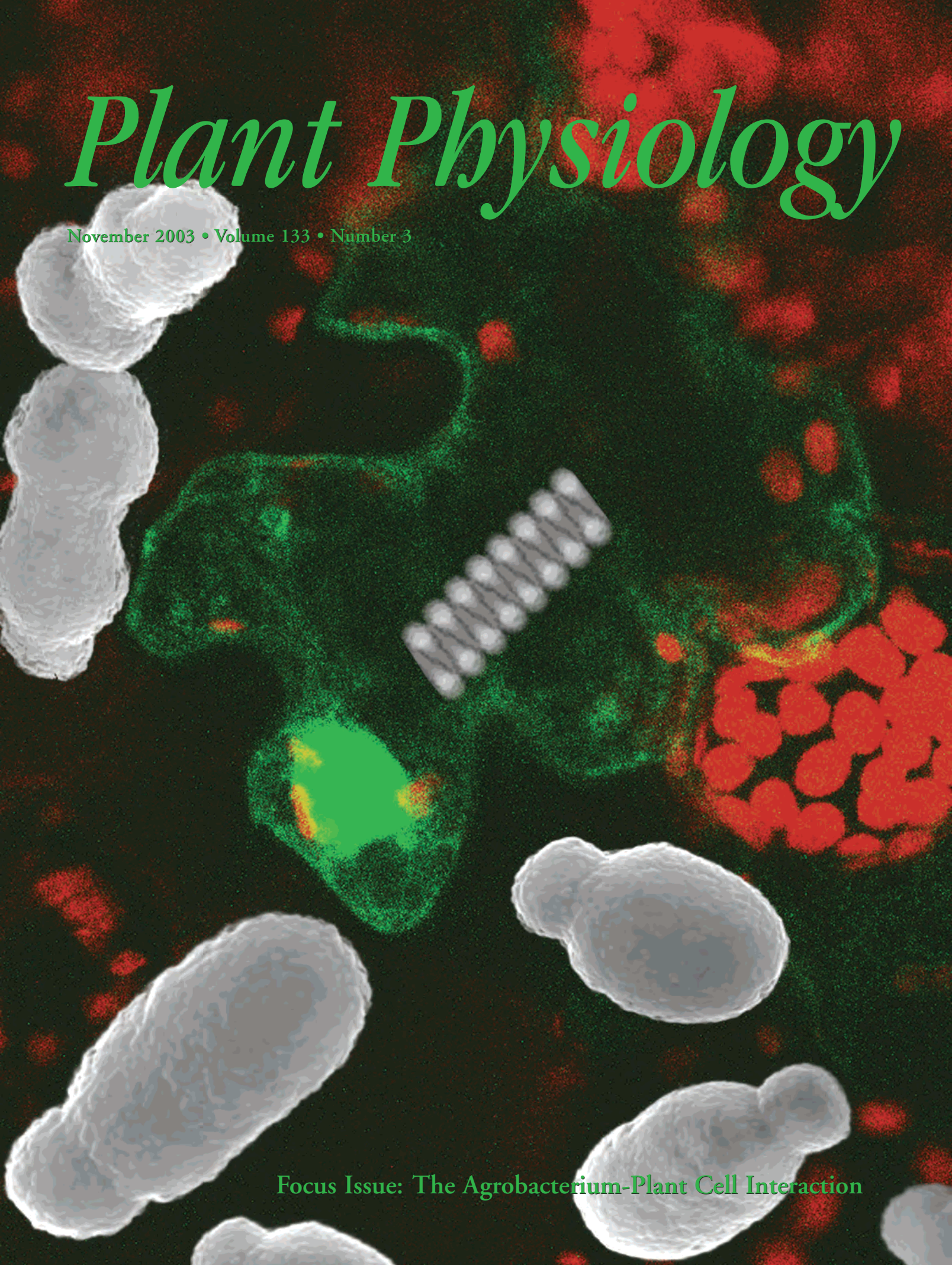

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Focus Issue: The Agrobacterium-Plant Cell Interaction

The Agrobacterium-Plant Cell Interaction. Taking Biology Lessons from a Bug[1,2]

Tzvi Tzfira and Vitaly Citovsky*

Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, New York 11794

Agrobacterium elicits neoplastic growths (called crown gall tumors) that affect most dicotyledonous plants. Moreover, although plants represent the natural hosts for Agrobacterium, this microorganism can also genetically transform a wide range of other eukaryotic species, from yeast (Bundock et al., 1995; Piers et al., 1996; Sawasaki et al., 1998) to mushrooms (de Groot et al., 1998; Chen et al., 2000) and filamentous fungi (de Groot et al., 1998; Gouka et al., 1999) to phytopathogenic fungi (Rho et al., 2001; Rolland et al., 2003) to human cells (Kunik et al., 2001). Most functions for Agrobacterium-host cell DNA transfer are coded by a large (200-kb) tumor-inducing (Ti) plasmid that resides in the bacterial cell and carries two important genetic components: the *vir* (virulence) region and the T-DNA delimited by two 25-bp direct repeats at its ends, termed the T-DNA borders (for review, see Citovsky et al., 1992a; Zupan et al., 2000; Gelvin, 2003). The *vir* region comprises seven major loci, *virA*, *virB*, *virC*, *virD*, *virE*, *virG*, and *virH*, which encode most of the bacterial protein machinery (Vir proteins) of the DNA transport. After induction of *vir* gene expression by small phenolic signal molecules secreted from wounded susceptible plant cells (Stachel et al., 1985), the T-DNA borders are nicked by the bacterial VirD2 endonuclease (Wang et al., 1987), generating a transferable single-stranded (ss) copy of the bottom strand of the T-DNA region, designated the T strand (Stachel et al., 1986).

Interestingly, the T strand does not travel alone but is thought to directly associate with two Agrobacterium proteins, VirD2 and VirE2, forming a transport (T) complex (Zupan and Zambryski, 1997) in which one molecule of VirD2 is covalently attached to the 5′-end of the T strand, whereas VirE2, an ssDNA-binding protein, is presumed to cooperatively coat the rest of the T strand molecule (for review, see Zupan and Zambryski, 1997; Tzfira et al., 2000; Zupan et al., 2000). Although only the wild-type T-DNA carries Ti genes, any DNA placed between the T-DNA borders will be transferred to the plant host (for review, see Zambryski, 1992; Sheng and Citovsky, 1996). This lack of sequence specificity implies that a T-DNA molecule itself does not encode protein machinery for its transport from the bacterial cell into the host cell, import into the host cell nucleus, and integration into the host cell genome. Instead, these functions are fulfilled by the bacterial Vir proteins and their host cell partners (for review, see Gelvin, 2000; Tzfira et al., 2000).

In the last quarter of a century, since the discovery of the stable integration of the bacterial DNA in crown gall tumors (Chilton et al., 1977), Agrobacterium has served as a primary a tool for plant genetic engineering. Furthermore, the Agrobacterium-host cell interaction also represents a unique and powerful experimental system to study a wide spectrum of basic biological processes such as cell-cell recognition and cell-to-cell transport, nuclear import, assembly and disassembly of protein-DNA complexes, DNA recombination, and regulation of gene expression. This special Focus Issue reviews the use of Agrobacterium as a gene vector for plants and reports new insights into the processes of cell-cell recognition and attachment, intercellular transport, DNA integration, and transgene expression gained from the Agrobacterium research.

GENETIC ENGINEERING

Predating the science fiction visions of nanomachines performing genetic engineering and other biotechnological tasks (Modesitt Jr., 2000), Agrobacterium is a present-day microscopic but extremely complex machine routinely used to alter genotypes of higher plants. This use of Agrobacterium is based on its unique capacity for "trans-kingdom sex" (Stachel and Zambryski, 1989), i.e. transfer of genetic material between prokaryotic and eukaryotic cells. Decades of research altered, augmented, and vastly improved this natural capacity, resulting in ingeniously modified Agrobacterium strains that can transfer and stably integrate virtually any gene to a variety of plant species, from research model plants such as Arabidopsis (Clough and Bent, 1998) to agriculturally important rice (Hiei et al., 1994) and corn (Ishida et al., 1996). In this issue, Valentine (pp. 948–

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* Corresponding author; e-mail vitaly.citovsky@stonybrook.edu; fax 631–632–8575.

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955) reviews the molecular mechanisms of T-DNA transfer that underlie the use of Agrobacterium as gene vector. Importantly, a special emphasis is made on the ethical and political aspects of the release of Agrobacterium-generated genetically modified plants into the environment and on the attitudes of different and often opposing geopolitical and economic forces to these matters (Valentine, 2003).

CELL-CELL RECOGNITION AND ATTACHMENT

Agrobacterium recognition of and attachment to the host cells is an early and essential step of the infection process. The bacterial proteins participating in these events are encoded by several loci, e.g. *chvA*, *chvB*, *pscA*, and *att* (Douglas et al., 1985; Matthysse, 1987; Thomashow et al., 1987). In particular, the *att* genes (Matthysse, 1987; Matthysse et al., 2000), such as *attR* and *attD*, are located on a cryptic plasmid pAtC58 (Goodner et al., 2001). Although pAtC58 is about twice as large as the Ti plasmid and, thus, likely encodes numerous bacterial functions, it has been considered dispensable for infection (Hooykaas et al., 1977; Rosenberg and Huguet, 1984; Hynes et al., 1985). In this issue, Nair et al. (pp. 989–999) revisit this dogma and demonstrate that pAtC58 increases Agrobacterium virulence. Surprisingly, however, this increase was likely due to the enhancement of the *vir* gene expression rather than to the presence of the *attR* gene on the pATC58 plasmid (Nair et al., 2003).

Although the bacterial genes participating in Agrobacterium attachment to the host plants are relatively well characterized (Douglas et al., 1985; Matthysse, 1987; Thomashow et al., 1987; Matthysse et al., 2000), the involvement of host factors remains largely obscure. Recent progress in this direction stemmed from identification of T-DNA-tagged Arabidopsis mutants defective in their ability to bind Agrobacterium (Nam et al., 1999; Zhu et al., 2003b). In this issue, Zhu et al. (2003) report characterization of one such mutant, *rat4*, which contains a T-DNA insertion in the 3′-untranslated region of cellulose synthase-like gene *CSLA9*. Their data indicate that *CSLA9* is involved in development and growth of lateral roots, determination of sugar composition of plant cell walls, and the ability of the roots to bind Agrobacterium (Zhu et al., 2003a). Furthermore, the *CSLA9* promoter exhibited enhanced expression in the root elongation zone (Zhu et al., 2003a), previously shown to be most susceptible to Agrobacterium-mediated transformation (Yi et al., 2002). Similarly, preferential expression in the elongation zones of Arabidopsis roots has been reported for a plant histone gene, *H2A-1*, which is required for T-DNA integration (Yi et al., 2002).

CELL-TO-CELL TRANSPORT

T strands with their cognate VirD2 are exported into the host cell by a type IV secretion system, which in Agrobacterium is assembled from proteins encoded by the *virD4* gene and *virB* operon, with 11 open reading frames (for review, see Christie, 1997; Zupan et al., 1998). Interestingly, this system also exports other Agrobacterium proteins, such as VirE3 (Schrammeijer et al., 2003), VirF, and VirE2 (Vergunst et al., 2000). Although VirE2 most likely packages the T strand into the T complex (for review, see Zupan and Zambryski, 1997; Tzfira et al., 2000; Zupan et al., 2000), this binding is thought to occur within the cytoplasm of the host cell (Citovsky et al., 1992b; Gelvin, 1998) after independent export of the T strands and VirE2 (Binns et al., 1995; Lee et al., 1999). But what prevents VirE2 from binding to the T strands already within Agrobacterium? This role has been assigned to the VirE1 chaperone protein that associates with VirE2 and blocks its binding to ssDNA (Sundberg et al., 1996; Deng et al., 1999; Sundberg and Ream, 1999; Zhou and Christie, 1999). Furthermore, VirE1 has been suggested also to participate in the VirE2 export (Sundberg et al., 1996; Sundberg and Ream, 1999). However, convincing evidence to the contrary is presented by Vergunst et al. (2003). Using the Cre Recombinase Reporter Assay for Translocation to study protein export from Agrobacterium into plant and yeast cells, they demonstrate that recognition of VirE2 by the bacterial export machinery and its subsequent translocation into host cells does not depend on the presence of VirE1 (Vergunst et al., 2003). Cre Recombinase Reporter Assay for Translocation was then utilized to show that VirE3, another product of the *virE* locus earlier reported to be exported into the yeast cells (Schrammeijer et al., 2003), is transferred from Agrobacterium to plants (Vergunst et al., 2003). The function of VirE3 in the plant cell, however, remains completely unknown. Vergunst et al. (2003) also localized the Agrobacterium-to-plant export signal to the C-terminal 50 amino acids of VirE2 and VirE3. These and previous studies (Vergunst et al., 2000, 2003; Schrammeijer et al., 2003) clearly demonstrate that in addition to DNA, Agrobacterium transfers a variety of its own proteins to the host cell; the challenge to researchers now is to understand how these bacterial proteins participate in the genetic transformation process from within the host cell.

DNA INTEGRATION AND EXPRESSION

T-DNA integration is the culmination point of the entire process of the Agrobacterium-plant cell DNA transfer. But how does a T-DNA molecule insinuate itself into the molecule of the plant genomic DNA? Because the T-DNA does not encode enzymatic activities required for integration, the protein components of the T-complex, i.e. VirD2 (Tinland et al.,

1995; Mysore et al., 1998) and VirE2 (Rossi et al., 1996) and/or host nuclear factors, such as AtKu80 (Friesner and Britt, 2003) and plant DNA ligases (Ziemienowicz et al., 2000; Friesner and Britt, 2003), must provide these functions. T-DNA integration was proposed to initiate with ligation of the 5′ end of the T strand to the genomic DNA followed by second strand synthesis by the plant DNA repair machinery (Tinland et al., 1995). However, another study suggested that the T strand is converted into a double-stranded form before integration (De Neve et al., 1997).

Two papers published in this issue (Chilton and Que, pp. 956–965; Tzfira et al., pp. 1011–1023) shed new and exciting light on the integration mechanism. Chilton and Que (2003) provide strong evidence for T-DNA integration into double-stranded breaks created in the plant genome by a transiently expressed rare cutting endonuclease I-*Ceu*I. Nucleotide sequence analysis of the plant DNA/T-DNA junctions indicated that T-DNA integration occurred by a non-homologous end-joining mechanism (Chilton and Que, 2003). These findings are consistent with the recently reported involvement of Arabidopsis AtKu80—known to be required for the initiation of non-homologous end-joining—in the T-DNA integration process (Friesner and Britt, 2003). Tzfira et al. (2003) utilized the double-stranded DNA breaks created by transient expression of another endonuclease, I-*Sce*I, to demonstrate preferential T-DNA integration into these 18-bp-long I-*Sce*I recognition sites as determined by sequencing analyses of integration junctions from 620 independent transgenic lines (Tzfira et al., 2003). The efficiency of targeted integration in these experiments was 2.58%, significantly higher than 4×10^{-7}% predicted for the probability of random integration into a 18-bp region of the approximately 4.5-gigabase tobacco (*Nicotiana tabacum*) genome (Tzfira et al., 2003). Both studies suggested that T strands are first converted to double-stranded intermediates and only then are integrated into the plant DNA (Chilton and Que, 2003; Tzfira et al., 2003).

Obviously, it is the expression of the integrated transgenes that produces tumors in the wild-type infection or desired transgenic phenotypes in genetic engineering experiments. However, some transgenes, although stably integrated in the plant genome, often are not expressed due to their posttranscriptional gene silencing (PTGS), which is characterized by a reduction in transcript levels without affecting the rate of transcription (for review, see Fagard and Vaucheret, 2000; Vaucheret et al., 2001). Furthermore, PTGS of a transgene can also silence its endogenous cellular homologs, resulting in co-suppression (van der Boogaart et al., 1998). Agrobacterium-mediated gene delivery has served as a valuable experimental tool to study the mechanisms of induction and cell-to-cell spread of PTGS (Voinnet et al., 1998; Johansen and Carrington, 2001; Ueki and Citovsky, 2001). Continuing this trend, Lee et al. (2003) utilized silencing of Agrobacterium oncogenes contained within the T-DNA to demonstrate that transgene sequences influence the effectiveness of PTGS and that sequences required for oncogene silencing must include a translation start site (Lee et al., 2003). Unexpectedly, unlike several other cases of PTGS (Palauqui et al., 1997), oncogene silencing was not graft transmissible (Escobar et al., 2003; Lee et al., 2003). In addition to helping us better understand the mechanisms of PTGS, silencing of Agrobacterium oncogenes (Escobar et al., 2001, 2003; Lee et al., 2003) represents a novel approach to control the crown gall disease, which affects such agronomically important plants as grape, rose, apple, cherry, and others.

Finally, Agrobacterium infection was also used as a model system to study cellular processes required for establishment of plant tumors. Wächter et al. (2003) showed that vascular differentiation and disruption of epidermal cell layers play key roles in tumor formation, allowing delivery of water and Suc to the proliferating tumor cells. This work suggested that Agrobacterium-induced tumors represent nutritional sinks to which a high-volume flow of solutes with essential inorganic nutrients is directed in an ethylene-dependent fashion, followed by a Suc-to-hexose shift in sugar balance caused by vacuolar invertases within the growing tumors (Wächter et al., 2003).

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LITERATURE CITED

Binns AN, Beaupre CE, Dale EM (1995) Inhibition of VirB-mediated transfer of diverse substrates from *Agrobacterium tumefaciens* by the InQ plasmid RSF1010. J Bacteriol **177:** 4890–4899

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

Chen X, Stone M, Schlagnhaufer C, Romaine CP (2000) A fruiting body tissue method for efficient *Agrobacterium*-mediated transformation of *Agaricus bisporus*. Appl Environ Microbiol **66:** 4510–4513

Chilton MD, Drummond MH, Merio DJ, Sciaky D, Montoya AL, Gordon MP, Nester EW (1977) Stable incorporation of plasmid DNA into higher plant cells: the molecular basis of crown gall tumorigenesis. Cell **11:** 263–271

Chilton MD, Que Q (2003) Targeted integration of T-DNA into the tobacco genome at double-strand breaks: new insights on the mechanism of T-DNA integration. Plant Physiol **133:** 956–965

Christie PJ (1997) *Agrobacterium tumefaciens* T-complex transport apparatus: a paradigm for a new family of multifunctional transporters in eubacteria. J Bacteriol **179:**3085–3094,

Citovsky V, McLean BG, Greene E, Howard E, Kuldau G, Thorstenson Y, Zupan J, Zambryski PC (1992a) *Agrobacterium*-plant cell interaction: induction of *vir* genes and T-DNA transfer. *In* DPS Verma, ed, Molecular Signals in Plant-Microbe Communications. CRC Press, Inc., Boca Raton FL, pp 169–198

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

Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J **16:** 735–743

de Groot MJ, Bundock P, Hooykaas PJJ, Beijersbergen AG (1998) *Agrobacterium tumefaciens*-mediated transformation of filamentous fungi. Nat Biotechnol **16:**839–842; erratum **de Groot MJ, Bundock P, Hooykaas PJJ, Beijersbergen AG** (1998) Nat Biotechnol **16:** 1074

De Neve M, De Buck S, Jacobs A, Van Montagu M, Depicker A (1997) T-DNA integration patterns in co-transformed plant cells suggest that T-DNA repeats originate from co-integration of separate T-DNAs. Plant J **11:** 15–29

Deng W, Chen L, Peng WT, Liang X, Sekiguchi S, Gordon MP, Comai L, Nester EW (1999) VirE1 is a specific molecular chaperone for the exported single-stranded-DNA-binding protein VirE2 in *Agrobacterium*. Mol Microbiol **31:** 1795–1807

Douglas CJ, Staneloni RJ, Rubin RA, Nester EW (1985) Identification and genetic analysis of an *Agrobacterium tumefaciens* chromsomal virulence region. J Bacteriol **161:** 850–860

Escobar MA, Civerolo EL, Politito VS, Pinney KA, Dandekar AM (2003) Characterization of oncogene-silenced transgenic plants: implications for *Agrobacterium* biology and post-transcriptional gene silencing. Mol Plant Pathol **4:** 57–65

Escobar MA, Civerolo EL, Summerfelt KR, Dandekar AM (2001) RNAi-mediated oncogene silencing confers resistance to crown gall tumorigenesis. Proc Natl Acad Sci USA **98:** 13437–13442

Fagard M, Vaucheret H (2000) (Trans)gene silencing in plants: How many mechanisms? Annu Rev Plant Physiol Plant Mol Biol **51:** 167–194

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

Gelvin SB (1998) *Agrobacterium* VirE2 proteins can form a complex with T strands in the plant cytoplasm. J Bacteriol **180:** 4300–4302

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*-mediated plant transformation: the biology behind the "gene-jockeying" tool. Microbiol Mol Biol Rev **67:** 16–37

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

Gouka RJ, Gerk C, Hooykaas PJ, Bundock P, Musters W, Verrips CT, de Groot MJ (1999) Transformation of *Aspergillus awamori* by *Agrobacterium tumefaciens*-mediated homologous recombination. Nat Biotechnol **17:** 598–601

Hiei Y, Ohta S, Komari T, Kumashiro T (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant J **6:** 271–282

Hooykaas PJ, Klapwijk PM, Nuti MP, Schilperoort RA, Rorsch A (1977) Transfer of the *Agrobacterium tumefaciens* Ti plasmid to avirulent agrobacteria and to explanta. J Gen Microbiol **98:** 477–484

Hynes MF, Simon R, Puhler A (1985) The development of plasmid-free strains of *Agrobacterium tumefaciens* by using incompatibility with a *Rhizobium meliloti* plasmid to eliminate pAtC58. Plasmid **13:** 99–105

Ishida Y, Saito H, Ohta S, Hiei Y, Komari T, Kumashiro T (1996) High efficiency transformation of maize (*Zea mays* L.) mediated by *Agrobacterium tumefaciens*. Nat Biotechnol **14:** 745–750

Johansen LK, Carrington JC (2001) Silencing on the spot: induction and suppression of RNA silencing in the *Agrobacterium*-mediated transient expression system. Plant Physiol **126:** 930–938

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

Lee H, Humann J, Pitrak J, Cuperus J, Parks TD, Whistler C, Mok M, Ream W (2003) Translation start sequences affect the efficiency of silencing of *Agrobacterium tumefaciens* T-DNA oncogenes. Plant Physiol **133:** 966–977

Lee LY, Gelvin SB, Kado CI (1999) pSa causes oncogenic suppression of *Agrobacterium* by inhibiting VirE2 protein export. J Bacteriol **181:** 186–196

Matthysse AG (1987) Characterization of nonattaching mutants of *Agrobacterium tumefaciens*. J Bacteriol **169:** 313–323

Matthysse AG, Yarnall H, Boles SB, McMahan S (2000) A region of the *Agrobacterium tumefaciens* chromosome containing genes required for virulence and attachment to host cells. Biochim Biophys Acta **1490:** 208–212

Modesitt Jr LE (2000) Gravity Dreams. Tor Books, New York

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

Nair GR, Liu Z, Binns AN (2003) Re-examining the role of the cryptic plasmid pAtC58 in the virulence of *Agrobacterium tumefaciens* strain C58. Plant Physiol **133:** 989–999

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

Palauqui JC, Elmayan T, Pollien JM, Vaucheret H (1997) Systemic acquired silencing: transgene-specific post-translational silencing is transmitted by grafting from silenced stocks to non-silenced scions. EMBO J **16:** 4738–4745

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

Rho HS, Kang S, Lee YH (2001) *Agrobacterium tumefaciens*-mediated transformation of plant pathogenic fungus, *Magnaporthe grisea*. Mol Cells **12:** 407–411

Rolland S, Jobic C, Fevre M, Bruel C (2003) *Agrobacterium*-mediated transformation of *Botrytis cinerea*, simple purification of monokaryotic transformants and rapid conidia-based identification of the transfer-DNA host genomic DNA flanking sequences. Curr Genet (in press)

Rosenberg C, Huguet T (1984) The pAtC58 plasmid of *Agrobacterium tumefaciens* is not essential for tumour induction. Mol Gen Genet **196:** 533–536

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*. Proc Natl Acad Sci USA **93:** 126–130

Sawasaki Y, Inomata K, Yoshida K (1998) Trans-kingdom conjugation between *Agrobacterium tumefaciens* and *Saccharomyces cerevisiae*, a bacterium and a yeast. Plant Cell Physiol **37:** 103–106

Schrammeijer B, Dulk-Ras Ad A, Vergunst AC, Jurado Jacome E, Hooykaas PJ (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

Sheng J, Citovsky V (1996) *Agrobacterium*-plant cell interaction: have virulence proteins, will travel. Plant Cell **8:** 1699–1710

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

Stachel SE, Timmerman B, Zambryski PC (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 SE, Zambryski PC (1989) Generic trans-kingdom sex? Nature **340:** 190–191

Sundberg C, Meek L, Carrol K, Das A, Ream W (1996) VirE1 protein mediates export of single-stranded DNA binding protein VirE2 from *Agrobacterium tumefaciens* into plant cells. J Bacteriol **178:** 1207–1212

Sundberg CD, Ream W (1999) The *Agrobacterium tumefaciens* chaperone-like protein, VirE1, interacts with VirE2 at domains required for single-stranded DNA binding and cooperative interaction. J Bacteriol **181:** 6850–6855

Thomashow MF, Karlinsey JE, Marks JR, Hurlbert RE (1987) Identification of a new virulence locus in *Agrobacterium tumefaciens* that affects polysaccharide composition and plant cell attachment. J Bacteriol **169:** 3209–3216

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, Frankmen L, Vaidya M, Citovsky V (2003) Site-specific integration of *Agrobacterium* T-DNA via double-stranded intermediates. Plant Physiol **133:** 1011–1023

Tzfira T, Rhee Y, Chen M-H, Citovsky V (2000) Nucleic acid transport in plant-microbe interactions: the molecules that walk through the walls. Annu Rev Microbiol **54:** 187–219

Ueki S, Citovsky V (2001) Inhibition of post transcriptional gene silencing by non-toxic concentrations of cadmium. Plant J **28:** 283–291

Valentine L (2003) *Agrobacterium tumefaciens*: the David and Goliath of modern genetics. Plant Physiol **133:** 948–955

van der Boogaart T, Lomonossoff GP, Davies JW (1998) Can we explain RNA-mediated virus resistance by homology-dependent gene silencing? Mol Plant-Microbe Interact **11:** 717–723

Vaucheret H, Bèclin C, Fagard M (2001) Post-transcriptional gene silencing in plants. J Cell Sci **114:** 3083–3091

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

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

Voinnet O, Vain P, Angell S, Baulcombe DC (1998) Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoterless DNA. Cell **95:** 177–187

Wächter R, Langhans M, Aloni R, Götz G, Weilmünster A, Koops A, Temguia L, Mistrik I, Pavlovkin J, Rascher U et al. (2003) Vascularization, high-volume solution flow, and localized roles for enzymes of sucrose metabolism during tumorigenesis by *Agrobacterium tumefaciens*. Plant Physiol **133:** 1024–1037

Wang K, Stachel SE, Timmerman B, Van Montagu M, Zambryski PC (1987) Site-specific nick occurs within the 25 bp transfer promoting border sequence following induction of vir gene expression in *Agrobacterium tumefaciens*. Science **235:** 587–591

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

Zambryski PC (1992) Chronicles from the *Agrobacterium*-plant cell DNA transfer story. Annu Rev Plant Physiol Plant Mol Biol **43:** 465–490

Zhou XR, Christie PJ (1999) Mutagenesis of the *Agrobacterium* VirE2 single-stranded DNA-binding protein identifies regions required for self-association and interaction with VirE1 and a permissive site for hybrid protein construction. J Bacteriol **181:** 4342–4352

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:** xxx–xxx

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

Ziemienowicz A, Tinland B, Bryant J, Gloeckler V, Hohn B (2000) Plant enzymes but not *Agrobacterium* VirD2 mediate T-DNA ligation *in vitro*. Mol Cell Biol **20:** 6317–6322

Zupan J, Muth TR, Draper O, Zambryski PC (2000) The transfer of DNA from *Agrobacterium tumefaciens* into plants: a feast of fundamental insights. Plant J **23:** 11–28

Zupan J, Ward D, Zambryski PC (1998) Assembly of the VirB transport complex for DNA transfer from *Agrobacterium tumefacien*s to plant cells. Curr Opin Microbiol **1:** 649–655

Zupan J, Zambryski PC (1997) The *Agrobacterium* DNA transfer complex. Crit Rev Plant Sci **16:** 279–295