

# The Role of the Ubiquitin-Proteasome System in *Agrobacterium tumefaciens*-Mediated Genetic Transformation of Plants<sup>1</sup>

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*Agrobacterium tumefaciens*-mediated genetic transformation of plants is the first example of trans-kingdom gene transfer and had been considered the only known natural example of such a case until the recent discovery of *Bartonella henselae*-mediated transformation of human cells under laboratory conditions (Schröder et al., 2011). In nature, the pathogenic soil bacterium *A. tumefaciens* induces neoplastic growths (crown gall tumors) on various plant species, including many agronomically important crops. During its infection, *A. tumefaciens* mobilizes a single-stranded copy of the bacterial transferred DNA (T-DNA) into the host cell and subsequently integrates it into the host genome (Gelvin, 2000, 2010; Tzfira and Citovsky, 2002; Pitzschke and Hirt, 2010). The wild-type T-DNA encodes several genes involved in auxin and cytokinin biosynthesis, and their expression in the infected plant cells leads to abnormal cell proliferation and the formation of tumors. With the help of other genes encoded by the T-DNA, the tumors then synthesize and secrete opines, amino acid derivatives that can be metabolized mainly by *A. tumefaciens*. This unique infection strategy allows *A. tumefaciens* to hijack the host cell machinery and turn it into its own “food factory.” Although *A. tumefaciens* mainly infects dicotyledonous plants in nature (De Cleene and De Ley, 1976), it can genetically transform virtually any eukaryotic species under laboratory conditions (Lacroix et al., 2006). Because of this broad host range, *A. tumefaciens* serves as a transformation vehicle of choice for the genetic manipulation of most plant species as well as numerous fungal species (Lacroix et al., 2006). Thus, understanding the molecular mechanism of *A. tumefaciens* infection is important not only to protect crops from the crown gall disease and to improve the efficiency of *A. tumefaciens*-mediated genetic engineering, but it also

substantially advances our knowledge of fundamental aspects of genetic transformation and bacterial pathogen-host interactions.

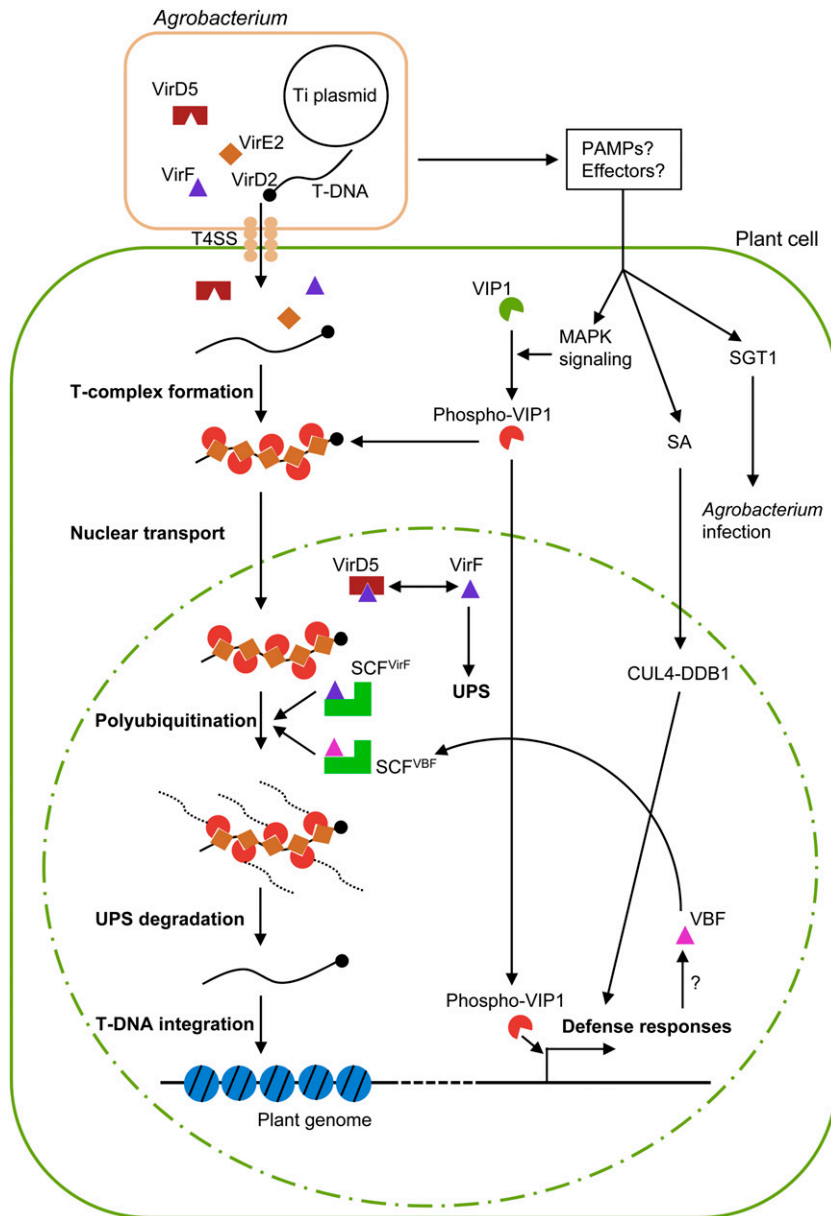
Recently, the ubiquitin-proteasome system (UPS) has emerged as a critical player in plant-pathogen interactions (Citovsky et al., 2009; Dielen et al., 2010; Trujillo and Shirasu, 2010). Numerous studies have shown that the plant UPS regulates the host defense responses, presumably by controlling the stability of the host and/or pathogen proteins. Moreover, increasing evidence suggests that several plant pathogens exploit the host UPS for efficient infection, further emphasizing the importance of the UPS in plant-pathogen interactions (Magori and Citovsky, 2011b). Consistent with this notion, the host UPS plays a critical role in the *A. tumefaciens*-plant interaction. Recent studies have shown that, upon *A. tumefaciens* infection, the host plants up-regulate or down-regulate several UPS-associated genes and proteins (Ditt et al., 2006; Anand et al., 2007, 2012; Zhao et al., 2011; Tie et al., 2012), some of which likely affect the efficiency of the *A. tumefaciens* infection (Zaltsman et al., 2010; Anand et al., 2012). In addition, *A. tumefaciens* is known to export into the host cell an F-box protein, a component of the SCF (for S-PHASE KINASE-ASSOCIATED PROTEIN1 (SKP1)-CULLIN1 (CUL1)-F-box protein) ubiquitin ligase complex, and facilitate infection via the UPS-mediated protein degradation (Tzfira et al., 2004). Thus, *A. tumefaciens* represents a powerful model system to study how plants defend against invading pathogens via their UPS and how pathogens exploit the host UPS during infection. In this review, we focus on recent advances in understanding the role of the UPS in *A. tumefaciens*-mediated genetic transformation (summarized in Fig. 1).

## THE *A. TUMEFACIENS* F-BOX PROTEIN VIRF

In addition to its T-DNA, *A. tumefaciens* translocates several bacterial proteins, termed virulence (Vir) proteins, into the host cell via its type IV secretion system (Vergunst et al., 2000, 2005). These exported bacterial effectors are thought to mediate, directly or indirectly, the nuclear transport of the T-DNA as well

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**Figure 1.** Involvement of the UPS and UPS-associated factors in *A. tumefaciens*-mediated genetic transformation. *A. tumefaciens* processes a single-stranded T-DNA from the tumor-inducing (Ti) plasmid and exports it to the plant cell via the type IV secretion system (T4SS) along with several virulence (Vir) proteins. Within the plant cytoplasm, the T-DNA is packaged into the T-complex, in which the T-DNA molecule is covalently associated with a single molecule of VirD2 and cooperatively coated with numerous VirE2 molecules. In addition, the host factor VIP1 directly interacts with VirE2. The plant mitogen-activated protein kinase (MAPK) defense signaling induces the phosphorylation of VIP1, leading to nuclear import of the phospho-VIP1. The nucleus-localized phospho-VIP1 then activates the expression of defense genes. Meanwhile, *A. tumefaciens* exploits the phospho-VIP1 to translocate the T-complex to the cell nucleus and target it to the host chromatin. Once the T-complex reaches the host chromatin, VIP1 becomes polyubiquitinated by the VirF-containing SCF complex (SCF<sup>VirF</sup>). Another effector protein, VirD5, stabilizes VirF, which otherwise undergoes degradation via the host UPS. In addition to VirF, *A. tumefaciens* utilizes the host F-box protein VBF and presumably polyubiquitinates VIP1 via SCF<sup>VBF</sup>. The VBF-encoding gene is induced upon *A. tumefaciens* infection, most likely as part of the host defense responses. The polyubiquitinated VIP1 as well as its associated protein VirE2 are degraded via the host UPS, resulting in exposure of the T-DNA molecule. This T-complex proteasomal uncoating likely occurs in the vicinity of the host chromatin just prior to T-DNA integration. Finally, the T-DNA becomes integrated into the plant genome via largely uncharacterized mechanisms. The host plants may activate multiple defense signaling pathways in response to *A. tumefaciens*-derived pathogen-associated molecular patterns or effector proteins. Among such defense signaling events, the plant hormone salicylic acid (SA) activates the CUL4-DDB1 ubiquitin ligase complex, leading to defense responses against *A. tumefaciens*. The SCF complex-associated protein SGT1 is induced upon *A. tumefaciens* infection and promotes the *A. tumefaciens*-mediated transformation via an unknown mechanism.

as its integration into the host genome. Among the exported proteins, VirE2 serves to package the mobile single-stranded T-DNA molecule, covalently associated with a single molecule of the bacterial endonuclease VirD2 (Dürrenberger et al., 1989), into a nucleoprotein complex (T-complex), in which numerous VirE2 molecules cover the entire length of the T-DNA molecule (Citovsky et al., 1997; Abu-Arish et al., 2004). Moreover, *A. tumefaciens* also utilizes the plant factor VIP1 (for VirE2-interacting protein1), which directly binds to VirE2 and facilitates the nuclear import and chromatin targeting of the entire T-complex (Tzfira et al., 2001; Li et al., 2005; Djamei et al., 2007; Lacroix et al., 2008). In the current model, the T-complex is most likely uncoated of its protein components by the VirF-mediated proteasomal degradation before the T-DNA becomes integrated into the host genome (Tzfira et al., 2004). It has been shown that VirF, the first F-box protein found to be encoded by a prokaryotic genome, interacts with the Arabidopsis (*Arabidopsis thaliana*) SKP1 proteins, indicating that VirF functions in the SCF ubiquitin ligase complex (Schrammeijer et al., 2001; Tzfira et al., 2004). As a subunit of SCF, VirF targets at least VIP1 and its associated VirE2 for proteasomal degradation (Tzfira et al., 2004). This VirF function appears important for the infection process, as mutations in the *virF* gene substantially reduce infection efficiency in many, but not all, hosts (Melchers et al., 1990; Regensburg-Tuink and Hooykaas, 1993; Schrammeijer et al., 2001).

However, we cannot exclude the possibility that VirF harbors multiple functions during *A. tumefaciens* transformation. A fascinating hypothesis is that this F-box protein might also be necessary to subvert the host defense system against *A. tumefaciens*. A recent study has demonstrated that, upon *A. tumefaciens* infection, VIP1 becomes phosphorylated via the mitogen-activated protein kinase defense signaling (Djamei et al., 2007). The phosphorylated form of VIP1 then enters the cell nucleus and serves as a transcription factor that activates the expression of stress-dependent genes, including *PATHOGENESIS-RELATED GENE1* (*PR1*; Djamei et al., 2007; Pitzschke et al., 2009). These observations suggest that the role of VirF is not restricted to the T-complex uncoating but, rather, that this bacterial F-box effector also acts to down-regulate the VIP1-mediated host defense responses through the degradation of VIP1.

This scenario, however, involves a paradox where VIP1 seemingly plays a dual role in the *A. tumefaciens*-mediated genetic transformation: one as a positive regulator and another as a negative regulator. As a positive regulator, VIP1 helps the nuclear import and chromatin targeting of the T-complex, and as a negative regulator, it presumably reduces *A. tumefaciens* infectivity via the host defense system. Thus, excessive or premature degradation of VIP1 by VirF would hamper the T-DNA nuclear uptake and chromatin localization and, hence, the bacterial infection. How does

*A. tumefaciens* resolve this conundrum? The answer to this question may lie in as-yet-unidentified plant factors that regulate the timing of VirF-mediated VIP1 degradation through the posttranslational modification of VIP1. Indeed, posttranslational protein modifications, such as phosphorylation and glycosylation, are often required for a substrate protein to be recognized by the SCF complex (Cardozo and Pagano, 2004). By analogy, it is tempting to speculate that an unknown host factor, such as chromatin-associated protein kinases, chemically modifies the nuclear VIP1 molecules into a substrate form favorable for the VirF-containing SCF complex (SCF<sup>VirF</sup>). In this model, VIP1 ubiquitination and the subsequent degradation do not happen until the T-complex reaches the host chromatin.

#### VIRF ITSELF IS TARGETED FOR DEGRADATION BY UPS AND PROTECTED BY ANOTHER BACTERIAL EFFECTOR

Many F-box proteins are inherently unstable due to their own proteolysis, which is mediated by autoubiquitination activity (Zhou and Howley, 1998; Galan and Peter, 1999) or other E3 ligases (Ayad et al., 2003; Guardavaccaro et al., 2003; Margottin-Goguet et al., 2003). This is also the case for the *A. tumefaciens* F-box protein VirF (Magori and Citovsky, 2011a). Specifically, VirF is rapidly degraded in plant extracts via the host UPS (Magori and Citovsky, 2011a). This degradation of VirF does not occur by an autocatalytic mechanism, because mutations in the F-box, a motif essential for the autoubiquitination of F-box proteins, do not stabilize VirF (Magori and Citovsky, 2011a). Instead, an unknown plant SCF complex may destabilize VirF. Consistent with this idea, coexpression of a dominant negative CUL1, which is expected to disrupt the activity of the host SCF complexes, led to the stabilization of VirF in plant extracts (Magori and Citovsky, 2011a). This observation suggests that the host plants may possess a defense system that wards off *A. tumefaciens* infection via the UPS-mediated VirF degradation.

To counteract the host-mediated VirF degradation, *A. tumefaciens* exports to plant cells another bacterial effector protein called VirD5 (Magori and Citovsky, 2011a). Due to the lack of known functional domains, the role of VirD5 had been completely unknown. However, a recent study revealed that VirD5 directly interacts with VirF in plant cells and protects it from UPS-mediated degradation (Magori and Citovsky, 2011a). The role of VirD5 in *A. tumefaciens* infection is significant because a mutation in the VirD5-encoding locus substantially attenuates tumor formation (Magori and Citovsky, 2011a). Together, these data reveal a novel type of host-pathogen molecular arms race, in which both sides compete to control the stability of pathogen-encoded F-box effectors.

## A. TUMEFACIENS INFECTION AFFECTS THE EXPRESSION OF SEVERAL UPS-ASSOCIATED GENES

The bacterial F-box protein VirF casts a spotlight on the role of the host UPS during *A. tumefaciens* infection, but it may be just the tip of the iceberg in the entire complexity of molecular reactions involved in the *A. tumefaciens*-plant cell interaction. A transcriptome analysis using Arabidopsis revealed that at least three F-box genes are induced and at least one F-box gene is repressed within 48 h after *A. tumefaciens* inoculation (Ditt et al., 2006; Table I). More recent studies expanded this analysis and identified 24 F-box genes that are up-regulated in Arabidopsis leaves upon *A. tumefaciens* inoculation (Anand et al., 2007, 2012; Table I). Furthermore, another transcriptome analysis using indica rice (*Oryza sativa*) revealed that several UPS-associated genes (i.e. those coding for the SKP1-like protein 1B, cullin, ubiquitin-activating enzyme, ubiquitin-conjugating enzyme, ubiquitin C-terminal hydrolase, and SKP1 family dimerization domain-containing protein) are down-regulated upon *A. tumefaciens* infection (Tie et al., 2012; Table I).

In addition, a recent proteome study using grapevine (*Vitis vinifera*) embryogenic callus revealed that *A. tumefaciens* inoculation affects the protein abundance of several 26S proteasome components in the host cell (Zhao et al., 2011; Table I). For example, the  $\alpha 6$  subunit of the 20S proteasome, the catalytic core particle of the 26S proteasome, was down-regulated, while the 26S proteasome regulatory subunit 7 was up-regulated within 3 d after *A. tumefaciens* inoculation

(Zhao et al., 2011). Moreover, the Ddi1 (for DNA damage-inducible1)-like protein was down-regulated upon *A. tumefaciens* inoculation (Zhao et al., 2011; Table I). The yeast (*Saccharomyces cerevisiae*) Ddi1 protein is a ubiquitin receptor that binds to polyubiquitinated proteins as well as to the 26S proteasome (Saeki et al., 2002). In yeast, the Ddi1 protein is thought to facilitate degradation of the homothallic-switching endonuclease, the crucial enzyme that induces a site-specific DNA double-strand break during mating-type switching (Kaplun et al., 2005); the molecular function of Ddi1 in plants remains to be investigated. Interestingly, *A. tumefaciens* infection also induces the accumulation of polyubiquitinated proteins in grapevine cells, further implicating UPS in the *A. tumefaciens*-host plant interaction (Zhao et al., 2011).

## INVOLVEMENT OF A HOST F-BOX PROTEIN IN A. TUMEFACIENS INFECTION

It remains largely unknown whether the UPS-associated factors, induced or repressed upon inoculation with *A. tumefaciens*, are directly involved in the infection process. However, at least one of the induced genes, *VIP1-BINDING F-BOX PROTEIN (VBF)* of Arabidopsis, was suggested to play a crucial role in *A. tumefaciens* infection (Ditt et al., 2006; Zaltsman et al., 2010). Like the bacterial F-box protein VirF, the plant F-box protein VBF interacts with VIP1 and targets it for proteasomal degradation via the SCF<sup>VBF</sup> complex (Zaltsman et al., 2010). The *VBF* transcripts are up-regulated in Arabidopsis

**Table I.** Summary of the UPS-associated factors that are differentially expressed upon *A. tumefaciens* infection

Gene/Protein <sup>a</sup>	Plant <sup>b</sup>	Tissue	Response <sup>c</sup>	Reference
26S proteasome components				
$\alpha 6$ subunit	Grapevine	Embryogenic callus	Down	Zhao et al. (2011)
$\beta$ -type 6 subunit	Grapevine	Embryogenic callus	Down	Zhao et al. (2011)
Regulatory subunit 7	Grapevine	Embryogenic callus	Up	Zhao et al. (2011)
$\alpha$ -type subunit	Grapevine	Embryogenic callus	Up	Zhao et al. (2011)
Ubiquitin-related				
<i>Ubiquitin-activating enzyme</i>	Rice	Embryogenic callus	Down	Tie et al. (2012)
<i>Ubiquitin-conjugating enzyme</i>	Rice	Embryogenic callus	Down	Tie et al. (2012)
<i>Ubiquitin C-terminal hydrolase</i>	Rice	Embryogenic callus	Down	Tie et al. (2012)
Ddi1-like (ubiquitin receptor)	Grapevine	Embryogenic callus	Down	Zhao et al. (2011)
SCF complex components				
<i>VBF</i> (F-box)	Arabidopsis	Cell culture, root	Up	Ditt et al. (2006); Zaltsman et al. (2010)
<i>At5g42350</i> (F-box)	Arabidopsis	Cell culture	Up	Ditt et al. (2006)
<i>At3g58890</i> (F-box)	Arabidopsis	Cell culture	Up	Ditt et al. (2006)
<i>At1g31350</i> (F-box)	Arabidopsis	Cell culture	Down	Ditt et al. (2006)
<i>SKIPs</i> (F-box)	Arabidopsis	Leaf	Up	Anand et al. (2007, 2012)
<i>ASK1, ASK2, ASK20</i> (SKP1-like)	Arabidopsis	Leaf	Up	Anand et al. (2007, 2012)
<i>SKP1-like protein1B</i>	Rice	Embryogenic callus	Down	Tie et al. (2012)
<i>SKP1 family dimerization domain-containing protein</i> (SKP1-like)	Rice	Embryogenic callus	Down	Tie et al. (2012)
<i>Cullin-like</i>	Rice	Embryogenic callus	Down	Tie et al. (2012)
<i>SGT1</i> (SCF accessory protein)	Arabidopsis	Leaf	Up	Anand et al. (2007, 2012)

<sup>a</sup>Gene names are in italic type and protein names are in roman type. <sup>b</sup>Plant species used in each study. <sup>c</sup>Up and Down indicate up-regulation and down-regulation, respectively, of the corresponding gene/protein upon *A. tumefaciens* infection.

upon inoculation with not only *A. tumefaciens* (Ditt et al., 2006) but also fungal pathogens (Li et al., 2006). This suggests that *A. tumefaciens* may co-opt the plant defense responses to promote the T-complex uncoating and the subsequent T-DNA integration.

The host factor VBF may be the key to understanding why *A. tumefaciens* strains lacking the *virF* gene can still induce tumors on some plant species (Hooykaas et al., 1984; Melchers et al., 1990; Jarchow et al., 1991; Regensburg-Tuink and Hooykaas, 1993). In such plants, *A. tumefaciens* may exploit a host VBF-like protein during infection as an alternative to VirF. Consistent with this model, expression of VBF in the VirF-lacking strain and its export into a plant cell functionally complemented tumor formation on tomato (*Solanum lycopersicum*), which is usually recalcitrant to the VirF-lacking strain (Zaltsman et al., 2010).

#### DISSECTING THE ROLE OF THE SCF COMPLEX IN THE *A. TUMEFACIENS*-PLANT INTERACTION

The SCF complex is composed of CUL1, SKP1, RING-BOX1 (RBX1), and an F-box protein (Petroski and Deshaies, 2005; Hua and Vierstra, 2011). Considering that two F-box proteins, VirF and VBF, play important roles in the *A. tumefaciens*-mediated genetic transformation, other subunits of the SCF complex most likely are also involved in the infection process. Indeed, a microarray analysis revealed that among 21 Arabidopsis *SKP1-LIKE* (*ASK*) genes (Marrocco et al., 2003), only *ASK1*, *ASK2*, and *ASK20* are specifically induced in leaves following *A. tumefaciens* inoculation (Anand et al., 2007, 2012; Table I). This induction of specific *ASK* genes appears to be important because at least the Arabidopsis *ask1* and *ask2* mutants exhibited low transformation efficiency when their root segments were infected with *A. tumefaciens* (Anand et al., 2012). Similarly, gene silencing of the *SKP1* homolog in *Nicotiana benthamiana* led to reduced efficiency in *A. tumefaciens*-induced tumor formation on leaves (Anand et al., 2012). These observations suggest that a specific set of SKP1 proteins may positively regulate the *A. tumefaciens*-mediated transformation. On the other hand, quite unexpectedly, knockdown of the *CUL1* or *RBX1* homolog of *N. benthamiana* did not affect transformation efficiency (Anand et al., 2012).

The SCF complex-associated protein SGT1 (a suppressor of the G2 allele of SKP1) is another factor required for *A. tumefaciens* infection (Anand et al., 2012). Both *SGT1a* and *SGT1b*, two paralogous genes encoding SGT1, are up-regulated in Arabidopsis leaves upon *A. tumefaciens* infection (Anand et al., 2007, 2012; Table I). However, only the *sgt1b* mutant, but not *sgt1a*, exhibited reduced tumor formation on roots when infected with *A. tumefaciens* (Anand et al., 2012). Gene silencing of the *SGT1* homolog in *N. benthamiana* also attenuated *A. tumefaciens* infectivity in leaves, further supporting the essential role of SGT1 in *A. tumefaciens*-plant interaction. It is well known that SGT1 regulates

plant defense responses against diverse pathogens, presumably by modulating the ubiquitination activity of the SCF complex (Muskett and Parker, 2003). Thus, SGT1 might be a missing link that connects *A. tumefaciens* infection and plant innate immunity. Although in many cases SGT1 is considered to be an activator of host defense responses, it may also function as a negative regulator of plant resistance against *A. tumefaciens*. Alternatively, the involvement of SGT1 in *A. tumefaciens* infection may represent another "Trojan horse strategy" (Djamei et al., 2007), in which *A. tumefaciens* induces and abuses the SGT1-mediated defense system, for example, to promote T-DNA integration via an unknown mechanism. To elucidate the molecular role of SGT1 in *A. tumefaciens* infection, further molecular and genetic analyses are needed.

#### OTHER UBIQUITIN E3 LIGASES AND *A. TUMEFACIENS*

The SCF complex belongs to the Cullin-RING Ligase (CRL) family, the largest known class of ubiquitin ligases (Petroski and Deshaies, 2005; Hua and Vierstra, 2011). In plants, CRLs are classified into at least three major groups based on the type of the scaffold subunit cullin (CUL), namely the CUL1-based SCF, the CUL3-BTB (for Broad complex, Tramtrack, Bric-a-brac), and the CUL4-DDB1 (for DNA damage-binding protein1) complexes (Hua and Vierstra, 2011). In addition to the SCF complex, the CUL4-DDB1 complex has been implicated in *A. tumefaciens* plant transformation (Liu et al., 2012). The CUL4-DDB1 complex is composed of the scaffold protein CUL4, DDB1, a DDB1-binding/WD-40 domain-containing protein (DWD), and RBX1 (Hua and Vierstra, 2011). DWD proteins function as the substrate recognition module of the complex, while the adaptor protein DDB1 tethers a DWD protein to the CUL4/RBX1 catalytic core (Hua and Vierstra, 2011). Among these subunits, DDB1 has been shown to affect *A. tumefaciens* infection on tomato via the plant defense system (Liu et al., 2012). A tomato DDB1-deficient mutant (*high pigment1* [*hp1*]) exhibited hypersensitivity against even nontumorigenic *A. tumefaciens* strains, which do not usually cause disease symptoms on plants (Liu et al., 2012). The excised cotyledons of the *hp1* mutant manifested severe necrosis 10 d after inoculation of a nontumorigenic *A. tumefaciens* strain, while the infected wild-type tomato rarely showed such cell death (Liu et al., 2012). Furthermore, the leaves of *hp1* developed wilting-like disease symptoms upon *A. tumefaciens* infection (Liu et al., 2012). This hypersusceptible phenotype of *hp1* may be the consequence of compromised pathogen-associated molecular pattern-triggered immunity (PTI). Indeed, unlike the wild-type plants, *hp1* could not induce the expression of a PTI marker gene, *PR1*, upon *A. tumefaciens* inoculation (Liu et al., 2012). Consistent with this finding, the plant hormone salicylic acid, a critical inducer of PTI, failed to enhance resistance

against *A. tumefaciens* in the *hp1* mutant (Liu et al., 2012). Thus, the CUL4-DDB1 ubiquitin ligase complex likely inhibits *A. tumefaciens* infection via salicylic acid-regulated defense signaling. It would be interesting to examine whether the tomato DDB1 actually functions in the CUL4-DDB1 complex and to determine the type of proteins this ubiquitin ligase complex potentially targets for degradation.

## CONCLUSION

With the discovery and functional characterization of the first bacterial F-box protein, VirF, *A. tumefaciens* has developed into a valuable model system for analyses of the regulation of plant-pathogen interactions by the host UPS. In the recent years, we have witnessed substantial advances in our understanding of the importance of the UPS in *A. tumefaciens*-mediated genetic transformation of plants. However, many questions still remain to be answered. For example, does *A. tumefaciens* modulate the activity of SCF<sup>VirF</sup> and SCF<sup>VirB</sup> in the host cell, and if so, how is this modulation achieved? What other host factors may be targeted for degradation by SCF<sup>VirF</sup> or SCF<sup>VirB</sup>? How does the plant defense system recognize VirF and promote its degradation? Are the induced or repressed UPS-associated factors directly involved in *A. tumefaciens* infection? Future studies will likely decipher the precise molecular role of the UPS in the *A. tumefaciens*-plant interaction, uncovering new fundamental aspects of the genetic transformation process and paving the way to the improvement of plant genetic engineering as well as the prevention of crown gall disease on crops.

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