CHAPTER ONE

Genetic factors governing bacterial virulence and host plant susceptibility during *Agrobacterium* infection

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Abstract

Several species of the *Agrobacterium* genus represent unique bacterial pathogens able to genetically transform plants, by transferring and integrating a segment of their own DNA (T-DNA, transferred DNA) in their host genome. Whereas in nature this process results in uncontrolled growth of the infected plant cells (tumors), this capability of *Agrobacterium* has been widely used as a crucial tool to generate transgenic plants,

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for research and biotechnology. The virulence of *Agrobacterium* relies on a series of virulence genes, mostly encoded on a large plasmid (Ti-plasmid, tumor inducing plasmid), involved in the different steps of the DNA transfer to the host cell genome: activation of bacterial virulence, synthesis and export of the T-DNA and its associated proteins, intracellular trafficking of the T-DNA and effector proteins in the host cell, and integration of the T-DNA in the host genomic DNA. Multiple interactions between these bacterial encoded proteins and host factors occur during the infection process, which determine the outcome of the infection. Here, we review our current knowledge of the mechanisms by which bacterial and plant factors control *Agrobacterium* virulence and host plant susceptibility.

1. Introduction

Agrobacterium tumefaciens was discovered more than a century ago as the causing agent of the crown-gall disease, which results in uncontrolled cell division (tumors) mostly at the roots and base of the stem of the infected plants (Kado, 2014). Different species or strains of *Agrobacterium* may cause different diseases in various host plants: *A. tumefaciens* causes crown galls, *A. vitis* causes galls on the stem of grape species and *A. rhizogenes* causes root proliferation (hairy root). The infection of plants by *Agrobacterium* species represents a unique case of active horizontal gene transfer in the living world. Indeed, *Agrobacterium* virulence relies mostly on two essential regions of its Ti-plasmid (tumor-inducing plasmid): the virulence region (containing the *vir* genes) and the T-DNA region (containing the T-DNA, a segment of DNA transferred and integrated into the genome of the host plant cell). In wild-type *Agrobacterium* strains, the T-DNA contains several genes, which will be expressed in the transformed plant cells. Some of these gene products will affect host cell division and cause ectopic growth (such as the crown gall), while another series of genes encode proteins responsible for the synthesis of small molecules (opines) that are exported out of the host tissues and used by *Agrobacterium* as a source of nutrition (Escobar & Dandekar, 2003). Because the T-DNA transfer does not depend on its sequence, T-DNA genes can be replaced by any sequence of interest, which made possible the use of *Agrobacterium* as a tool for plant genetic transformation. Since the discovery of the T-DNA as the "tumor-inducing agent," numerous studies have focused on identifying the bacterial and plant genetic factors involved in the infection process and on deciphering the molecular mechanism of plant genetic transformation mediated by *Agrobacterium* (Gelvin, 2003a; Lacroix & Citovsky, 2019). Most of our current knowledge of the

infection mechanisms is derived from the experiments performed with *A. tumefaciens*; thus, we will use the term *Agrobacterium* in this chapter, although we will refer mostly to *A. tumefaciens*.

Susceptibility of plants to *Agrobacterium* infection varies widely between plant species; most notably monocotyledon species are generally recalcitrant to transformation (De Cleene & De Ley, 1976). Variations are also observed within a species, between varieties, or accessions (Chateau, Sangwan, & Sangwan-Norreel, 2000). Moreover, organs, tissues, or cell types of a plant differ in their susceptibility, and different treatments (nutritional, hormonal) of the plant cells or tissues affect transformation efficiency, suggesting that the physiological status of the cells also alters their susceptibility. Under laboratory conditions, *Agrobacterium*-mediated genetic transformation can be achieved with most plant species, with variable efficiency, and non-plant species (yeast, fungi, and even mammalian cells) can also be transformed (Lacroix, Tzfira, Vainstein, & Citovsky, 2006). Many genetic factors affecting the susceptibility of plants to *Agrobacterium* have been identified (Gelvin, 2003b; Lacroix & Citovsky, 2019); their presence and activity may represent the determinants for the outcome of the infection process. In this review, we will focus on our current understanding of bacterial and plant factors controlling *Agrobacterium* virulence and host plant susceptibility, from the first cellular interactions to the integration of T-DNA into the host genome.

2. Bacterial factors defining *Agrobacterium* virulence

Agrobacterium virulence relies mostly on a series of genes (the *vir* genes), located on the Ti plasmid, of which expression may be activated in response to plant and environmental signals. These genes encode proteins involved at different stages of plant infection and can be classified into different groups according to their level of requirement for *Agrobacterium*-mediated transformation (Table 1). The first group represents a core of essential genes, absolutely required for infection: *vir*A and *vir*G (main system for *vir* gene induction), the *vir*B operon, and *vir*D4 (export of macromolecules from the bacterial cells), and *vir*D1 and *vir*D2 (synthesis and transport of the T-DNA). The second group of *vir* genes can be defined as important but not absolutely essential (transformation occurs only at a very low rate with *Agrobacterium* mutated in these genes): *vir*C1 and *vir*C2 (T-DNA synthesis), *vir*E1 and *vir*E2 (protection and nuclear import of the T-DNA). A third group, sometimes qualified as host-range genes, corresponds to genes that

Table 1 *Agrobacterium* virulence genes (see text for references).

Requirement for transformation	Gene name	Function
Essential	*vir*A	Sensor, part of the two-component regulator of *vir* gene expression
	*vir*B1–11	Main components of the T4SS responsible for export of T-complex and effector proteins
	*vir*D1	T-strand synthesis
	*vir*D2	T-strand synthesis, protein component of the T-complex, nuclear import of the T-DNA
	*vir*D4	Part of the T4SS (coupling factor)
	*vir*G	Part of two-component system, transcription activator of *vir* genes
Important	*vir*C1–2	Enhance T-strand synthesis
	*vir*E1	Chaperone for VirE2 protein
	*vir*E2	Effector protein, protection of the T-strand
Host range	*vir*D3	Unknown
	*vir*D5	Effector protein, prevents VirF degradation
	*vir*E3	Effector protein, interacts with VirE2, anchor for VirE2 after its entry in the host cell, transcription regulator
	*vir*F	Effector protein, F-box protein, proteasomal degradation of several host plant target proteins
Unknown	*vir*H1–2	Detoxification
	*vir*J	Homolog of chromosomally encoded *acv*B
	*vir*K	Unknown
	*vir*L	Unknown
	*vir*M	Unknown

may be required as an enhancer of transformation efficiency only with a certain host or in specific conditions; this group includes *vir*D5, *vir*E3, and *vir*F. Finally, some of the *vir* genes are not found in all *Agrobacterium* strains (*vir*D3, *vir*H1 and *vir*H 2, *vir*J, *vir*K, *vir*L, *vir*M); although they belong to the *vir* regulon, their potential role in *Agrobacterium* infection is generally

unknown. Originally, the importance of virulence genes was assessed by testing the ability of *Agrobacterium* insertion mutants for different genes to induce tumor formation after infection of plants highly susceptible to *Agrobacterium* (usually tobacco or kalanchoe) (Horsch et al., 1986; Stachel, An, Flores, & Nester, 1985). It is likely that non-essential or accessory genes play a role in the infection of other, less susceptible, plant species. In addition, the importance of these genes may be undetectable under laboratory conditions, but play a role under natural conditions, in the highly competitive environment of the rhizosphere. Besides the *vir* genes present on the Ti-plasmid, some chromosomally encoded genes are required for *Agrobacterium* virulence. These genes are involved mostly in sensing environmental conditions and modulation of virulence induction (*chv*G, *chv*E, *chv*I, *exo*R), or in the attachment of *Agrobacterium* cells to plant cell/tissue surface (*chv*A, *chv*B, *exo*C).

3. Virulence factors in non-*Agrobacterium* species

Interestingly, homologs of virulence genes have been found in many bacterial species related to *Agrobacterium*, within plasmids of bacteria belonging to the *Rhizobiaceae* family. Indeed, the study of many plasmids from species of the *agrobacteria-rhizobia* complex shows that partial or complete *vir* regions are often found in these plasmids and that combination of these mobile genetic elements in one strain may lead to the assembling of a functional DNA transfer machinery (Weisberg et al., 2020), which raises the question of whether other, non-*Agrobacterium*, species can transfer DNA to eukaryotic hosts. Because most of the virulence determinants are found on a mobile genetic element (Ti-plasmid), a strain harboring no Ti-plasmid or only an incomplete virulence system may become virulent by acquiring a plasmid from another strain by conjugative plasmid transfer.

It has been known for a long time that transferring a plasmid(s) containing a functional virulence region and a T-DNA to several species closely related to *Agrobacterium* (belonging to the *Rhizobiaceae* and *Phyllobacteriaceae* families) could confer the ability to transfer T-DNA to the recipient species (Broothaerts et al., 2005; Hooykaas, Klapwijk, Nuti, Schilperoort, & Rorsch, 1977; Wendt, Doohan, & Mullins, 2012; Zuniga-Soto, Mullins, & Dedicova, 2015). These results indicate that these bacterial strains harbor all the chromosomally encoded factors required for T-DNA transfer. More recently, it was shown that a *vir* region of the native plasmid p42a of *Rhizobium etli* CFN42 strain is functional, and able to transfer T-DNA to host

plant cells (albeit with low efficiency), when that bacterial strain was transformed with a plasmid containing a T-DNA region only, (Lacroix & Citovsky, 2016). It was later shown that *R. etli vir* gene expression was regulated by phenolics, similarly to *Agrobacterium* (Wang, Lacroix, Guo, & Citovsky, 2017).

4. Regulation of virulence gene expression

All the *vir* gene or operon promoters contain at least one specific sequence, 10- to 12-bp sequences (*vir* box) located between 200 and 50 bp upstream of the transcription initiation site, which is required for the coordinated *vir* gene induction (Steck, Morel, & Kado, 1988). The activity of these promoters, and thus the expression of the *vir* genes, is mostly under the control of a two-component receptor system composed of the VirA and VirG proteins (Stachel & Zambryski, 1986). VirA is an integral membrane sensor protein that integrates several signals; once activated, VirA mediates the phosphorylation of VirG, which then binds to the *vir* box containing promoters and promotes *vir* gene expression (Fig. 1). The

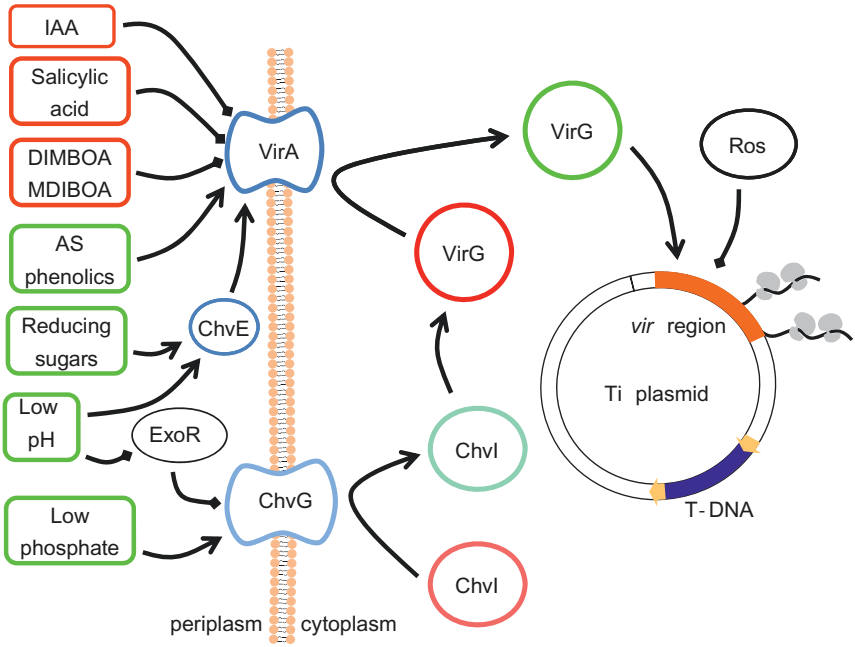

Fig. 1 Regulation of *vir* gene expression by plant and environmental factors (see text for details).

most important of these activating signals is a plant-produced phenolic compound, acetosyringone (AS, 3,5-dimethoxyacetophenone) (Bolton, Nester, & Gordon, 1986; Stachel, Messens, Van Montagu, & Zambryski, 1985). Several other phenolic compounds related to AS (including phenolic glycosides) are also able to activate the VirA/VirG system (Joubert et al., 2004; Melchers, Regensburg-Tuink, Schilperoort, & Hooykaas, 1989). Genetic studies suggest that AS and other phenolics bind directly to VirA (Lee, Jin, Sim, & Nester, 1995), although it cannot be completely excluded that a yet unknown intermediary protein bind phenolics in the periplasm before activating VirA. A range of reducing monosaccharides (e.g., D-glucose and D-galactose) can bind to ChvE, a chromosome-encoded periplasmic protein, which then enhances *vir* gene expression by directly binding to VirA (Cangelosi, Ankenbauer, & Nester, 1990; Shimoda, Toyoda-Yamamoto, Aoki, & Machida, 1993). This interaction results in an increase of VirA/VirG sensitivity and of saturating concentration for the *vir* gene induction by phenolics (Shimoda et al., 1990). Other environmental features, such as low pH and low phosphate concentration, also affect *vir* gene expression. Together, they activate ChvG/ChvI, another two-component regulatory system, which in turn increases the expression of *virG* (Charles & Nester, 1993). Low pH results in the degradation of ExoR, a periplasmic inhibitor of ChvG (Heckel, Tomlinson, Morton, Choi, & Fuqua, 2014). Moreover, low pH (between pH 5 and 6) enhances VirA activity directly (Melchers et al., 1989) or through ChvE (Gao & Lynn, 2005).

Because the expression of *vir* genes is costly in energy for the bacterial cells (Platt, Bever, & Fuqua, 2012), it is also important that their expression is repressed in later infectious stages when Vir proteins are not required anymore. Several mechanisms could play this role in *Agrobacterium*. Indeed, *Agrobacterium* virulence is inhibited in response to the auxin IAA (indole acetic acid), produced at high levels by developing *Agrobacterium*-induced tumors (Liu & Nester, 2006). IAA likely can bind VirA, acting as a competitive inhibitor of AS. Another pathway capable of turning off the *vir* gene expression was recently suggested. In this pathway, sucrose would bind to and inactivate *Agrobacterium* SghR, resulting in the expression of SghA; SghA would then free SA (salicylic acid) from its storage form SAG (SA β-glucoside), and SA would inhibit VirA (Wang et al., 2019). However, this model relies on two yet unproved assumptions: the massive release of sucrose from plants to the extracellular space and the export of SAG (usually stored in vacuoles) from plant cells.

In addition, the ability to inhibit *vir* gene expression may represent a mechanism of plant defense to prevent infection by *Agrobacterium*, and consequently a source of variability of susceptibility to *Agrobacterium* between plant species. For example, two chemicals isolated from corn seedling homogenates (DIMBOA and MDIBOA) were shown to inhibit both *vir* gene induction and *Agrobacterium* growth (Sahi, Chilton, & Chilton, 1990; Zhang et al., 2000). Like IAA, these two molecules derive from the tryptophan biosynthetic pathway (Melanson, Chilton, Masters-Moore, & Chilton, 1997), and could also inhibit *vir* gene expression by binding to VirA. In studies with plants deficient or overexpressing genes for the synthesis of salicylic acid (SA, the major signal molecule of the systemic acquired resistance pathway), it was shown that SA inhibited *vir* gene expression, most likely by interfering with VirA activity (Anand et al., 2008; Yuan et al., 2007). The plant gaseous growth regulator ethylene was also able to inhibit *Agrobacterium* virulence (Nonaka, Sugawara, Minamisawa, Yuhashi, & Ezura, 2008; Nonaka, Yuhashi, et al., 2008), although it is not known if ethylene has a direct effect on *vir* gene expression.

Whereas most of the regulation pathways cited above converge on the VirA/VirG two-component system, *vir* gene expression can also be altered via pathways independent of this system. Indeed, a mutation in Ros, a transcription regulator encoded by the *Agrobacterium* chromosome, resulted in the activation of expression of the *vir*C and *vir*D operons (Close et al., 1987). Furthermore, an investigation of the small RNA-dependent gene regulations in *Agrobacterium* showed that some of the *vir* genes were regulated via this pathway (Dequivre et al., 2015).

5. T-DNA synthesis

The T-DNA synthesis corresponds to the production of the single strand T-DNA segment via a mechanism of strand-replacement (Stachel, Timmerman, & Zambryski, 1986). Two 24–25 bp sequences, known as the left border (LB) and the right border (RB), present as a direct repeat on the Ti-plasmid, mark the beginning and the end of the T-DNA (Peralta & Ream, 1985; Yadav, Vanderleyden, Bennett, Barnes, & Chilton, 1982), which can be mobilized from the Ti-plasmid in the form of a single-stranded DNA intermediate (the T-strand). The processing of the T-strand is mediated by VirD2, acting as an endonuclease (Albright, Yanofsky, Leroux, Ma, & Nester, 1987; Yanofsky et al., 1986), associated

with VirD1, most likely acting as a DNA topoisomerase (Ghai & Das, 1989). The result of this process is the immature T-complex, comprised of the T-strand and of a molecule of the VirD2 protein that remains covalently linked to its 5′-end (RB) (Young & Nester, 1988). VirC1 and VirC2 were also shown to bind to "overdrive" sequences located close to the T-DNA borders, causing an increase in the amount of processed T-strand molecules (De Vos & Zambryski, 1989).

6. T-DNA and associated protein export

Export of T-DNA and associated proteins from the bacterial cells relies on a Type IV secretion system (T4SS) encoded by the *vir*B operon and *vir*D4, via a mechanism close to the plasmid translocation during bacterial conjugation (Li & Christie, 2018). In addition to the VirD2-T-strand complex, four other proteins are translocated to the host cell: VirE2, VirE3, VirD5, and VirF. Firstly, the VirD2-T-strand complex and the translocated proteins must be targeted to the T4SS within the bacterial cell. All these proteins display an arginine-rich C-terminal sequence required for their export (Vergunst et al., 2000, 2005), although it is not an exact signal sequence (identical for all these proteins). The presence of a similar signal sequence shared between the different exported proteins suggests a unique pathway of recognition of the translocated proteins within bacterial cells, but such a unique pathway has not been identified yet. Instead, several different factors have been suggested for each of the exported proteins. Interaction with the T4SS coupling protein VirD4 seemed required to recruit VirE2 to the cell poles, where T4SS is assembled (Atmakuri, Ding, & Christie, 2003). VirC1 and VirC2 likely assist the targeting of VirD2 (and thus the T-complex) to the cell poles (Atmakuri, Cascales, Burton, Banta, & Christie, 2007). More recently, VBPs (VirD2-binding proteins) were identified as bacterial factors able to recruit VirD2 and the associated T-strand to the T4SS energizing components (VirD4, VirB4, and VirB11) (Guo, Hou, Hew, & Pan, 2007; Guo, Jin, Sun, Hew, & Pan, 2007). In other systems, VBPs can recruit relaxase proteins and conjugating DNA intermediates to the T4SS during conjugation. However, VBPs did not interact with the other exported effector proteins of *Agrobacterium*, thus they do not represent the only factors recognizing C-terminal sequences of the exported proteins.

Agrobacterium represents a model for the study of T4SSs; thus, the structure of its T4SS is known in detail (Christie, 2004), and the sequence of

interactions between the different subunits of the T4SS and the T-DNA transport substrate was elucidated (Cascales & Christie, 2004).

7. Attachment and biofilm formation

Generally, interactions between pathogenic bacteria and their eukaryotic host require a close interaction between bacteria and the plant cells/tissue is necessary. The attachment of the bacterial cell relies on several bacterial and plant factors and often results in the formation of a biofilm. In the case of *Agrobacterium*, this process of transition from motile planktonic bacterial cells to biofilm can be divided into three steps (Heindl et al., 2014). First, bacterial cells are attracted toward the plant cell surface by chemotaxis, which is triggered by the same plant exudates that induce virulence, i.e., phenolic compounds and reducing sugars (Guo, Huang, & Yang, 2017). Bacteria approach the surface of plant tissue via flagellum-dependent motility, relying on the ChvA sensor (Merritt, Danhorn, & Fuqua, 2007; Wright, Deakin, & Shaw, 1998). Second, there is initial contact and reversible attachment between the bacterial cells and the host tissue surface. Third, bacterial attachment is stabilized, and bacteria are embedded within a biofilm. *Agrobacterium* synthesizes several exocellular polysaccharides known to play a role in attachment and biofilm formation: 1,2-β-D-glucan produced and exported by the activity of ChvA, ChvB, and ExoC, for attachment and virulence (Cangelosi et al., 1989; de Iannino & Ugalde, 1989); unipolar polysaccharides (UPPs) involved in attachment (Xu, Kim, Danhorn, Merritt, & Fuqua, 2012); and cellulose for attachment consolidation and biofilm formation (Matthysse, 1983). Potential plant cell surface receptors able to bind these exopolysacharides (such as the lectins known to be involved in *Rhizobium*-host cell attachment) have not yet been identified for *Agrobacterium* host cell attachment. Proteins exposed at the surface of *Agrobacterium* cells could also play a role in attachment. Indeed, several *Arabidopsis* genes were shown to encode proteins interacting with VirB2, the main component of the T4SS pilus (Hwang & Gelvin, 2004). Although T-DNA transfer efficiency was affected in *Arabidopsis* lines mutants in these genes, it is not known whether this change in efficiency reflects a disruption of attachment or of another step (passage of T-DNA or proteins from bacterial to plant cell, or cell signaling). VirB5 is a minor component of the T4SS located at the tip of the VirB2 pilus (Aly & Baron, 2007); although it was shown that the addition of free extracellular VirB5 enhances the T-DNA transfer (Lacroix & Citovsky, 2011), there is no indication that it is involved in attachment. Whereas their requirement for

virulence is not clear, CtpA and PilA (two other extracellular *Agrobacterium* proteins) were shown to play a role in the first steps of *Agrobacterium* attachment to plant tissue surface (Wang, Haitjema, & Fuqua, 2014).

Two Arabidopsis mutant lines resistant to *Agrobacterium* could potentially be deficient in proteins involved in bacterial attachment, although their function was not completely elucidated. This is the case of CSLA9 (Zhu, Nam, Carpita, Matthysse, & Gelvin, 2003), encoding an enzyme that is likely synthesizing cell wall polysaccharides, and of AGP17 (an extracellular arabinogalactan-protein) (Gaspar et al., 2004).

8. T-DNA entry in the plant cell

The mechanism of the entry of T-DNA and associated proteins through the host cell membrane and the nature of the plant factors involved in this process are not completely understood. Theoretically, there are three ways by which the exported macromolecules could pass through the membrane and enter the host cell cytoplasm. Similar to a mechanism occurring in the type III secretion system (T3SS) (Notti & Stebbins, 2016), the transported macromolecules could pass through the VirB2 pilus, their entry into the plant cell relying on interactions between pilus proteins and host-cell membrane-associated proteins. In another scenario, close to a proposed mechanism for bacterial conjugation (Cabezón, Ripoll-Rozada, Peña, de la Cruz, & Arechaga, 2015), after depolymerization of the VirB2 pilus, the membranes of the bacterial and host cells would be close enough to fusion together, allowing the transfer of macromolecules. Finally, the macromolecules exported from *Agrobacterium* cells could be deposited at the surface of the host cell and internalized via interactions with host membrane factors and potentially via the endocytosis pathway. Recent studies have shown that the transport of VirE2 probably takes advantage of the host cell endocytosis pathway (Li & Pan, 2017); indeed, VirE2 entry into plant cells was associated with early endosome formation, and VirE2 interacted with AP2M, a protein located on the cytoplasmic side of the clathrin-coated vesicles. So far, there is no evidence that the internalization of other translocated macromolecules is also associated with the endocytotic pathway.

9. T-DNA intracellular transport

The transport of the T-DNA within the plant cell relies on interactions between translocated Vir proteins and several host factors. Prior to the potential integration of the T-DNA in the host genome, the

VirD2-T-strand complex and translocated proteins playing a role in the host nucleus must be imported into the nucleus. The T-complex depends on its associated proteins to reach its host cell nucleus. VirD2, attached covalently to the 5′-end of the T-strand, is targeted to the nucleus via its interaction with a specific importin alpha (Ballas & Citovsky, 1997), and other members of the importin alpha family (Bakó, Umeda, Tiburcio, Schell, & Koncz, 2003). VirD2 likely represents the "pilot" protein mediating the targeting of T-complex toward the nucleus. However, the transport of a large DNA molecule (such as the T-DNA) through the cytoplasm and the nuclear pore likely requires more than a single protein molecule. Many studies suggest that VirE2, as an ssDNA-binding protein, can bind and coat the T-DNA, efficiently leading to the formation of a mature T-complex (Citovsky, Wong, & Zambryski, 1989; Gelvin, 1998). Indeed, VirE2 binds to ssDNA with a strong affinity (Christie, Ward, Winans, & Nester, 1988; Citovsky et al., 1989), which results in ssDNA-VirE2 filament with a helical structure (Abu-Arish et al., 2004). Transformation experiments using *Agrobacterium vir*E2 mutant strains result in an increased level of truncations in the integrated T-DNA (Rossi, Hohn, & Tinland, 1996), consistent with the role of VirE2 in protecting the T-DNA against nucleolytic degradation. However, so far, such a mature T-complex has not been visualized in living plant cells, suggesting that it might not exist as a stable macromolecular assembly. Early studies showed a nuclear targeting for VirE2 labeled with different markers (Citovsky, Zupan, Warnick, & Zambryski, 1992; Ziemienowicz, Görlich, Lanka, Hohn, & Rossi, 1999). In later studies, VirE2 fused with autofluorescent proteins remained mostly cytoplasmic (Lee, Fang, Kuang, & Gelvin, 2008; Shi, Lee, & Gelvin, 2014), with a tendency to form aggregates consistent with the strong VirE2 homopolymerization. In a different experimental setting, where VirE2 fusion with a partial GFP sequence was expressed in *Agrobacterium* and fluorescence was reconstituted after *Agrobacterium*-mediated transfer in plant cell expressing the other part of GFP, VirE2 was at least partially nuclear (Li, Yang, Tu, Lim, & Pan, 2014). The intracellular targeting of VirE2 may depend on its interaction with several host proteins. Indeed, VirE2 was shown to interact with VIP1 (VirE2 interacting protein 1) (Tzfira, Vaidya, & Citovsky, 2001), VIP2 (VirE2 interacting protein 2) (Anand et al., 2007), core histones (Lacroix, Loyter, & Citovsky, 2008; Loyter et al., 2005), and importins alpha (Bhattacharjee et al., 2008). Other translocated proteins (VirE3, VirD5, and VirF) are also targeted to the nucleus, after binding to host importins. Interestingly, VirE3 interacts with VirE2, which is likely involved in two steps of VirE2 intracellular transport:

accumulation of VirE2 on the cytoplasmic side of the host cell plasma membrane immediately after it enters the host cell (Li, Tu, & Pan, 2018), and VirE2 subsequent nuclear targeting (Lacroix, Vaidya, Tzfira, & Citovsky, 2005). A more recent study suggests that VirE2 nuclear import depends on the presence of the T-DNA and that VirE2 interaction with the plant nucleoporin CG1 facilitates the passage of the T-complex through nuclear pores (Li et al., 2020).

10. T-DNA integration

Several studies have shown that the sites of integration of *Agrobacterium* T-DNA are not targeted to a specific region of the host genome. In the first studies, T-DNA integration sites were found throughout the genome but mostly in the region of the active expression (Alonso et al., 2003). However, this observation was based on an experimental bias because the transgenic plants were recovered after selection and required expression of the gene encoding resistance agent to the selection pressure. Indeed, a similar experiment realized without selection pressure revealed that integration occurs truly randomly in the host genome (Kim, Veena, & Gelvin, 2007), whereas there is a possible local bias toward some epigenetic markers in the chromatin (Shilo et al., 2017). *Agrobacterium* does not encode a dedicated integrase among its effector proteins, thus the integration of the T-DNA into the host genome depends on the activity of several host pathways. Indeed, VirD2 was once suspected to mediate T-DNA integration (Pansegrau, Schoumacher, Hohn, & Lanka, 1993; Tinland, Schoumacher, Gloeckler, Bravo-Angel, & Hohn, 1995), but it was later shown not to be the case (Ziemienowicz, Tinland, Bryant, Gloeckler, & Hohn, 2000). It is likely, however, that interactions between *Agrobacterium* effector and host proteins are required for integration. It has been shown that induction of DSBs in plant tissue prior to *Agrobacterium* infection results in an increase in stable transformation (Salomon & Puchta, 1998), suggesting that DSBs in the host genome might be a target for T-DNA integration and that the activation of DNA damage reaction following DSB induction might also enhance T-DNA integration. Experiments realized with yeast (*Saccharomyces cerevisiae*) as host cells demonstrate that the outcome of integration indeed depends on the activity of host factors. Using yeast as host cells has two advantages: many viable mutants in different DSB repair pathways are available, and DSB repair occurs via either HR (homologous recombination) or NHEJ (non-homologous end joining) pathways. With yeast mutants in genes essential for the HR pathway (Rad51 or Rad52), only integration

via NHEJ was observed, whereas when the NHEJ was disrupted (mutation in Ku70 or Mre11) all integration events resulted from HR (van Attikum, Bundock, & Hooykaas, 2001; van Attikum et al., 2003). In plants (where DSB repair is mostly mediated by NHEJ), the situation seems more complex. Conflicted data resulted from the analysis of T-DNA integration efficiency in *Arabidopsis* lines mutated in genes representing the different DSB repair pathways. In *Arabidopsis* mutant deficient in Ku80 and Lig4, it was found that T-DNA integration was inhibited in two studies (Friesner & Britt, 2003; Li et al., 2005), while it was reported in another study that mutation in Ku80 did not affect integration efficiency (Gallego, Bleuyard, Daoudal-Cotterell, Jallut, & White, 2003). In a similar study with *Arabidopsis* mutants in different genes representing the known pathway of DSB repair, it was reported that none of these mutants displayed inhibition of the T-DNA integration (Park et al., 2015). However, only very low levels of integration were observed in *Arabidopsis* lines disrupted in several of these pathways (Mestiri, Norre, Gallego, & White, 2014). In rice plants where expression of Ku70, Ku80, and Lig4 was downregulated, lower rates of T-DNA integration were measured (Nishizawa-Yokoi et al., 2012). Redundancy between the different DNA repair pathways existing in plants may explain partially the discrepancy between the results reported in these different studies. It is also possible that other pathways are involved in T-DNA integration.

Recently, the role of polymerase theta (also known as Tebichi in plants) in T-DNA integration was investigated. Indeed, sequence analysis of a large number of T-DNA integration sites showed that many of them displayed a signature of ligation by polymerase theta, and T-DNA integration was impaired in an *Arabidopsis* line deficient in this gene (van Kregten et al., 2016). Because polymerase theta, first discovered as a suppressor of genome instability, is also involved in microhomology-mediated end joining (MMEJ), or alternative end-joining (alt-EJ) (Black, Kashkina, Kent, & Pomerantz, 2016), it was suspected that this alternative pathway of DNA repair is involved in T-DNA integration. In a more recent study, it was shown that in addition to polymerase theta ligating the 3′-end of the T-DNA to the genomic DNA, other proteins were required to remove the VirD2 protein from the T-complex before the ligation (Kralemann et al., 2022). Indeed, while attachment of the 3′-end resulted from the polymerase theta activity exclusively, removal of VirD2 and ligation of the 5′-end could occur via two different mechanisms. Either VirD2 was removed by TDP2 and ligation was mediated by canonical NHEJ, or VirD2 removal relied on MRE11 (part of the MRN complex), and attachment to the genomic DNA occurred via the polymerase theta pathway.

Moreover, in *Arabidopsis* double mutants in TDP2 and Mre11 very low rate of stable transformation was observed, suggesting the requirement for several DNA repair pathways for T-DNA integration.

11. Host factors interacting with *Agrobacterium* effector proteins

Besides the main roles of *Agrobacterium* essential effectors for intracellular transport and, potentially, integration, many other bacterial-host protein interactions occur during the infection process (Table 2). These interactions may represent either the targets of *Agrobacterium* effector proteins (their interaction being part of a mechanism by which the effector

Table 2 *Agrobacterium* encoded effector proteins and their host interacting proteins (see text for references).

Effector	Host protein	Host species	Known or suspected functions
VirD2	Importin α	Arabidopsis	Nuclear import
	Cyclophilins	Arabidopsis	Unknown
	2C protein phosphatase	Tomato	Regulation of nuclear import
	CAK2M	Alfalfa	Interaction with host chromatin, integration
	TBP	Alfalfa	
	Core histones	Yeast	Interaction with host chromatin
VirE2	Importin α	Arabidopsis	Nuclear import
	VIP1	Arabidopsis	Nuclear import, plant transcriptional regulation
	RSG (VIP1 ortholog)	Tobacco	
	bZIP proteins (related to VIP1)	Arabidopsis	
	VIP2	Arabidopsis benthamiana	Required for integration, plant transcriptional regulation
	XRCC4	Arabidopsis	Integration
	GST	Rice	Regulation of nuclear import

Continued

Table 2 *Agrobacterium* encoded effector proteins and their host interacting proteins (see text for references).—cont'd

Effector	Host protein	Host species	Known or suspected functions
VirE3	Importin α	Arabidopsis	Nuclear import
	Csn5	Arabidopsis	Plant transcriptional regulation
	Brp	Arabidopsis	
	JAZ8	Arabidopsis	Regulation of plant defense reaction
VirD5	Spt4	Yeast	Mitotic destabilization
	Aurora kinase	Yeast and Arabidopsis	
	VIP1	Arabidopsis	Prevents VBF binding and VIP1 proteasomal degradation
	VIP2	Arabidopsis	Plant transcriptional regulation
VirF	ASK1	Arabidopsis	Proteasomal degradation (part of the SCF complex)
	VIP1	Arabidopsis	Target for degradation, T-complex uncoating, plant transcriptional regulation
	VFP3	Arabidopsis	Plant transcriptional regulation
	VFP5	Arabidopsis	Plant transcriptional regulation
	VFP4	Arabidopsis	Target for degradation, plant transcriptional regulation

protein enhances the efficiency of *Agrobacterium*-mediated transformation) or pathways of plant "defense" against *Agrobacterium* (resulting in inhibition of *Agrobacterium*-mediated transformation). Until now, the search for host factors involved in *Agrobacterium*-plant interactions was done mostly with susceptible plant species (such as *Arabidopsis* and *Nicotiana* species). It is likely that if more investigations were performed with resistant host plants, more of the second category host factors would be uncovered.

11.1 VirD2

VirD2 interacts with several members of a subgroup of *Arabidopsis* cyclophilins, single domain cyclophilins (Bakó et al., 2003; Deng et al., 1998). Cyclophilins represent a large family of proteins (21 in *Arabidopsis*), present in different subcellular compartments and involved in a wide variety of cellular

processes (Romano, Horton, & Gray, 2004). Their potential function in *Agrobacterium* T-DNA transfer is not known, whereas treatment with cyclosporine A (which binds cyclophilins and inhibited interaction between cyclophilin and VirD2) resulted in inhibition of T-DNA transfer. A type 2C serine/threonine protein phosphatase from tomato was also found to interact with VirD2 (Tao, Rao, Bhattacharjee, & Gelvin, 2004), this phosphatase is thought to inhibit VirD2 nuclear targeting via dephosphorylation of VirD2. Furthermore, it was reported that VirD2 interacts and is phosphorylated by CAK2M (a cyclin-dependent kinase-activating kinase) in alfalfa cells; CAK2M also phosphorylates a subunit of RNA-polymerase II able to recruit TATA-binding protein (TBP), and VirD2 was found to associate with TBP. Because orthologs of CAK2M and TBP are involved in transcription-coupled DNA repair, it was suggested that they are involved in T-DNA integration. Finally, VirD2 interacted with yeast core histones (Wolterink-van Loo, Escamilla Ayala, Hooykaas, & van Heusden, 2015), which might also play a role in the interaction between T-complex and host chromatin during integration.

11.2 VirE2

The first series of studies uncovered the interaction between VirE2 and VIP1 (VirE2 interacting protein 1) by yeast-two-hybrid screening (Kunik et al., 2001); and that increased transformation levels were observed in tobacco plants overexpressing *Arabidopsis* VIP1 (AtVIP1), likely by the role of VIP1 in facilitating VirE2 nuclear targeting (Tzfira et al., 2001; Tzfira, Vaidya, & Citovsky, 2002). However, a later study, using *Arabidopsis* mutants, concluded that VIP1 was not required for the *Agrobacterium*-mediated transformation (Shi et al., 2014). VIP1 belongs to a family of bZIP (basic Leucine zipper) transcription factors found in *Arabidopsis* and most plant species. Recently, it was shown that VirE2 could interact not only with AtVIP1 but also with several of its close *Arabidopsis* homologs, as well as with the AtVIP1 tobacco ortholog NtRSG (Wang et al., 2018). Moreover, VirE2 proteins from different *Agrobacterium* strains displayed variable binding efficiency with the different AtVIP1 homologs. Another study confirmed the interactions between VirE2 and several AtVIP1 homologs and demonstrated that disrupting VIP1 transcription activator ability (but not its VirE2 binding ability) did not affect T-DNA transfer efficiency (Lapham et al., 2018). The second interactor of VirE2 (VIP2, or VirE2 interacting protein 2) was discovered after yeast two-hybrid screening of

an *Arabidopsis* cDNA library. It was demonstrated that VIP2 (a transcription regulator) is required for *Agrobacterium* T-DNA integration (stable transformation) but not for its transient expression, both in *Arabidopsis* and *N. benthamiana* (Anand et al., 2007). VirE2 also interacted with *Arabidopsis* XRCC4 (X-ray cross complementation group 4), a protein involved in the NHEJ DNA repair pathway, potentially interfering with the host plant DSB repair pathway to facilitate the T-DNA integration (Vaghchhipawala, Vasudevan, Lee, Morsy, & Mysore, 2012). Finally, it was reported that VirE2 interacted with the rice protein OsGSTU5, a tau class GST (glutathione S-transferase). That interaction occurred in the host cell cytoplasm and likely resulted in VirE2 glutathionylation and inhibition of *Agrobacterium*-mediated transformation, potentially by reducing VirE2 affinity for single-stranded T-DNA (Tiwari et al., 2022).

11.3 VirD5

Agrobacterium VirD5 was first found to interact with VirF, another effector protein; VirF being naturally unstable in the plant cell, its association with VirD5 resulted in the protection of VirF against degradation (Magori & Citovsky, 2011). Moreover, VirD5 expression was shown to induce cell toxicity in yeast and plant cells via its interaction with kinetochore proteins (Spt4 in yeast and Aurora kinases in yeast and plants), causing chromosomal instability (Zhang & Hooykaas, 2019; Zhang, van Heusden, & Hooykaas, 2017). How this interaction could be involved in the *Agrobacterium* infection process is still unknown. It was also reported that VirD5 interacted with VIP1 (VirE2 interacting protein 1), competing with VBF and potentially preventing VIP1 degradation (Wang et al., 2014), as well as with VIP2 (VirE2 interacting protein 2), potentially preventing VIP2 interaction with Cap-binding proteins (proteins involved in mRNA biosynthesis) (Wang et al., 2018). In the latter case, it is not clear how this interaction would play a role in *Agrobacterium* T-DNA transfer and integration.

11.4 VirE3

Besides binding to VirE2 and being targeted to the host cell nucleus via the interaction with the importin alpha (García-Rodríguez, Schrammeijer, & Hooykaas, 2006; Lacroix et al., 2005), VirE3 was shown to interact with several Arabidopsis proteins, such as Csn5 (a component of the COP9 signalosome) and Brp (a member of the TFIIB family that binds to TATA box) (García-Rodríguez et al., 2006). Although a potential role of CSn5 in *Agrobacterium*-mediated T-DNA transfer is not known, the

interaction with Brp correlates with the demonstrated function of VirE3 in the transcriptional regulation (Niu, Zhou, Henkel, van Heusden, & Hooykaas, 2015). Recently, it was reported that VirE3 interacts with *Arabidopsis* JAZ8, a transcription regulator involved in the plant defense reaction (Li et al., 2021). It seems that VirE3 was able to interfere with JAZ8 transcription regulator activity to mitigate plant defense response via the SA pathway, whereas overexpression of JAZ8 reduced the efficiency of *Agrobacterium*-mediated transformation.

11.5 VirF

VirF contains an F-box domain and was shown to interact with several ASK proteins (plant homologs of Skp1 functioning in the SCF pathway of proteasomal degradation) from *Arabidopsis* (Schrammeijer et al., 2001). VirF activity as a component of the SCF pathway was demonstrated in yeast and plant cells, and VirF interacted with one of its target *Arabidopsis* VIP1 (Tzfira, Vaidya, & Citovsky, 2004). Indeed, VirF was able to induce destabilization of VIP1 (and of its associated VirE2, potentially stripping the T-DNA from VirE2 coating) via proteasomal degradation. Later, several other targets of VirF were discovered, including VFP4 a transcription regulator involved in the plant defense response (García-Cano, Hak, Magori, Lazarowitz, & Citovsky, 2018); VirF also interacted with two closely related trihelix-domain transcription factors (VFP3 and VFP5) but without activating the host UPS pathway (García-Cano et al., 2015). Interestingly, *Agrobacterium* induces the expression of a plant F-box protein (VBF, VIP1 binding F-box), which could partially substitute for VirF activity in plant cells by targeting VIP1 (Zaltsman, Krichevsky, Loyter, & Citovsky, 2010). It was originally believed that, unlike the *Agrobacterium* octopine strain A6, the nopaline strain C58 did not encode a functional VirF. However, it was shown that C58-VirF is most likely a functional F-box protein because it contains an F-box domain and was able to bind ASK1 (Lacroix & Citovsky, 2015). C58-VirF did not interact with VIP1, suggesting that it could have a set of target host proteins different from A6-VirF.

12. Plant transcriptional response to *Agrobacterium* infection

Transcriptomic studies have shown that the expression of many host genes is modified upon *Agrobacterium* infection. Among the genes of which expression is regulated, many of them are involved in plant defense reactions (reviewed in (Willig, Duan, & Zhang, 2018)).

Like most of the higher eukaryotes, plants can sense bacteria through the perception of PAMPs (pathogen-associated molecular patterns), via a receptor that triggers a plant defense response when activated. For example, *Arabidopsis* was able to detect *Agrobacterium* via one of these PAMPs called EF-Tu (elongation factor thermo unstable), resulting in a defense response (Zipfel et al., 2006). Plants usually recognize another PAMP, flagellin 22 (flg22, a fragment of bacterial flagellin) via the receptor FLS2 (flagellin sensitive 2), but *Agrobacterium* harbors a highly divergent flg22 that evades detection by most plants (Felix, Duran, Volko, & Boller, 1999). Interestingly, some plant species, such as the wild grape *Vitis riparia*, may encode a different FLS2 receptor that can recognize *Agrobacterium* flg22; tobacco plants expressing this receptor displayed increased resistance to *Agrobacterium* (Fürst et al., 2020). Several elements of the signaling cascade involved in pathogen-triggered immunity in *Arabidopsis* were shown to be important for *Agrobacterium* infection. Indeed, two mitogen-activated protein kinase kinases MKK4/MKK5 and their downstream mitogen-activated protein kinases MPK3/MPK6 were shown to play a crucial role in the induction of various plant defense pathways by *Agrobacterium*, and their activity affected the efficiency of *Agrobacterium*-mediated transformation (Liu et al., 2021).

Although a plant defense response to *Agrobacterium* infection is observed at the transcriptional level, in most plant species no extensive defense reaction is observed upon *Agrobacterium* infection. As shown in the previous section, several of the translocated effectors were suggested to act as transcriptional regulators themselves or to interfere with host transcription regulation pathways, which could mitigate the plant defense reaction. For example, VirE2 interaction with two plant transcription regulators involved in plant defense reaction (VIP1 and VIP2) might alter their activity. VirE3 was shown to act as a transcription regulator itself, as well as to interact with Arabidopsis JAZ8 (affecting JAZ8 role as a SA pathway activator). VirD5 is also suspected to induce a transcriptional response, either directly or via its interaction with VIP2. Finally, VirF can interact with several transcription regulators, and in some cases (e.g., VIP1, VFP4) induce their proteasomal degradation. In fact, it was demonstrated that VirF-dependent degradation of VFP4 resulted in mitigating the host defense response. Globally, *Agrobacterium* encodes effector proteins that can in many ways interfere with the transcriptional response of its host plant.

For the most part, the plant transcriptional response to *Agrobacterium* seems to be related to the regulation of the general plant defense response.

In a few cases, however, the activation of a plant factor specifically involved in *Agrobacterium*-mediated T-DNA transfer was shown. For example, VirE3 induced the expression of *Arabidopsis* VBF, a functional equivalent of *Agrobacterium* VirF (Niu et al., 2015). It was reported in two recent studies that several genes involved in plant DNA repair pathways are transcriptionally activated in tobacco and *Arabidopsis* plants challenged by *Agrobacterium* infection, and this activation seemed to depend on the presence of the *vir* genes (Hu, Lacroix, & Citovsky, 2021; Joseph, Chandhini, Das, Mysore, & Shah, 2021). We can speculate that this increased expression of DNA repair-related genes plays a role in T-DNA integration.

13. Conclusions

The study of *Agrobacterium*-mediated genetic transformation of plants has revealed a complex network of interactions between bacterial and host factors. On the bacterial side, *Agrobacterium* encoded proteins are either directly mediating the transfer of T-DNA or enhancing this transfer, for example, by interfering with plant defense response. On the plant side, many plant proteins interact with bacterial factors, and a plant defense reaction is triggered. *Agrobacterium* has evolved many strategies to use host pathways to its advantage and to mitigate the plant defense response. Most research so far was performed in plant species susceptible to *Agrobacterium*, and it is likely that more host factors will be discovered in the future, particularly those that render some plant species resistant to *Agrobacterium*. Furthermore, there is a certain level of variability of the virulence factors between *Agrobacterium* strains and species, to which correspond different capabilities of the factors from different host species to interact with bacterial factors. The adequation and the nature of these interactions between host and bacterial species determine the outcome of infection and are responsible for the differences in susceptibility of host plants to different *Agrobacterium* strains.

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References

Abu-Arish, A., Frenkiel-Krispin, D., Fricke, T., Tzfira, T., Citovsky, V., Wolf, S. G., et al. (2004). Three-dimensional reconstruction of *Agrobacterium* VirE2 protein with single-stranded DNA. *The Journal of Biological Chemistry*, *279*, 25359–25363.

Albright, L. M., Yanofsky, M. F., Leroux, B., Ma, D. Q., & Nester, E. W. (1987). Processing of the T-DNA of *Agrobacterium tumefaciens* generates border nicks and linear, single-stranded T-DNA. *Journal of Bacteriology*, *169*, 1046–1055.

Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., et al. (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science*, *301*, 653–657.

Aly, K. A., & Baron, C. (2007). The VirB5 protein localizes to the T-pilus tips in *Agrobacterium tumefaciens*. *Microbiology*, *153*, 3766–3775.

Anand, A., Krichevsky, A., Schornack, S., Lahaye, T., Tzfira, T., Tang, Y., et al. (2007). *Arabidopsis* VIRE2 INTERACTING PROTEIN2 is required for *Agrobacterium* T-DNA integration in plants. *Plant Cell*, *19*, 1695–1708.

Anand, A., Uppalapati, S. R., Ryu, C. M., Allen, S. N., Kang, L., Tang, Y., et al. (2008). Salicylic acid and systemic acquired resistance play a role in attenuating crown gall disease caused by *Agrobacterium tumefaciens*. *Plant Physiology*, *146*, 703–715.

Atmakuri, K., Cascales, E., Burton, O. T., Banta, L. M., & Christie, P. J. (2007). *Agrobacterium* ParA/MinD-like VirC1 spatially coordinates early conjugative DNA transfer reactions. *The EMBO Journal*, *26*, 2540–2551.

Atmakuri, K., Ding, Z., & Christie, P. J. (2003). VirE2, a type IV secretion substrate, interacts with the VirD4 transfer protein at cell poles of *Agrobacterium tumefaciens*. *Molecular Microbiology*, *49*, 1699–1713.

Bakó, L., Umeda, M., Tiburcio, A. F., Schell, J., & Koncz, C. (2003). The VirD2 pilot protein of *Agrobacterium*-transferred DNA interacts with the TATA box-binding protein and a nuclear protein kinase in plants. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 10108–10113.

Ballas, N., & Citovsky, V. (1997). Nuclear localization signal binding protein from *Arabidopsis* mediates nuclear import of *Agrobacterium* VirD2 protein. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 10723–10728.

Bhattacharjee, S., Lee, L. Y., Oltmanns, H., Cao, H., Veena, C., & J., and Gelvin, S. B. (2008). IMPa-4, an *Arabidopsis* importin alpha isoform, is preferentially involved in *Agrobacterium*-mediated plant transformation. *Plant Cell*, *20*, 2661–2680.

Black, S. J., Kashkina, E., Kent, T., & Pomerantz, R. T. (2016). DNA polymerase theta: A unique multifunctional end-joining machine. *Genes*, *7*, 67.

Bolton, G. W., Nester, E. W., & Gordon, M. P. (1986). Plant phenolic compounds induce expression of the *Agrobacterium tumefaciens* loci needed for virulence. *Science*, *232*, 983–985.

Broothaerts, W., Mitchell, H. J., Weir, B., Kaines, S., Smith, L. M., Yang, W., et al. (2005). Gene transfer to plants by diverse species of bacteria. *Nature*, *433*, 629–633.

Cabezón, E., Ripoll-Rozada, J., Peña, A., de la Cruz, F., & Arechaga, I. (2015). Towards an integrated model of bacterial conjugation. *FEMS Microbiology Reviews*, *39*, 81–95.

Cangelosi, G. A., Ankenbauer, R. G., & Nester, E. W. (1990). Sugars induce the *Agrobacterium* virulence genes through a periplasmic binding protein and a transmembrane signal protein. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 6708–6712.

Cangelosi, G. A., Martinetti, G., Leigh, J. A., Lee, C. C., Thienes, C., & Nester, E. W. (1989). Role for *Agrobacterium tumefaciens* ChvA protein in export of beta-1,2-glucan. *Journal of Bacteriology*, *171*, 1609–1615.

Cascales, E., & Christie, P. J. (2004). Definition of a bacterial type IV secretion pathway for a DNA substrate. *Science*, *304*, 1170–1173.

Charles, T. C., & Nester, E. W. (1993). A chromosomally encoded two-component sensory transduction system is required for virulence of *Agrobacterium tumefaciens*. *Journal of Bacteriology*, *175*, 6614–6625.

Chateau, S., Sangwan, R. S., & Sangwan-Norreel, B. S. (2000). Competence of *Arabidopsis thaliana* genotypes and mutants for *Agrobacterium tumefaciens*-mediated gene transfer: Role of phytohormones. *Journal of Experimental Botany*, *51*, 1961–1968.

Christie, P. J. (2004). Type IV secretion: The *Agrobacterium* VirB/D4 and related conjugation systems. *Biochimica et Biophysica Acta*, *1694*, 219–234.

Christie, P. J., Ward, J. E., Winans, S. C., & Nester, E. W. (1988). The *Agrobacterium tumefaciens virE2* gene product is a single-stranded-DNA-binding protein that associates with T-DNA. *Journal of Bacteriology*, *170*, 2659–2667.

Citovsky, V., Wong, M. L., & Zambryski, P. C. (1989). Cooperative interaction of *Agrobacterium* VirE2 protein with single stranded DNA: Implications for the T-DNA transfer process. *Proceedings of the National Academy of Sciences of the United States of America*, *86*, 1193–1197.

Citovsky, V., Zupan, J., Warnick, D., & Zambryski, P. C. (1992). Nuclear localization of *Agrobacterium* VirE2 protein in plant cells. *Science*, *256*, 1802–1805.

Close, T. J., Rogowsky, P. M., Kado, C. I., Winans, S. C., Yanofsky, M. F., & Nester, E. W. (1987). Dual control of *Agrobacterium tumefaciens* Ti plasmid virulence genes. *Journal of Bacteriology*, *169*, 5113–5118.

De Cleene, M., & De Ley, J. (1976). The host range of crown gall. *The Botanical Review*, *42*, 389–466.

de Iannino, N. I., & Ugalde, R. A. (1989). Biochemical characterization of avirulent *Agrobacterium tumefaciens chvA* mutants: Synthesis and excretion of beta-(1-2)glucan. *Journal of Bacteriology*, *171*, 2842–2849.

De Vos, G., & Zambryski, P. C. (1989). Expression of *Agrobacterium* nopaline specific VirD1, VirD2, and VirC1 proteins and their requirement for T-strand production in *E. coli*. *Molecular Plant-Microbe Interactions*, *2*, 43–52.

Deng, W., Chen, L., Wood, D. W., Metcalfe, T., Liang, X., Gordon, M. P., et al. (1998). *Agrobacterium* VirD2 protein interacts with plant host cyclophilins. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 7040–7045.

Dequivre, M., Diel, B., Villard, C., Sismeiro, O., Durot, M., Coppée, J. Y., et al. (2015). Small RNA deep-sequencing analyses reveal a new regulator of virulence in *Agrobacterium fabrum* C58. *Molecular Plant-Microbe Interactions*, *28*, 580–589.

Escobar, M. A., & Dandekar, A. M. (2003). Agrobacterium tumefaciens as an agent of disease. *Trends in Plant Science*, *8*, 380–386.

Felix, G., Duran, J. D., Volko, S., & Boller, T. (1999). Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *The Plant Journal*, *18*, 265–276.

Friesner, J., & Britt, A. B. (2003). Ku80- and DNA ligase IV-deficient plants are sensitive to ionizing radiation and defective in T-DNA integration. *The Plant Journal*, *34*, 427–440.

Fürst, U., Zeng, Y., Albert, M., Witte, A. K., Fliegmann, J., & Felix, G. (2020). Perception of *Agrobacterium tumefaciens* flagellin by FLS2(XL) confers resistance to crown gall disease. *Nature Plants*, *6*, 22–27.

Gallego, M. E., Bleuyard, J. Y., Daoudal-Cotterell, S., Jallut, N., & White, C. I. (2003). Ku80 plays a role in non-homologous recombination but is not required for T-DNA integration in *Arabidopsis*. *The Plant Journal*, *35*, 557–565.

Gao, R., & Lynn, D. G. (2005). Environmental pH sensing: Resolving the VirA/VirG two-component system inputs for *Agrobacterium* pathogenesis. *Journal of Bacteriology*, *187*, 2182–2189.

García-Cano, E., Hak, H., Magori, S., Lazarowitz, S. G., & Citovsky, V. (2018). The *Agrobacterium* F-box protein effector VirF destabilizes the *Arabidopsis* GLABROUS1

enhancer/binding protein-like transcription factor VFP4, a transcriptional activator of defense response genes. *Molecular Plant-Microbe Interactions*, *31*, 576–586.
García-Cano, E., Magori, S., Sun, Q., Zhang, S., Lazarowitz, S. G., & Citovsky, V. (2015). Interaction of *Arabidopsis* trihelix-domain transcription factors VFP3 and VFP5 with *Agrobacterium* virulence protein VirF. *PLoS One*, *10*, e014212.
García-Rodríguez, F. M., Schrammeijer, B., & Hooykaas, P. J. J. (2006). The *Agrobacterium* VirE3 effector protein: A potential plant transcriptional activator. *Nucleic Acids Research*, *34*, 6496–6504.
Gaspar, Y. M., Nam, J., Schultz, C. J., Lee, L. Y., Gilson, P. R., Gelvin, S. B., et al. (2004). Characterization of the *Arabidopsis* lysine-rich arabinogalactan-protein *AtAGP17* mutant (*rat1*) that results in a decreased efficiency of *Agrobacterium* transformation. *Plant Physiology*, *135*, 2162–2171.
Gelvin, S. B. (1998). *Agrobacterium* VirE2 proteins can form a complex with T strands in the plant cytoplasm. *Journal of Bacteriology*, *180*, 4300–4302.
Gelvin, S. B. (2003a). *Agrobacterium*-mediated plant transformation: The biology behind the "gene-jockeying" tool. *Microbiology and Molecular Biology Reviews*, *67*, 16–37.
Gelvin, S. B. (2003b). Improving plant genetic engineering by manipulating the host. *Trends in Biotechnology*, *21*, 95–98.
Ghai, J., & Das, A. (1989). The *virD* operon of *Agrobacterium tumefaciens* Ti plasmid encodes a DNA-relaxing enzyme. *Proceedings of the National Academy of Sciences of the United States of America*, *86*, 3109–3113.
Guo, M., Hou, Q., Hew, C. L., & Pan, S. Q. (2007). *Agrobacterium* VirD2-binding protein is involved in tumorigenesis and redundantly encoded in conjugative transfer gene clusters. *Molecular Plant-Microbe Interactions*, *20*, 1201–1212.
Guo, M., Huang, Z., & Yang, J. (2017). Is there any crosstalk between the chemotaxis and virulence induction signaling in *Agrobacterium tumefaciens*? *Biotechnology Advances*, *35*, 505–511.
Guo, M., Jin, S., Sun, D., Hew, C. L., & Pan, S. Q. (2007). Recruitment of conjugative DNA transfer substrate to *Agrobacterium* type IV secretion apparatus. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 20019–20024.
Heckel, B. C., Tomlinson, A. D., Morton, E. R., Choi, J. H., & Fuqua, C. (2014). *Agrobacterium tumefaciens exoR* controls acid response genes and impacts exopolysaccharide synthesis, horizontal gene transfer, and virulence gene expression. *Journal of Bacteriology*, *196*, 3221–3233.
Heindl, J. E., Wang, Y., Heckel, B. C., Mohari, B., Feirer, N., & Fuqua, C. (2014). Mechanisms and regulation of surface interactions and biofilm formation in *Agrobacterium*. *Frontiers in Plant Science*, *5*, 176.
Hooykaas, P. J. J., Klapwijk, P. M., Nuti, M. P., Schilperoort, R. A., & Rorsch, A. (1977). Transfer of the *Agrobacterium tumefaciens* Ti plasmid to avirulent agrobacteria and to *Rhizobium ex planta*. *Journal of General Microbiology*, *98*, 477–484.
Horsch, R. B., Klee, H. J., Stachel, S., Winans, S. C., Nester, E. W., Rogers, S. G., et al. (1986). Analysis of A*grobacterium tumefaciens* virulence mutants in leaf discs. *Proceedings of the National Academy of Sciences of the United States of America*, *83*, 2571–2575.
Hu, Y., Lacroix, B., & Citovsky, V. (2021). Modulation of plant DNA damage response gene expression during *Agrobacterium* infection. *Biochemical and Biophysical Research Communications*, *554*, 7–12.
Hwang, H. H., & Gelvin, S. B. (2004). Plant proteins that interact with VirB2, the *Agrobacterium tumefaciens* pilin protein, mediate plant transformation. *Plant Cell*, *16*, 3148–3167.
Joseph, J. T., Chandhini, S., Das, S., Mysore, K. S., & Shah, J. M. (2021). Methylation status of *Arabidopsis* DNA repair gene promoters during *Agrobacterium* infection reveals epigenetic changes in three generations. *Plant Molecular Biology Reporter*, *39*, 773–791.

Joubert, P., Beaupère, D., Wadouachi, A., Chateau, S., Sangwan, R. S., & Sangwan-Norreel, B. S. (2004). Effect of phenolic glycosides on *Agrobacterium tumefaciens virH* gene induction and plant transformation. *Journal of Natural Products*, *67*, 348–351.

Kado, C. I. (2014). Historical account on gaining insights on the mechanism of crown gall tumorigenesis induced by *Agrobacterium tumefaciens*. *Frontiers in Microbiology*, *5*, 340.

Kim, S. I., Veena, & Gelvin, S. B. (2007). Genome-wide analysis of *Agrobacterium* T-DNA integration sites in the *Arabidopsis* genome generated under non-selective conditions. *The Plant Journal*, *51*, 779–791.

Kralemann, L. E. M., de Pater, S., Shen, H., Kloet, S. L., van Schendel, R., Hooykaas, P. J. J., et al. (2022). Distinct mechanisms for genomic attachment of the 5′ and 3′ ends of *Agrobacterium* T-DNA in plants. *Nature Plants*, *8*, 526–534.

Kunik, T., Tzfira, T., Kapulnik, Y., Gafni, Y., Dingwall, C., & Citovsky, V. (2001). Genetic transformation of HeLa cells by *Agrobacterium*. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 1871–1876.

Lacroix, B., & Citovsky, V. (2011). Extracellular VirB5 enhances T-DNA transfer from *Agrobacterium* to the host plant. *PLoS One*, *6*, e25578.

Lacroix, B., & Citovsky, V. (2015). Nopaline-type Ti plasmid of *Agrobacterium* encodes a VirF-like functional F-box protein. *Scientific Reports*, *5*, 16610.

Lacroix, B., & Citovsky, V. (2016). A functional bacterium-to-plant DNA transfer machinery of *Rhizobium etli*. *PLoS Pathogens*, *12*, e1005502.

Lacroix, B., & Citovsky, V. (2019). Pathways of DNA transfer to plants from *Agrobacterium tumefaciens* and related bacterial species. *Annual Review of Phytopathology*, *57*, 231–251.

Lacroix, B., Loyter, A., & Citovsky, V. (2008). Association of the *Agrobacterium* T-DNA-protein complex with plant nucleosomes. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 15429–15434.

Lacroix, B., Tzfira, T., Vainstein, A., & Citovsky, V. (2006). A case of promiscuity: *Agrobacterium*'s endless hunt for new partners. *Trends in Genetics*, *22*, 29–37.

Lacroix, B., Vaidya, M., Tzfira, T., & Citovsky, V. (2005). The VirE3 protein of *Agrobacterium* mimics a host cell function required for plant genetic transformation. *The EMBO Journal*, *24*, 428–437.

Lapham, R. A., Lee, L. Y., Tsugama, D., Lee, S., Mengiste, T., & Gelvin, S. B. (2018). VIP1 and its homologs are not required for *Agrobacterium*-mediated transformation, but play a role in *Botrytis* and salt stress responses. *Frontiers in Plant Science*, *9*, 749.

Lee, L. Y., Fang, M. J., Kuang, L. Y., & Gelvin, S. B. (2008). Vectors for multi-color bimolecular fluorescence complementation to investigate protein-protein interactions in living plant cells. *Plant Methods*, *4*, 24.

Lee, Y. W., Jin, S., Sim, W. S., & Nester, E. W. (1995). Genetic evidence for direct sensing of phenolic compounds by the VirA protein of *Agrobacterium tumefaciens*. *Proceedings of the National Academy of Sciences of the United States of America*, *92*, 12245–12249.

Li, Y. G., & Christie, P. J. (2018). The *Agrobacterium* VirB/VirD4 T4SS: Mechanism and architecture defined through *in vivo* mutagenesis and chimeric systems. *Current Topics in Microbiology and Immunology*, *418*, 233–260.

Li, X., & Pan, S. Q. (2017). *Agrobacterium* delivers VirE2 protein into host cells *via* clathrin-mediated endocytosis. *Science Advances*, *3*, e1601528.

Li, X., Tu, H., & Pan, S. Q. (2018). *Agrobacterium* delivers anchorage protein VirE3 for companion VirE2 to aggregate at host entry sites for T-DNA protection. *Cell Reports*, *25*, 302–311.

Li, J., Vaidya, M., White, C., Vainstein, A., Citovsky, V., & Tzfira, T. (2005). Involvement of KU80 in T-DNA integration in plant cells. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 19231–19236.

Li, S., Xu, B., Niu, X., Lu, X., Cheng, J., Zhou, M., et al. (2021). JAZ8 interacts with VirE3 attenuating *Agrobacterium*-mediated root tumorigenesis. *Frontiers in Plant Science*, *12*, 685533.

Li, X., Yang, Q., Peng, L., Tu, H., Lee, L. Y., Gelvin, S. B., et al. (2020). *Agrobacterium*-delivered VirE2 interacts with host nucleoporin CG1 to facilitate the nuclear import of VirE2-coated T complex. *Proceedings of the National Academy of Sciences of the United States of America*, *117*, 26389–26397.

Li, X., Yang, Q., Tu, H., Lim, Z., & Pan, S. Q. (2014). Direct visualization of *Agrobacterium*-delivered VirE2 in recipient cells. *The Plant Journal*, 77, 487–495.

Liu, T., Cao, L., Cheng, Y., Ji, J., Wei, Y., Wang, C., et al. (2021). MKK4/5-MPK3/6 cascade regulates *Agrobacterium*-mediated transformation by modulating plant immunity in *Arabidopsis*. *Frontiers in Plant Science*, *12*, 731690.

Liu, P., & Nester, E. W. (2006). Indoleacetic acid, a product of transferred DNA, inhibits *vir* gene expression and growth of A*grobacterium tumefaciens* C58. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 4658–4662.

Loyter, A., Rosenbluh, J., Zakai, N., Li, J., Kozlovsky, S. V., Tzfira, T., et al. (2005). The plant VirE2 interacting protein 1. A molecular link between the *Agrobacterium* T-complex and the host cell chromatin? *Plant Physiology*, *138*, 1318–1321.

Magori, S., & Citovsky, V. (2011). *Agrobacterium* counteracts host-induced degradation of its F-box protein effector. *Science Signaling*, *4*. ra69.

Matthysse, A. G. (1983). Role of bacterial cellulose fibrils in *Agrobacterium tumefaciens* infection. *Journal of Bacteriology*, *154*, 906–915.

Melanson, D., Chilton, M. D., Masters-Moore, D., & Chilton, W. S. (1997). A deletion in an indole synthase gene is responsible for the DIMBOA-deficient phenotype of *bxbx* maize. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 13345–13350.

Melchers, L. S., Regensburg-Tuink, A. J., Schilperoort, R. A., & Hooykaas, P. J. J. (1989). Specificity of signal molecules in the activation of *Agrobacterium* virulence gene expression. *Molecular Microbiology*, *3*, 969–977.

Merritt, P. M., Danhorn, T., & Fuqua, C. (2007). Motility and chemotaxis in *Agrobacterium tumefaciens* surface attachment and biofilm formation. *Journal of Bacteriology*, *189*, 8005–8014.

Mestiri, I., Norre, F., Gallego, M. E., & White, C. I. (2014). Multiple host-cell recombination pathways act in *Agrobacterium* mediated transformation of plant cells. *The Plant Journal*, *77*, 511–520.

Nishizawa-Yokoi, A., Nonaka, S., Saika, H., Kwon, Y. I., Osakabe, K., & Toki, S. (2012). Suppression of Ku70/80 or Lig4 leads to decreased stable transformation and enhanced homologous recombination in rice. *The New Phytologist*, *196*, 1048–1059.

Niu, X., Zhou, M., Henkel, C. V., van Heusden, G. P., & Hooykaas, P. J. J. (2015). The *Agrobacterium tumefaciens* virulence protein VirE3 is a transcriptional activator of the F-box gene VBF. *The Plant Journal*, *84*, 914–924.

Nonaka, S., Sugawara, M., Minamisawa, K., Yuhashi, K., & Ezura, H. (2008). 1-Aminocyclopropane-1-carboxylate deaminase enhances *Agrobacterium tumefaciens*-mediated gene transfer into plant cells. *Applied and Environmental Microbiology*, *74*, 2526–2528.

Nonaka, S., Yuhashi, K., Takada, K., Sugaware, M., Minamisawa, K., & Ezura, H. (2008). Ethylene production in plants during transformation suppresses *vir* gene expression in *Agrobacterium tumefaciens*. *The New Phytologist*, *178*, 647–656.

Notti, R. Q., & Stebbins, C. E. (2016). The structure and function of type III secretion systems. *Microbiology Spectrum*, *4*. VMBF-0004-2015.

Pansegrau, W., Schoumacher, F., Hohn, B., & Lanka, E. (1993). Site-specific cleavage and joining of single-stranded DNA by VirD2 protein of *Agrobacterium tumefaciens* Ti plasmids: Analogy to bacterial conjugation. *Proceedings of the National Academy of Sciences of the United States of America*, *90*, 11538–11542.

Park, S. Y., Vaghchhipawala, Z., Vasudevan, B., Lee, L. Y., Shen, Y., Singer, K., et al. (2015). *Agrobacterium* T-DNA integration into the plant genome can occur without

the activity of key non-homologous end-joining proteins. *The Plant Journal*, *81*, 934–946.
Peralta, E. G., & Ream, L. W. (1985). T-DNA border sequences required for crown gall tumorigenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *82*, 5112–5116.
Platt, T. G., Bever, J. D., & Fuqua, C. (2012). A cooperative virulence plasmid imposes a high fitness cost under conditions that induce pathogenesis. *Proceedings of the Royal Society B*, *279*, 1691–1699.
Romano, P. G., Horton, P., & Gray, J. E. (2004). The *Arabidopsis* cyclophilin gene family. *Plant Physiology*, *134*, 1268–1282.
Rossi, L., Hohn, B., & Tinland, B. (1996). Integration of complete transferred DNA units is dependent on the activity of virulence E2 protein of *Agrobacterium tumefaciens*. *Proceedings of the National Academy of Sciences of the United States of America*, *93*, 126–130.
Sahi, S. V., Chilton, M. D., & Chilton, W. S. (1990). Corn metabolites affect growth and virulence of *Agrobacterium tumefaciens*. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 3879–3883.
Salomon, S., & Puchta, H. (1998). Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. *The EMBO Journal*, *17*, 6086–6095.
Schrammeijer, B., Risseeuw, E., Pansegrau, W., Regensburg-Tuïnk, T. J. G., Crosby, W. L., & Hooykaas, P. J. J. (2001). Interaction of the virulence protein VirF of *Agrobacterium tumefaciens* with plant homologs of the yeast Skp1 protein. *Current Biology*, *11*, 258–262.
Shi, Y., Lee, L. Y., & Gelvin, S. B. (2014). Is VIP1 important for *Agrobacterium*-mediated transformation? *The Plant Journal*, *79*, 848–860.
Shilo, S., Tripathi, P., Melamed-Bessudo, C., Tzfadia, O., Muth, T. R., & Levy, A. A. (2017). T-DNA-genome junctions form early after infection and are influenced by the chromatin state of the host genome. *PLoS Genetics*, *13*, e1006875.
Shimoda, N., Toyoda-Yamamoto, A., Aoki, S., & Machida, Y. (1993). Genetic evidence for an interaction between the VirA sensor protein and the ChvE sugar-binding protein of *Agrobacterium*. *The Journal of Biological Chemistry*, *268*, 26552–26558.
Shimoda, N., Toyoda-Yamamoto, A., Nagamine, J., Usami, S., Katayama, M., Sakagami, Y., et al. (1990). Control of expression of *Agrobacterium vir* genes by synergistic actions of phenolic signal molecules and monosaccharides. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 6684–6688.
Stachel, S. E., An, G., Flores, C., & Nester, E. W. (1985). A Tn3 *lacZ* transposon for the random generation of beta-galactosidase gene fusions: Application to the analysis of gene expression in *Agrobacterium*. *The EMBO Journal*, *4*, 891–898.
Stachel, S. E., Messens, E., Van Montagu, M., & Zambryski, P. C. (1985). Identification of the signal molecules produced by wounded plant cell that activate T-DNA transfer in *Agrobacterium tumefaciens*. *Nature*, *318*, 624–629.
Stachel, S. E., Timmerman, B., & Zambryski, P. C. (1986). Generation of single-stranded T-DNA molecules during the initial stages of T-DNA transfer for *Agrobacterium tumefaciens* to plant cells. *Nature*, *322*, 706–712.
Stachel, S. E., & Zambryski, P. C. (1986). *virA* and *virG* control the plant-induced activation of the T-DNA transfer process of *A. tumefaciens*. *Cell*, *46*, 325–333.
Steck, T. R., Morel, P., & Kado, C. I. (1988). Vir box sequences in *Agrobacterium tumefaciens* pTiC58 and A6. *Nucleic Acids Research*, *16*, 8736.
Tao, Y., Rao, P. K., Bhattacharjee, S., & Gelvin, S. B. (2004). Expression of plant protein phosphatase 2C interferes with nuclear import of the *Agrobacterium* T-complex protein VirD2. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 5164–5169.
Tinland, B., Schoumacher, F., Gloeckler, V., Bravo-Angel, A. M., & Hohn, B. (1995). The *Agrobacterium tumefaciens* virulence D2 protein is responsible for precise integration of T-DNA into the plant genome. *The EMBO Journal*, *14*, 3585–3595.

Tiwari, M., Gautam, N., Indoliya, Y., Kidwai, M., Mishra, A. K., & Chakrabarty, D. (2022). A tau class GST, OsGSTU5, interacts with VirE2 and modulates the *Agrobacterium*-mediated transformation in rice. *Plant Cell Reports*, *41*, 873–891.

Tzfira, T., Vaidya, M., & Citovsky, V. (2001). VIP1, an *Arabidopsis* protein that interacts with *Agrobacterium* VirE2, is involved in VirE2 nuclear import and *Agrobacterium* infectivity. *The EMBO Journal*, *20*, 3596–3607.

Tzfira, T., Vaidya, M., & Citovsky, V. (2002). Increasing plant susceptibility to *Agrobacterium* infection by overexpression of the *Arabidopsis VIP1* gene. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 10435–10440.

Tzfira, T., Vaidya, M., & Citovsky, V. (2004). Involvement of targeted proteolysis in plant genetic transformation by *Agrobacterium*. *Nature*, *431*, 87–92.

Vaghchhipawala, Z. E., Vasudevan, B., Lee, S., Morsy, M. R., & Mysore, K. S. (2012). *Agrobacterium* may delay plant nonhomologous end-joining DNA repair via XRCC4 to favor T-DNA integration. *Plant Cell*, *24*, 4110–4123.

van Attikum, H., Bundock, P., & Hooykaas, P. J. J. (2001). Non-homologous end-joining proteins are required for *Agrobacterium* T-DNA integration. *The EMBO Journal*, *20*, 6550–6558.

van Attikum, H., Bundock, P., Overmeer, R. M., Lee, L. Y., Gelvin, S. B., & Hooykaas, P. J. J. (2003). The *Arabidopsis AtLIG4* gene is required for the repair of DNA damage, but not for the integration of *Agrobacterium* T-DNA. *Nucleic Acids Research*, *31*, 4247–4255.

van Kregten, M., de Pater, S., Romeijn, R., van Schendel, R., Hooykaas, P. J. J., & Tijsterman, M. (2016). T-DNA integration in plants results from polymerase-theta-mediated DNA repair. *Nature Plants*, *2*, 16164.

Vergunst, A. C., Schrammeijer, B., den Dulk-Ras, A., de Vlaam, C. M. T., Regensburg-Tuink, T. J., & Hooykaas, P. J. J. (2000). VirB/D4-dependent protein translocation from *Agrobacterium* into plant cells. *Science*, *290*, 979–982.

Vergunst, A. C., van Lier, M. C. M., den Dulk-Ras, A., Stüve, T. A., Ouwehand, A., & Hooykaas, P. J. J. (2005). Positive charge is an important feature of the C-terminal transport signal of the VirB/D4-translocated proteins of *Agrobacterium*. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 832–837.

Wang, Y., Haitjema, C. H., & Fuqua, C. (2014). The Ctp type IVb pilus locus of *Agrobacterium tumefaciens* directs formation of the common pili and contributes to reversible surface attachment. *Journal of Bacteriology*, *196*, 2979–2988.

Wang, L., Lacroix, B., Guo, J., & Citovsky, V. (2017). Transcriptional activation of virulence genes of *Rhizobium etli*. *Journal of Bacteriology*, *199*. e00841-16.

Wang, Y., Peng, W., Zhou, X., Huang, F., Shao, L., & Luo, M. (2014). The putative *Agrobacterium* transcriptional activator-like virulence protein VirD5 may target T-complex to prevent the degradation of coat proteins in the plant cell nucleus. *The New Phytologist*, *203*, 1266–1281.

Wang, C., Ye, F., Chang, C., Liu, X., Wang, J., Wang, J., et al. (2019). Agrobacteria reprogram virulence gene expression by controlled release of host-conjugated signals. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 22331–22340.

Wang, L., Zhang, S., Huang, F., Zhou, X., Chen, Z., Peng, W., et al. (2018). VirD5 is required for efficient *Agrobacterium* infection and interacts with Arabidopsis VIP2. *The New Phytologist*, *217*, 726–738.

Weisberg, A. J., Davis, E. W., 2nd, Tabima, J., Belcher, M. S., Miller, M., Kuo, C. H., et al. (2020). Unexpected conservation and global transmission of agrobacterial virulence plasmids. *Science*, *368*. eaba5256.

Wendt, T., Doohan, F., & Mullins, E. (2012). Production of *Phytophthora infestans*-resistant potato (*Solanum tuberosum*) utilising *Ensifer adhaerens* OV14. *Transgenic Research*, *21*, 567–578.

Willig, C. J., Duan, K., & Zhang, Z. J. (2018). Transcriptome profiling of plant genes in response to *Agrobacterium tumefaciens*-mediated transformation. *Current Topics in Microbiology and Immunology*, *418*, 319–348.

Wolterink-van Loo, S., Escamilla Ayala, A. A., Hooykaas, P. J. J., & van Heusden, G. P. (2015). Interaction of the *Agrobacterium tumefaciens* virulence protein VirD2 with histones. *Microbiology*, *161*, 401–410.

Wright, E. L., Deakin, W. J., & Shaw, C. H. (1998). A chemotaxis cluster from *Agrobacterium tumefaciens*. *Gene*, *220*, 83–89.

Xu, J., Kim, J., Danhorn, T., Merritt, P. M., & Fuqua, C. (2012). Phosphorus limitation increases attachment in *Agrobacterium tumefaciens* and reveals a conditional functional redundancy in adhesin biosynthesis. *Research in Microbiology*, *163*, 674–684.

Yadav, N. S., Vanderleyden, J., Bennett, D. R., Barnes, W. M., & Chilton, M. D. (1982). Short direct repeats flank the T-DNA on a nopaline Ti plasmid. *Proceedings of the National Academy of Sciences of the United States of America*, *79*, 6322–6326.

Yanofsky, M. F., Porter, S. G., Young, C., Albright, L. M., Gordon, M. P., & Nester, E. W. (1986). The *virD* operon of *Agrobacterium tumefaciens* encodes a site-specific endonuclease. *Cell*, *47*, 471–477.

Young, C., & Nester, E. W. (1988). Association of the VirD2 protein with the 5′ end of T-strands in *Agrobacterium tumefaciens*. *Journal of Bacteriology*, *170*, 3367–3374.

Yuan, Z. C., Edlind, M. P., Liu, P., Saenkham, P., Banta, L. M., Wise, A. A., et al. (2007). The plant signal salicylic acid shuts down expression of the *vir* regulon and activates quormone-quenching genes in *Agrobacterium*. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 11790–11795.

Zaltsman, A., Krichevsky, A., Loyter, A., & Citovsky, V. (2010). *Agrobacterium* induces expression of a plant host F-box protein required for tumorigenicity. *Cell Host & Microbe*, *7*, 197–209.

Zhang, J., Boone, L., Kocz, R., Zhang, C., Binns, A. N., & Lynn, D. G. (2000). At the maize/*Agrobacterium* interface: Natural factors limiting host transformation. *Chemistry & Biology*, *7*, 611–621.

Zhang, X., & Hooykaas, P. J. J. (2019). The *Agrobacterium* VirD5 protein hyper-activates the mitotic Aurora kinase in host cells. *The New Phytologist*, *222*, 1551–1560.

Zhang, X., van Heusden, G. P. H., & Hooykaas, P. J. J. (2017). Virulence protein VirD5 of *Agrobacterium tumefaciens* binds to kinetochores in host cells *via* an interaction with Spt4. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, 10238–10243.

Zhu, Y., Nam, J., Carpita, N. C., Matthysse, A. G., & Gelvin, S. B. (2003). *Agrobacterium*-mediated root transformation is inhibited by mutation of an *Arabidopsis* cellulose synthase-like gene. *Plant Physiology*, *133*, 1000–1010.

Ziemienowicz, A., Görlich, D., Lanka, E., Hohn, B., & Rossi, L. (1999). Import of DNA into mammalian nuclei by proteins originating from a plant pathogenic bacterium. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 3729–3733.

Ziemienowicz, A., Tinland, B., Bryant, J., Gloeckler, V., & Hohn, B. (2000). Plant enzymes but not *Agrobacterium* VirD2 mediate T-DNA ligation *in vitro*. *Molecular and Cellular Biology*, *20*, 6317–6322.

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J. D., Boller, T., et al. (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell*, *125*, 749–760.

Zuniga-Soto, E., Mullins, E., & Dedicova, B. (2015). *Ensifer*-mediated transformation: An efficient non-*Agrobacterium* protocol for the genetic modification of rice. *Springerplus*, *4*, 600.