Agrobacterium in the Genomics Age

Stanton B. Gelvin*

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907–1392

Members of the genus *Agrobacterium* cause the neoplastic diseases crown gall, hairy root, and cane gall on numerous plant species. Extensive genetic analyses conducted in the 1980s identified key bacterial genes involved in virulence. During the past decade, however, genomic technologies have revealed numerous additional bacterial genes that more subtly influence transformation. The results of these genomic analyses allowed scientists to develop a more integrated view of how *Agrobacterium* interacts with host plants. In a similar manner, genomic technologies have identified numerous plant genes important for *Agrobacterium*-mediated genetic transformation. Knowledge of these genes and their roles in transformation has revealed how *Agrobacterium* manipulates its hosts to increase the probability of a successful transformation outcome. In this article, I review our current knowledge of *Agrobacterium*-plant interactions and how genomic and proteomic technologies have increased our understanding of this unique plant-microbe interaction.

Agrobacterium species are phytopathogens that cause a variety of neoplastic diseases, including crown gall (*Agrobacterium tumefaciens* and *Agrobacterium vitis*), hairy root (*Agrobacterium rhizogenes*), and cane gall (*Agrobacterium rubi*). Virulent strains of *Agrobacterium* contain tumor-inducing (Ti) or root-inducing (Ri) plasmids. During infection, enzymes encoded by plasmid-localized virulence (*vir*) genes process the T-DNA region of these plasmids. The resulting single-strand DNA (T-strand) linked to VirD2 protein exits the bacterium via a type IV protein secretion system and enters the plant cell. Within the plant, T-strands likely form complexes with other secreted virulence effector proteins, including VirE2, VirE3, VirD5, and VirF, and supercomplexes with plant proteins as they traverse the cytoplasm and target the nucleus. Once inside the nucleus, T-strands integrate randomly into the plant genome and express T-DNA-encoded transgenes. Two classes of T-DNA genes mediate the pathology of *Agrobacterium* infection. The first group, the oncogenes, either effect phytohormone production (*iaa* and *ipt*; Akiyoshi et al., 1984; Schroder et al., 1984), sensitize the plant to endogenous hormone levels (*rol* and other genes of pRi, *gene5* and *gene6* of pTi; Shen et al., 1988; Spanier et al., 1989; Tinland et al., 1990; Korber et al., 1991), or may be involved in chromatin remodeling (*gene6b*; Terakura et al., 2007). Expression of these genes results in tumorigenic or rhizogenic growth. A second set of genes directs the synthesis of various low M_r compounds, opines, that can serve as energy sources for the inciting bacterial strain and can perhaps affect virulence (Veluthambi et al., 1989). For reviews, the reader should see Gelvin (2000, 2003), Tzfira and Citovsky (2001, 2003), McCullen and Binns (2006), and Citovsky et al. (2007). In addition, the reader is directed to an excellent new book on *Agrobacterium* biology (Tzfira and Citovsky, 2008).

Most plant biologists, however, best know *Agrobacterium* as an agent of horizontal gene transfer that plays an essential role in basic scientific research and in agricultural biotechnology. In the 1980s, scientists learned to disarm (delete the oncogenes and, usually, the opine synthase genes) virulent *Agrobacterium* strains such that tissues infected by the bacteria could regenerate into normal plants (Bevan et al., 1983; Fraley et al., 1983; Herrera-Estrella et al., 1983). Substituting genes of interest for oncogenes and opine synthase genes resulted in plants expressing these novel transgenes and, thus, novel phenotypes. Although transgene substitution for oncogenes within T-DNA was initially conducted in cis (i.e. novel transgenes were placed within T-DNA of Ti-plasmids; Caplan et al., 1983; Fraley et al., 1985), the development of binary systems, in which T-DNA and virulence helper plasmids were separated into two different replicons (de Framond et al., 1983; Hoekema et al., 1983), greatly increased the utility of *Agrobacterium* as a vehicle for gene transfer in plant biology laboratories.

Throughout its development as a gene jockeying tool, genomic studies on *Agrobacterium* and its plant hosts guided scientists in basic science and agricultural biotechnology developments. In this article, I review some of the key genomic methodologies and findings that have contributed to our knowledge of how *Agrobacterium* works and will contribute in the future better to utilize *Agrobacterium*'s amazing gene transfer abilities in the laboratory and in the agricultural biotechnology industry.

GENOMICS OF *AGROBACTERIUM*

Whole-Genome Mutagenesis

Although not frequently considered genomics, important early studies on *A. tumefaciens* and *A. rhizogenes* utilized whole-genome mutagenesis and mass

* E-mail gelvin@bilbo.bio.purdue.edu.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Stanton B. Gelvin (gelvin@bilbo.bio.purdue.edu).

www.plantphysiol.org/cgi/doi/10.1104/pp.109.139873

phenotypic screening to define *Agrobacterium* genes important for transformation (i.e. T-DNA and Vir protein transfer) and tumorigenesis. Transposon mutagenesis was generally the method of choice because of the relatively random integration pattern of transposons in the bacterial genome and because the positions of transposon insertions could easily be determined by restriction endonuclease mapping. Thus, scientists localized genes involved in opine catabolism to a specific region of the Ti-plasmid and identified genes involved in crown gall tumorigenesis in the T-DNA, in regions of the Ti-plasmid not within the T-DNA (later to be identified as the virulence region), and in the bacterial chromosome (chromosomal virulence [*chv*] genes; Garfinkel and Nester, 1980; Holsters et al., 1980; Ooms et al., 1980; De Greve et al., 1981). More directed mutagenesis studies identified opine synthase genes and oncogenes in T-DNA regions of Ti- and Ri-plasmids and specific virulence genes within the *vir* gene region (Garfinkel et al., 1981; Leemans et al., 1981; Ooms et al., 1981; Ream et al., 1983; Inze et al., 1984; White et al., 1985; Stachel and Nester, 1986; Stachel and Zambryski, 1986). These studies involved testing of hundreds or thousands of individually mutagenized *Agrobacterium* strains for virulence, opine catabolism and synthesis, and tumor morphology and may thus be categorized as early *Agrobacterium* genomic studies.

Rong et al. (1990) conducted a second type of genetic screening, using the promoter-less *lacZ*-containing transposon MuDI-1681, to identify plant-inducible *Agrobacterium* genes on the chromosome of *A. tumefaciens* A136 (C58 chromosomal background lacking a Ti-plasmid). These authors assayed several thousand randomly mutagenized *Agrobacterium* strains for induced gene expression on plates containing carrot root extract and X-gal. Insertion of the transposon into the *picA* gene revealed that this gene was >10-fold inducible by the root extract. The *picA* gene, currently identified as encoding a polygalacturonase-like protein (Atu3129), was the first identified plant-inducible *Agrobacterium* chromosomal gene.

Agrobacterium Whole-Genome Sequencing

Scientists had sequenced large portions of the *Agrobacterium* genome, including entire Ti-plasmids, by the late 1990s and the following years (Barker et al., 1983; Gielen et al., 1984; Slightom et al., 1986; Thompson et al., 1988; Ward et al., 1988; Rogowsky et al., 1990; Suzuki et al., 2000; Moriguchi et al., 2001; Oger et al., NC_010929; Kalogeroki and Winans, NC_002377). Generation of a complete nucleotide sequence of the nopaline-type strain *A. tumefaciens* C58 (Goodner et al., 2001; Wood et al., 2001), from which many *Agrobacterium* strains commonly used for plant genetic engineering are derived [e.g. GV3010::pMP90, C58-Z707, NT1(pKPSF2), EHA101/105, AGL-0/-1; see Lee and Gelvin (2008) for characteristics of these strains], opened the door for more extensive analyses of this important phytopathogen. *A. tumefaciens* C58 contains four replicons: a circular and a linear chromosome and two plasmids (pTiC58 and pAtC58). The genome contains approximately 30 insertion sequence elements and encodes an unusually large number of transporters (at least 153) and two-component regulatory systems (at least 25). Recently, Ulker et al. (2008) described the surprising observation that *Agrobacterium* can transfer its chromosomal DNA to plants. Interestingly, particular insertion sequence elements and transporter gene sequences are hot spots for chromosomal DNA transfer. The preferential appearance of these chromosomal sequences associated with T-DNA in Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*) T-DNA/plant DNA junctions suggests multiple mechanisms for chromosomal DNA mobilization during T-DNA transfer (Gelvin, 2008).

The complete genome sequence and annotation of *A. tumefaciens* C58 is posted on http://depts.washington.edu/agro/. In addition to this biovar I *A. tumefaciens* strain, DNA sequence analysis of the *Agrobacterium radiobacter* biovar II strain K84 and the *A. vitis* biovar III strain S4 has recently appeared (Slater et al., 2009). *A. radiobacter* K84 is an especially important strain because it and its derivatives are widely used as biocontrol agents against tumorigenic *Agrobacterium* strains (Kerr and Panagopoulos, 1977; Jones et al., 1988). Comparative analysis of the sequences of the three *Agrobacterium* strains and several other species of the family *Rhizobiaceae* indicate a complex genome evolution, including the migration of gene blocks among replicons within and between species. Sequencing of other *Agrobacterium* strains (the biovar III strain *A. vitis* F5R19 and the biovar II strain *A. rhizogenes* A4) is in progress.

Agrobacterium Transcriptional Profiling

Based upon the *A. tumefaciens* C58 sequence, scientists have generated microarrays to probe the response of bacterial genes to environmental and chemical conditions important for *Agrobacterium* virulence and plant defense.

The first such study investigated genes on the octopine-type Ti-plasmid pTiA6 and the nopaline-type Ti-plasmid pTiC58. Cho and Winans (2005) incubated bacteria individually containing these Ti-plasmids with acetosyringone (AS), a potent inducer of the *vir* gene regulon synthesized by wounded plant cells (Stachel et al., 1985; Stachel and Nester, 1986; Stachel and Zambryski, 1986). They used RNA extracted from induced and noninduced cells as probes of microarrays containing all Ti-plasmid genes. As expected, they observed an increase in all previously identified *vir* genes, along with several other Ti-plasmid genes previously not identified as part of the *vir* regulon. Most interestingly, they noted an increase in expression of all Ti-plasmid-encoded genes, suggesting that AS induction of the *vir* regulon increases the copy number of the Ti-plasmid relative to that of the bac-

terial chromosomes. Veluthambi et al. (1988) previously observed a similar increase in Ti-plasmid copy number in bacteria cocultivated with plant cells. Further investigation by Cho and Winans (2005) demonstrated that the *repAB*C operon, essential for replication of these Ti-plasmids, was induced by AS. Induction was under the control of the two-component VirA/VirG regulatory system also responsible for *vir* gene induction. Thus, when *Agrobacterium* is in the environment of a wounded plant cell, the Ti-plasmid overreplicates, perhaps increasing the probability of T-DNA transfer to the plant.

The plant wound environment in which *Agrobacterium* effects horizontal gene transfer is acidic (Fierer and Jackson, 2006), and the bacterium must maintain pH homeostasis. An acidic environment is also essential for efficient *vir* gene induction (Stachel et al., 1986), and two promoters regulate *virG*, one of which is acid inducible (Mantis and Winans, 1992; Chang and Winans, 1996). In addition, two chromosomal genes important for *vir* gene induction and transformation, *chvG* and *chvI*, are acid inducible (Charles and Nester, 1993; Li et al., 2002). With these facts in mind, Yuan et al. (2008a) conducted a microarray-based, whole-genome transcriptional profiling study of all *Agrobacterium* genes responding to acidic conditions. These authors identified 152 acid-responsive genes. These included previously identified acid-induced genes, genes involved in cell envelope synthesis, genes involved in exopolysaccharide (succinoglycan) synthesis and metabolism, several newly recognized acid-inducible *vir* genes (*virE0*, *virE1*, *virH1*, and *virH2*), and genes encoding a recently described type VI secretion system (Wu et al., 2008). Acidic conditions repressed a number of genes, including some involved in motility, chemotaxis, and cellular metabolism.

Salicylic acid (SA) is a major signaling molecule that is important for plant defense responses. Although induction of SA and downstream plant defense genes by bacterial elicitation is well studied, fewer reports have investigated the effect of plant-derived SA on pathogen gene expression. Two groups used microarray analysis to investigate the effect of SA on the accumulation of *Agrobacterium* transcripts. Yuan et al. (2007) showed that SA, at concentrations that do not influence bacterial growth (2–8 μM), inhibits *vir* gene expression in acidified medium containing AS. At higher concentrations (>10 μM), SA inhibits bacterial growth in acidic medium. Transcriptional profiling of RNA from bacteria incubated for 6 h with AS and 6 μM SA indicated that expression of Ti-plasmid-localized *vir* genes and the *repABC* genes was repressed. However, SA induced a number of *Agrobacterium* genes, including *attKLM*, which encodes a quormone degradation system. Because plants deficient in SA production are hypersusceptible to *Agrobacterium* transformation, whereas elicitation with SA decreased virulence (Yuan et al., 2007; Anand et al., 2008; Veena and S.B. Gelvin, unpublished data), these data suggest that the plant signaling molecule SA may inhibit transformation by shutting down *vir* gene expression and consequently T-DNA transfer. Anand et al. (2008) noted another effect of SA on *Agrobacterium*. Bacteria treated with 100 μM SA did not efficiently attach to plant cells. Thus, bacterial attachment may be yet another process that is disrupted by this plant hormone.

To explore further the effects of plant-released signal molecules on *Agrobacterium* gene expression, Yuan et al. (2008b) incubated bacteria with physiological levels of SA, indole-3-acetic acid (IAA), and γ-amino butyric acid (GABA) that do not inhibit *Agrobacterium* growth. Previous data had indicated that each of these compounds affect *Agrobacterium* virulence (Chevrot et al., 2006; Liu and Nester, 2006; Yuan et al., 2007; Anand et al., 2008). Incubation of *Agrobacterium* at acid pH with each of these compounds, followed by microarray analysis, revealed 100 to 200 genes for each treatment whose expression was modulated. In some instances, different compounds affected the same genes, whereas numerous *Agrobacterium* genes showed differential regulation by one compound only. IAA inhibited expression of the entire Ti-plasmid-localized *vir* regulon but did not have appreciable effects on expression of chromosomal virulence genes. Thus, the effect of IAA on *vir* regulon induction was similar to that of SA. However, the effects of SA and GABA on *Agrobacterium* gene expression were generally very different. In a most interesting exception, SA and GABA both induced the *attKLM* operon, which is involved in destroying the quorum sensing homoserine lactone that serves as a signaling molecule between *Agrobacterium* cells. In addition, seven genes were coregulated by IAA, SA, and GABA. Most of these were transporters, and mutation of some of these resulted in altered AttM lactonase activity. Taken together, these data suggest that at later times during *Agrobacterium* infection, plant signal molecules shut down *vir* gene expression (which is no longer needed once infection has been established) and may destroy quorum sensing signals.

Agrobacterium Proteomics

Engstrom et al. (1987) conducted the first proteomic study of *Agrobacterium*. Using one-dimensional SDS-PAGE, they identified 10 to 15 protein bands that appeared in various *Agrobacterium* strains following incubation with the *vir* gene inducer AS. Several of these bands corresponded to VirB membrane proteins comprising the type IV secretion system that transfers T-DNA and virulence effector proteins to plants. They also identified VirF and VirE2, two proteins of the *vir* regulon. In addition, they detected a number of other AS-induced proteins encoded by the Ti-plasmid or by the *Agrobacterium* chromosome. Similarly, Rong et al. (1990) detected by one-dimensional SDS-PAGE 10 plant-induced *Agrobacterium* chromosomal protein bands.

Rosen et al. (2004) made the first attempt at experimentally defining the *Agrobacterium* proteome. Using two-dimensional gel electrophoresis, they detected ap-

proximately 300 proteins from exponentially growing bacteria. Interestingly, approximately 10% of the proteins were represented by multiple spots on the gel. The authors suggested that a high level of protein modification of the proteome occurs. Similar studies by this group (Rosen et al., 2001, 2002) investigated stress (high temperature, oxidative, and mild acid conditions) and heat shock-induced proteins of *Agrobacterium*. This group also identified proteins induced when the bacteria were incubated with and bound to cut tomato (*Solanum lycopersicum*) root segments, simulating plant infection conditions (Rosen et al., 2003). As controls, they examined, by two-dimensional gel electrophoresis, proteins from unbound bacteria and bacteria not incubated with root segments. Incubation of bacteria with roots induced approximately 30 proteins, regardless of whether the bacteria bound to the root segments or not. Although incubation with root segments induced ChvE, AttK, and AttM (all proteins involved in virulence), their experiments detected no induced Ti-plasmid-encoded virulence proteins. Because, for example, VirE2 is a major virulence protein induced by phenolic molecules such as AS (Engstrom et al., 1987; Lai et al., 2006), the results of this study indicate either that *vir* gene induction did not efficiently occur or that it occurred in only a small percentage of the bacteria.

More recently, Lai et al. (2006) investigated *Agrobacterium* proteins induced by the phenolic *vir* regulon inducer AS. Using two-dimensional gel electrophoresis coupled with mass spectrometry, they identified 11 AS-induced proteins. Nine of these proteins were well-known Ti-plasmid-encoded Vir proteins (VirE2, several VirB proteins, Tzs, VirH1, and VirK), thus verifying their *vir* regulon induction conditions. In addition, they identified two proteins encoded by chromosomal genes, HspL (a small heat shock protein) and Y4mC (a protein of unknown function). Reverse transcription-PCR analysis indicated that transcripts of the genes encoding these proteins also were AS inducible and that induction was dependent upon the two-component sensing system VirA/VirG that mediates induction of the *vir* regulon. All *vir* regulon genes previously identified contain a *vir* box in their promoter regions (Das et al., 1986). The *y4mC* gene promoter similarly contains a *vir* box, but, interestingly, the *hspL* promoter does not. Thus, *hspL* activation by AS may be an indirect consequence of expression of Vir proteins. In addition, Wu et al. (2008) analyzed proteins secreted by *Agrobacterium* into the medium. They identified 12 proteins, including VirB1* (a cleaved fragment of VirB1 protein) and Hcp (hemolysin-coregulated protein). Hcp is secreted by a newly discovered type VI secretion system.

GENOMICS OF PLANT GENES IMPORTANT FOR *AGROBACTERIUM*-MEDIATED GENETIC TRANSFORMATION

Scientists have used a variety of genomic techniques to investigate plant genes important for *Agrobacterium*-mediated transformation. These include forward genetic screens to identify mutant plants with altered transformation susceptibility, yeast two-hybrid studies to detect plant proteins that interact with Virulence effector proteins, and transcriptional profiling to discover plant genes whose expression is altered following *Agrobacterium* infection. In addition, reverse genetic analyses have been used to probe the importance of candidate genes in the transformation process.

Forward Genetic Screens for Plant Mutants with Altered Transformation Characteristics

Plant species, and even different cultivars/genotypes of the same species, are notoriously varied in their transformation susceptibility (DeCleene and DeLey, 1976; Anderson and Moore, 1979; Conner and Commisse, 1992; van Wordragen and Dons, 1992; Bliss et al., 1999; Pena and Seguin, 2001; Somers et al., 2003; Shrawat and Lorz, 2006). In addition, *Agrobacterium* can transform *Streptomyces*, yeast, and other fungal species (Bundock et al., 1995, 2002; Piers et al., 1996; de Groot et al., 1998; Abuodeh et al., 2000; Kelly and Kado, 2002; Roberts et al., 2003; Schrammeijer et al., 2003; van Attikum and Hooykaas, 2003; Michielse et al., 2004), HeLa cells (Kunik et al., 2001), and sea urchin embryos (Bulgakov et al., 2006). Thus, *Agrobacterium* is incredibly promiscuous in its ability to mediate horizontal gene flow among numerous species of different phylogenetic kingdoms. A genetic basis for susceptibility to *Agrobacterium* exists in many crop species (Owens and Cress, 1984; Szegedi and Kozma, 1984; Smarrelli et al., 1986; Robbs et al., 1991; Bailey et al., 1994; Mauro et al., 1995), and Nam et al. (1997) also described a genetic basis for various degrees of susceptibility among approximately 40 Arabidopsis ecotypes.

Large-scale forward genetic screening of approximately 20,000 T-DNA mutagenized Arabidopsis lines resulted in the first identification of plant genes involved in *Agrobacterium*-mediated transformation (Nam et al., 1999; Zhu et al., 2003b). These forward genetic analyses revealed >120 genes encoding proteins involved in transformation and, because the screen was not saturating (e.g. no gene was discovered more than once), the authors suggested that >200 Arabidopsis genes likely influence plant transformation susceptibility (Zhu et al., 2003b). The authors termed mutants with greatly decreased susceptibility to transformation *rat* (for resistant to *Agrobacterium* transformation) mutants and the corresponding mutant genes, *rat* genes. The identified genes represent most of the proposed transformation events that occur in the plant (bacterial attachment/biofilm formation, T-DNA and Virulence protein transfer to the plant, cytoplasmic trafficking and targeting of the proposed T-complex to the nucleus, virulence protein removal from the T-strand, T-DNA integration into the plant genome, and transgene expression).

Examples of plant proteins identified in these initial genetic screens and mediating transformation include those involved in cell wall structure and biosynthesis (Rat1 and Rat4, and arabinogalactan and cellulose synthase-like [CslA9] proteins, respectively; Zhu et al., 2003a; Gaspar et al., 2004), cytoskeleton proteins potentially involved in cytoplasmic trafficking of T-complex components (actins and a kinesin; Zhu et al., 2003b), importin α and β proteins that may mediate nuclear targeting of T-complex components (Ballas and Citovsky, 1997; Bakó et al., 2003; Bhattacharjee et al., 2008), chromatin proteins such as various histones, histone acetyltransferases, histone deacetylases, and histone chaperones that may facilitate T-DNA integration into the plant genome (Nam et al., 1999; Mysore et al., 2000; Yi et al., 2002, 2006; Tian et al., 2003; Zhu et al., 2003b; Gelvin and Kim, 2007), and histone proteins that can increase transgene expression (G. Tenea and S.B. Gelvin, unpublished data). The nature of these *rat* genes has stimulated reverse genetic experiments to determine the potential roles of candidate genes in the transformation process (see below).

Recently, the Gelvin laboratory further identified several Arabidopsis mutants that are hypersusceptible to *Agrobacterium* transformation (*hat* mutants and, therefore, *hat* genes; Fig. 1; N. Sardesai and S.B. Gelvin, unpublished data). Arabidopsis lines containing T-DNA activation tags (Weigel et al., 2000) provide a resource for overexpressed genes that may influence transformation susceptibility. When roots of these mutagenized plants were assayed at low bacterial inoculum conditions (10^2- to 10^3-fold lower than that usually used to screen for *rat* mutants), we identified seven independent lines that displayed increased levels of transformation relative to that of wild-type control plants. T-DNA/plant DNA junction sequences from five *hat* mutants identified several new genes involved in transformation susceptibility, including a cellulose synthase-like protein (CslB5), a potassium transporter family protein (two independent T-DNA insertion lines), a UDP-glucosyltransferase (UGT), and a myb transcription factor (MTF).

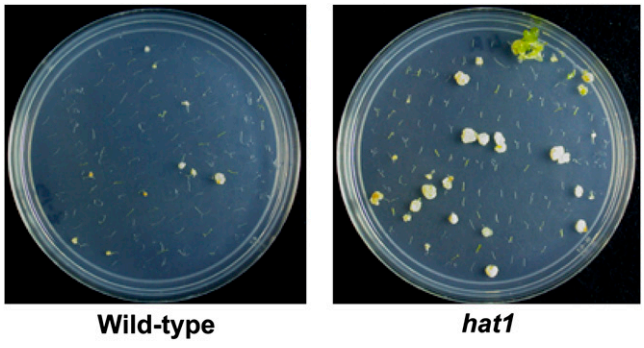

Figure 1. Activation tagging identifies Arabidopsis mutants that are hypersusceptible to *Agrobacterium* transformation (*hat* mutants). Root segments of wild-type (ecotype Wassilewskija) and T-DNA activation-tagged mutants (Weigel et al., 2000) were inoculated with the tumorigenic strain *A. tumefaciens* A208 at low inoculum density (10^6 colony forming units/mL). After 2 d of cocultivation, the root segments were transferred to Murashige and Skoog medium lacking phytohormones and tumors were allowed to develop (Zhu et al., 2003b). The plates were photographed after 4 weeks. Note the larger and more numerous tumors formed on root segments of the *hat1* mutant line, compared to the tumors formed on wild-type roots. The *hat1* mutant has a T-DNA activation tag inserted into a cellulose synthase-like gene. Expression of a neighboring UGT gene is greatly enhanced in the *hat1* mutant.

Overexpression of the UGT cDNA in wild-type plants confirmed that this gene is a *hat* gene. Interestingly, metabolic profiling of roots from UGT overexpressing plants indicated alterations in the levels of key defense compounds, and microarray analyses of these plants revealed decreased expression of most genes in the phenypropanoid biosynthetic and SA signaling pathways (N. Sardesai, A. Perera, R. Doerge, and S.B. Gelvin, unpublished data). These results further indicate that plant defense response signaling pathways are involved in susceptibility to *Agrobacterium*-mediated transformation (see the discussion of transcriptional profiling below).

The *hat3* mutant has a T-DNA activation tag inserted into the 5′ untranslated region of an MTF gene. Although we could not isolate any homozygous *hat3* mutants (suggesting that this MTF is essential for normal plant growth and development), heterozygous *hat3* mutants are approximately 10-fold more susceptible to *Agrobacterium*-mediated transformation than are wild-type control plants (Fig. 2A; N. Sardesai and S.B. Gelvin, unpublished data). Three additional independent T-DNA insertions in this gene are also *hat* mutants (Fig. 2B), indicating that this MTF is a negative regulator of *Agrobacterium*-mediated transformation. Microarray analysis of RNA isolated from roots of *mtf* mutant plants indicated that a WRKY transcription factor gene was expressed to a lower level in the mutant. A homozygous T-DNA insertion into this WRKY transcription factor gene also resulted in a hat phenotype. This WRKY transcription factor is involved in regulating plant defense responses, once again implicating plant defense responses as a component of transformation susceptibility.

As an alternative to screening T-DNA insertion mutants for hat and rat phenotypes, Anand et al. (2007b) used virus-induced gene silencing to investigate *Nicotiana benthamiana* genes important for *Agrobacterium*-mediated transformation. The authors identified 21 genes whose expression, when lowered, resulted in an altered crown gall phenotype. Proteins encoded by these genes include a nodulin-like protein, α-expansin, VIP1, importin-α, and histones H2A and H3.

Identification of *rat* and *hat* mutants emphasizes the utility of large-scale forward genetic screens to understand the plant contribution to the *Agrobacterium*-mediated transformation process.

Yeast Two-Hybrid Screening for Plant Proteins That Interact with Virulence Effector Proteins

A. tumefaciens transfers at least five Virulence effector proteins to plants (VirD2 attached to the T-strand,

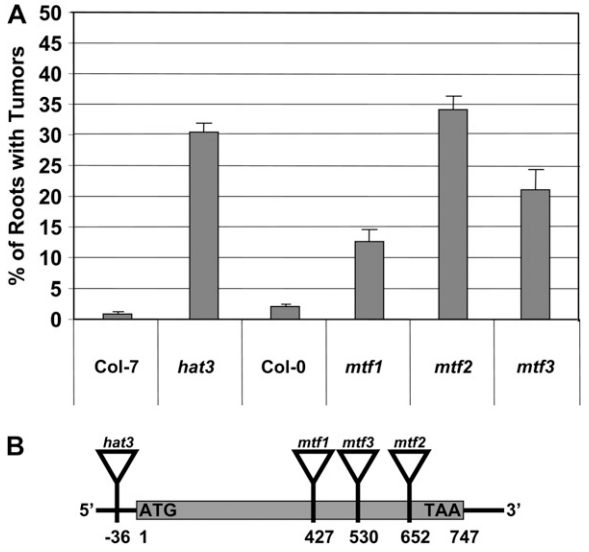

Figure 2. An MTF negatively affects transformation susceptibility. A, Transformation efficiency of the *hat3* (MTF) mutant and its wild-type control (ecotype Columbia-7 [Col-7]) and three independent T-DNA insertion mutants in the MTF gene (*mtf1*, *-2*, and *-3*) and its wild-type control (ecotype Columbia-0 [Col-0]). Root segments were inoculated with the tumorigenic strain *A. tumefaciens* A208 at low inoculum density (10^5 colony forming units/mL). After 2 d of cocultivation, the root segments were transferred to Murashige and Skoog medium lacking phytohormones and tumors were allowed to develop (Zhu et al., 2003b). The plates were photographed after 4 weeks. Note the more numerous tumors formed on root segments of MTF mutant lines compared to the tumors formed on wild-type roots. B, Map of the MTF gene mutated in the *hat3* mutant. Numbers below the bar indicate nucleotides (+1 is the start site of translation). *hat3*, *mtf1*, *mtf2*, and *mtf3* indicate the positions of three independent T-DNA insertions into the gene.

VirD5, VirE2, VirE3, and VirF; Otten et al., 1984; Stahl et al., 1998; Vergunst et al., 2000, 2003, 2005; Schrammeijer et al., 2003). In addition, *A. rhizogenes* transfers GALLS-FL (full-length) and GALLS-CT (C-terminal) to plant cells (Hodges et al., 2006). Several laboratories have used yeast two-hybrid systems to search for plant proteins that interact with these effector proteins or with other proteins that appear on the bacterial surface. The rationale for these experiments is that if a plant protein interacts with an *Agrobacterium* protein, it is likely that this plant protein is involved in the transformation process.

VirB2 is the major constituent protein of the *Agrobacterium* T-pilus (Lai and Kado, 1998). The T-pilus is an important bacterial structure that may come into contact with the plant during T-DNA and Vir protein transfer. Although proteins on the plant cell surface had previously been implicated in bacterial adhesion (Neff and Binns, 1985; Gurlitz et al., 1987; Neff et al., 1987; Wagner and Matthysse, 1992; Swart et al., 1994; Clauce-Coupel et al., 2008), no previously identified plant surface protein directly influenced bacterial virulence. Hwang and Gelvin (2004) used the processed form of VirB2 (Lai and Kado, 1998) as a bait protein to screen in yeast for Arabidopsis VirB2 interacting proteins. In addition to a RAB8 GTPase, they identified three reticulon domain proteins termed BTI1, -2, and -3 (for VirB2 Interacting proteins 1, 2, and 3). Decreasing expression of the Arabidopsis *BTI* genes by T-DNA mutagenesis or RNA interference (RNAi) resulted in reduced susceptibility to *Agrobacterium*-mediated transformation, whereas overexpression of *BTI1* made the plant hypersusceptible to transformation. As would be expected of a protein that interacts with the T-pilus, the BTI proteins localize to the plant surface. Although more experiments need to be conducted, the BTI proteins may serve as receptors for VirB2 protein on the T-pilus.

In addition to VirB2, the role of VirB5 (a minor T-pilus constituent) needs further exploration. In animal pathogens that have type IV secretion systems, VirB5 orthologs, such as CagL, may serve as specialized adhesins that interacts with human integrin β1 and fibronectin during bacterial/animal cell contact (Backert et al., 2008). It would be interesting to determine, using yeast two-hybrid systems, whether *Agrobacterium* VirB5 interacts with a specific plant surface protein.

VirD2 is the pilot protein that guides the T-strand through the type IV secretion system into the plant cell, through the plant cytoplasm, and into the nucleus. VirD2 may also influence T-DNA integration into the plant genome (Tinland et al., 1995; Mysore et al., 1998). It is therefore likely that VirD2 interacts with plant proteins during this journey, and yeast two-hybrid analyses have identified a number of these proteins. The first of these was the nuclear transfer importin α protein AtKAPα (Ballas and Citovsky, 1997), now known as IMPa-1 (Bhattacharjee et al., 2008). The Arabidopsis genome encodes nine importin α proteins, and VirD2 interacts in yeast with all tested importin α isoforms (Ballas and Citovsky, 1997; Bakó et al., 2003; Bhattacharjee et al., 2008). Additionally, bimolecular fluorescence complementation studies in planta indicated that each of these isoforms interacts with VirD2 and localizes the complex to the nucleus (Bhattacharjee et al., 2008).

Yeast two-hybrid screening additionally identified several other plant proteins that interact with VirD2. These include several cyclophilins (Deng et al., 1998; Bakó et al., 2003), the kinase CAK2Ms (Bakó et al., 2003), and a protein phosphatase PP2C (Tao et al., 2004). Interaction with these latter two proteins suggested that VirD2 may be a phosphoprotein. Bakó et al. (2003) confirmed this hypothesis, and Tao et al. (2004) showed that PP2C can regulate nuclear entry of VirD2.

The single-strand DNA binding protein VirE2 is important for transformation. *Agrobacterium* strains mutant for *virE2* are highly attenuated in virulence (Stachel and Nester, 1986). VirE2 plays numerous important roles within the plant cell (Citovsky et al., 1992; Ward and Zambryski, 2001) and therefore likely interacts with numerous plant proteins. Yeast two-hybrid analyses have confirmed these interactions. VirE2 interacts with numerous importin α isoforms; however, only interaction with IMPa-4 results in nu-

clear localization (Bhattacharjee et al., 2008; Lee et al., 2008). VirE2 also interacts in yeast with the VirE2 interacting proteins VIP1 and VIP2 (Tzfira et al., 2001; Anand et al., 2007a). Interaction of VirE2 with these proteins likely contributes to nuclear targeting and genomic integration of T-strands (Tzfira et al., 2001; Citovsky et al., 2004; Li et al., 2005; Loyter et al., 2005; Anand et al., 2007a; Bhattacharjee et al., 2008; Lacroix et al., 2008)

VirF is a nonessential virulence protein for infection of most plant species. However, it is required for efficient transformation of a few species (Melchers et al., 1990; Regensburg-Tuink and Hooykaas, 1993). Schrammeijer et al. (2001) screened for VirF interacting proteins in yeast and identified a plant Skp1 ortholog. Skp1 (ASK1) is a component of the SCF ubiquitin ligase complex that identifies and marks proteins for degradation via the 26S proteosome. Indeed, experiments in both yeast and in planta indicated the importance of VirF in proteolysis of VirE2, suggesting that VirF plays a role in stripping VirE2 from T-strands prior to integration (Tzfira et al., 2004).

VirE3 is a nuclear-localized *Agrobacterium* effector protein that may serve as a plant transcription factor (Schrammeijer et al., 2003; Garcia-Rodriguez et al., 2006). VirE3 may also substitute for plant-encoded VIP1 when this latter protein is limiting (Lacroix et al., 2005). In yeast, VirE3 interacts with several importin α isoforms, with pCsn5-1 (also known as AJH1), a component of the COP9 signalosome involved in protein degradation, and with pBrp, a plant transcriptional activator. An intriguing potential function for VirE3 may be as a molecular bridge to transport plant transcription factors to the nucleus where they may activate plant genes involved in tumorigenesis or transformation (Garcia-Rodriguez et al., 2006).

GALLS-FL and GALLS-CT are two effector proteins encoded by some *A. rhizogenes* Ri-plasmids (Hodges et al., 2006). Although these proteins do not share sequence homology with *A. tumefaciens* VirE2, they can substitute for this essential *A. tumefaciens* virulence effector protein (Hodges et al., 2004, 2009). Recent yeast two-hybrid analysis using GALLS-FL as the bait identified a specific interacting plant protein (GALLS interacting protein [GIP]; Y. Wang and S.B. Gelvin, unpublished data). Bimolecular fluorescence complementation experiments confirmed GALLS-FL and GALLS-CT interaction with GIP in planta (L.-Y. Lee and S.B. Gelvin, unpublished data). GIP is encoded by one of an eight-member multigene family whose functions are unknown. Research in this author's laboratory is aimed at defining the role of GIP in both *A. rhizogenes*- and *A. tumefaciens*-mediated plant transformation.

Host Transcriptional Profiling and *Agrobacterium* Infection

Several recent studies have investigated host transcriptional responses to *Agrobacterium* infection or to crown gall tumorigenesis. Veena et al. (2003) used suppressive subtractive hybridization and DNA macroarrays to investigate the transcriptional response of tobacco BY-2 cells to infection by several nontumorigenic *Agrobacterium* strains. The authors used nontumorigenic strains to avoid complications resulting from phytohormone overproduction by expression of oncogenes and studied the initial plant response by limiting sampling to times $<$36 h after infection. The results of these experiments indicated that *Agrobacterium* exquisitely manipulates expression of the plant genome to facilitate transformation: plant genes important for transformation, such as those encoding histone proteins, were induced by the bacterium, whereas expression of genes involved in host defense responses was suppressed. Interestingly, Anand et al. (2007a) later showed that expression of numerous Arabidopsis histone genes was higher in wild-type Arabidopsis plants than in *vip2* (VirE2 interacting protein 2) mutant plants. Arabidopsis *vip2* mutant plants are highly recalcitrant to *Agrobacterium*-mediated transformation. These authors suggested that VIP2, a putative transcription factor, may play a role in maintaining high-level expression of histone genes important for transformation.

Ditt et al. (2001) used a disarmed *Agrobacterium* strain to infect *Ageratum* cell cultures. Using cDNA/amplified fragment length polymorphism analyses of RNA extracted at relatively long times after infection (48 h), the authors identified a few genes whose expression was either repressed or induced by cocultivation with *Agrobacterium*. Whereas expression of most of the identified genes was similarly affected by cocultivation with *Escherichia coli*, expression of two genes (encoding a nodulin-like protein and a lectin-like protein kinase) was specifically induced by *Agrobacterium* infection.

Ditt et al. (2006) also used Arabidopsis Affymetrix ATH1 microarrays to investigate host gene expression changes following infection of Arabidopsis suspension cell cultures with a tumorigenic *Agrobacterium* strain. Interestingly, the authors were only able to detect transcriptional changes 48 h after infection. In contrast to the results of Veena et al. (2003), the study of Ditt et al. (2006) indicated that *Agrobacterium* infection induced, rather than repressed, defense gene expression and that infection repressed expression of genes encoding proteins involved in cell proliferation. This latter observation was rather surprising considering that growth of the cell cultures was not slowed by bacterial infection. The seemingly opposing results of Ditt et al. (2006) and those of Veena et al. (2003) may be explained by the different plant culture systems used (tobacco and Arabidopsis) and the fact that one group used disarmed strains, whereas the other used tumorigenic strains that would result in the overproduction of phytohormones by the host and, eventually, production of tumors. Differential gene expression occurs in Arabidopsis crown gall tumors (Deeken et al., 2006). The expression of numerous genes, in-

cluding those involved in cell wall biosynthesis, Suc degradation, transport, and glycolysis, is up-regulated in tumors, whereas expression of genes involved in photosynthesis, nitrogen metabolism, lipid metabolism, and amino acid synthesis is down-regulated. Differential gene expression in crown gall tumors correlated with altered solute profiles, leading the authors to speculate that metabolism in mature crown gall tumors occurs mainly anaerobically (Deeken et al., 2006).

In addition to examining host transcriptional responses following *Agrobacterium*-mediated transformation or the development of crown gall tumors, Kim et al. (2007) used Arabidopsis Affymetrix ATH1 microarrays and custom macroarrays to investigate the transcriptional and methylation status of host T-DNA integration sites. The results of these assays indicated that, in the absence of selection, T-DNA target sites were not preferentially transcribed to a greater extent than were Arabidopsis genes in general and that T-DNA integration occurred without regard to the methylation status of the target DNA.

Reverse Genetic Screening for Genes Required for *Agrobacterium*-Mediated Transformation

A large number of studies have employed reverse genetic strategies to determine the role of candidate genes in the transformation process. Candidate genes include those identified by yeast two-hybrid and transcriptional profiling analyses, as well as additional members of multigene families when one family member clearly plays a role in virulence. Gene/expression disruption techniques have included T-DNA insertional mutagenesis and RNAi and antisense inhibition of gene expression. Overexpression of several plant genes has also resulted in a hat phenotype (Mysore et al., 2000; Tzfira et al., 2002; Hwang and Gelvin, 2004; Yi et al., 2006; G. Tenea, J. Spantzel, and S.B. Gelvin, unpublished data). Some genes confirmed as *rat* genes by these studies include those encoding BTI proteins (Hwang and Gelvin, 2004), various importin α family members (Bhattacharjee et al., 2008), VIP1 (Tzfira et al., 2001, 2002; Li et al., 2005), VIP2 (Anand et al., 2007a), Ku80 (West et al., 2002; Friesner and Britt, 2003; Li et al., 2005), DNA ligase IV (Friesner and Britt, 2003; van Attikum et al., 2003), SGA1 (G. Tenea and S.B. Gelvin, unpublished data), and various histones (Mysore et al., 2000; Yi et al., 2006; Anand et al., 2007b; G. Tenea and S.B. Gelvin, unpublished data). In addition, mutation of several genes involved in plant defense responses and signal transduction results in altered susceptibility to *Agrobacterium*-mediated transformation (Veena, N. Sardesai, and S.B. Gelvin, unpublished data).

Crane and Gelvin (2007) conducted a large-scale reverse genetic screen for Arabidopsis *rat* mutants. Using 340 independent mutant lines containing RNAi constructions targeted against 109 chromatin genes, they identified 24 genes important, to various extents, for transformation. These genes encoded histone acetyltransferases, histone deacetylases, chromatin remodeling proteins, DNA methyltransferases, global transcription factors, histone H1, nucleosome assembly factors, SET domain proteins, and antisilencing group proteins. Some of these genes, such as *HDT19*, were previously implicated in the transformation process (Tian et al., 2003). Most interesting were three genes whose expression is important for T-DNA integration: *HDT1*, *HDT2*, and *SGA1*. *HDT1* and *HDT2* encode histone deacetylases, whereas *SGA* encodes a histone H3 chaperone/chromatin assembly protein also known as ASF1 in yeast and animals.

CONCLUSIONS AND PROSPECTIVE

In addition to its long-described history as a plant pathogen (Smith and Townsend, 1907), *Agrobacterium* is a natural genetic engineer that scientists have used for gene transfer experiments for the past 25 years. Whole-genome saturation mutagenesis studies in the early 1980s defined many *Agrobacterium* genes important for transformation, but only during the past decade have scientists applied modern genomic technologies to unravel the full complement of bacterial and host proteins important for transformation. In the near future, advances in molecular biology combined with novel imaging (e.g. Lee et al., 2008) and genetic (e.g. House et al., 2004) techniques will give scientists a considerably more refined view of bacterial and host proteins involved in transformation. This knowledge will likely result in improved transformation technologies, both to increase our ability to control *Agrobacterium* host range and to improve the quality (e.g. single-copy T-DNA insertions that result in predictable and stable transgene expression) of transformation events.

Numerous important questions need to be answered to understand *Agrobacterium*-mediated plant genetic transformation more fully. Many of these questions beg genome-wide answers: (1) What roles do plant defense responses, and *Agrobacterium*'s ability to overcome these responses, play in transformation? (2) How does the transferred VirD2/T-strand assemble with other virulence effector proteins and host proteins to traverse the plant cytoplasm and nucleus? (3) What roles do plant proteins play in T-strand targeting to plant chromatin and in T-DNA integration into the genome? Can we manipulate *Agrobacterium* for gene targeting (site-directed integration) purposes? (4) How can we best manipulate both the bacterium and the host to obtain high-quality transformation events? (5) How does *Agrobacterium* manipulate host metabolism for its advantage? (6) How does *Agrobacterium* interact with other organisms in the rhizosphere? (7) To what extent do the lessons we have learned about transformation using laboratory conditions apply to transformation in nature? (8) Has horizontal gene transfer effected by *Agrobacterium* species influenced plant

evolution? These and other questions will likely be answered using genomic, proteomic, and metabolomic approaches.

ACKNOWLEDGMENTS

I thank Drs. Erh-Min Lai and Walt Ream for critical reading of this manuscript and for helpful suggestions. Research in the author's laboratory is funded by the National Science Foundation, the Department of Energy through the Corporation for Plant Biotechnology Research, the Biotechnology Research and Development Corporation, and Dow Agrosciences.

Received April 11, 2009; accepted May 6, 2009; published May 13, 2009.

LITERATURE CITED

Abuodeh RO, Orbach MJ, Mandel MA, Das A, Galgiani JN (2000) Genetic transformation of *Coccidioides immitis* facilitated by *Agrobacterium tumefaciens*. J Infect Dis **181:** 2106–2110

Akiyoshi DE, Klee H, Amasino RM, Nester EW, Gordon MP (1984) T-DNA of *Agrobacterium tumefaciens* encodes an enzyme of cytokinin biosynthesis. Proc Natl Acad Sci USA **81:** 5994–5998

Anand A, Krichevsky A, Schomack S, Lahaye T, Tzfira T, Tang Y, Citovsky V, Mysore KS (2007a) *Arabidopsis* VirE2 Interacting Protein2 is required for *Agrobacterium* T-DNA integration in plants. Plant Cell **19:** 1695–1708

Anand A, Uppalapati SR, Ryu CM, Allen SN, Kang L, Tang Y, Mysore KS (2008) Salicylic acid and systemic acquired resistance play a role in attenuating crown gall disease caused by *Agrobacterium tumefaciens*. Plant Physiol **146:** 703–715

Anand A, Vaghchhipawala Z, Ryu CM, Kang L, Wang K, del-Pozo O, Martin GB, Mysore KS (2007b) Identification and characterization of plant genes involved in *Agrobacterium-mediated* plant transformation by virus-induced gene silencing. Mol Plant Microbe Interact **20:** 41–52

Anderson A, Moore L (1979) Host specificity in the genus *Agrobacterium*. Phytopathology **69:** 320–323

Backert S, Fronzes R, Waksman G (2008) VirB2 and VirB5 proteins: specialized adhesins in bacterial type-IV secretion systems? Trends Microbiol **16:** 409–413

Bailey MA, Boerma HR, Parrott WA (1994) Inheritance of *Agrobacterium tumefaciens*-induced tumorigenesis of soybean. Crop Sci **34:** 514–519

Bakó L, Umeda M, Tiburcio AF, 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. Proc Natl Acad Sci USA **100:** 10108–10113

Ballas N, Citovsky V (1997) Nuclear localization signal binding protein from *Arabidopsis* mediates nuclear import of *Agrobacterium* VirD2 protein. Proc Natl Acad Sci USA **94:** 10723–10728

Barker RF, Idler KB, Thompson DV, Kemp JD (1983) Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti-plasmid pTi15955. Plant Mol Biol **2:** 335–350

Bevan MW, Flavell RB, Chilton MD (1983) A chimeric antibiotic resistance gene as a selectable marker for plant cell transformation. Nature **304:** 184–187

Bhattacharjee S, Lee L-Y, Oltmanns H, Cao H, Veena, Cuperus J, Gelvin SB (2008) AtImpa-4, an *Arabidopsis* importin α isoform, is preferentially involved in *Agrobacterium*-mediated plant transformation. Plant Cell **20:** 2661–2680

Bliss FA, Almehdi AA, Dandekar AM, Schuerman PL, Bellaloui N (1999) Crown gall resistance in accessions of 20 *Prunus* species. HortScience **34:** 326–330

Bulgakov VP, Kisselev KV, Yakovlev KV, Zhuravlev YN, Gontcharov AA, Odintsova NA (2006) *Agrobacterium*-mediated transformation of sea urchin embryos. Biotechnol J **1:** 454–461

Bundock P, den Dulk-Ras A, Beijersbergen A, Hooykaas PJJ (1995) Trans-kingdom T-DNA transfer from *Agrobacterium tumefaciens* to *Saccharomyces cerevisiae*. EMBO J **14:** 3206–3214

Bundock P, van Attikum H, den Dulk-Ras A, Hooykaas PJJ (2002) Insertional mutagenesis in yeasts using T-DNA from *Agrobacterium tumefaciens*. Yeast **19:** 529–536

Caplan A, Herrera-Estrella L, Inze D, van Haute E, van Montagu M, Schell J, Zambryski P (1983) Introduction of genetic material into plant cells. Science **222:** 815–821

Chang CH, Winans SC (1996) Resection and mutagenesis of the acid pH-inducible P2 promoter of the *Agrobacterium tumefaciens virG* gene. J Bacteriol **178:** 4717–4720

Charles TC, Nester EW (1993) A chromosomally encoded two-component sensory transduction system is required for virulence of *Agrobacterium tumefaciens*. J Bacteriol **175:** 6614–6625

Chevrot R, Rosen R, Haudecoeur E, Cirou A, Shelp BJ, Ron E, Faure D (2006) GABA controls the level of quorum-sensing signal in *Agrobacterium tumefaciens*. Proc Natl Acad Sci USA **103:** 7460–7464

Cho H, Winans SC (2005) VirA and VirG activate the Ti plasmid *repABC* operon, elevating plasmid copy number in response to wound-released chemical signals. Proc Natl Acad Sci USA **102:** 14843–14848

Citovsky V, Kapelnikov A, Oliel S, Zakai N, Rojas MR, Gilbertson RL, Tzfira T, Loyter A (2004) Protein interactions involved in nuclear import of the *Agrobacterium* VirE2 protein in vivo and in vitro. J Biol Chem **279:** 29528–29533

Citovsky V, Kozlovsky SV, Lacriox B, Zaltsman A, Dafny-Yelin M, Vyas S, Tovkach A, Tzfira T (2007) Biological systems of the host cell involved in *Agrobacterium* infection. Cell Microbiol **9:** 9–20

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

Clauce-Coupel H, Chateau S, Ducrocq C, Niot V, Kaveri S, Dubois F, Sangwan-Norreel B, Sangwan RS (2008) Role of vitronectin-like protein in *Agrobacterium* attachment and transformation of *Arabidopsis* cells. Protoplasma **234:** 65–75

Conner AJ, Commisse EM (1992) Monocotyledonous plants as hosts for *Agrobacterium*. Int J Plant Sci **153:** 550–555

Crane YM, Gelvin SB (2007) RNAi-mediated gene silencing reveals involvement of *Arabidopsis* chromatin-related genes in *Agrobacterium-mediated* root transformation. Proc Natl Acad Sci USA **104:** 15156–15161

Das A, Stachel S, Ebert P, Allenza P, Montoya A, Nester E (1986) Promoters of *Agrobacterium tumefaciens* Ti-plasmid virulence genes. Nucleic Acids Res **14:** 1355–1364

DeCleene M, DeLey J (1976) The host range of crown gall. Bot Rev **42:** 389–466

Deeken R, Engelmann JC, Efetova M, Czirjak T, Muller T, Kaiser WM, Tietz O, Krischke M, Mueller MJ, Palme K, et al (2006) An integrated view of gene expression and solute profiles of *Arabidopsis* tumors: a genome-wide approach. Plant Cell **18:** 3617–3634

de Framond AJ, Barton KA, Chilton MD (1983) Mini-Ti: a new vector strategy for plant genetic engineering. Biotechnology **1:** 262–269

DeGreve H, Decraemer H, Seurinck J, Van Montagu M, Schell J (1981) The functional organization of the octopine *Agrobacterium tumefaciens* plasmid pTiB6S3. Plasmid **6:** 235–248

de Groot MJA, Bundock P, Hooykaas PJJ, Beijersbergen AGM (1998) *Agrobacterium tumefaciens*-mediated transformation of filamentous fungi. Nat Biotechnol **16:** 839–842

Deng W, Chen L, Wood DW, Metcalfe T, Liang X, Gordon MP, Comai L, Nester EW (1998) *Agrobacterium* VirD2 protein interacts with plant host cyclophilins. Proc Natl Acad Sci USA **95:** 7040–7045

Ditt RF, Kerr KF, de Figueiredo P, Delrow J, Comai L, Nester EW (2006) The *Arabidopsis thaliana* transcriptome in response to *Agrobacterium tumefaciens*. Mol Plant Microbe Interact **19:** 665–681

Ditt RF, Nester EW, Comai L (2001) Plant gene expression response to *Agrobacterium tumefaciens*. Proc Natl Acad Sci USA **98:** 10954–10959

Engstrom P, Zambryski P, Van Montagu M, Stachel S (1987) Characterization of *Agrobacterium tumefaciens* virulence proteins induced by the plant factor acetosyringone. J Mol Biol **197:** 635–645

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci USA **103:** 626–631

Fraley RT, Rogers SG, Horsch RB, Eichholtz DA, Flick JS, Fink CL, Hoffmann NL, Sanders PR (1985) The SEV system: a new disarmed Ti plasmid vector system for plant transformation. Biotechnology **3:** 629–635

Fraley RT, Rogers SG, Horsch RB, Sanders PR, Flick JS, Adams SP, Bittner ML, Brand LA, Fink CL, Fry JS, et al (1983) Expression of bacterial genes in plant cells. Proc Natl Acad Sci USA **80:** 4803–4807

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

Garcia-Rodriguez FM, Schrammeijer B, Hooykaas PJJ (2006) The *Agrobacterium* VirE3 effector protein: a potential plant transcriptional activator. Nucleic Acids Res **34:** 6496–6504

Garfinkel DJ, Nester EW (1980) *Agrobacterium tumefaciens* mutants affected in crown gall tumorigenesis and octopine catabolism. J Bacteriol **144:** 732–743

Garfinkel DJ, Simpson RB, Ream LW, White FF, Gordon MP, Nester EW (1981) Genetic analysis of crown gall: fine structure map of the T-DNA by site-directed mutagenesis. Cell **27:** 143–153

Gaspar YM, Nam J, Schultz CJ, Lee L-Y, Gilson PR, Gelvin SB, Bacic A (2004) Characterization of the *Arabidopsis* lysine-rich arabinogalactan-protein AtAGP17 mutant (*rat1*) that results in a decreased efficiency of *Agrobacterium* transformation. Plant Physiol **135:** 2162–2171

Gelvin SB (2000) *Agrobacterium* and plant genes involved in T-DNA transfer and integration. Annu Rev Plant Physiol Plant Mol Biol **51:** 223–256

Gelvin SB (2003) *Agrobacterium* and plant transformation: the biology behind the "gene-jockeying" tool. Microbiol Mol Biol Rev **67:** 16–37

Gelvin SB (2008) *Agrobacterium*-mediated DNA transfer, and then some. Nat Biotechnol **26:** 998–1000

Gelvin SB, Kim SI (2007) Effect of chromatin upon *Agrobacterium* T-DNA integration and transgene expression. Biochim Biophys Acta **1769:** 410–421

Gielen JM, DeBeuckeleer M, Seurinc J, Deboeck F, DeGreve H, Lemmers M, Van Montagu M, Schell J (1984) The complete nucleotide sequence of the TL-DNA of the *Agrobacterium tumefaciens* plasmid pTiAch5. EMBO J **3:** 835–846

Goodner B, Hinkle G, Gattung S, Miller N, Blanchard M, Qurollo B, Goldman BS, Cao Y, Askenazi M, Halling C, et al (2001) Genome sequence of the plant pathogen and biotechnology agent *Agrobacterium tumefaciens* C58. Science **294:** 2323–2328

Gurlitz RHG, Lamb PW, Matthysse AG (1987) Involvement of carrot cell surface proteins in attachment of *Agrobacterium tumefaciens*. Plant Physiol **83:** 564–568

Herrera-Estrella L, DeBlock M, Messens E, Hernalsteens JP, Van Montagu M, Schell J (1983) Chimeric genes as dominant selectable markers in plant cells. EMBO J **2:** 987–996

Hodges L, Cuperus J, Ream W (2004) *Agrobacterium rhizogenes* GALLS protein substitutes for *Agrobacterium tumefaciens* single-stranded DNA-binding protein VirE2. J Bacteriol **186:** 3065–3077

Hodges LD, Lee LY, McNett H, Gelvin SB, Ream W (2009) The *Agrobacterium rhizogenes* GALLS gene encodes two secreted proteins required for genetic transformation of plants. J Bacteriol **191:** 355–364

Hodges LD, Vergunst AC, Neal-McKinney J, den Dulk-Ras A, Moyer DM, Hooykaas PJJ, Ream W (2006) *Agrobacterium rhizogenes* GALLS protein contains domains for ATP binding, nuclear localization, and type IV secretion. J Bacteriol **188:** 8222–8230

Hoekema A, Hirsh PR, Hooykaas PJJ, Schilperoort RA (1983) A binary plant vector strategy based on separation of vir- and T-region of the *Agrobacterium tumefaciens* Ti-plasmid. Nature **303:** 179–180

Holsters M, Silva B, Van Vliet F, Genetello C, DeBlock M, Dhaese P, Depicker A, Inze D, Engler G, Villarroel R, et al (1980) The functional organization of the nopaline *A. tumefaciens* plasmid pTiC58. Plasmid **3:** 212–230

House BL, Mortimer MW, Kahn ML (2004) New recombination methods for *Sinorhizobium meliloti* genetics. Appl Environ Microbiol **70:** 2806–2815

Hwang HH, Gelvin SB (2004) Plant proteins that interact with VirB2, the *Agrobacterium tumefaciens* pilin protein, mediate plant transformation. Plant Cell **16:** 3148–3167

Inze D, Follin A, Van Lijsebettens M, Simoens C, Genetello C, Van Montagu M, Schell J (1984) Genetic analysis of the individual T-DNA genes of *Agrobacterium tumefaciens*; further evidence that two genes are involved in indole-3-acetic acid synthesis. Mol Gen Genet **194:** 265–274

Jones DA, Ryder MH, Clare BG, Farrand SK, Kerr A (1988) Construction of a Tra- deletion mutant of pAgK84 to safeguard the biological control of crown gall. Mol Gen Genet **212:** 207–214

Kelly BA, Kado CI (2002) *Agrobacterium*-mediated T-DNA transfer and integration into the chromosome of *Streptomyces lividans*. Mol Plant Pathol **3:** 125–134

Kerr A, Panagopoulos CG (1977) Biotypes of *Agrobacterium radiobacter* var. *tumefaciens* and their biological control. Phytopathol Z **90:** 172–179

Kim SI, Veena, Gelvin SB (2007) Genome-wide analysis of *Agrobacterium* T-DNA integration sites in the *Arabidopsis* genome generated under non-selective conditions. Plant J **51:** 779–791

Korber H, Strizhov N, Staiger D, Feldwisch J, Olsson O, Sandberg G, Palme K, Schell J, Koncz C (1991) T-DNA gene 5 of *Agrobacterium* modulates auxin response by autoregulated synthesis of a growth hormone antagonist in plants. EMBO J **10:** 3983–3991

Kunik T, Tzfira T, Kapulnik Y, Gafni Y, Dingwall C, Citovsky V (2001) Genetic transformation of HeLa cells by *Agrobacterium*. Proc Natl Acad Sci USA **98:** 1871–1876

Lacroix B, Loyter A, Citovsky V (2008) Association of the *Agrobacterium* T-DNA-protein complex with plant nucleosomes. Proc Natl Acad Sci USA **105:** 15429–15434

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

Lai EM, Shih HW, Wen SR, Cheng MW, Hwang HH, Chiu SH (2006) Proteomic analysis of *Agrobacterium tumefaciens* response to the *vir* gene inducer acetosyringone. Proteomics **6:** 4130–4136

Lai E-M, Kado CI (1998) Processed VirB2 is the major subunit of the promiscuous pilus of *Agrobacterium tumefaciens*. J Bacteriol **180:** 2711–2717

Lee LY, Fang MJ, Kuang LY, Gelvin SB (2008) Vectors for multi-color bimolecular fluorescence complementation to investigate protein-protein interactions in living plant cells. Plant Methods **4:** 24

Lee LY, Gelvin SB (2008) T-DNA binary vectors and systems. Plant Physiol **146:** 325–332

Leemans J, Shaw C, Deblaere R, DeGreve H, Hernalsteens JP, Maes M, Van Montagu M, Schell J (1981) Site-specific mutagenesis of *Agrobacterium* Ti plasmids and transfer of genes to plant cells. J Mol Appl Genet **1:** 149–164

Li J, Krichevsky A, Vaidya M, Tzfira T, Citovsky V (2005) Uncoupling of the functions of the *Arabidopsis* VIP1 protein in transient and stable plant genetic transformation by *Agrobacterium*. Proc Natl Acad Sci USA **102:** 5733–5738

Li L, Jia Y, Hou Q, Charles TC, Nester EW, Pan SQ (2002) A global pH sensor: *Agrobacterium* sensor protein ChvG regulates acid-inducibile genes on its two chromosomes and Ti plasmid. Proc Natl Acad Sci USA **99:** 12369–12374

Liu P, Nester EW (2006) Indoleacetic acid, a product of transferred DNA, inhibits *vir* gene expression and growth of *Agrobacterium tumefaciens* C58. Proc Natl Acad Sci USA **103:** 4658–4662

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

Mantis NJ, Winans SC (1992) The *Agrobacterium tumefaciens vir* gene transcriptional activator *virG* is transcriptionally induced by acid pH and other stress stimuli. J Bacteriol **174:** 1189–1196

Mauro AO, Pfeiffer TW, Collins GB (1995) Inheritance of soybean susceptibility to *Agrobacterium tumefaciens* and its relationship to transformation. Crop Sci **35:** 1152–1156

McCullen CA, Binns AN (2006) *Agrobacterium tumefaciens* and plant cell interactions and activities required for interkingdom macromolecular transfer. Annu Rev Cell Dev Biol **22:** 101–127

Melchers LS, Maroney MJ, den Dulk-Ras A, Thompson DV, van Vuuren HAJ, Schilperoort RA, Hooykaas PJJ (1990) Octopine and nopaline strains of *Agrobacterium tumefaciens* differ in virulence; molecular characterization of the *virF* locus. Plant Mol Biol **14:** 249–259

Michielse CB, Ram AFJ, Hooykaas PJJ, van den Hondal CAMJJ (2004) *Agrobacterium*-mediated transformation of *Aspergillus awamori* in the absence of full-length VirD2, VirC2, or VirE2 leads to insertion of aberrant T-DNA structures. J Bacteriol **186:** 2038–2045

Moriguchi K, Maeda Y, Satou M, Hardayani NSN, Kataoka M, Tanaka N, Yoshida K (2001) The complete nucleotide sequence of a plant root-inducing (Ri) plasmid indicates its chimeric structure and evolutionary relationship between tumor-inducing (Ti) and symbiotic (Sym) plasmids in *Rhizobiaceae*. J Mol Biol **307:** 771–784

Mysore KS, Bassuner B, Deng XB, Darbinian NS, Motchoulski A, Ream W, Gelvin SB (1998) Role of the *Agrobacterium tumefaciens* VirD2 protein in T-DNA transfer and integration. Mol Plant Microbe Interact **11:** 668–683

Mysore KS, Nam J, Gelvin SB (2000) An *Arabidopsis* histone H2A mutant is deficient in *Agrobacterium* T-DNA integration. Proc Natl Acad Sci USA **97:** 948–953

Nam J, Matthysse AG, Gelvin SB (1997) Differences in susceptibility of *Arabidopsis* ecotypes to crown gall disease may result from a deficiency in T-DNA integration. Plant Cell **9:** 317–333

Nam J, Mysore KS, Zheng C, Knue MK, Matthysse AG, Gelvin SB (1999) Identification of T-DNA tagged *Arabidopsis* mutants that are resistant to transformation by *Agrobacterium*. Mol Gen Genet **261:** 429–438

Neff NT, Binns AN (1985) *Agrobacterium tumefaciens* interaction with suspension-cultured tomato cells. Plant Physiol **77:** 35–42

Neff NT, Binns AN, Brandt C (1987) Inhibitory effects of a pectin-enriched tomato cell wall fraction on *Agrobacterium tumefaciens* binding and tumor formation. Plant Physiol **83:** 525–528

Ooms G, Hooykaas PJJ, Moolenaar G, Schilperoort RA (1981) Crown gall plant tumors of abnormal morphology, induced by *Agrobacterium tumefaciens* carrying mutated octopine Ti plasmids; analysis of T-DNA functions. Gene **14:** 33–50

Ooms G, Klapwijk M, Poulis JA, Schilperoort RA (1980) Characterization of Tn*904* insertions in octopine Ti-plasmid mutants of *Agrobacterium tumefaciens*. J Bacteriol **144:** 82–91

Otten L, DeGreve H, Leemans J, Hain R, Hooykaas P, Schell J (1984) Restoration of virulence of vir region mutants of *Agrobacterium tumefaciens* strain B6S3 by coinfection with normal and mutant *Agrobacterium* strains. Mol Gen Genet **195:** 159–163

Owens LD, Cress DE (1984) Genotypic variability of soybean response to *Agrobacterium* strains harboring the Ti or Ri plasmids. Plant Physiol **77:** 87–94

Pena L, Seguin A (2001) Recent advances in the genetic transformation of trees. Trends Biotechnol **19:** 500–506

Piers KL, Heath JD, Liang X, Stephens KM, Nester EW (1996) *Agrobacterium tumefaciens*-mediated transformation of yeast. Proc Natl Acad Sci USA **93:** 1613–1618

Ream LW, Gordon MP, Nester EW (1983) Multiple mutations in the T region of the *Agrobacterium tumefaciens* tumor-inducing plasmid. Proc Natl Acad Sci USA **80:** 1660–1664

Regensburg-Tuink AJG, Hooykaas PJJ (1993) Transgenic *N. glauca* plants expressing bacterial virulence gene *virF* are converted into hosts for nopaline strains of *A. tumefaciens*. Nature **363:** 69–71

Robbs SL, Hawes MC, Lin HJ, Pueppke SG, Smith LY (1991) Inheritance of resistance to crown gall in *Pisum sativum*. Plant Physiol **95:** 52–57

Roberts RL, Metz M, Monks DE, Mullaney ML, Hall T, Nester EW (2003) Purine synthesis and increased *Agrobacterium tumefaciens* transformation of yeast and plants. Proc Natl Acad Sci USA **100:** 6634–6639

Rogowsky PM, Powell BS, Shirasu K, Lin TS, Morel P, Zyprian EM, Steck TR, Kado CI (1990) Molecular characterization of the *vir* regulon of *Agrobacterium tumefaciens*: complete nucleotide sequence and gene organization of the 28.63-kbp regulon clones as a single unit. Plasmid **23:** 85–106

Rong L, Karcher SJ, O'Neal K, Hawes MC, Yerkes CD, Jayaswal RK, Hallberg CA, Gelvin SB (1990) *picA*, a novel plant-inducible locus on the *Agrobacterium tumefaciens* chromosome. J Bacteriol **172:** 5828–5836

Rosen R, Buttner K, Becher D, Nakahigashi K, Yura T, Hecker M, Ron EZ (2002) Heat shock proteome of *Agrobacterium tumefaciens*: evidence for new control systems. J Bacteriol **184:** 1772–1778

Rosen R, Buttner K, Schmid R, Hecker M, Ron EZ (2001) Stress-induced proteins of *Agrobacterium tumefaciens*. FEMS Microbiol Ecol **35:** 277–285

Rosen R, Matthysse AG, Becher D, Biran D, Yura T, Hecker M, Ron EZ (2003) Proteome analysis of plant-induced proteins of *Agrobacterium tumefaciens*. FEMS Microbiol Ecol **44:** 355–360

Rosen R, Sacher A, Shechter N, Becher D, Buttner K, Biran D, Hecker M, Ron EZ (2004) Two-dimensional reference map of *Agrobacterium tumefaciens* proteins. Proteomics **4:** 1061–1073

Schrammeijer B, den Dulk-Ras A, Vergunst AC, Jacome EJ, Hooykaas PJJ (2003) Analysis of Vir protein translocation from *Agrobacterium tumefaciens* using *Saccharomyces cerevisiae* as a model: evidence for transport of a novel effector protein VirE3. Nucleic Acids Res **31:** 860–868

Schrammeijer B, Risseeuw E, Pansegrau W, Regensburg-Tuink TJG, Crosby WL, Hooykaas PJJ (2001) Interaction of the virulence protein VirF of *Agrobacterium tumefaciens* with plant homologs of the yeast Skp1 protein. Curr Biol **11:** 258–262

Schroder G, Waffenschmidt S, Weiler EW, Schroder J (1984) The T-region of Ti plasmids codes for an enzyme synthesizing indole-3-acetic acid. Eur J Biochem **138:** 387–391

Shen WH, Petit A, Guern J, Tempe J (1988) Hairy roots are more sensitive to auxin than normal roots. Proc Natl Acad Sci USA **85:** 3417–3421

Shrawat AK, Lorz H (2006) *Agrobacterium*-mediated transformation of cereals: a promising approach crossing barriers. Plant Biotechnol J **4:** 575–603

Slater SC, Goldman BS, Goodner B, Setubal JC, Farrand SK, Nester EW, Burr TJ, Banta LM, Dickerman AW, Paulsen I, et al (2009) Genome sequences of three *Agrobacterium* biovars help elucidate the evolution of multichromosome genomes in bacteria. J Bacteriol **191:** 2501–2511

Slightom JL, Durand-Tardif M, Jouanin L, Tepfer D (1986) Nucleotide sequence analysis of TL-DNA of *Agrobacterium rhizogenes* agropine type plasmid. J Biol Chem **261:** 108–112

Smarrelli J, Watters MT, Diba LH (1986) Response of various cucurbits to infection by plasmid-harboring strains of *Agrobacterium*. Plant Physiol **82:** 622–624

Smith EF, Townsend CO (1907) A plant-tumor of bacterial origin. Science **25:** 671–673

Somers DA, Samac DA, Olhoft PM (2003) Recent advances in legume transformation. Plant Physiol **131:** 892–899

Spanier K, Schell J, Schreier PH (1989) A functional analysis of T-DNA gene 6b: the fine tuning of cytokinin effects on shoot development. Mol Gen Genet **219:** 209–216

Stachel SE, Messens E, Van Montagu M, Zambryski P (1985) Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in *Agrobacterium tumefaciens*. Nature **318:** 624–629

Stachel SE, Nester EW (1986) The genetic and transcriptional organization of the *vir* region of the A6 Ti plasmid of *Agrobacterium tumefaciens*. EMBO J **5:** 1445–1454

Stachel SE, Nester EW, Zambryski PC (1986) A plant cell factor induces *Agrobacterium tumefaciens vir* gene expression. Proc Natl Acad Sci USA **83:** 379–383

Stachel SE, Zambryski PC (1986) *virA* and *virG* control the plant-induced activation of the T-DNA transfer process of *A. tumefaciens*. Cell **46:** 325–333

Stahl LE, Jacobs A, Binns AN (1998) The conjugal intermediate of plasmid RSF1010 inhibits *Agrobacterium tumefaciens* virulence and VirB-dependent export of VirE2. J Bacteriol **180:** 3933–3939

Suzuki K, Hattori Y, Uraji M, Ohta N, Iwata K, Murata K, Kato A, Yoshida K (2000) Complete nucleotide sequence of a plant tumor-inducing Ti plasmid. Gene **242:** 331–336

Swart S, Logman TJJ, Smit G, Lugtenberg BJJ, Kijne JW (1994) Purification and partial characterization of a glycoprotein from pea (*Pisum sativum*) with receptor activity for rhicadhesin, an attachment protein of *Rhizobiaceae*. Plant Mol Biol **24:** 171–183

Szegedi E, Kozma P (1984) Studies on the inheritance of resistance to crown gall disease of grapevine. Vitis **23:** 121–126

Tao Y, Rao PK, Bhattacharjee S, Gelvin SB (2004) Expression of plant protein phosphatase 2C interferes with nuclear import of the *Agrobacterium* T-complex protein VirD2. Proc Natl Acad Sci USA **101:** 5164–5169

Terakura S, Ueno Y, Tagami H, Kitakura S, Machida C, Wabiko H, Aiba H, Otten L, Tsukagoshi H, Nakamura K, et al (2007) An oncoprotein from the plant pathogen *Agrobacterium* has histone chaperone-like activity. Plant Cell **19:** 2855–2865

Thompson DV, Melchers LS, Idler KB, Schilperoort RA, Hooykaas PJJ (1988) Analysis of the complete nucleotide sequence of the *Agrobacterium tumefaciens virB* operon. Nucleic Acids Res **16:** 4621–4636

Tian L, Wang J, Fong MP, Chen M, Cao H, Gelvin SB, Chen ZJ (2003) Genetic control of developmental changes induced by disruption of *Arabidopsis* histone deacetylase 1 (*AtHD1*) expression. Genetics **165:** 399–409

Tinland B, Rohgritsch O, Michler P, Otten L (1990) *Agrobacterium tumefaciens* T-DNA gene 6b stimulates rol-induced root formation, permits growth at high auxin concentrations and increases root size. Mol Gen Genet **223:** 1–10

Tinland B, Schoumacher F, Gloeckler V, Bravo-Angel AM, Hohn B (1995) The *Agrobacterium tumefaciens* virulence D2 protein is responsible for precise integration of T-DNA into the plant genome. EMBO J **14:** 3585–3595

Tzfira T, Citovsky V (2001) Partners-in-infection: host proteins involved in the transformation of plant cells by *Agrobacterium*. Trends Cell Biol **12:** 121–128

Tzfira T, Citovsky V (2003) The *Agrobacterium*-plant cell interaction. Taking biology lessons from a bug. Plant Physiol **133:** 943–947

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. EMBO J **20:** 3596–3607

Tzfira T, Vaidya M, Citovsky V (2002) Increasing plant susceptibility to *Agrobacterium* infection by over-expression of the *Arabidopsis* nuclear protein VIP1. Proc Natl Acad Sci USA **99:** 10435–10440

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

Tzfira T, Citovsky V, editors (2008) *Agrobacterium*: From Biology to Biotechnology. Springer, New York

Ulker B, Li Y, Rosso MG, Logemann E, Somssich IE, Weisshaar B (2008) T-DNA-mediated transfer of *Agrobacterium tumefaciens* chromosomal DNA into plants. Nat Biotechnol **26:** 1015–1017

van Attikum H, Bundock P, Lee LY, Gelvin SB, Hooykaas PJJ (2003) The *Arabidopsis AtLIG4* gene is involved in the repair of DNA damage, but not in the integration of *Agrobacterium* T-DNA. Nucleic Acids Res **31:** 4247–4255

van Attikum H, Hooykaas PJJ (2003) Genetic requirements for the targeted integration of *Agrobacterium* T-DNA in *Saccharomyces cerevisiae*. Nucleic Acids Res **31:** 826–832

van Wordragen MF, Dons HJM (1992) *Agrobacterium tumefaciens*-mediated transformation of recalcitrant crops. Plant Mol Biol Rep **10:** 12–36

Veena, Jiang H, Doerge RW, Gelvin SB (2003) Transfer of T-DNA and Vir proteins to plant cells by *Agrobacterium tumefaciens* induces expression of host genes involved in mediating transformation and suppresses host defense gene expression. Plant J **35:** 219–236

Veluthambi K, Krishnan M, Gould JH, Smith RH, Gelvin SB (1989) Opines stimulates induction of the *vir* genes of the *Agrobacterium tumefaciens* Ti plasmid. J Bacteriol **171:** 3696–3703

Veluthambi K, Ream W, Gelvin SB (1988) Virulence genes, borders, and overdrive generate single-stranded T-DNA molecules from the A6 Ti plasmid of *Agrobacterium tumefaciens*. J Bacteriol **170:** 1523–1532

Vergunst AC, Schrammeijer B, den Dulk-Ras A, de Vlaam CMT, Regensburg-Tuink TJG, Hooykaas PJJ (2000) VirB/D4-dependent protein translocation from *Agrobacterium* into plant cells. Science **290:** 979–982

Vergunst AC, van Lier MCM, den Duld-Ras A, Hooykaas PJJ (2003) Recognition of the *Agrobacterium tumefaciens* VirE2 translocation signal by the VirB/D4 transport system does not require VirE1. Plant Physiol **133:** 978–988

Vergunst AC, van Lier MCM, den Dulk-Ras A, Stuve TAG, Ouwehand A, Hooykaas PJJ (2005) Positive charge is an important feature of the C-terminal transport signal of the VirB/D4-translocated proteins of *Agrobacterium*. Proc Natl Acad Sci USA **102:** 832–837

Wagner VT, Matthysse AG (1992) Involvement of a vitronectin-like protein in attachment of *Agrobacterium tumefaciens* to carrot suspension culture cells. J Bacteriol **174:** 5999–6003

Ward DV, Zambryski PC (2001) The six functions of *Agrobacterium* VirE2. Proc Natl Acad Sci USA **98:** 385–386

Ward JE, Akiyoshi DE, Regier D, Datta A, Gordon MP, Nester EW (1988) Characterization of the *virB* operon from an *Agrobacterium tumefaciens* Ti plasmid. J Biol Chem **263:** 5804–5814

Weigel D, Ahn JH, Blázquez MA, Borevitz JO, Christensen SK, Fankhauser C, Ferrándiz C, Kardailsky I, Malancharuvil EJ, Neff MM, et al (2000) Activation tagging in *Arabidopsis*. Plant Physiol **122:** 1003–1013

West CE, Waterworth WM, Story GW, Sunderland PA, Jiang Q, Bray CM (2002) Disruption of the *Arabidopsis AtKu80* gene demonstrates an essential role for AtKu80 protein in efficient repair of DNA double-strand breaks in vivo. Plant J **31:** 517–528

White FF, Taylor BH, Huffman GA, Gordon MP, Nester EW (1985) Molecular and genetic analysis of the transferred DNA regions of the root-inducing plasmid of *Agrobacterium rhizogenes*. J Bacteriol **164:** 33–44

Wood DW, Setubal JC, Kaul R, Monks DE, Kitajima JP, Okura VK, Zhou Y, Chen L, Wood GE, Almeida NF, et al (2001) The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. Science **294:** 2317–2323

Wu HY, Chung PC, Shih HW, Wen SR, Lai EM (2008) Secretome analysis uncovers an Hcp-family protein secreted via a Type VI secretion sytem in *Agrobacterium tumefaciens*. J Bacteriol **190:** 2841–2850

Yi H, Mysore KS, Gelvin S (2002) Expression of the *Arabidopsis* histone *H2A-1* gene correlates with susceptibility to *Agrobacterium* transformation. Plant J **32:** 285–298

Yi H, Sardesai N, Fujinuma T, Chan CW, Veena Gelvin SB (2006) Constitutive expression exposes functional redundancy between the *Arabidopsis* histone H2A gene *HTA1* and other H2A gene family members. Plant Cell **18:** 1575–1589

Yuan ZC, Edlind MP, Liu P, Saenkham P, Banta LM, Wise AA, Ronzone E, Binns AN, Kerr K, Nester EW (2007) The plant signal salicylic acid shuts down the expression of the *vir* regulon and activates quarmone-quenching genes in *Agrobacterium*. Proc Natl Acad Sci USA **104:** 11790–11795

Yuan ZC, Haudecoeur E, Faure D, Kerr KF, Nester EW (2008a) Comparative transcriptome analysis of *Agrobacterium tumefaciens* in response to plant signal salicylic acid, indole-3-acetic acid and gamma-amino butyric acid reveals signalling cross-talk and *Agrobacterium*-plant co-evolution. Cell Microbiol **10:** 2339–2354

Yuan ZC, Liu P, Saenkham P, Kerr K, Nester EW (2008b) Transcriptome profiling and functional analysis of *Agrobacterium tumefaciens* reveals a general conserved response to acidic conditions (pH 5.5) and a complex acid-mediated signaling involved in *Agrobacterium*-plant interactions. J Bacteriol **190:** 494–507

Zhu Y, Nam J, Carpita NC, Matthysse AG, Gelvin SB (2003a) *Agrobacterium*-mediated root transformation is inhibited by mutation of an *Arabidopsis* cellulose synthase-like gene. Plant Physiol **133:** 1000–1010

Zhu Y, Nam J, Humara JM, Mysore KS, Lee LY, Cao H, Valentine L, Li J, Kaiser AD, Kopecky AL, et al (2003b) Identification of Arabidopsis *rat* mutants. Plant Physiol **132:** 494–505