerge (Bozkurt et al. 2012; Lindeberg et al. 2012). As discussed above, filamentous pathogens and nematodes have adapted to the proteolytic environment of the plant apoplast by evolving diverse protease inhibitor effectors (Rooney et al. 2005; Song et al. 2009; Lozano-Torres et al. 2012).

ETPs are a direct reflection of the pathogenic strategy used by a given pathogen and the obstacles that the pathogen faces during host infection. In the future, as we discover ETPs for a wider range of plant-interacting taxa, we should be able to classify and define plant pathogens and pests by the nature of the host processes that they must impair.

RECOGNITION OF PATHOGEN EFFECTORS BY PLANT IMMUNORECEPTORS

Plants have evolved a potent immune system to recognize invading organisms. This ubiquitous immune response uses conserved pathways to respond to a variety of parasites, including viruses, bacteria, fungi, oomycetes, nematodes, insects, and parasitic plants. Plant immunoreceptors can turn the table on the parasite by recognizing effector molecules, therefore turning the effector into a liability for the pathogen (Figs. 1B and 2B).

Plant perception of invading organisms can occur via cell surface receptors, receptor-like kinases or receptor-like proteins, or NB-LRR proteins, a hugely diverse class of plant intracellular immunoreceptors. These immune receptors can perceive all types of pathogen molecules, ranging from conserved microbial patterns (also known as PAMPs) to highly polymorphic and rapidly evolving effectors. Recognition of pathogen molecules can be direct or indirect. The cell surface PRRs FLS2 and EFR directly bind their ligands to the PAMPs flagellin and EF-Tu, respectively (Chinchilla et al. 2006; Zipfel et al. 2006). Another example of direct recognition is the direct binding between the flax NB-LRR receptors L5, L6, and L7 and the flax rust fungus AvrL567 effector (Dodds et al. 2006). In contrast, in plant–bacteria interactions, intracellular immunoreceptors often recognize effectors indirectly through the perception of modifications in the host protein targeted by the effector. For instance, *Arabidopsis* NB-LRR protein RPM1 detects modifications in the host protein RIN4, which is targeted by the *P. syringae* effector proteins AvrB and AvrRpm1 (Mackey et al. 2002). With no universal recognition mechanism evident, several key models have been proposed to encapsulate the complexity of the recognition process.

Historically, indirect recognition was viewed as a guard process, with immunoreceptors acting as sentinels that "guard" particular plant proteins and detect pathogen-induced perturbations (Jones and Dangl 2006). This model had to be expanded with the discovery that most effectors bind multiple host targets, some of which having no apparent role in ETS. One theory is that the plant deploys decoy proteins to sequester the effectors and activate innate immunity. In the decoy model, decoy proteins act exclusively in effector recognition, mimicking the operative target (van der Hoorn and Kamoun 2008). For instance, indirect recognition of Avr2 by tomato Cf-2 is mediated by the selective inhibition of two papain-like cysteine proteases, RCR3 and PIP1, by Avr2 (Rooney et al. 2005; Shabab et al. 2008). Shabab et al. (2008)

proposed that PIP1 is the operative target of Avr2 and that the decoy RCR3 acts to exclusively mediate recognition by Cf-2. Accordingly, RCR3 accumulates in the tomato apoplast at much lower levels than PIP1 during infection, and inhibition of RCR3 does not contribute to enhanced virulence (Dixon et al. 2000).

In addition to models involving NB-LRR immunoreceptors, the recognition of bacterial TAL effectors can present a distinct mechanism. Some resistance genes that recognize TAL effectors are transcriptionally activated through the interaction of corresponding effectors with elements within their promoters (see above). For example, the pepper resistance gene *Bs3* is targeted by the *Xanthomonas* AvrBs3 effector that directly activates its transcription and thereby confers resistance (Romer et al. 2007).

EFFECTORS AS MOLECULAR PROBES TO STUDY PLANT BIOLOGY

Research on plant–microbe interactions has yielded many key discoveries that impacted all of plant biology. For example, the plant hormone gibberellin was first identified as the molecule that triggers the "foolish rice" symptoms caused by the plant pathogenic fungus *Gibberella fujikuroi* (Tamura 1990). Strigolactones, which are now known to inhibit plant shoot branching, were first discovered as stimulants of seed germination of parasitic plants and growth activators of arbuscular mycorrhizal fungi (Xie and Yoneyama 2010). Other areas of plant biology that have been significantly influenced by the study of plant–biotic interactions include developmental biology, receptor biology, and epigenetics (Speth et al. 2007).

The study of the effectors of plant-associated organisms promises to continue this tradition of influential and wide-reaching discoveries. Effectors can be used as molecular probes to unravel unknown aspects of plant immunity and biology (Bozkurt et al. 2012; Feng and Zhou 2012). By definition, studying the function of an effector is a leap into the unknown. One has little clue regarding where the research will lead. But the targeted host process must be important enough to enable the effector activity to emerge and become selected. In essence, effectors have evolved as a result of an awesome, comprehensive genetic screen that has taken place in nature over millions of years.

Effectors have been used as molecular probes in multiple ways. The type-III effectors of pathogenic bacteria turned out to be an exquisite toolkit to dissect plant immune pathways (Feng and Zhou 2012; Wei et al. 2012). Effectors are also useful probes for plant cell biology. Plant vesicular trafficking and focal immunity have been difficult processes to dissect using standard genetic approaches, mainly because mutants tend to be pleiotropic. Effectors promise to be a useful toolkit to dissect such dynamic cellular processes, potentially helping to unravel the diversity of secretory vesicles and their cargo (Bozkurt et al. 2011).

SYNTHETIC EFFECTORS: NEW TOOLS FOR PLANT BIOTECHNOLOGY

Plant pathogen effectors have proven to be a rich source of genetic innovation, often evolving independent mechanisms that mimic plant processes. However, effectors have evolved to benefit the invading organism. Can we turn this around and engineer beneficial synthetic effectors that can be used in biotechnology? This has already happened with TAL effectors, which can be custom designed and engineered to bind any target DNA sequence (Bogdanove and Voytas 2011). Indeed, TAL effectors have emerged as key tools for synthetic biology. TAL effectors can be fused to DNA nucleases to target a precise site in a genome to produce genetic variations. Such TAL nucleases have already been used to produce targeted variations in the genomes of mammals, flies, worms, and plants (Bogdanove and Voytas 2011). In a recent landmark study, Li et al. (2012) used TAL nucleases to generate 3–57 base deletions in a specific susceptibility gene of rice to engineer bacterial blight resistance. Other type-III effectors can also be used as reagents to manipulate cellular pathways. Wei et al. (2012) engineered two type-III effector proteins from animal parasitic bacteria to rewire kinase pathways in yeast and mammalian cells. In the future, as we learn more about structure–function relationships and mechanisms of action of different classes of effectors, we can further exploit effectors as synthetic biology reagents.

CONCLUDING REMARKS

The effectors of plant-associated organisms are a source of biological innovation; they include some of the most remarkable proteins known to function in plant cells and tissue. Nonetheless, the field of effector biology remains in its infancy. Our knowledge of effectors is mostly limited to a few taxa of plant pathogens and to immunosuppression processes. We know little about the identity and function of effectors from deep lineages of plant-associated organisms, including obligate plant pathogens, such as powdery mildews and rust fungi, and mutualists, such as mycorrhizal fungi. In addition, the occurrence of effectors in insect herbivores and parasitic plants is only starting to be appreciated (Gheysen and Mitchum 2011; Hogenhout and Bos 2011). Clearly, this plethora of effectors from a wide range of plant-associated organisms are likely to have unexpected functions and should prove to be a rich source of scientific discovery. Effector biology is, therefore, poised to continue to broadly impact the biological sciences.

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