A plethora of virulence strategies hidden behind nuclear targeting of microbial effectors

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Plant immune responses depend on the ability to couple rapid recognition of the invading microbe to an efficient response. During evolution, plant pathogens have acquired the ability to deliver effector molecules inside host cells in order to manipulate cellular and molecular processes and establish pathogenicity. Following translocation into plant cells, microbial effectors may be addressed to different subcellular compartments. Intriguingly, a significant number of effector proteins from different pathogenic microorganisms, including viruses, oomycetes, fungi, nematodes, and bacteria, is targeted to the nucleus of host cells. In agreement with this observation, increasing evidence highlights the crucial role played by nuclear dynamics, and nucleocytoplasmic protein trafficking during a great variety of analyzed plant–pathogen interactions. Once in the nucleus, effector proteins are able to manipulate host transcription or directly subvert essential host components to promote virulence. Along these lines, it has been suggested that some effectors may affect histone packing and, thereby, chromatin configuration. In addition, microbial effectors may either directly activate transcription or target host transcription factors to alter their regular molecular functions. Alternatively, nuclear translocation of effectors may affect subcellular localization of their cognate resistance proteins in a process that is essential for resistance protein-mediated plant immunity. Here, we review recent progress in our field on the identification of microbial effectors that are targeted to the nucleus of host plant cells. In addition, we discuss different virulence strategies deployed by microbes, which have been uncovered through examination of the mechanisms that guide nuclear localization of effector proteins.

Keywords: microbial effector, NES, NLS, nuclear pore complex, plant immunity, nucleocytoplasmic protein translocation, resistance protein, transcription factor

INTRODUCTION

As sessile organisms living in a microbe-rich environment, plants have developed an intricate defense network to fight off invading pathogens. The first layer of plant defense involves recognition of pathogen-associated molecular patterns (PAMPs), defined as invariant epitopes within molecules that are fundamental to pathogen fitness and widely distributed among different microbes. Examples of PAMPs are flagellin from bacteria and chitin from fungi and their recognition, historically known as basal defense, is now referred to as PAMP-triggered immunity (PTI; Jones and Dangl, 2006). PTI is associated to the production of reactive oxygen species and antimicrobial compounds, the induction of mitogen-activated protein kinase (MAPK) cascades, the modulation of host gene transcription, and the deposition of lignin and callose at the plant cell wall (Asai et al., 2002; Torres et al., 2002; Hauck et al., 2003; Tao et al., 2003). Thriving pathogens evolved to suppress PTI and promote successful infection by delivering a plethora of small molecules, referred to as effectors, in the apoplastic space, and inside host cells (Gordeeva et al., 2003; Alfano and Collmer, 2004; Chisholm et al., 2006). In turn, plants have evolved to gain the ability to recognize directly or indirectly effectors through resistance (R) proteins. This recognition response, which leads to resistance of the plant, is associated with the long-standing gene-for-gene hypothesis and, more recently, with the guard hypothesis (van der Biezen and Jones, 1998), and is now known as effector-triggered immunity (ETI). ETI is frequently associated with development of the hypersensitive response (HR), a form of programmed cell death localized at the infection site, which prevents the spread of the pathogen inside the plant (Mur et al., 2008). In most cases, the onset of the HR results in the activation of systemic acquired resistance (SAR), which provides protection to the plant against a wide range of pathogens (Durrant and Dong, 2004). The co-evolutionary arms race between plants and pathogens has resulted in the generation of highly polymorphic repertoires of R proteins and microbial effectors.

Plant pathogenic bacteria, viruses, fungi, oomycetes, and nematodes engage varied mechanisms to deliver effector proteins inside host cells. To achieve this goal, bacteria use specialized secretion systems, such as the type III and type IV secretion systems (Galan and Wolf-Watz, 2006; Block et al., 2008; McCann and Guttman, 2008). Biotrophic fungi and oomycetes have evolved specialized

structures called haustoria that penetrate the host tissue, invaginating the host plasma membrane. Haustoria probably play a dual role as they have been proposed to enable effector secretion and uptake of nutrients, although this idea remains to be demonstrated (Panstruga and Dodds, 2009; de Jonge et al., 2011). In contrast, several fungal and oomycete effectors can enter plant cells independently of the pathogen, probably *via* receptor-mediated endocytosis (Kale and Tyler, 2011). Finally, plant parasitic nematodes use a specialized feeding organ, referred to as the stylet, to inject their effector proteins into parasitized plant vascular cells (Davis et al., 2008; Gheysen and Mitchum, 2011).

Following their translocation into plant cells, microbial effectors may be addressed to different subcellular compartments where they may manipulate a variety of host cellular functions. A particularly important role in plant defense responses has been attributed to nuclear dynamics since a growing number of reports has revealed that nuclear localization of not only pathogen

effectors, but also of R proteins and key host components, including transcription factors (TFs) and regulators, is essential for plant immunity (Wiermer et al., 2007; Liu and Coaker, 2008; Deslandes and Rivas, 2011; Rivas, 2011). The fact that a significant number of effector proteins is translocated into the host cell nucleus (**Table 1**) suggests that effectors may manipulate host transcription or directly target essential nuclear host components for the benefit of the pathogen. Along these lines, it has been additionally proposed that some effectors may affect histone modification and chromatin remodeling (Kay and Bonas, 2009). Indeed, chromatin configuration allows or prevents protein access to specific DNA regions and regulates essential cellular processes such as DNA replication, DNA repair, and transcription (Clapier and Cairns, 2009). Although chromatin remodeling has only been formally demonstrated in the case of effector proteins from *Agrobacterium tumefaciens* (Citovsky et al., 2007), it is now well accepted that modulation of chromatin configuration is a strategy employed by

Table 1 | Examples of effector proteins with demonstrated nuclear localization from different phytopathogenic microorganisms.

Effector	Species	Function/features	Reference
VIRUSES			
2b	*Cucumber mosaic virus*	PTGS suppression	Lucy et al. (2000)
NIa	*Tobacco etch potyvirus*	protease	Carrington et al. (1988), Restrepo et al. (1990)
NIb	*Tobacco etch potyvirus*	RNA-dependent RNA polymerase	Allison et al. (1986), Li et al. (1997)
p25	*Beet necrotic yellow vein virus*	Symptom development	Tamada et al. (1999), Vetter et al. (2004)
ORF3	*Groundnut rosette virus*	RNA protection/movement	Ryabov et al. (2004)
P6	*Cauliflower mosaic virus*	Symptom development	Daubert and Routh (1990), Haas et al. (2005)
p50	*Tobacco mosaic virus*	Viral replicase	Burch-Smith et al. (2007)
OOMYCETES			
Nuks	*Phytophthora infestans*	Unknown	Kanneganti et al. (2007)
CRNs	*P. infestans*	Unknown	Schornack et al. (2010)
*Ae*CRN5	*Aphanomyces euteiches*	Unknown	Schornack et al. (2010)
FUNGI			
Uf-RTP1	*Uromyces fabae*	Unknown	Kemen et al. (2005)
NEMATODES			
Hs-UBI1	*Heterodera schachtii*	Mono-ubiquitin domain	Tytgat et al. (2004)
SPRYSEC	*Globodera pallida*	Defence suppression	Jones et al. (2009)
BACTERIA			
SAP11	Aster Yellows phytoplasma strain Witches' Broom	Unknown	Bai et al. (2009)
VirE2	*Agrobacterium tumefaciens*	T-strand coating/integration	Tinland et al. (1992), Tzfira and Citovsky (2002)
VirD2	*A. tumefaciens*	T-strand capping	Tzfira and Citovsky (2002)
VirF	*A. tumefaciens*	T-strand uncoating/integration	Tzfira et al. (2004)
VirE3	*A. tumefaciens*	Tumor formation	Garcia-Rodriguez et al. (2006)
6b	*A. tumefaciens*	ADP-ribosyltransferase; histone chaperone	Kitakura et al. (2002), Tinland et al. (1990)
HopU1	*Pseudomonas syringae*	mono-ADP-ribosyltransferase	Fu et al. (2007)
HopAI1	*P. syringae*	phospho-Thr lyase	Zhang et al. (2007)
AvrBs3	*Xanthomonas campestris* pv. *vesicatoria*	TAL; cell hypertrophy	Boch et al. (2009), Kay et al. (2007), Moscou and Bogdanove (2009)
PthXo1	*Xanthomonas oryzae* pv. *oryzae*	TAL; nutrient (sugar) acquisition/copper resistance	Chen et al. (2010), Yuan et al. (2010)
AvrXa7	*X. oryzae* pv. *oryzae*	TAL; nutrient (sugar) acquisition	Chen et al. (2010), Yuan et al. (2010)
HsvG	*Pantoea agglomerans*	TAL	Nissan et al. (2011)
PopP2	*Ralstonia solanacearum*	Acetyltransferase	Deslandes et al. (2003), Tasset et al. (2010)
XopD	*X. campestris*	SUMO protease; TF targeting	Canonne et al. (2011), Kim et al. (2008)

bacterial virulence proteins to subvert plant immunity (Ma et al., 2011). Alternatively, nuclear translocation of effectors may affect subcellular localization of their cognate R proteins in a process that is essential for R protein-mediated plant immunity (Burch-Smith and Dinesh-Kumar, 2007; Shen and Schulze-Lefert, 2007).

In eukaryotic cells, trafficking of protein and RNA molecules between the cytoplasm and the nucleus occurs through nuclear pore complexes (NPCs). NPCs are composed of multiple subunits of nucleoporins (Nups) organized in a donut-shaped complex of eightfold symmetry that spans the double membrane of the nuclear envelope (Meier, 2007; **Figure 1**). Nucleocytoplasmic transport of macromolecules depends on import and export receptors (importins and exportins), which translocate cargo through the nuclear pore following their respective recognition of exposed nuclear localization signals (NLSs) and nuclear export signals (NESs) on cargo proteins (Merkle, 2001; Meier, 2007; Patel et al., 2007). NLSs are typically classified as either monopartite NLSs composed of a continuous stretch of basic amino acids (Kalderon et al., 1984a,b), or bipartite NLSs composed of two sets of two to three positively charged amino acids separated by a 10-amino acid linker region (Dingwall et al., 1982; Robbins et al., 1991). α-importins are adapter proteins that bind to NLS-containing cargo proteins and bridge their interaction with importin β. The trimeric complex importin-α/importin-β/cargo

FIGURE 1 | Model for nucleocytoplasmic transport of macromolecules through the nuclear pore complex. Cytoplasmic proteins with a nuclear localization signal (NLS) are translocated into the nucleus through nuclear pore complexes that are composed of nucleoporins and span the double membrane of the nuclear envelope. The NLS in cargo proteins is recognized by importin-α (Impα) that bridges the interaction of cargo proteins with importin-β (Impβ) and promote their transport into the nucleus. The directionality of transport is maintained by the Ras-related nuclear (Ran) protein through its binding to GDP (cytoplasmic side) or GTP (nuclear side). Export receptors or exportins (Exp) recognize nuclear export signals (NES) in cargo proteins, promoting their nuclear export to the cytoplasm.

protein is translocated into the nucleus across the NPC, thanks to the interaction between Nups and importin β (**Figure 1**). The directionality of the nuclear transport is maintained by the ratio of additional import factors such as Ran-GDP (cytoplasmic side) and Ran-GTP (nuclear side). Upon binding of Ran-GTP in the nucleus, the complex is disassembled and importin-α and β shuttle back to the cytoplasm to allow additional rounds of transport (Merkle, 2001; Meier, 2007; **Figure 1**). Nuclear export of proteins classically occurs through the nuclear export pathway mediated by an evolutionarily conserved CRM1/exportin protein that belongs to the importin-β family. The CRM1–Ran-GTP complex binds directly to the leucine-rich NES contained in cargo and directs the export of the ternary complex from the nucleus. The cargo is released from the complex by hydrolysis of Ran-GTP to Ran-GDP in the cytoplasm (Ossareh-Nazari et al., 2001; **Figure 1**).

Mounting evidence suggests that pathogen effectors co-opt the host cell nuclear transport machinery, including α-importins, to target plant cell nuclei (Vetter et al., 2004; Kay and Bonas, 2009; Schornack et al., 2010). In other cases, α-importin-independent translocation of effector proteins into the nucleus has been reported (Kanneganti et al., 2007). In addition, mutations in cellular factors involved in the transport of macromolecules through the nuclear envelope, compromise plant resistance signaling, underlining the importance of nucleocytoplasmic trafficking during plant innate immunity (Kinkema et al., 2000; Mou et al., 2003; Zhang and Li, 2005).

Here, we provide an overview on our current knowledge about translocation of microbial effectors into the host cell nucleus. We first discuss nuclear-targeted effectors from bacteria and viruses, whose functional characterization is rather well documented. Second, we summarize recent data involving nuclear effectors from nematodes and filamentous pathogens, for which further functional studies are required before concluding on the specificities and commonalities of the virulence strategies that are hidden behind nuclear targeting of effector proteins.

AGROBACTERIUM TUMEFACIENS

Agrobacterium tumefaciens is a soil phytopathogenic bacterium with the unique ability to transfer a segment of its Ti plasmid (T-DNA) into plant cells and integrate it into the chromosomal DNA. As a result, *Agrobacterium* infection induces the formation of crown gall tumors on dicotyledonous plants (Gelvin, 1998). T-DNA is transported from the bacterium to the host cell as a single-stranded DNA (ssDNA) molecule, named the T-strand, *via* the so-called type IV secretion system (T4SS), which is ancestrally related to bacterial conjugation machines (Christie, 2004; Christie et al., 2005). Conjugation systems enable bacteria to adapt to changing environments through acquisition of fitness traits. Throughout evolution, conjugation has substantially contributed to genome rearrangement and plasticity. The genes responsible for the processing and transfer of the T-DNA to plant cells are present in the Virulence region of the Ti plasmid and are called *vir* genes. The translocated virulence proteins are effector proteins, also delivered into the host cell through the T4SS. Once in the plant cell, Vir proteins form a nucleoprotein complex (T-complex) with the T-strand, as well as with host proteins, to mediate the
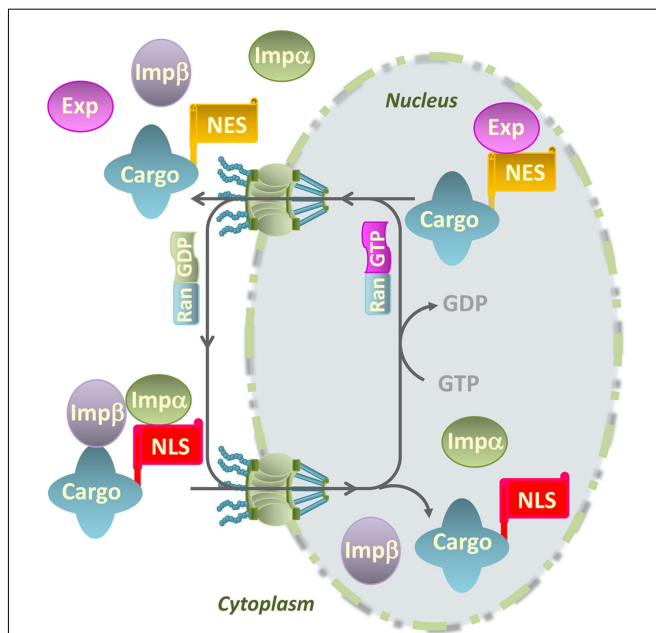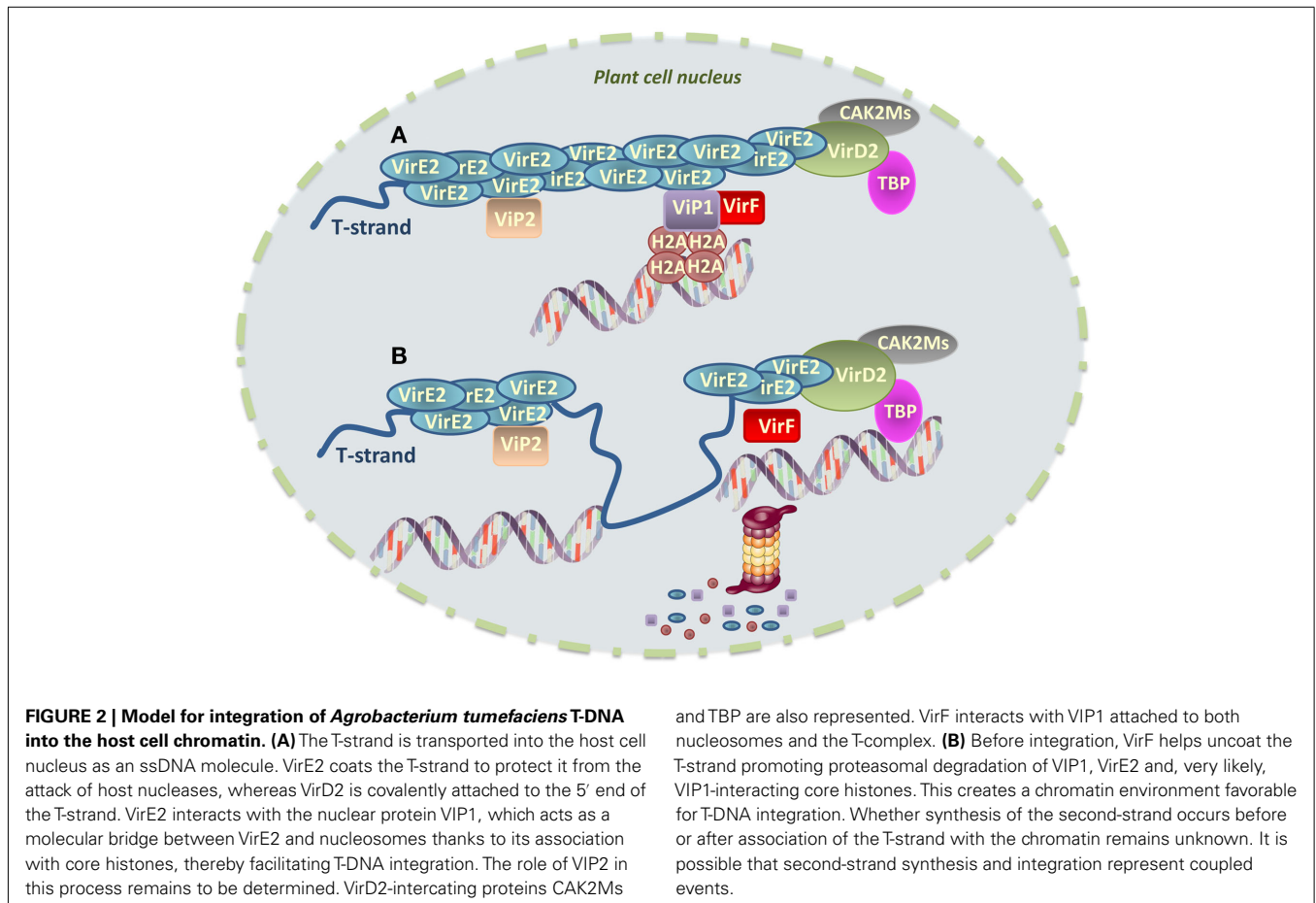
transformation of a normal plant cell into a crown gall tumor cell (Lacroix and Citovsky, 2009).

A subset of the Vir proteins that are translocated into plant cells is targeted to the nucleus and historically Vir proteins were the first nuclear-localized effectors described (Tinland et al., 1992). Studies on nuclear-targeted proteins from *Agrobacterium* provide a fascinating illustration of the great diversity of molecular activities that bacterial effectors are able to display in the host nucleus to subvert basic cellular processes. Among the Vir proteins involved in the processing and/or transfer of the T-DNA to the plant cell, VirE2 is the most abundant protein produced after induction of the *vir* genes. It binds in an unspecific and highly cooperative manner to ssDNA, coating the T-strand to protect it from the attack of host nucleases (**Figure 2**). Another Vir protein, VirD2, is covalently attached to the 5′ end of the T-strand following generation of nicks at the border repeats surrounding the T-DNA in the Ti plasmid (Tzfira and Citovsky, 2002; **Figure 2**). VirE2 is translocated into plant cells independently of the T-strand-VirD2 complex (Vergunst et al., 2000). Both VirD2 and VirE2 have NLSs, which helps target the T-strand to the plant cell nucleus. However, nuclear import of the T-complex is mediated by VirD2, following its binding to the host importin-α (Ballas and Citovsky, 1997; Bako et al., 2003). VirE2, which has low binding affinity for importin-α (Citovsky et al., 2004), interacts with the nuclear protein VIP1 (VirE2-interacting protein 1; Tzfira et al., 2001) that then binds

to importin-α and guides VirE2 nuclear import, and association with the host chromatin (Tzfira and Citovsky, 2002; Citovsky et al., 2004; Lacroix et al., 2008). VIP1 is a basic domain-leucine zipper (bZIP) protein that may act as a TF involved in plant defense (Tzfira et al., 2001; Djamei et al., 2007).

VirE2 has been shown to modulate chromatin functions and facilitate T-DNA integration. In *Arabidopsis*, VIP1 directly interacts with various core histones, such as H2A, and it may therefore bridge the association of VirE2 with the plant nucleosome, facilitating T-DNA integration (Li et al., 2005; Lacroix et al., 2008; **Figure 2**). Furthermore, another VirE2-interacting protein, VIP2, may regulate histone gene transcription (Anand et al., 2007). In agreement with this observation, expression of several histone genes is induced upon *Agrobacterium* infection (Veena et al., 2003). It is thus tempting to speculate that VirE2 and/or other *Agrobacterium* effectors may modulate histone gene expression to facilitate infection.

VirD2-interacting proteins have also been described. For example, the nuclear kinase CAK2Ms, a member of the conserved cyclin-dependent kinase-activating kinase family, interacts with, and phosphorylates VirD2, perhaps modulating its activity. In addition, a TATA box-binding protein (TBP) also interacts with VirD2, possibly guiding the T-DNA to transcription-prone sites in the host genome, which are preferential for T-DNA integration (Bako et al., 2003; **Figure 2**).

FIGURE 2 | Model for integration of *Agrobacterium tumefaciens* T-DNA into the host cell chromatin. (A) The T-strand is transported into the host cell nucleus as an ssDNA molecule. VirE2 coats the T-strand to protect it from the attack of host nucleases, whereas VirD2 is covalently attached to the 5′ end of the T-strand. VirE2 interacts with the nuclear protein VIP1, which acts as a molecular bridge between VirE2 and nucleosomes thanks to its association with core histones, thereby facilitating T-DNA integration. The role of VIP2 in this process remains to be determined. VirD2-intercating proteins CAK2Ms

and TBP are also represented. VirF interacts with VIP1 attached to both nucleosomes and the T-complex. **(B)** Before integration, VirF helps uncoat the T-strand promoting proteasomal degradation of VIP1, VirE2 and, very likely, VIP1-interacting core histones. This creates a chromatin environment favorable for T-DNA integration. Whether synthesis of the second-strand occurs before or after association of the T-strand with the chromatin remains unknown. It is possible that second-strand synthesis and integration represent coupled events.

An additional group of Vir proteins is involved in hijacking the host cell metabolism to allow the integration and expression of the genes carried on the T-DNA. Before integration, VirF, an F-box protein translocated into the host cell, helps uncoat the T-DNA from VirE2 and VIP1 proteins (Schrammeijer et al., 2001). Indeed, as part of the Skp1-Cdc53-cullin-F-box (SCF) complex, VirF binds VIP1 that is attached both to nucleosomes and to the T-complex, promoting proteasomal degradation of both VIP1 and its associated VirE2 (Tzfira et al., 2004; **Figure 2**). It has been proposed that VirF may additionally induce degradation of the core histones that are bound to VIP1 (Li et al., 2005; Loyter et al., 2005; Lacroix et al., 2008), thereby inducing their local destabilization and creating a chromatin environment favorable for T-DNA integration (**Figure 2**). In agreement with the observation that some plant species do not require VirF for transformation (Hirooka et al., 1987), recent data show that a plant F-box protein, named VBF (VIP1-binding F-box protein), is able to functionally replace VirF. VBF is induced by *Agrobacterium* infection and, as VirF, regulates the protein levels of VIP1 and its associated VirE2 after binding to VIP1 (Zaltsman et al., 2010). VBF expression in and export from *Agrobacterium* lead to increased tumorigenesis, indicating that *Agrobacterium* subverts a component of the host cell proteasome, whose expression is induced during pathogen infection, to promote plant genetic transformation.

VirE3 is conserved among all the *Agrobacterium* Ti plasmids studied so far. Even the *Agrobacterium rhizogenes* Ri-plasmid that lacks the genes *virE1* and *virE2* contains a copy of *virE3*, suggesting that the VirE3 protein plays an important role during transformation (Garcia-Rodriguez et al., 2006). Indeed, mutations in *virE3* diminish tumor formation on tobacco, tomato, and sunflower (Garcia-Rodriguez et al., 2006). The VirE3 protein has NLSs that mediate its interaction with importin-α and nuclear localization (Garcia-Rodriguez et al., 2006). VirE3 additionally interacts with pCsn5, a component of the COP9 signalosome and pBrp, a plant-specific general TF that belongs to the TFIIB family. When bound to DNA, VirE3 is able to promote gene transcription. These data suggest that nuclear VirE3 may act as a transcriptional activator to induce the expression of genes needed for tumor development (Garcia-Rodriguez et al., 2006).

Finally, the *6b* gene in the T-DNA from *A. tumefaciens* has oncogenic activity in plant cells, inducing tumor formation, and alterations in leaf morphology (Tinland et al., 1990). The tobacco protein NtSIP1 was identified in a yeast two-hybrid screen as a 6b-interacting protein (Kitakura et al., 2002). NtSIP1 is localized in the nucleus and appears to act as a TF because its predicted amino acid sequence includes two predicted NLSs and a putative DNA-binding motif, which is similar to the triple helix motif of rice TF GT-2 (Dehesh et al., 1992). Nuclear localization of 6b was enhanced by co-expression with NtSIP1 in tobacco cells. In addition, a fusion protein between the DNA-binding domain of yeast GAL4 and 6b activated the transcription of a reporter gene in tobacco (Kitakura et al., 2002). Finally, an acidic C-terminal domain of 6b is required for its nuclear localization and trans-activation as well as for hormone-independent proliferation of tobacco cells. These data suggest that 6b may affect transcription of plant genes controlled by NtSIP1 and function in the proliferation of plant cells through an association with NtSIP1. In addition

to NtSIP1, 6b associates with other *Arabidopsis* proteins in the nucleus, including key components of the microRNA pathway and the core histone H3 (Terakura et al., 2007; Wang et al., 2011). Based on this finding, 6b has been proposed to act as a histone chaperone, which works together with other chromatin remodelers to affect nucleosome assembly, histone displacement and transcription in a gene-specific manner (Terakura et al., 2007). Recent structural analysis suggests that 6b displays an ADP-ribosyltransferase activity (Wang et al., 2011). Since 6b directly interacts with H3, it will be interesting to determine whether H3 can be modified by 6b and how potential 6b-mediated ribosylation of H3 may affect transcription.

OTHER GRAM-NEGATIVE BACTERIA
Gram-negative bacteria have evolved a sophisticated mechanism to deliver effector proteins into host cells. Indeed, the so-called type III secretion system (T3SS) provides a continuous channel for Type III effectors (T3Es) to travel from the bacterial cytoplasm directly into the cytoplasm of eukaryotic cells in a process that involves transport across (i) the two bacterial membranes separated by a peptidoglycan layer and (ii) the plasma membrane of the plant cell, which is surrounded by a thick cell wall (Buttner and He, 2009). In plant pathogenic bacteria, T3SSs are encoded by *hrp* (for HR and pathogenicity) genes that are required by bacteria to elicit the HR in resistant plants and to cause disease in susceptible plants (Lindgren et al., 1986)

In *Ralstonia solanacearum*, PopB is a small basic T3SS-secreted protein that carries a functional C-terminal bipartite NLS and a predicted helix forming a coiled-coil domain, suggesting that PopB might interact with other proteins (Gueneron et al., 2000). In addition to PopB and PopP2 (see below), at least three other *R. solanacearum* T3Es of unknown function (RSp0216, RSc1349, and RSc3272) appear to be nuclear localized when expressed in plant cells (Anne-Claire Cazalé, personal communication).

The T3E from *Xanthomonas campestris* pathovar *vesicatoria* (*Xcv*) HpaA (for *Hrp*-associated) is specifically required for disease development in pepper plants. *hpaA* mutants are affected in pathogenicity whereas they partially retain the ability to induce an HR (Huguet et al., 1998). HpaA presents two functional NLSs that are important for full HpaA-mediated disease development in the plant (Huguet et al., 1998).

Effector mining in *Pseudomonas syringae* pv. *tomato* DC3000, which infects *Arabidopsis thaliana* and tomato, has identified more than 30 candidate effector genes (Grant et al., 2006; Lindeberg et al., 2006; Cunnac et al., 2009). Systematic survey of *P. syringae* T3Es containing putative NLSs has identified AvrE, HopI1, and HopY1, although their nuclear localization in plant cells remains to be demonstrated (Lionel Navarro, personal communication). The *P. syringae* T3E HopU1 displays mono-ADP-ribosyltransferase (ADP-RT) activity and mutation of HopU1 catalytic site abolishes HopU1-mediated suppression of plant innate immunity (Fu et al., 2007). HopU1 targets *Arabidopsis* RNA-binding proteins with RNA-recognition motifs (RRMs), including GRP7, a glycine-rich RNA-binding protein (GR-RBP). *Arabidopsis grp7* mutant displays increased susceptibility to *P. syringae* inoculation as compared to wild-type plants. Both HopU1 and GRP7 present a dual nucleo-cytoplasmatic localization (Fu et al., 2007). The

observation that ADP-ribosylation of GRP7 by HopU1 requires two arginine residues within the RRM indicates that this modification may affect the RNA-binding ability of GRP7. By disabling the function of GR-RBPs, the pathogen may modify the host RNA status, resulting in reduced amounts of immunity-related mRNAs available in the plant and suppression of host immunity.

The *P. syringae* T3E HopAI1 displays phospho-Thr lyase activity and disrupts defense signal transduction by directly inactivating MAPKs in plants (Zhang et al., 2007). A nuclear localization of HopAI1 has not been reported. However, based on the functional similarities between HopAI1 and the T3E OspF of the animal pathogen *Shigella flexneri*, which also displays phospho-Thr lyase activity that targets the phosphorylated MAPKs in the nucleus (Li et al., 2007), it is tempting to speculate that HopAI1 may be targeted to host cell nuclei. Interestingly, OspF remodels host chromatin by inducing dephosphorylation and deacetylation of H3, which leads to decreased expression of specific immunity-related genes (Arbibe et al., 2007). This function is accomplished through the interaction of OspF with host retinoblastoma protein, which has been linked to histone modification (Zurawski et al., 2009). Whether HopAI1 is also able to target nuclear MAPKs and/or modulate histone modifications in plant cells is an exciting perspective for future research.

Although in most cases the cellular activities that are manipulated by nuclear effectors remain to be determined, the action of the following nuclear T3Es has been relatively well characterized and illustrates varied pathogen strategies that lead to the establishment of a cellular environment that favors pathogen proliferation.

TAL EFFECTORS FROM *XANTHOMONAS* AND *RALSTONIA*

Transcription activator-like (TAL) proteins, also called AvrBs3 family members, are T3Es only identified to date in plant pathogenic *Xanthomonas* spp. and *R. solanacearum* (for recent reviews, see Boch and Bonas, 2010; Bogdanove et al., 2010; Scholze and Boch, 2010). Historically, TAL effectors were the first plant pathogen T3Es shown to be specifically addressed to the nuclear compartment (Yang and Gabriel, 1995; Van den Ackerveken et al., 1996). Moreover, AvrBs3 is also a rare example of a plant pathogen T3E for which translocation into the plant nucleus was evidenced by immunodetection after bacterial infection (Szurek et al., 2002).
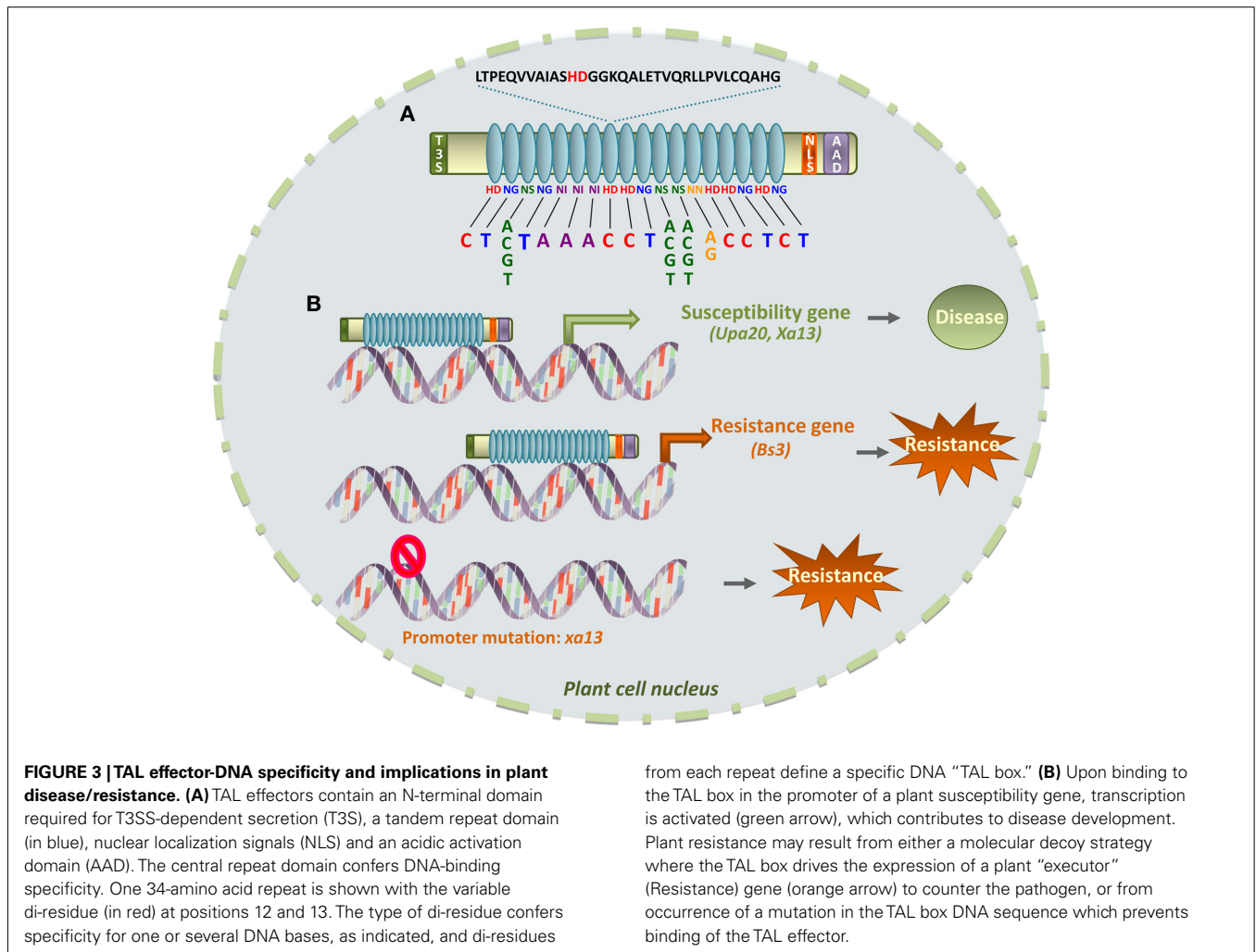
Transcription activator-like effectors act as transcriptional activators in the plant cell nucleus and provide a fascinating example of manipulation of the eukaryotic transcriptional machinery by directly promoting specific host gene reprogramming. More than a hundred TAL candidate sequences have been identified to date, mostly in *Xanthomonas* spp. genomes. Most TAL proteins contain NLSs and an acidic domain involved in transcriptional activation and localize to the nucleus through their interaction with importin-α (Szurek et al., 2001). However, the signature domain of TAL effectors is located in the central part of the proteins and consists of tandemly arranged nearly identical repeat units (**Figure 3A**). These repeats are 34- or more rarely 35-amino acid long and the number of repeats may vary from 1.5 to 33.5 repeats (Boch and Bonas, 2010) although a minimum of 6.5 repeats is required to detect a transcription activator function (Boch et al.,

2009). These TAL repeats were shown to be a novel type of DNA-binding domain (Kay et al., 2007; Romer et al., 2007) in which the target DNA-recognition specificity results from a one-repeat-to-one-bp correlation with different repeat types exhibiting a different DNA base pair specificity (Boch et al., 2009; Moscou and Bogdanove, 2009). Each repeat domain of a TAL effector contains two hypervariable residues at positions 12 and 13 per repeat which have been termed RVDs (for repeat-variable di-residues) and that are directly involved in the pairing to one specific nucleotide of the target DNA sequence. Some RVDs are specific for a particular DNA bp whereas others recognize more than one bp (**Figure 3A**; Boch et al., 2009; Moscou and Bogdanove, 2009). The succession of RVDs in each repeat therefore determines the nature of the target promoter sequence that has been defined as a "TAL box" element (Scholze and Boch, 2011). Quite remarkably, this specific recognition property of TAL effectors can be used to create artificial TAL effectors with novel DNA-recognition specificities and opens the way to various biotechnological applications (Boch et al., 2009; Geissler et al., 2011).

First structural data on TAL repeats revealed that a repeat folds into a helix-turn-helix structure reminiscent of a tetratrico peptide repeat (TPR; Murakami et al., 2010). TPRs are 34-amino acid long but are known to be involved in protein–protein interactions in prokaryotes and eukaryotes rather than protein–DNA interactions. The evolutionary origin of TAL effectors remains unknown but several types of TAL repeats exist in nature: whereas 34 aa repeats are prevalent in *Xanthomonas* spp., 35 aa repeats are found in *R. solanacearum* (Cunnac et al., 2004) or some *Xanthomonas* strains (Kay et al., 2005; Schornack et al., 2008). The biological implications of such differences are unclear but raise intriguing evolutionary questions since they suggest that TAL effectors containing 35-amino acid repeats independently arose by successive duplications of an initial variant repeat.

The pathogenic strategy of some *Xanthomonas* spp. appears to rely massively on host gene reprogramming by TAL effectors since some *X. oryzae* strains can harbor up to 28 TAL family members (including pseudogenes; Gonzalez et al., 2007), several of them being essential virulence factors for infection of rice (White et al., 2009). No TAL effector candidates were identified in the *X. campestris* pv. *campestris* (*Xcc*) strains B100, 8004, and ATCC 39913 for which a full sequence is available (da Silva et al., 2002; Vorhölter et al., 2003; Qian et al., 2005). However, recent work has identified between 1 and 4 TAL effector sequences in the genomes of 60% of a total of 55 surveyed *Xcc* strains (Boris Szurek and Laurent Noël, personal communication). The molecular functions of these newly identified TAL effector candidates are unknown but this finding suggests that TAL-based virulence strategies are widespread among xanthomonads.

The role of most TAL effectors in virulence is still unknown but there is some evidence of their role in pathogen proliferation and dispersal. For example, AvrBs3 from *X. campestris* pv. *vesicatoria* induces cell hypertrophy in leaves of susceptible pepper hosts, leading to epidermal rupture that is thought to be involved in bacterial dissemination (Marois et al., 2002). AvrBs3 binds to the *UPA* box, a TAL box found in the promoters of some pepper genes including *UPA20* (**Figure 3B**). *UPA20* encodes a TF that controls expression of auxin-induced genes and α-expansins and

FIGURE 3 | TAL effector-DNA specificity and implications in plant disease/resistance. (A) TAL effectors contain an N-terminal domain required for T3SS-dependent secretion (T3S), a tandem repeat domain (in blue), nuclear localization signals (NLS) and an acidic activation domain (AAD). The central repeat domain confers DNA-binding specificity. One 34-amino acid repeat is shown with the variable di-residue (in red) at positions 12 and 13. The type of di-residue confers specificity for one or several DNA bases, as indicated, and di-residues from each repeat define a specific DNA "TAL box." **(B)** Upon binding to the TAL box in the promoter of a plant susceptibility gene, transcription is activated (green arrow), which contributes to disease development. Plant resistance may result from either a molecular decoy strategy where the TAL box drives the expression of a plant "executor" (Resistance) gene (orange arrow) to counter the pathogen, or from occurrence of a mutation in the TAL box DNA sequence which prevents binding of the TAL effector.

is crucial for the development of plant cell hypertrophy (Kay et al., 2007; Romer et al., 2007).

A second example illustrating how bacteria use a TAL effector to manipulate host transcription to its own benefit involves the rice gene *Xa13*. *Xa13*, which is transcriptionally activated by the TAL effector PthXo1 (Yang et al., 2006; Romer et al., 2010), is defined as a susceptibility gene since its expression facilitates *X. oryzae* pv. *oryzae* (*Xoo*) infection. It was recently shown that Xa13 corresponds to OsSWEET11 that belongs to a recently discovered family of sugar transporters mediating sugar efflux in plants (Chen et al., 2010). PthXo1-mediated induction of OsSweet11 transcription most probably causes glucose efflux from rice cells in order to feed bacteria. An additional TAL effector from *Xoo*, AvrXa7, triggers induction of OsSWEET14, and consequently allows overcoming xa13-mediated resistance by inducing sugar release through another SWEET transporter (Chen et al., 2010). A separate report showed that Xa13 is a plasma membrane protein interacting with two copper transporter proteins that contribute to decrease the copper content of xylem sap (Yuan et al., 2010). Since *Xoo* is a vascular pathogen that spreads through xylem vessels, PthXo1-dependent increased transcription of *Xa13* might

pave the way for *Xoo* successful infection by clearing its path of toxic amounts of copper (Yuan et al., 2010).

In order to circumvent TAL-mediated pathogenic strategies, resistant plants have developed defense strategies based on subverting the recognition specificities of TAL effectors to trap the pathogen. Indeed, point mutations in the promoter of a key susceptibility gene may alter TAL effector recognition, and subsequent gene activation, leading to plant resistance. For example, the resistance *xa13* and susceptibility *Xa13* alleles show sequence polymorphisms only in their promoter regions, resulting in induced expression of *Xa13* but not recessive *xa13* upon *Xoo* infection (**Figure 3B**; Chu et al., 2006). However, such a resistance strategy would be much limited (or presumably take longer to be effective) in some other cases where TAL effectors target multiple host susceptibility genes. The pepper *Bs3* resistance gene illustrates a more subtle mechanism to promote resistance since, in this case, TAL effectors are lured into inducing the expression of a gene that promotes plant defense (**Figure 3B**; Boch and Bonas, 2010). Induction of Bs3 expression specifically triggers resistance and HR responses to *Xanthomonas* strains carrying the TAL T3E AvrBs3 (or related effectors displaying the same DNA-recognition

specificity; Romer et al., 2007). Bs3 encodes a protein homologous to flavine-dependent mono-oxygenases, which play roles in auxin biosynthesis and glucosinolate metabolism, therefore contributing to plant defense against pathogens (Schlaich, 2007). Although it is not known whether Bs3-triggered cell death is due to toxin production or induction of defense signaling, resistant plants in this case have evolved a molecular trap to detect AvrBs3 activity. Indeed, by mimicking the *upa20* promoter, BS3 subverts the virulence function of AvrBs3 and instead initiates plant immunity (Romer et al., 2007). Interestingly, this strategy should prove useful to engineer durable resistance to multiple pathogenic strains (or species). Along these lines, it has been already demonstrated that different TAL boxes combined into one promoter render this promoter responsive to several TAL effectors (Romer et al., 2009).

Finally, similar to *Xanthomonas* TAL proteins, HsvG and HsvB effectors of gall-forming *Pantoea agglomerans* act as transcriptional activators, although they are structurally distinct from TAL effectors (Nissan et al., 2006). HsvG and HsvB determine host specificity on gypsophila and beet, respectively. Both proteins present two functional NLSs required for their nuclear targeting and are able to bind DNA and activate transcription (Nissan et al., 2006; Weinthal et al., 2011). Although the mode of action of HsvG and HsvB remains unknown, it has been hypothesized that it involves modulation of host phytohormones associated with gall formation. A recent report showed that HsvG induces the transcriptional activation of a gene named *HSVGT* in *Gypsophila*

paniculata (Nissan et al., 2011). *HSVGT* encodes a predicted acidic protein of the DnaJ family, which presents a bipartite NLS as well as zinc-finger and leucine zipper DNA-binding motifs, typical of TFs. HsvG binds to the *HSVGT* promoter indicating that *HSVGT* is a direct target of HsvG (Nissan et al., 2011). These data indicate that HsvG functions as a TF in gypsophila.

XopD FROM *XANTHOMONAS CAMPESTRIS*

XopD from the bacterial strain *Xcv* (XopD$_{Xcv}$) promotes bacterial growth and delays the onset of leaf chlorosis and necrosis in late infection stages of tomato, presumably to sustain bacterial populations in infected tissues (Kim et al., 2008). XopD$_{Xcv}$ is a modular protein of 760 amino acids that shows different biochemical activities and contains (i) a recently identified N-terminal domain of 215 amino acids (Canonne et al., 2010), (ii) a helix-loop-helix domain (HLH), (iii) two tandemly repeated EAR (ERF-associated amphiphilic repression) motifs, previously described in plant transcriptional repressors during defense responses (Kazan, 2006), and (iv) a C-terminal cysteine protease domain with structural similarity with the yeast ubiquitin-like protease 1 (ULP1; **Figure 4A**). Consistent with its protein structure, XopD$_{Xcv}$ displays small ubiquitin-like modifier (SUMO) protease (Canonne et al., 2010) and non-specific DNA-binding activities (Kim et al., 2008). XopD$_{Xcv}$ has been additionally shown to repress transcription of defense- and senescence-associated plant genes in an EAR-dependent manner, suggesting that XopD$_{Xcv}$ may target host TFs (Kim et al., 2008).
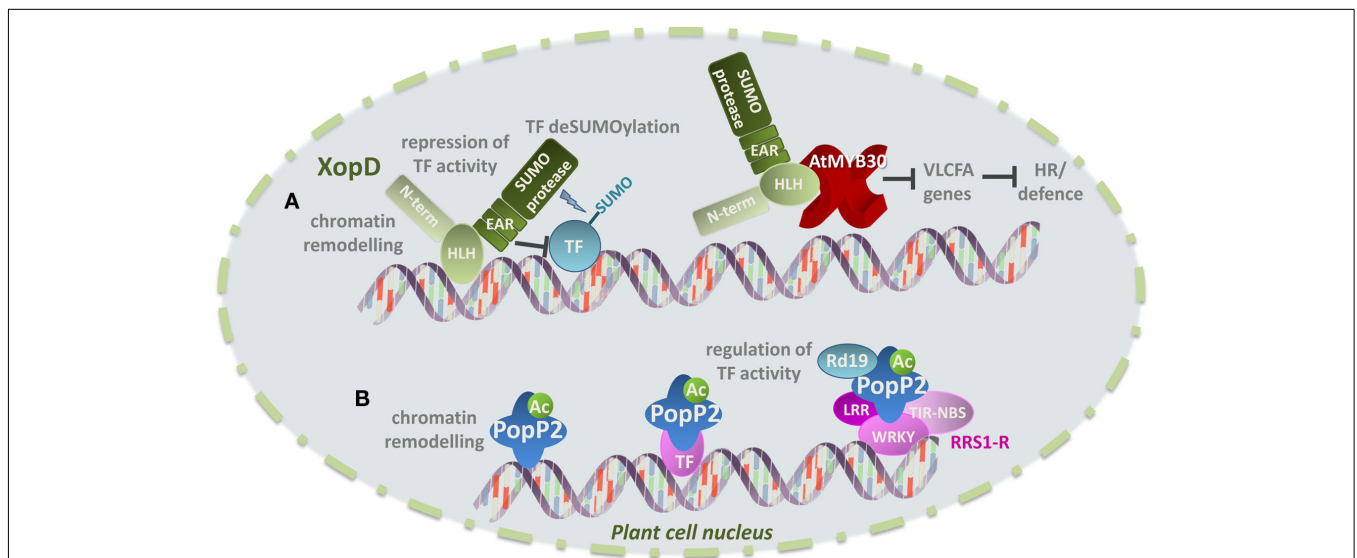

FIGURE 4 | Examples of virulence strategies displayed by bacterial effectors in the plant cell nucleus. (A) XopD from the strain B100 of *Xcc* is a modular protein with an N-terminal domain of unknown function, a helix-loop-helix domain (HLH), three tandemly repeated transcriptional repressor domain of the EAR type and a C-terminal SUMO protease domain. It has been suggested that XopD DNA-binding activity through its HLH domain may provide access to chromatin and that XopD may thus modulate host transcription by altering chromatin remodeling. XopD may additionally interact with host TFs and repress their transcriptional activity directly via its EAR domains and/or by TF deSUMOylation. Recent work shows that the HLH domain of XopD targets the *Arabidopsis* TF AtMYB30 leading to repression of AtMYB30 trasncriptional activity and suppression of the plant HR and defense responses. **(B)** The *R. solanacearum* effector protein PopP2 displays acetyltransferase activity, suggesting that PopP2 may directly manipulate host transcription through chromating remodeling following acetylation of histone residues. PopP2 acetyltransferase activity may also promote the recruitment of TFs and enhance their DNA-binding affinity, resulting in modulation of host transcription. Finally, the interaction of PopP2 with host proteins, such as the cysteine protease RD19 and the resistance gene RRS1-R may additionally affect RRS1-R-meadited transcriptional reprogramming.

XopD is targeted to plant cell subnuclear structures named nuclear bodies (or nuclear foci; Hotson et al., 2003; Canonne et al., 2010). The molecular mechanism allowing XopD nuclear import remains unknown but a truncated XopD version containing only the HLH domain of XopD (amino acids 216–405), and not comprising its putative NLS, has been shown to be necessary and sufficient for XopD nuclear import and subnuclear targeting (Canonne et al., 2011). Intriguingly, expression of XopD appears to induce reorganization of the nuclear structure in host cells, leading to non-specific relocalization of all tested nuclear proteins into nuclear bodies. In addition, DAPI staining showed that DNA accumulation is weaker in nuclear bodies, where XopD is expressed, compared to the nucleoplasm, where DNA distribution seems to remain unaltered (Canonne et al., 2011). It is thus enticing to speculate that XopD-induced modification of the nuclear structure and protein distribution may be part of a general virulence strategy, which allows *Xanthomonas* to perturb plant cell responses to bacterial infection. Along these lines, it has been proposed that the presence of the HLH and EAR domains in XopD may provide access to chromatin and/or transcriptional units, leading to modulation of host transcription by affecting chromatin remodeling and/or TF activity (Kay and Bonas, 2009; **Figure 4A**).

In agreement with the idea that plant TFs and/or regulators might be direct targets of XopD, recent data show that XopD from the strain B100 of *X. campestris* pathovar *campestris* (XopD$_{Xcc}$B100), which presents a similar protein structure to XopD$_{Xcv}$ (Canonne et al., 2012), is able to target the *Arabidopsis* MYB TF AtMYB30. AtMYB30 is a positive regulator of defense and cell death associated responses through the activation of the lipid biosynthesis pathway that leads to the production of very long chain fatty acids (VLCFAs; Raffaele et al., 2008). In agreement with the finding that transcriptional activation of VLCFA-related genes by AtMYB30 is required to mount an efficient defense response during bacterial infection, it has been demonstrated that AtMYB30 transcriptional activity is tightly controlled by the plant cell. Indeed, AtMYB30 is able to induce partial nuclear relocalization of the secreted phospholipase *At*sPLA$_2$-α, which is otherwise localized intracellularly in Golgi-associated vesicles before being secreted to the extracellular space. The physical interaction between AtMYB30 and *At*sPLA$_2$-α leads to repression of the AtMYB30-mediated transcriptional activity and negative regulation of plant HR and defense responses (Froidure et al., 2010). These data highlight the importance of dynamic nucleocytoplasmic protein trafficking for the regulation of defense-related transcription. Interestingly, in addition to the control of AtMYB30 activity exerted by the plant cell, AtMYB30 transcriptional activation is additionally controlled by bacteria. Indeed, XopD$_{Xcc}$B100 specifically interacts with AtMYB30. This protein association, which appears to be independent of nuclear foci formation, leads to inhibition of the transcriptional activation of AtMYB30 target genes and suppression of plant defense during *Xanthomonas* infection (Canonne et al., 2011; **Figure 4A**). The HLH domain of XopD$_{Xcc}$B100 is necessary and sufficient to mediate interaction with AtMYB30 and repression of AtMYB30 transcriptional activation and plant resistance responses.

Considering XopD modular structure and varied biochemical activities, it is likely that XopD mediates multiple protein–DNA and protein–protein interactions to modulate host transcription. In addition, XopD-dependent bacterial strategies used to subvert plant resistance may vary depending on the *Xanthomonas*/host plant interaction. XopD SUMO protease, EAR transcription repressor, and DNA-binding activities are at least partially involved in promoting virulence in tomato during *Xcv* infection (Kim et al., 2008). As previously mentioned, the HLH domain of XopD is necessary and sufficient to suppress AtMYB30-mediated defense during *Arabidopsis* infection by *Xcc*, while the EAR motifs and Cys protease domains are not involved in this process. It is thus tempting to speculate that the EAR and the Cys protease domains in XopD are likely involved in targeting host defense-related components other than MYB30. Together, these data suggest that different XopD host targets are involved in the outcome of the interaction between *Xcc* and *Arabidopsis*. Future work should uncover additional XopD-related molecular interactions during *Xanthomonas* infection.

PopP2 FROM *RALSTONIA SOLANACEARUM*

The YopJ/AvrRxv family of T3Es is present in both mammalian and plant pathogens, indicating that they play important roles in the interaction with the host (Orth, 2002; Roden et al., 2004). YopJ/AvrRxv-like effectors belong to the C55 peptidase family of the clan CE of cysteine proteases, which share a nucleophile cysteine and a predicted catalytic core composed of three conserved amino acid residues (H,D/E,C; Hotson and Mudgett, 2004; Rawlings et al., 2006). YopJ/AvrRxv family members differ in their subcellular localization, indicating that their host targets may be diverse. For example in *Xcv*, AvrRxv is a cytoplasmic protein (Bonshtien et al., 2005), XopJ is targeted to the plasma membrane (Thieme et al., 2007) and AvrBsT localizes to both cytoplasm and nucleus, although the biological significance of AvrBsT nuclear localization remains to be elucidated (Szczesny et al., 2010).

PopP2, a member of the YopJ/AvrRxv T3E family, is injected in plant cells by *R. solanacearum*, the causal agent of bacterial wilt in more than 200 plant species (Hayward, 1991). The *RRS1-R* resistance gene from *Arabidopsis* plants of the Nd-1 ecotype confers broad-spectrum resistance to several strains of *R. solanacearum*. RRS1-R encodes an R protein with original structure since it belongs to the Toll/interleukin1 receptor (TIR)-NBS-LRR subclass of R proteins and presents a C-terminal WRKY motif that is characteristic of the WKRY class of zinc-finger plant TFs (Deslandes et al., 2003; **Figure 4B**). RRS1-R presents a putative bipartite NLS and is localized in the nucleus. Intriguingly, PopP2, which presents an NLS that is required for its nuclear targeting (Deslandes et al., 2003), promotes nuclear accumulation of RRS1-R, possibly by preventing its proteasomal degradation (Tasset et al., 2010). PopP2 and RRS1-R physically interact in the nucleus of *Arabidopsis* cells but whether and how this protein interaction affects host transcription remains to be determined. The *Arabidopsis* cysteine protease RESPONSIVE TO DEHYDRATATION19 (RD19) was identified in a yeast two-hybrid screen as a PopP2-interacting protein (**Figure 4B**). In plant cells, PopP2 induces nuclear targeting of RD19, which is otherwise localized to mobile vacuole-associated vesicles and destined to the lytic vacuole (Bernoux et al., 2008).

RD19 does not contain a predicted NLS and the molecular mechanism allowing its nuclear recruitment is still unknown. It has been proposed that a PopP2-induced membrane permeabilization process would lead to the release of RD19 from mobile vacuole-associated compartments into the cytoplasm, where it would become available for SUMOylation (Bernoux et al., 2008). Indeed, lysine residues with high probability of being SUMOylated are present in RD19. Therefore, it has been suggested that RD19 SUMOylation may generate the signal required for its nuclear translocation. Alternatively, PopP2 may intercept RD19 on its way to the vacuole through retrograde transport from the endomembrane system, which has some continuity with the nuclear envelope. RD19 interacts with PopP2, but not RRS1-R, in the plant cell nucleus. Since RD19, whose expression is induced by *R. solanacearum* infection, is required for *Arabidopsis* resistance to *Ralstonia*, it was proposed that RD19 associates with PopP2 to form a nuclear complex that is required for activation of the plant resistance response (**Figure 4B**). PopP2 interaction with RRS1-R may lead to regulation of defense-related gene expression either directly via RRS1-R WKRY domain or through the action of additional plant TFs (Deslandes et al., 2003; Tasset et al., 2010). In this context, it has been suggested that, once in the nucleus, RD19 may function as a transcriptional activator and/or compete with RRS1-R for similar or overlapping *cis*-elements in the promoters of defense-related genes (Bernoux et al., 2008).

The conserved cysteine residue in PopP2 catalytic triad is required to trigger RRS1-R-mediated resistance. Since PopP2 is able to display acetyltransferase activity, in addition to the proposed indirect modulation of transcription by PopP2 *via* its interaction with RRS1-R or RD19, it has been suggested that PopP2 may directly manipulate host transcription (Tasset et al., 2010; **Figure 4B**). Indeed, acetylation of lysine residues of histone tails facilitates access of TFs to DNA by disrupting higher-order packaging of the chromatin (Kornberg and Lorch, 1999). Addition of acetyl groups may also neutralize the positive charge of histones, thereby reducing their affinity for DNA (Hong et al., 1993). In addition, acetylation forms docking sites for recruitment of transcriptional co-activators and impairs the ability of the lysine side chain to form hydrogen bonds thereby enhancing specific or inhibiting non-specific DNA-binding activities of TFs (Mujtaba et al., 2004; Friedler et al., 2005). It has been therefore proposed that PopP2 autoacetylation and/or acetylation of its host targets may affect gene transcription in host cells (Tasset et al., 2010). Identification of host targets of PopP2 should provide insight into the molecular mechanisms developed by *R. solanacearum* to suppress plant resistance.

PHYTOPLASMA

Phytoplasma are specialized bacteria that are intracellular obligate parasites of plant phloem tissue and their transmitting insect vectors. Phytoplasmas secrete effectors into cells of plants and insects to target host molecules that modulate plant development and increase phytoplasma fitness (Sugio et al., 2011b). As phytoplasma are located intracellularly for much of their life cycle, it is likely that their effectors are released into the cytoplasm of host cells *via* Sec-dependent translocation. Fifty-six secreted effector proteins were identified in AY-WB (Aster Yellows phytoplasma strain Witches'

Broom) and four contain NLSs. For one of these four effectors, named SAP11, it was further shown that the NLS sequence is required for SAP11 accumulation in *Nicotiana benthamiana* cell nuclei (Bai et al., 2009). Phytoplasmas are limited to the phloem sieve cells of their plant hosts, which have no nuclei, suggesting the possibility that SAP11 targets tissues beyond the phloem. Consistent with SAP11 nuclear targeting, SAP11 binds and destabilizes a class I and a subset of class II TCP TFs to manipulate plant development and jasmonic acid biosynthesis (Sugio et al., 2011a). As a result, fitness of the leafhopper insect vector is improved, which ensures efficient transmission of the phytoplasma to other plants (Sugio et al., 2011a).

VIRUSES

Several viral encoded proteins have been shown to translocate into the nuclei during infection. For example, nuclear targeting in infected cells has been observed for various proteins of the rhabdoviruses *Sonchus yellow net virus* (SYNV) and *Maize fine streak virus* (MFSV; Goodin et al., 2002; Tsai et al., 2005). An additional example of a viral protein addressed to nuclei is the 2b protein, a virulence determinant encoded by *Cucumber mosaic virus* (CMV) that suppresses initiation of post-transcriptional gene silencing (PTGS) in transgenic *N. benthamiana*. CMV 2b protein contains an NLS that is essential for translocation of 2b to the nuclei of tobacco cells. Furthermore, nuclear targeting of 2b protein is required for efficient suppression of PTGS (Lucy et al., 2000).

The NIa and NIb proteins of *Tobacco etch potyvirus* (TEV) contain two NLSs and have been shown to be translocated to the nucleus of infected cells (Restrepo et al., 1990; Carrington et al., 1991; Li et al., 1997). The NIa proteinase is required for proteolytic maturation of most TEV proteins (Carrington et al., 1988) whereas NIb functions as the RNA-dependent RNA polymerase (Allison et al., 1986). Mutations in the NLSs of NIb disrupt its nuclear translocation and RNA amplification of TEV (Li and Carrington, 1995; Li et al., 1997). It has been additionally demonstrated that NIa interacts with NIb and that this protein interaction is important during TEV genome replication (Li et al., 1997).

The protein p25 encoded by *Beet necrotic yellow vein virus* (BNYVV) is an important determinant of leaf symptom development and also governs BNYVV invasion of plant roots and induction of rootlet proliferation in sugar beet (Jupin et al., 1992; Tamada et al., 1999). p25 actively shuttles between the cytoplasm and the nucleus of infected cells (Haeberle and Stussi-Garaud, 1995; Vetter et al., 2004). An N-terminal monopartite NLS in p25 is responsible for nuclear accumulation of the protein *via* its interaction with importin-α, whereas a NES sequence in the C-terminus of p25 mediates its active nuclear export in a CRM1/exportin1-dependent manner. Importantly, modification of the wild-type distribution of p25 between the nuclear and cytoplasmic compartments is accompanied by alterations in p25-related symptoms during virus infection (Vetter et al., 2004). These data highlight the importance of nucleocytoplasmic protein trafficking on the production of necrotic symptoms during BNYVV infection.

Similarly, the regions involved in nucleocytoplasmic shuttling of the ORF3 protein of *Groundnut rosette virus* (GRV) have been

mapped (Ryabov et al., 2004). ORF3 is required for viral RNA protection and movement through the phloem. In infected cells, ORF3 is localized in cytoplasmic granules and also in nuclei, preferentially targeting nucleoli, indicating that this protein can be transported between cytoplasm and nucleus during the course of virus infection (Ryabov et al., 1998, 2004). An arginine-rich NLS in ORF3 is responsible for its nuclear targeting whereas mutations in a leucine-rich NES disrupt nuclear import. The importance of nuclear import and export of GRV ORF3 protein is underlined by the high sequence conservation of the NLS and NES regions among different viruses (Ryabov et al., 2004).

The *Cauliflower mosaic virus* (CaMV) open reading frame VI product (P6) is a multifunctional protein essential for the viral infection cycle. P6 is a translational reinitiation factor that associates with the host translational machinery and thus permits translation of downstream ORFs (Park et al., 2001). The P6 protein is the major determinant of host specificity in CaMV and determines symptom severity (Daubert and Routh, 1990). P6 is an abundantly synthesized CaMV protein that in the cytoplasm of infected cells forms electron-dense inclusion bodies, also referred to as viroplasms, where virus replication and assembly occur (Mazzolini et al., 1989). Viroplasms have been additionally detected at the periphery of the nucleus and it has been shown that P6 is able to enter the nucleus during viral infection, suggesting that P6 is a nucleocytoplasmic shuttling protein (Haas et al., 2005). Nuclear localization of P6 is consistent with the finding that P6 interacts with nuclear-localized proteins (Park et al., 2001; Bureau et al., 2004). The N-terminal region of P6 presents an amphipatic α-helix containing a leucine zipper motif that is predicted to form a parallel coiled-coil structure. P6 N-terminal region is well conserved among CaMV strains and mediates the interaction between P6 molecules *in vitro*, making of this region an essential determinant for the formation of viroplasms (Haas et al., 2005). An NES has been identified in P6 Leu-rich sequence that bears some resemblance to NES sequences found in the BR1 protein of the geminivirus *Squash leaf curl virus* (Ward and Lazarowitz, 1999) and in several shuttling nuclear proteins, such as HIV Rev protein (Pollard and Malim, 1998). The NES in P6 determines its CRM1-dependent nuclear export and thus P6 localization in the cytoplasm (Haas et al., 2005). It has been suggested that P6 nuclear export probably occurs very rapidly in infected cells, so that only low amounts are present in the nucleus at any time. Therefore, activity of CRM1-dependent export pathway would limit the extent of P6 nuclear accumulation in the nucleus that could be deleterious for the CaMV infectious cycle. Different hypotheses regarding the role of P6 nuclear accumulation have been formulated. Since P6 is able to bind single- and double-stranded RNA (De Tapia et al., 1993; Cerritelli et al., 1998), it has been proposed that, similar to the Rev protein of HIV-1 (Pollard and Malim, 1998), P6 may control export of CaMV 35S RNA and its spliced versions. Additionally, P6 has been detected in the nucleolus where assembly of ribosomal subunits occurs, suggesting that P6 may interact directly with ribosomes before their export to render them competent for translation of the CaMV polycistronic mRNA. In this context, the P6-interacting ribosomal proteins L18 and L24 (Leh et al., 2000; Park et al., 2001) could be targets for

P6 since they participate in the formation of the 60S ribosomal subunit in the nucleolus (Andersen et al., 2002). Finally, as a nucleocytoplasmic protein, P6 may play a role in inhibition of nonsense-mediated mRNA decay to prevent degradation of the 35S RNA and its spliced versions (Maquat and Carmichael, 2001). Indeed, P6 nuclear export is mediated by the CRM1 pathway (Kudo et al., 1998), which is known to be specifically used for export of the ribosomal subunits and of some cellular mRNAs (Weis, 2002).

The TIR-NBS-LRR immune receptor N is localized to the cytoplasm and the nucleus of uninfected tobacco cells. During *Tobacco mosaic virus* (TMV) infection, the viral replicase p50 is delivered into the plant cell cytoplasm, where it is recognized by the N protein, leading to the establishment of plant resistance (Burch-Smith et al., 2007). In TMV-infected cells, cytoplasmic p50 induces recruitment to the cytoplasm of NRIP1, a tobacco rhodanase sulfurtransferase that otherwise localizes to the stroma of chloroplasts. NRIP1 is able to interact with N but only in the presence of p50 and NRIP1 rhodanase sulfurtransferase activity is not necessary of its association with N or p50. In this context, a cytoplasmic pre-recognition complex is formed that contains NRIP1, p50, and possible additional host proteins (Caplan et al., 2008). Interaction of N with this pre-recognition complex would lead to its activation and, once activated, N would be either translocated into the nucleus or able to send a signal that activates the N nuclear pool, resulting in a successful defense response. Shuttling of p50-activated N from the cytoplasm to the nucleus appears to be required for an efficient defense response (Burch-Smith et al., 2007). Similarly, NRIP1 nuclear relocalization is necessary to provide full resistance to TMV infection (Caplan et al., 2008). Although the molecular mechanism behind p50-mediated NRIP1 nuclear relocalization remains to be determined, different hypothesis have been proposed to explain this finding. p50 might disrupt global chloroplast import by an unknown mechanism that would affect translocation of NRIP1. Otherwise, interaction with p50 might mask the chloroplast targeting signal in NRIP1 facilitating its nuclear import. Alternatively, NRIP1 may be released from chloroplasts into the cytoplasm and the nucleus following p50-induced permeabilization of the outer membrane. Finally, the close physical association between stromules and nuclei might enhance the nuclear import of chloroplastic factors, including NRIP1.

FILAMENTOUS PATHOGENS

Plant pathogenic fungi and oomycetes, collectively referred to as filamentous pathogens, are responsible for a variety of diseases in natural populations and agricultural crops. As sophisticated manipulators of plant cell functions, filamentous pathogens are intimately associated with host plants cells. As previously mentioned, haustoria are highly specialized structures that develop within plant cells and appear to be involved in nutrient acquisition (Hahn and Mendgen, 2001). In addition, haustoria contain specific membrane proteins required for pathogenicity (Avrova et al., 2008), are highly enriched in secreted effector proteins and most probably play a role in mediating effector translocation into host cells, although this hypothesis remains to be formally demonstrated (Panstruga and Dodds, 2009). Following secretion from the

pathogen, effectors that carry host-translocation signals are transported into the plant cell. However, in order to reach the host cytoplasm, effectors need to travel across two membranes, one pathogen-derived and one host cell-derived membrane surrounding the haustorium, and the nature of the mechanism directing effector delivery is still unclear.

Catalogs of the complete set of secreted proteins for a number of filamentous pathogens have been recently generated thanks to extensive genome sequencing programs coupled with robust computational predictions of secretion signals and other sequence motifs characteristic of effectors (Dean et al., 2005; Kamper et al., 2006; Haas et al., 2009; Duplessis et al., 2011). From these studies, it is clear that oomycetes and phytopathogenic fungi secrete a suite of effector molecules that is considerably larger than the effector repertoires of phytopathogenic bacteria. These candidate effector proteins are predicted to modulate host innate immunity and enable parasitic infection (Kamoun, 2007; Hogenhout et al., 2009).

OOMYCETES

Oomycota or oomycetes form a distinct phylogenetic lineage of fungus-like eukaryotic microorganisms. Species of the oomycete genus *Phytophthora* are devastating pathogens of dicotyledoneous plants. Sequencing and analysis of oomycete genomes has revealed that the predicted proteomes include a large repertoire of candidate host-translocated effector proteins that have been classified in two main classes, named RXLR and CRN (for crinkling and necrosis; Haas et al., 2009). Both RXLR and CRN effector proteins present a modular architecture and include a signal peptide, conserved N termini functioning in host delivery and highly diverse C-terminal domains directing the effector activity (Haas et al., 2009; Schornack et al., 2010). Indeed, RXLR effectors are defined by a conserved N-terminal RXLR motif, flanked by a high frequency of acidic (D/E) residues, that enables delivery of effector proteins inside plant cells (Whisson et al., 2007; Dou et al., 2008). It has been proposed that RxLR motifs enable effectors to bind to host cell surface phosphatidylinositol-3-phosphate (PI3P) and subsequently enter host cells through lipid raft-mediated endocytosis (Kale et al., 2010). However, this mechanism of effector translocation and the experiments that support it are still under debate (de Jonge et al., 2011; Stassen and Van den Ackerveken, 2011). Similar to RXLR effectors, N termini of CRN proteins present a conserved but not invariant LXLFLAK motif, which is required for effector targeting and translocation (Schornack et al., 2010). *Aphanomyces euteiches* CRN-like sequences carry a conserved N-terminal LQLYLALK motif similar to the *Phytophthora* LXLFLAK sequence (Gaulin et al., 2008). It has been demonstrated that the LQLYLALK motif in *A. euteiches* CRNs is able to mediate effector translocation (Schornack et al., 2010).

Five hundred sixty-three RXLR effectors are predicted for the strain T30-4 of *Phytophthora infestans* (Haas et al., 2009), whereas they appear to be absent in *Pythium ultimum* (Levesque et al., 2010) and *A. euteiches* (Gaulin et al., 2008), suggesting that these effectors have evolved only recently within the Peronosporales, coinciding with the appearance of haustoria (Levesque et al., 2010). In contrast, the CRN family is ubiquitous in plant pathogenic oomycetes (Gaulin et al., 2008; Levesque et al., 2010),

suggesting that the CRNs belong to an ancient effector family that arose early in oomycete evolution before the emergence of haustoria. Recent evidence however indicates that a significant part of the oomycete secretome was probably acquired from fungi through horizontal gene transfer events, and this may have facilitated the spread of oomycetes to plant hosts (Richards et al., 2011). Conservation of the RXLR motif, which has not been described in fungal effectors, has enabled the computational development of genome-wide catalogs of candidate RXLR effectors from several oomycete pathogens (Tyler et al., 2006; Win et al., 2007; Jiang et al., 2008; Haas et al., 2009). In addition, putative RXLR effector genes have been used in high-throughput screens to predict novel functional activities (Vleeshouwers et al., 2008; Oh et al., 2009). Similarly, LXLFLAK-type motifs may be used in genome-wide searches to identify oomycete effector proteins.

Subcellular localization studies of diverse CRN C termini unrelated in sequence and derived from two divergent species (CRN2, CRNR8, CRN15, and CRN16 from *P. infestans* and *Ae*CRN5 from *A. euteiches*) revealed that CRNs accumulate in plant cell nuclei (Schornack et al., 2010). NLS motifs were predicted in CRN8, CRN16, and *Ae*CRN5, suggesting that CRN proteins recruit the host nuclear import machinery to achieve nuclear accumulation. Indeed, CRN2, CRNR8, CRN16, and *Ae*CRN5 nuclear accumulation depends on the host nuclear import factor importin-α, and at least in the case of CRN8, on a functional NLS. Moreover, cell death induced by CRN8 requires its accumulation in host nuclei, suggesting that CRN proteins are targeted to the host nucleus during plant infection (Schornack et al., 2010). In *P. sojae*, CRN proteins PsCRN63 and PsCRN115, which are involved in virulence on soybean and suppression of host defense responses, contain an NLS motif (Liu et al., 2011). Despite their high sequence similarity (95.7% identity at the amino acid level), PsCRN63 triggers cell death in *N. benthamiana* whereas PsCRN115 is able to suppress cell death induced by the *P. sojae* necrosis-inducing protein PsojNIP (Qutob et al., 2002) and PsCRN63. A functional NLS is required for PsCRN63 induction of cell death and exclusion of PsCRN63 from the nucleus using a NES prevented cell death development, suggesting that PsCRN63 triggers cell death in the plant cell nucleus (Liu et al., 2011). In contrast, the NLS in PsCRN115 is not required for cell death suppression. Although the subcellular localization of PsCRN63 and PsCRN115 was not investigated, it was therefore proposed that PsCRN63 and PsCRN115 may share the same molecular host targets involved in cell death signaling and that their distinct activities are dependent or their nuclear localization (Liu et al., 2011).

Suppressor of necrosis1 (SNE1) and Avr3a are nuclear-targeted effectors from *P. infestans* that suppress plant cell death responses. SNE1 is a highly hydrophilic secreted protein that presents NLSs and is able to translocate to the cell nucleus and suppress the action of secreted cell death-inducing effectors that are expressed during the necrotrophic growth phase of *P. infestans*, as well as programmed cell death mediated by a range of Avr–R protein interactions (Kelley et al., 2010). Avr3a has been shown to interact with and stabilize the U-box E3 ligase CMPG1 that is required for cell death trigerred by *P. infestans* elicitin INF1 (ICD) and is degraded by the 26S proteasome (Bos et al., 2010). This protein interaction, which leads to suppression of ICD, occurs in the nucleus

and, more particularly, in the nucleolus of host cells (Gilroy et al., 2011). It has been therefore proposed that the nucleus is likely a major site of CMPG1 activity and 26S proteasome-mediated degradation. In an additional study, the authors searched for *P. infestans* proteins that contain a signal peptide and an NLS and identified four proteins called, Nuk6, Nuk7, Nuk10, and Nuk12. All four proteins were found in the nucleus of *N. benthamiana* cells in *Agrobacterium*-mediated transient expression assays. Nuclear localization of Nuk6, Nuk7, and Nuk10 is dependent on NbIMPα1 and NbIMPα2, whereas Nuk12 can target nuclei independently of these host α-importins (Kanneganti et al., 2007; Vleeshouwers et al., 2011). Interestingly, a mutation in the *MOS6* gene, encoding AtImpα3 (one of the nine *Arabidopsis* α-importins), enhanced susceptibility to an additional oomycete plant pathogen *Hyaloperonospora parasitica* (*Hpa*; Palma et al., 2005). This finding confirms the importance of protein nucleocytoplasmic trafficking during defense responses against oomycete pathogens.

Finally, sequencing of the *Hpa* genome predicted 134 high-confidence effector gene candidates of the RxLR type (HaRxLs), which is significantly fewer than in *Phytophthora* genomes (Baxter et al., 2010). Notably, a recent study showed association of the plant cell nucleus with developing haustoria in *Arabidopsis* mesophyll cells, possibly by moving through the actin cytoskeleton (Caillaud et al., 2011). In this context, it is tempting to speculate that haustoria directly influence the position of the nucleus in the cell in order to enhance delivery of effectors that compromise defense-related nuclear processes. Indeed, 66% of 49 tested HaRxLs target the nucleus, either as strictly nuclear or nuclear–cytoplasmic. However, only 37.5% of the 16 strictly nuclear HaRxLs carried a canonical NLS (Caillaud et al., 2011), suggesting that endogenous host proteins may facilitate nuclear delivery of these effectors. Interestingly, 21% of the HaRxLs tested localized to the nucleolus, indicating that *Hpa* hijacks the plant cell transcriptional machinery perhaps by acting on RNA biogenesis, transport, or splicing, on ribosome biogenesis and thereby on protein translation to prevent *de novo* induction of defense responses.

FUNGI

Similarly to oomycetes, pathogenic fungi have elaborated intricate parasitic relationships to feed from their host plants and the effector-dependent transcriptional reprogramming of host cells is much plausible. The production of apoplastic effectors by filamentous fungi is well documented (de Jonge et al., 2011; Stassen and Van den Ackerveken, 2011). Similar to what has been described for oomycete effectors, the N termini of various fungal effectors have been reported to carry degenerate RxLR motifs that bind to PI3P and mediate effector translocation, although this may not be a universal means of effector uptake (Kale et al., 2010). Indeed, powdery mildew and rust fungi encode small secreted proteins that share an N-terminal Y/F/WxC motif that is situated downstream of signal peptide and not found in effectors from non-haustorial fungi or oomycetes. It has been suggested that this motif mediates translocation of fungal haustorial effectors into plant cells (Godfrey et al., 2010).

The *Uf*-RTP1 protein from the rust fungus *Uromyces fabae* is the only effector from a pathogenic fungus for which a nuclear localization has been described to date. Indeed, *Uf*-RTP1 is a haustorial secreted effector that presents a signal peptide and a bipartite NLS that mediates *Uf*-RTP1 accumulation in plant cell nuclei (Kemen et al., 2005). Homologs of *Uf*-RTP1 have been found in other pathogenic rust fungi such as *Puccinia graminis*, *Melampsora* spp., *Uromyces appendiculatus*, or *Hemileia vastatrix* (Puthoff et al., 2008; Duplessis et al., 2011). However, the absence of obvious structural features within their protein sequences renders prediction of their putative function(s) a difficult task. Mining of effectors in the genome of *Ustilago maydis* identified among the secreted effector candidates 14 proteins that contain a putative NLS (Mueller et al., 2008), suggesting that virulence of this pathogen may also rely on nuclear targeting of effector proteins.

NEMATODES

Research aimed at the identification and characterization of pathogenicity effectors of plant nematodes has been intensively developed since genome sequences of these pathogenic organisms were first reported. Most nematode species are restricted to the roots in which they form specialized feeding sites to become sedentary endoparasites (Gheysen and Mitchum, 2011). Two groups of pathogens have been particularly studied: the root knot nematodes (*Meloidogyne* spp.) and the cyst nematodes (*Heterodera* spp. and *Globodera* spp.).

First suspicions about the existence of nuclear-localized proteins involved in nematode parasitism of plants came from analysis of first lists of genes encoding secretory proteins and identified as candidate effectors. These proteins are expressed and produced in pharyngeal glands and subsequently delivered into plant cells by the stylet, a protrusible hollow mouth spear (Bellafiore and Briggs, 2010). In *Heterodera glycines*, the identification of specific secretory gland-expressed genes revealed 51 candidate effectors, 15 of which displayed canonical NLSs (Gao et al., 2003), whereas inspection of the *Meloidogyne incognita* secretome yielded a total of 66 effectors putatively addressed to the plant nucleus (Bellafiore et al., 2008). These 66 candidates included 26 proteins with an NLS motif and 40 additional proteins with putative nucleotide binding activity such as DNA or chromatin interaction motifs. Similarly, three esophageal gland-specific gene products from *Meloidogyne chitwoodi* are predicted to be nuclear localized in host cells following cleavage of the leader peptide for protein secretion (Roze et al., 2008). These lists of potential NLS-effectors include many proteins of unknown function (Gao et al., 2003; Roze et al., 2008) but also helicases, histones, DNA-binding domain proteins, and the Nucleosome Assembly Protein, NAP-1 (Bellafiore et al., 2008).

Functional characterization of nematode effectors provided direct evidence of their localization to the plant nucleus. A GFP fusion to the *Heterodera schachtii* secreted protein Hs-UBI1, which encodes a protein with a mono-ubiquitin domain, was shown to be targeted to the nucleolus of tobacco cells (Tytgat et al., 2004). Interestingly, other nematode effectors with some similarity to components of E3-ubiquitin ligase complexes, such as a SKP1-homolog protein, have been identified in other species (Gao et al., 2003; Bellafiore et al., 2008) but their role during parasitic infection remains elusive.

A functional analysis of identified NLS-containing effectors in *H. glycines* demonstrated that two out of the eight tested effector proteins were imported into the nuclei of both onion epidermal

cells and *Arabidopsis* protoplasts (Elling et al., 2007). One of these protein fusions was further transported into the nucleolus. Mutation analysis of the NLS domains confirmed their role in effector nuclear uptake. The authors also showed that GFP fusions with three additional effectors also accumulated in the plant nucleus, but only with effector truncated versions (containing the NLS domain) and not with the full-length proteins (Elling et al., 2007). Whether this differential behavior is due to artifactual mislocalization or reflects the possible existence of an effector processing mechanism within the plant cell remains to be determined.

Another report also showed that some effectors encoded by genes belonging to the large SPRYSEC family in *Globodera pallida* are addressed to the host cell nucleus. These stylet-secreted effectors harbor a SPRY domain of 120 amino acids and unknown function that was first identified in some receptors from *Dictyostelium*. Two *G. pallida* SPRYSEC family proteins harbor NLSs and present nucleolar localization in both tobacco leaf and root cells whereas some other SPRYSEC proteins are cytoplasmic (Jones et al., 2009). It was hypothesized that SPRYSEC effectors suppress host defenses through interaction with a range of host targets in different cell compartments (Jones et al., 2009). Interestingly, similar to viral and bacterial effectors, two SPRYSEC proteins were also shown to interact with resistance proteins, including the potato R protein Gpa2 (Rehman et al., 2009; Sacco et al., 2009).

Since some stylet-secreted proteins have features of DNA-binding proteins (Bellafiore et al., 2008), it is tempting to speculate that some nuclear-localized nematode effectors might act in reprogramming host transcription. Although expression of some TFs involved in plant development have been shown to be induced in nematode feeding sites (Grunewald et al., 2008; Barcala et al., 2010), there is however no evidence supporting a direct role of nematode effectors in modifying plant gene expression. It has been reported that a 13-amino acid peptide, 16D10, secreted from the esophageal glands of *M. incognita*, stimulates root growth and the generation of extensive lateral roots in tobacco hairy roots. 16D10 has been shown to interact *in planta* with two putative plant SCARECROW-like TFs (Huang et al., 2006). However, 16D10 has not been visualized in the plant cell nucleus and its role in plant cell developmental processes, as well as the role of its interacting TFs, remains to be determined.

CONCLUDING REMARKS

Pathogenic microorganisms use an extremely diverse panoply of effector proteins to counteract plant defense and ensure successful colonization of their hosts. Although different subcellular compartments are targeted by effector proteins following their translocation into plant cells, it is now apparent that a significant number of these effector molecules are specifically addressed to the host plant cell nucleus. Nuclear targeting of effectors appears to be a general microbial strategy since examples have been documented in each main class of pathogenic organisms from viruses to nematodes, although no nuclear effectors from bacterial Gram-positive plant pathogens have been described to date.

As mentioned above, the search for NLSs in the available repertoires of predicted effector proteins from different microbes may help identify nuclear translocated effectors. However, it is worth mentioning that up to 45% of yeast proteins present a

predicted bipartite or monopartite NLS (and therefore the potential to enter the nucleus *via* the classical nuclear import pathway), although only 25.8% of proteins localize to the nucleus at steady state when chromosomally tagged with GFP (Huh et al., 2003). Moreover, only 25.8 and 30.9% of the yeast proteins that have been localized to the nucleus in the global GFP screen contain a putative bipartite or monopartite NLS, respectively (Lange et al., 2007). Therefore, about 43% of steady-state nuclear proteins in yeast may not use the classical nuclear import pathway to enter the nucleus. Similarly, *in silico* prediction of NLSs is not systematically associated to nuclear localization of effector proteins (Caillaud et al., 2011) and, in some cases, NLSs are dispensable for effector nuclear targeting (Canonne et al., 2011). Therefore, caution should be taken when interpreting data from *in silico* effector analysis and functional studies of predicted effector proteins are absolutely required to validate their subcellular localization.

Interestingly, nuclear translocation of effector proteins does not seem to be restricted to pathogenic microorganisms since targeting of effector proteins to the plant cell nucleus has been also recently discovered in the context of plant–microbe symbiotic interactions. Indeed, the secreted effector MiSSP7 from the ectomycorrhizal fungus *Laccaria bicolor* is detected in host cell nuclei where it induces reprogramming of the plant cell transcriptome to favor mutualism (Plett et al., 2011). Another report established that the arbuscular mycorrhizal fungus *Glomus intraradices* secretes a protein, SP7, which interacts with the pathogenesis-related TF ERF19 in the plant nucleus to promote symbiotic biotrophy (Kloppholz et al., 2011). These findings highlight the importance of the host nucleus in determining the fate of parasitic or mutualist interactions and the almost ubiquitous microbial strategy that involves direct interactions with host DNA and/or nuclear proteins. As illustrated above for pathogens, outputs resulting from these molecular interactions in the nucleus encompass various pathogenicity processes and multiple stages of host infection, such as the suppression of plant defense responses, the metabolic priming of host cells, or pathogen dispersal.

It is clear that, despite significant recent advances in our field describing nuclear targeting of effector proteins, a lot remains to be done to obtain a global view on how transcriptional reprogramming of host cells or specific targeting of nuclear proteins promote pathogen infection and plant disease. Oligonucleotide microarrays or mRNA sequencing approaches should shed light on the large-scale transcriptomic changes undergone by host cells during nuclear targeting of effector proteins. Finally, a whole cellular dynamics outlook, integrating the contribution to virulence of nuclear effectors and that of effectors targeted to additional subcellular compartments, represents an exciting perspective for future research.

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