Chromatin Configuration as a Battlefield in Plant-Bacteria Interactions[1]

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Plants and microbial pathogens are engaged in an endless arms race. For the interactions between plants and biotrophic pathogens (feeding on living plant tissue), the central concept is the activation of the plant defense response upon pathogen perception and the subversion of immunity by virulence factors produced by successful pathogens. The first layer of the plant innate immune system is based on the recognition of pathogen- or microbe-associated molecular patterns, which leads to a signal transduction cascade and eventually defense gene expression (Zipfel, 2009). Pathogen-associated molecular pattern-triggered immunity (PTI), broadly referred as the general defense response, restricts the growth of the vast majority of potential pathogens encountered by plants. However, successful pathogens produce virulence factors to effectively suppress PTI. For example, gram-negative bacteria inject type III secreted effectors (T3SEs) to inhibit PTI in plant cells (Block et al., 2008; Galán, 2009). As a counteraction, nucleotide-binding leucine-rich repeat (NB-LRR) proteins have evolved to perceive specific T3SEs and elicit a robust and localized programmed cell death, called effector-triggered immunity (ETI; Chisholm et al., 2006; Jones and Dangl, 2006; Boller and He, 2009). Both PTI and ETI also induce systemic acquired resistance (SAR), which is a long-lasting immunity protecting the plants from secondary infection by a broad range of microbial pathogens (Durrant and Dong, 2004).

PTI, ETI, and SAR all involve extensive transcription reprogramming. While there is substantial progress in our understanding of the early events in the defense response, including pathogen recognition and the initiation of signal transduction, significant gaps remain between these early events and the downstream transcription reprogramming. Furthermore, although many genes that are differentially expressed during PTI and ETI are the same, the transcriptional changes are stronger and more sustained during ETI (Tao et al., 2003; Wang et al., 2011b). It has become clear that the tight control of the kinetics and expression levels of defense genes is critical for plant immunity. However, the underlying molecular mechanisms are largely unknown.

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). Chromatin dynamics is orchestrated by ATP-dependent chromatin-remodeling complexes and histone-modifying enzymes. In conjunction with other coregulators, these chromatin remodelers modify histone-DNA interaction and regulate transcription at specific genomic loci. In this review, we summarize the experimental evidence supporting a role of chromatin configuration in regulating the plant immune response, especially the kinetics of defense gene expression. Modulation of chromatin configuration as a strategy employed by bacterial virulence proteins to subvert plant immunity is also discussed.

HISTONE-MODIFYING ENZYMES AND HISTONE MODIFICATIONS

Eukaryotic chromatin structure consists of multiple superimposed layers. DNA wound around the core histone octamers forms the fundamental packaging units called nucleosomes. The N-terminal tails of the core histones are subjected to posttranslational modifications, including acetylation, methylation, phosphorylation, ubiquitination, glycosylation, sumoylation, and ADP ribosylation (Fuchs et al., 2006; Berger, 2007; Bannister and Kouzarides, 2011; Hottiger, 2011). Together, histone-modifying enzymes generate a "histone code" in specific gene regions, leading to changes in chromatin configuration and gene expression.

Upon perception of pathogens, the mitogen-activated protein kinase (MAPK) signaling cascade is activated, leading to the phosphorylation of downstream nuclear targets including defense-related WRKY transcription factors and ethylene-responsive regulators (Andreasson and Ellis, 2010). Unlike in animals (Clayton and Mahadevan, 2003), direct histone phosphorylation by MAPK has not been reported in plants. Nonetheless, various histone-modifying enzymes and chromatin re-

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modelers are changed at transcriptional and/or post-translational levels in response to pathogen infection (Table I). These changes lead to the establishment of specific histone codes and the subsequent transcription reprogramming of defense-related genes.

Histone Acetylation

Acetylation of histone H3 and H4 usually associates with active genes (Berger, 2007). The level of acetylation is balanced by histone acetyltransferases and histone deacetylases (HDACs). Two HDACs have

Table I. *Chromatin-related proteins known for or potentially involved in resistance against bacterial infection*

Type	Gene	Phenotype or Function	Expression Change upon *Pto*DC3000 Infection[a]	Reference
Core ATPase belonging to the SNF2 family	SYD/SPLAYED/CHR3 (AT2G28290)	SNF2 subfamily member; regulating *MYC2*, *VSP*, *PDF1.2*, and *PR1* gene expression; directly shown to be recruited to promoters of *MYC2* and *VSP*	No change	Walley et al. (2008)
	BRM/BRAHMA/CHR2 (AT2G46020)	SNF2 subfamily member; regulating SA-responsive *PR1*, *PR2*, and *PR5* gene expression	No change	Bezhani et al. (2007)
	PIE1/CHR13 (AT3G12810)	SWR1 subfamily member; interacting with ACTIN-RELATED PROTEIN6 and SERRATED LEAVES, forming a large complex; negatively regulating defense against *Pto*DC3000; involved in H2A.Z histone replacement; regulating the expression of *EDS5*, *PR1*, *NIMIN1*, *WRKY18*, and *WRKY38*	No change	March-Díaz et al. (2008)
	DDM1/CHR1 (AT5G66750)	LSH subfamily member; maintaining DNA methylation and possibly regulating immunity through gene silencing	Down-regulated	Li et al. (2010b)
Homologous recombination related	SSN1/RAD51D (AT1G07745)	Forming a protein complex with the Leu-rich nuclear protein SNI1; positively regulating SAR and defense against *P. syringae* pv *maculicola* ES4326	Up-regulated	Durrant et al. (2007)
	SSN2 (AT4G33925)		No change	Song et al. (2011)
	SSN3/BRCA2A (AT4G00020)		No change	Wang et al. (2010b)
Histone-modifying enzymes	HDA19/HD1 (AT4G38130)	Histone deacetylase; regulating the transcriptional activation activity of WRKY38 and WRKY62; also regulating the expression of *DND1* and *DND2*, which negatively regulate defense against *Pto*DC3000	Up-regulated	Clough et al. (2000); Jurkowski et al. (2004); Kim et al. (2008b); Zhu et al. (2010)
	SRT2 (At5G09230)	Histone deacetylase; negatively regulating defense by suppressing SA biosynthetic genes *PAD4*, *EDS5*, and *SID2*	Down-regulated	Wang et al. (2010a)
	SDG8 (AT1G77300)	Histone methyltransferase; positively regulating defense against *P. syringae* pv *maculicola* ES4326, *Pto*DC3000, and strains expressing the type III effectors AvrB or AvrRpm1	No change	Palma et al. (2010)
	ATX1/SDG27 (AT2G31650)	Histone methyltransferase; positively regulating defense against *Pto*DC3000 by enhancing the expression of *WRKY70*	No change	Alvarez-Venegas et al. (2007)
Others	SRFR1 (AT4G37460)	Tetratricopeptide repeat domain-containing protein; suppressing AvrRps4-mediated ETI; involved in NB-LRR protein accumulation and negatively regulating defense gene expression	No change	Kim et al. (2009, 2010); Kwon et al. (2009)
	ELP2 (At1G49540)	Elongator subunit 2; positively regulating the kinetics of PTI and ETI	No change	DeFraia et al. (2010)

[a]Expression data are based on published results or, when not available, extracted from the Bio-Array Resource for Plant Biology (http://esc4037-shemp.csb.utoronto.ca/welcome.htm) based on change in gene expression at 24 h post inoculation of *Pto*DC3000 (10^8 colony-forming units mL^{-1}).

been reported to regulate plant immunity. HISTONE DEACETYLASE19 (HDA19) is induced in Arabidopsis (*Arabidopsis thaliana*) upon infection of the hemibiotrophic bacterial pathogen *Pseudomonas syringae* pv *tomato* strain DC3000 (*Pto*DC3000). As a positive regulator of defense, HDA19 interacts with and represses the activity of two transcription factors, WRKY38 and WRKY62, which negatively regulate the expression of *Pathogenesis-Related* (*PR*) genes (Kim et al., 2008b). Enhanced resistance to *Pto*DC3000 and repression of WRKY activities are abolished in an HDA19 catalytic mutant, suggesting that the deacetylase activity is required for HDA19-mediated plant defense (Kim et al., 2008b). Another HDAC in Arabidopsis, SIRTUIN2 (SRT2), suppresses the expression of salicylic acid (SA) biosynthetic genes (Wang et al., 2010a). SA is a crucial signaling molecule for resistance against *P. syringae* and other biotrophic pathogens (Vlot et al., 2009). SRT2 is down-regulated upon *Pto*DC3000 infection, thereby promoting SA production and the expression of defense-related genes (Wang et al., 2010a).

Histone Methylation

Comparing to acetylation, the effect of methylation is more diverse. Methylation of H3 can either enhance or repress transcription depending on the specific Lys or Arg residues that are modified and the number of methyl groups that are attached to these residues (Berger, 2007). Two histone methyltransferases are reported to regulate plant immunity. The histone methyltransferase ARABIDOPSIS HOMOLOG OF TRITHORAX (ATX1) is a positive regulator of basal resistance against a type III secretion system-deficient mutant strain of *Pto*DC3000. ATX1 positively regulates the expression of *WRKY70*, which is a key transcription factor regulating genes involved in SA and jasmonate/ethylene defense signaling pathways (Alvarez-Venegas et al., 2006, 2007). Another histone methyltransferase, SET DOMAIN GROUP8 (SDG8), is a positive regulator of *Pto*DC3000-triggered defense. SDG8 regulates the expression of *NB-LRR* genes in a gene-specific manner (Palma et al., 2010). For example, *LAZARUS5* (*LAZ5*) and *RESISTANCE TO P. SYRINGAE PV MACULICOLA1* (*RPM1*) are positively regulated by SDG8, which does not affect the transcription of *RESISTANT TO P. SYRINGAE2* (*RPS2*) and *RPS4*. Consistently, an activating mark trimethylation of H3K36 is enriched at the *LAZ5* locus during pathogen infection (Palma et al., 2010).

Although altered resistance has been reported for histone modification mutant plants, targeting specificity and the corresponding chromatin signatures associated with the targeted defense-related loci remain undefined for these histone-modifying enzymes. Despite a simple "on-or-off" histone code, a complex language of histone modifications is likely generated at defense genes upon pathogen infection, leading to a dynamic transcriptional regulation of immunity.

ATP-DEPENDENT CHROMATIN-REMODELING COMPLEXES

ATP-dependent chromatin-remodeling factors are multisubunit complexes with a catalytic subunit containing a conserved SUCROSE-NONFERMENTING2 (SNF2) ATPase domain. Other domains adjacent to the ATPase domain make each chromatin-remodeling ATPase unique (Mohrmann and Verrijzer, 2005). The specificity of remodeling complexes is also determined by the accompanying subunits of the ATPase. In Arabidopsis, 42 SNF2 family ATPases have been annotated and categorized into 24 distinct subfamilies (Flaus et al., 2006). Among them, SPLAYED (SYD) and BRAHMA (BRM) of the SNF2 subfamily, PHOTOPERIOD-INDEPENDENT EARLY FLOWERING1 (PIE1) of the SWI/SNF-RELATED1 (SWR1) subfamily, and DECREASED DNA METHYLATION1 (DDM1) of the LYMPHOID-SPECIFIC HELICASE (LSH) subfamily have been reported to regulate plant immunity (Fig. 1).

SYD and BRM have unique and overlapping targets, including some defense-related genes (Bezhani et al., 2007; Walley et al., 2008). The SA-responsive gene *PR1* is up-regulated in the *syd-2* mutant upon *Pto*DC3000 infection, suggesting that SYD is a negative regulator of the SA pathway. However, *syd-2* mutant plants did not exhibit altered resistance against *Pto*DC3000 (Walley et al., 2008). This could be due to the functions of T3SEs produced by *Pto*DC3000, which may mask potential SYD-regulated resistance. Various SA-responsive genes, including *PR1*, are also up-regulated in the *brm-101* mutant plants. However, BRM has not been examined for its phenotype in plant resistance. It would be interesting to use the type III secretion system-deficient mutant of *P. syringae* and strains triggering ETI to study the role of SYD and BRM in basal and effector-triggered immunity. Moreover, since SYD and BRM each has unique targets and protein partners, characterization of the double mutant will also be necessary to understand the role of the SNF2 subfamily ATPases in plant response to pathogen infection.

So far, the underlying mechanisms by which SYD and BRM regulate plant immunity remain largely unknown. SYD is recruited to the promoters of its target defense-related genes (Walley et al., 2008), but insufficient evidence is available to show that BRM is directly associated with defense-related gene regions. BRM contains a bromodomain and three DNA-binding regions with different binding specificities. A BRM mutant without the bromodomain and two of the DNA-binding regions showed an intermediate phenotype between the wild type and the null mutant, suggesting that these domains are required for the full function of BRM (Farrona et al., 2007). The bromodomain has been found in many chromatin-remodeling proteins and is believed to act as a functional unit for protein-protein interactions (Zeng and Zhou, 2002). Intriguingly, the bromodomains of histone acetyltransferase-

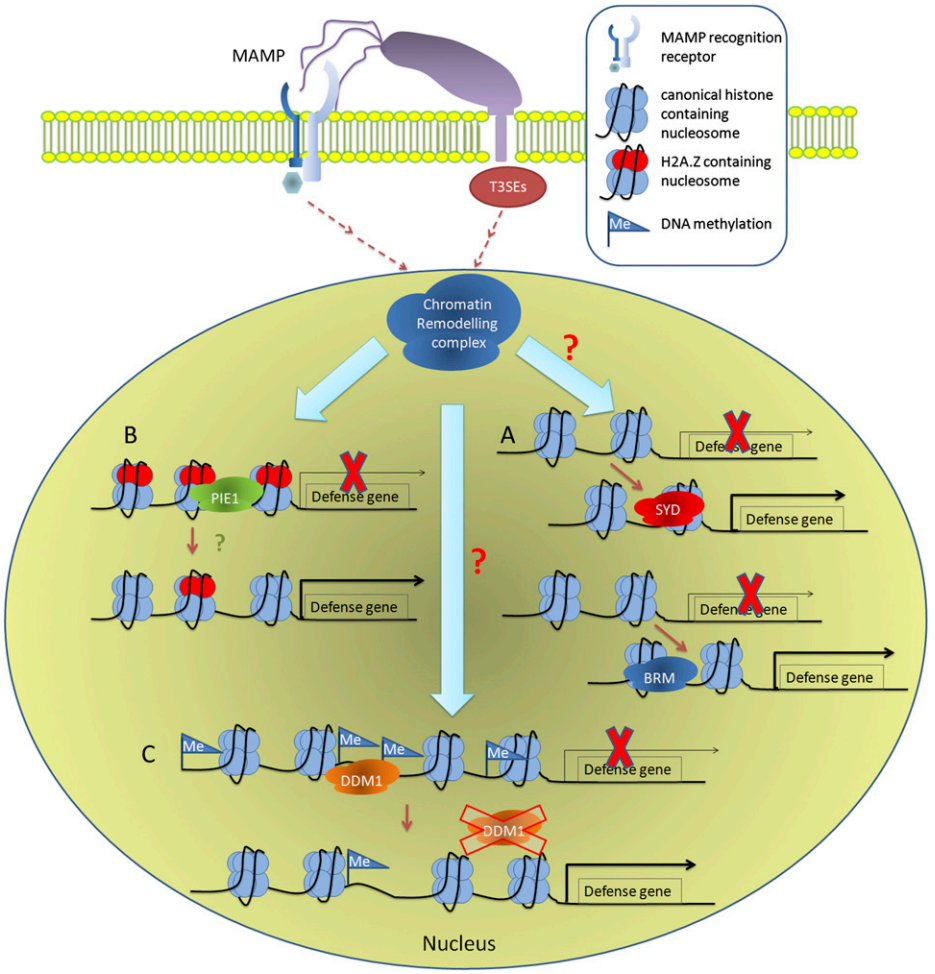

Figure 1. Possible mechanisms of ATP-dependent chromatin remodelers in regulating plant immunity upon bacterial infection. Chromatin remodelers are activated or repressed in response to pathogen perception by unknown pathways. A, SYD, and possibly BRM, are recruited to target loci and induces the expression of defense-related genes. B, PIE1 mediates H2A.Z deposition at target loci in the absence of pathogens to suppress defense gene expression. Upon pathogen infection, these genes might be activated due to a decrease of H2A.Z occupancy level. C, DNA methylation-associated gene silencing on defense genes might be alleviated due to the down-regulation of DDM1 at the transcriptional level upon pathogen infection. MAMP, Microbe-associated molecular pattern.

associated coactivators mediate direct interactions of these coactivators with histone, especially via the acetylated Lys residues (Dhalluin et al., 1999). BRM also interacts with H3 and H4 in vitro; however, its preferential binding to acetylated histones was not found (Farrona et al., 2007). Further study on the binding specificity of the bromodomain may help in constructing a mechanistic model of the functionality of BRM in plant immunity.

PIE1 is a negative regulator of plant resistance against *Pto*DC3000. Various defense-related genes that are repressed in the absence of the pathogen are constitutively expressed in the *pie1-5* mutant plants (March-Díaz et al., 2008). PIE1 interacts with the histone variant H2A.Z and was shown to be required for the deposition of H2A.Z at the PIE1-regulated loci (Deal et al., 2007; March-Díaz et al., 2008). Although deposition of H2A.Z at defense-related loci by PIE1 has not been reported, both *pie1-5* and the H2A.Z mutant plants show enhanced resistance to *Pto*DC3000 (March-Díaz et al., 2008). H2A.Z is usually found in nucleosomes flanking the transcription start sites and has been implicated in regulating the transcription of heat-responsive genes (Kumar and Wigge, 2010). It is likely that PIE1 deposits H2A.Z at the targeted defense-related loci and suppresses transcription in the absence of pathogen infection.

DDM1 contains a conserved SNF2 ATPase domain. Without a known methyltransferase domain or demonstrated methyltransferase activity (Jeddeloh et al., 1999), DDM1 is required to maintain DNA methylation, probably by modulating the access of DNA methyltransferases or DNA demethylases to the genome via its chromatin-remodeling activity (Brzeski and Jerzmanowski, 2003; Zemach et al., 2005). *ddm1* mutant plants exhibit global DNA hypomethylation, which leads to decreased gene silencing (Vongs et al., 1993). Although the role of DDM1 in the plant response to bacterial pathogens remains to be elucidated, another protein, ARGONAUTE4, which regulates small RNA-mediated DNA methylation and gene silencing, is a positive regulator of plant defense against *Pto*DC3000 (Agorio and Vera, 2007). A recent study (Li et al., 2010b) showed slightly increased (although not statistically significant) susceptibility of the *ddm1-8* mutant plants upon infection of the obligate biotrophic oomycete pathogen *Hyaloperonospora arabidopsidis* Noco2. This is consistent with the idea that DDM1 may act as a

positive regulator of plant resistance against biotrophic bacterial pathogens.

OTHER CHROMATIN-ASSOCIATED PROTEINS

Other chromatin-associated proteins have also been identified to regulate plant immunity. In genetic screens for the *SUPPRESSOR OF SNI1* (*SSN*), three genes, *RAD51D/SSN1*, *BRCA2A/SSN3*, and *SSN2*, were identified (Durrant et al., 2007; Wang et al., 2010b; Song et al., 2011). Arabidopsis *SSN* knocked out mutants exhibit reduced *PR* gene expression and dampened resistance against the infection of *P. syringae* pv *maculicola* ES4326. SSN2 contains a SWI2/SNF2 and MuDR domain and is mainly located to the nucleus (Song et al., 2011). RAD51D directly interacts with SSN2 and forms a complex with BRCA2A, suggesting that these three SSNs work together in one protein complex. Upon induction by SA, this protein complex can be recruited by TGA transcription factor(s) to the promoters of *PR* genes and regulate their transcription (Wang et al., 2010b; Song et al., 2011). BRCA2 promotes RAD51-ssDNA assembly in human (Jensen et al., 2010). During homologous DNA recombination, the RAD51-ssDNA nucleoprotein filament associates with and stimulates the chromatin-remodeling activity of RAD54, which is the core catalytic ATPase subunit of an ATP-dependent chromatin remodeler in yeast (Alexeev et al., 2003; Jensen et al., 2010). Although the identification of an RAD54 homolog was not reported in the genetic screen for *SSN*s, RAD51D, and possibly BRCA2A and SSN2, may associate with a chromatin-remodeling complex for the transcriptional regulation of *SNI1* and other *PR* genes.

INTERPLAY AMONG HISTONE-MODIFYING ENZYMES, ATP-DEPENDENT CHROMATIN REMODELERS, AND OTHER REGULATORS

It is generally accepted that while histone-modifying enzymes modify histone posttranslationally, ATP-dependent chromatin remodelers use the energy generated by ATP hydrolysis to move or eject nucleosomes along DNA (Hargreaves and Crabtree, 2011; Kasten et al., 2011). They are unlikely to work independently. Whether histone modifications, such as methylation and acetylation, and chromatin remodeling occur sequentially is still unresolved (Neely and Workman, 2002). Recently, the p65 subunit of the NF-κB protein complex was found to be reversibly methylated and demethylated by the histone methylase NSD1 and the demethylase FBXL11 (Lu et al., 2010), suggesting that histones are not the only substrates of histone-modifying enzymes. It would be exciting to investigate the possible roles of histone-modifying enzymes in regulating other transcription regulators, including transcription factors and chromatin-remodeling proteins.

POTENTIAL ROLE OF CHROMATIN REMODELERS IN REGULATING THE KINETICS OF DEFENSE GENE EXPRESSION

Without specialized immune cells, plants must tightly control the immune response so that the allocation of available resources is finely balanced with other biological processes, such as reproduction and growth (Kliebenstein and Rowe, 2008). The ability to switch fast between alternate states of gene transcription, therefore, is fundamental to an economic and efficient resistance. How plants regulate the timing of these switches remains a major challenge. Recent evidence suggests that chromatin-remodeling complexes might play an important role in determining the kinetics of defense-related gene expression in plants.

SRFR1 (for SUPPRESSOR OF RPS4-RLD1) was identified as a negative regulator of defense-related gene expression in Arabidopsis (Kim et al., 2009, 2010; Kwon et al., 2009). SRFR1 is located in both cytoplasm and nucleus and possibly possesses dual functions. In the cytoplasm, SRFR1 associates with the NB-LRR protein complexes and regulates NB-LRR protein accumulation (Kim et al., 2010; Li et al., 2010a). In the nucleus, SRFR1 suppresses defense gene expression and possibly sets up a threshold to switch on the immune response. Interestingly, SRFR1 shares sequence similarity with the yeast corepressor protein Ssn6, which is recruited by transcription factors to specific target genes. In yeast, Ssn6 primes these genes for rapid derepression by directing the preacetylation of nucleosomes (Desimone and Laney, 2010). It remains to be determined whether SRFR1 associates with chromatin-remodeling complexes and/or other transcription factors at specific gene regions.

Arabidopsis ELONGATOR SUBUNIT2 (ELP2) was recently shown to regulate the kinetics of defense gene expression (DeFraia et al., 2010). Elongator complex associates with RNA polymerase II and regulates transcription through various processes, including histone modification (Otero et al., 1999; Winkler et al., 2002). In *Atelp2* mutant plants, induction of defense-related genes during PTI and ETI is delayed and/or decreased, suggesting that AtELP2 is a positive regulator of immunity by accelerating the induction of defense-related genes (DeFraia et al., 2010). It will be interesting to test whether AtELP2 triggers histone modification or other chromatin configuration changes at its target genes.

BACTERIAL PATHOGENS MANIPULATE CHROMATIN-REMODELING PROCESSES TO FACILITATE INFECTION

Microbial pathogens employ a variety of strategies to suppress defense and successfully infect plants. The conserved nature of chromatin-remodeling complexes makes them attractive targets through which pathogens subvert innate immunity. This is particularly important for the biotrophic and hemibiotrophic pathogens, because they maintain a rather stable symbiotic

relationship with the hosts at least at the early stages of the infection.

The first example of virulence proteins directly modulating plant chromatin remodeling is the HC-toxin produced by the maize (*Zea mays*) fungal pathogen *Cochliobolus carbonum*. HC-toxin inhibits histone deacetylase activity, leading to hyperacetylation of histones during infection (Brosch et al., 1995). An HC-toxin reductase can detoxify HC-toxin and readily confer resistance of maize to the pathogen, suggesting that HC-toxin is a major virulence determinant in this interaction (Johal and Briggs, 1992). Thus far, how the inhibition of histone deacetylase activity promotes pathogen infection is not well understood. Since *C. carbonum* is a necrotrophic pathogen, HC-toxin could simply promote host cell death by targeting a fundamental cellular process.

Agrobacterium tumefaciens Virulence Proteins VirE2 and 6b

The *Agrobacterium*-Arabidopsis interaction represents an excellent example of the direct interaction between bacterial virulence factors and host chromatin-associating proteins. *Agrobacterium* causes plant diseases by transferring a segment of its own DNA (T-DNA) into the nucleus of plant cells. The T-DNA, carrying oncogenes coding for auxin and cytokinin biosynthetic enzymes, is then integrated into the plant genome. These phytohormones promote plant cell division and enlargement; thereby inducing uncontrolled growth of plant tissues to form galls. T-DNA also encodes opine biosynthetic enzymes, which produce opine that can be used as a nutrient source by *Agrobacterium* (Escobar and Dandekar, 2003). It is evident that multiple chromatin-related proteins, including core histones, histone-modifying enzymes, histone chaperones, and chromatin assembly proteins, function in the process of T-DNA integration (Lacroix and Citovsky, 2009; Gelvin, 2010).

The *Agrobacterium* virulence protein VirE2 has been shown to modulate the chromatin functions and facilitate T-DNA integration (Fig. 2). VirE2-INTERACTING PROTEIN1 (VIP1) directly interacts with various core histones, such as H2A, in Arabidopsis (Li et al., 2005). VIP1 may act as a bridge to reinforce the association of VirE2 with plant nucleosome. In this way, VirE2 may facilitate T-DNA integration by directing the T-DNA complex to host chromatin (Lacroix et al., 2008). Furthermore, another VirE2-interacting protein, VIP2, may regulate histone gene transcription (Anand et al., 2007). Consistent with this finding, several histone genes are up-regulated upon *Agrobacterium* infection (Veena et al., 2003). It is intriguing to speculate that VirE2 or other *Agrobacterium* effectors may modulate histone gene expression to facilitate infection. For example, VirE3 has been shown to be a transcription activator (García-Rodriguez et al., 2006).

The virulence protein 6b of *Agrobacterium* contributes to the induction of abnormal plant growth and tumor formation (Tinland et al., 1990). 6b physically associates with several Arabidopsis proteins in the nucleus, including key components of the microRNA pathway and the core histone H3 (Terakura et al., 2007; Wang et al., 2011a). 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 possesses an ADP-ribosyltransferase activity (Wang et al., 2011a). Since 6b directly interacts with H3, it will be interesting to examine whether H3 can be modified by 6b and how this potential 6b-mediated ribosylation of H3 might impact transcription.

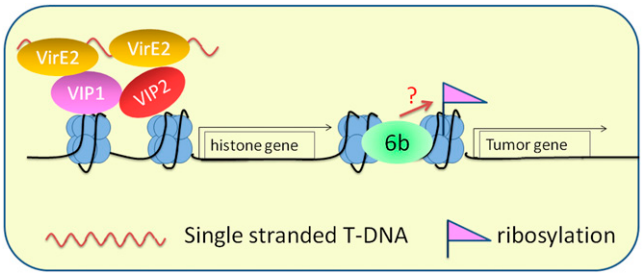

Figure 2. A model for the *Agrobacterium* effectors VirE2 and 6b in modulating chromatin configurations and gene expression in the plant nucleus. VirE2 directly interacts with VIP1 and VIP2, which also interact with each other. VIP1 facilitates the association of T-DNA complexes with histones and thereby promotes T-DNA integration. VIP2 may activate histone gene expression. 6b directly interacts with histone H3 and possesses an ADP-ribosyltransferase activity. 6b could potentially modulate histone modification and induce genes involved in abnormal cell growth.

T3SEs

T3SEs are well-studied virulence proteins produced by gram-negative bacteria to suppress host immunity (Galán, 2009). After being delivered into the host cytoplasm, some T3SEs can enter the nucleus either by the nuclear localization sequence in the effector sequence or via interaction with host nuclear proteins. Some of these nucleus-localized T3SEs have been shown to modulate transcription in the host. For example, the transcription activator-like (TAL) effectors produced by *Xanthomonas* species bind to specific promoter sequences and directly activate gene expression to promote bacterial infection and disease symptoms (Boch and Bonas, 2010). So far, TAL effectors have only been found in *Xanthomonas* species, and direct transcriptional regulation has not been reported for the other nucleus-localized T3SEs produced by plant pathogens. It remains to be determined whether any of these effectors interfere with chromatin-associated functions to indirectly modulate host gene expression.

The type III effector OspF of the animal pathogen *Shigella flexneri* 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; Fig. 3A). OspF also has the phospho-Thr lyase activity that targets the phosphorylated MAPKs in the nucleus (Li et al., 2007). The plant pathogen *P. syringae* produces an OspF-like T3SE, HopAI1, which possesses the same phospho-Thr lyase activity. HopAI1 also disrupts defense signal transduction by directly inactivating MAPKs in plants (Zhang et al., 2007). Since the nuclear localization of HopAI1 has not been reported, whether HopAI1 could also target nuclear MAPKs and/or modulate histone modifications in plant cells remains unknown.

Another T3SE with possible chromatin-remodeling activity is XopD from *Xanthomonas campestris* pv *vesicatoria*, which has small ubiquitin-like modifier (SUMO) protease activity. Genome-wide proteomic study in Arabidopsis identifies diverse SUMO substrates, including histone-modifying enzymes, chromatin-remodeling complex components, and defense-related transcription factors (Miller et al., 2010; van den Burg and Takken, 2010). A loss-of-function mutant of a SUMO E3 ligase, SIZ1, shows elevated accumulation of SA and constitutive defense response in Arabidopsis (Lee et al., 2007), implicating a role of sumoylation in defense regulation. XopD is located to the subnuclear foci in plant cells. In addition to the SUMO protease activity, XopD also contains an ERF-associated amphiphilic repressor motif (EAR), which is responsible for the transcriptional repression of defense- and senescence-related genes by XopD (Kim et al., 2008a). The EAR domain of the auxin regulator Aux/IAA directly interacts with the corepressor TOPLESS (TLP), which associates with HDA19 (Szemenyei et al., 2008). Since both TLP and HDA19 are substrates for sumoylation (Miller et al., 2010), it is intriguing to speculate that XopD could recruit and/or modify chromatin-remodeling proteins and regulate their activities through its SUMO protease activity (Fig. 3B). Identification of XopD-interacting proteins will help test this possibility.

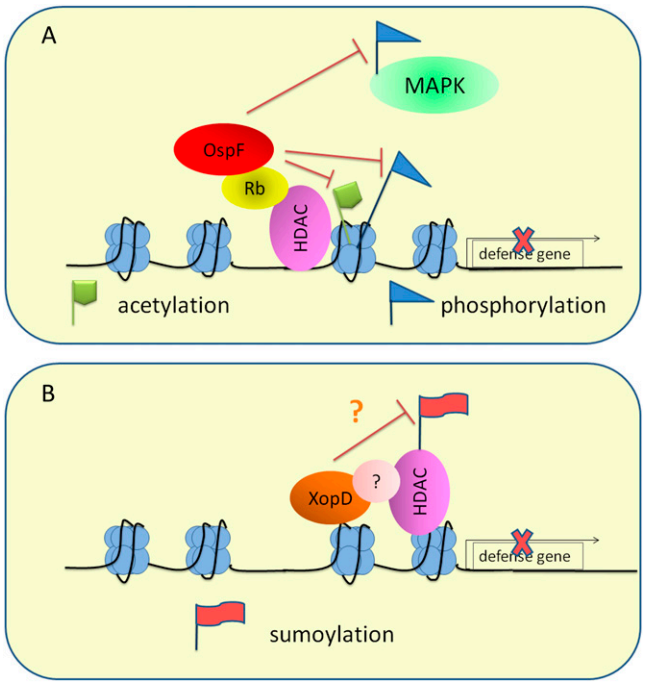

Figure 3. T3SEs suppress defense gene expression by modulating host chromatin configuration. A, OspF from the animal pathogen *S. flexneri* induces the dephosphorylation of MAPK and the dephosphorylation and deacetylation of histone, leading to reduced transcription of defense-related genes. The histone modification changes induced by OspF is dependent on its interaction with the retinoblastoma protein (Rb), which could interact with histone-modifying enzymes such as HDAC. B, XopD from the plant pathogen *X. campestris* pv *vesicatoria* has SUMO protease activity and acts as a transcriptional repressor. Through its EAR domain, XopD may associate with and modify histone-modifying enzymes, such as HDAC, or other chromatin-remodeling proteins that are substrates for sumoylation.

CONCLUSION AND PROSPECTS

The activation and suppression of innate immunity are central principles of plant-pathogen interaction. PTI and ETI involve large-scale gene expression changes in a quantitative manner. However, a significant gap of knowledge lies between the recognition of microbe-associated molecular patterns or effectors and the downstream transcription reprogramming. Emerging evidence suggests chromatin remodeling as an integral component linking these two events. Transcription regulation requires combined actions of sequence-specific DNA-binding transcription factors and other co-regulatory chromatin-remodeling machineries. Current data suggest that different chromatin-remodeling proteins control different sets of defense-related genes, probably upon the perception of specific elicitors. The specificity of chromatin-remodeling proteins with regard to their role in immunity may allow the integration of signals from different elicitors and generate the kinetic and quantitative regulation of defense gene expression.

The role of chromatin remodeling in plant immunity was not appreciated until recently. The rich resources available for the model system Arabidopsis-*P. syringae* provide an excellent opportunity to investigate the general function of chromatin-associating proteins in plant immunity. Systemic analyses of known chromatin-remodeling proteins by screening the corresponding Arabidopsis mutants for an altered resistance phenotype will provide a fundamental understanding of the significance and universality of chromatin-remodeling-mediated transcription regulation in innate immunity. However, as a fundamental biological process, evaluating the contribution of chromatin remodeling to a specific function could be challenging, since the knockout mutants will likely exhibit rather pleiotropic deficiencies. Therefore, it is important to determine the

target specificities of chromatin remodelers using chromatin immunoprecipitation sequencing, which is much more feasible and efficient with the development of the next-generation sequencing technology. Furthermore, the genome-wide charting of histone modifications, before and after pathogen infection, will also provide important hints on the role of chromatin remodelers in plant resistance.

Bacterial pathogens have evolved a fascinating number of virulence proteins, mainly T3SEs and toxins, to subvert plant immunity. In recent years, a great deal of effort has been invested to elucidate the molecular basis for T3SE-mediated suppression of innate immunity. This knowledge has greatly contributed to our basic understanding of bacterial pathogenicity and plant immunity. However, except for the TAL effectors, the functions of the nucleus-targeting T3SEs are largely unknown. It will be particularly interesting to examine whether any of these T3SEs directly target chromatin-remodeling processes and influence transcription at specific defense-related loci. This information will establish a clearer picture of chromatin configuration as a new battlefield between plants and bacterial pathogens.

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