REVIEW / SYNTHÈSE

Will you let me use your nucleus? How *Agrobacterium* gets its T-DNA expressed in the host plant cell[1]

Benoît Lacroix, Jianxiong Li, Tzvi Tzfira, and Vitaly Citovsky

Abstract: *Agrobacterium* is the only known bacterium capable of natural DNA transfer into a eukaryotic host. The genes transferred to host plants are contained on a T-DNA (transferred DNA) molecule, the transfer of which begins with its translocation, along with several effector proteins, from the bacterial cell to the host-cell cytoplasm. In the host cytoplasm, the T-complex is formed from a single-stranded copy of the T-DNA (T-strand) associated with several bacterial and host proteins and it is imported into the host nucleus via interactions with the host nuclear import machinery. Once inside the nucleus, the T-complex is most likely directed to the host genome by associating with histones. Finally, the chromatin-associated T-complex is uncoated from its escorting proteins prior to the conversion of the T-strand to a double-stranded form and its integration into the host genome.

Key words: Agrobacterium, T-DNA, nuclear import, integration.

Résumé : *Agrobacterium* est la seule bactérie capable de transférer naturellement de l'ADN vers un hôte eucaryote. Les gènes transférés vers les plantes hôtes sont situés sur l'ADN-T (ADN transféré), dont le transfert commence par sa translocation, en même temps que d'autres protéines effectrices, de la cellule bactérienne vers le cytoplasme de la cellule hôte. Dans le cytoplasme, le complexe-T est formé de l'ADN-T simple brin (brin-T) associé avec plusieurs protéines de la bactérie et de l'hôte, et est importé dans le noyau via des intéractions avec la machinerie d'importation nucléaire de l'hôte. A l'intérieur du noyau, le complexe-T est très probablement dirigé vers le génôme par association avec des histones. Finalement, le complexe-T associé a la chromatine est depouillé de ses protéines accompagnatrices avant la conversion du brin-T en une forme double brin, et son intégration dans le génôme de l'hôte.

Mots clés : Agrobacterium, ADN-T, importation nucléaire, intégration.

[Traduit par la Rédaction]

Introduction

The genus *Agrobacterium* comprises Gram-negative phytopathogenic bacteria, which infect plants via a unique mechanism of DNA transfer and integration of the transferred DNA (T-DNA) into the host-plant genome. The T-DNA carries gene-encoding enzymes implicated in growth-regulator synthesis that induce uncontrolled tissue proliferation, and enzymes responsible for the synthesis of opines, which are molecules that are secreted into the environment and can be used by the bacteria as a source of carbon and nitrogen (Gelvin 2000, 2003*b*; Tzfira and Citovsky 2002; Zupan et al. 2000). These modifications of plant cell growth and division and of cell metabolism provide an environment favorable to the proliferation of *Agrobacterium*. Depending upon the nature of the genes being transferred, the resultant disease will have different symptoms; for example, *A. tumefaciens* causes a neoplastic growth (crown gall), whereas the related species *A. rhizogenes* induces an abnormal proliferation of roots (hairy root) (Escobar and Dandekar 2003; Nilsson and Olsson 1997). The natural host range of *Agrobacterium* species is rather broad among higher plants, and the number of plant species successfully transformed by *Agrobacterium* under laboratory conditions continues to rise (Newell 2000). Recently, T-DNA transfer has also been demonstrated to non-plant hosts, such as yeast (Bundock et al. 1995; Piers et al. 1996), various filamentous fungi (de Groot et al. 1998; Michielse et al. 2005), cultivated mushrooms (de Groot et al. 1998), and even cultured human cells (Kunik et al. 2001).

The mechanism of DNA transfer has been studied extensively with *A. tumefaciens* and is described in several recent

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B. Lacroix,[2] J. Li, T. Tzfira,[3] and V. Citovsky. Department of Biochemistry and Cell Biology, State University of NY, Stony Brook, NY 11794-5212, USA.

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[2]Corresponding author (e-mail: lacroix@notes.cc.sunysb.edu).
[3]Present address: Department of Molecular, Cellular and Developmental Biology, The University of Michigan, Ann Arbor, MI 48109, USA.

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reviews (Gelvin 2000, 2003*b*; Tzfira and Citovsky 2002; Zupan et al. 2000). Briefly, the transformation process is initiated by the expression of a subset of *Agrobacterium* virulence (*vir*) genes located on a specialized Ti (tumor-inducing) plasmid and primarily induced by small phenolic compounds and sugars produced by wounded plant cells, via a VirA/VirG 2-component regulatory system. Subsequently, a DNA segment (T-DNA) is excised from the Ti-plasmid by the VirD2/VirD1 endonuclease complex, producing a single-stranded T-DNA (T-strand) that corresponds to the nontranscribed strand of T-DNA through a strand-replacement mechanism. The T-DNA segment is defined by its 2 borders, 25-bp direct repeats, and its transfer is not dependent on the nature of the DNA sequence between them. Consequently, the native T-DNA genes can be replaced at will by genes of interest, constituting the basis for the biotechnological uses of *Agrobacterium* for plant genetic transformation. The T-strand, with VirD2 covalently linked at its 5′ end, is then translocated to the plant cell via a type IV secretion system (T4SS) composed of the VirB and VirD4 proteins (Christie 2004). Other bacterial virulence proteins, namely VirE2, VirE3, VirF, and VirD5, are independently exported to the host plant cell, where they perform their respective functions (see below). After the T-DNA enters the plant-cell cytoplasm, the infection process proceeds in 4 major steps. The formation and nuclear import of the T-complex (T-strand with its cognate bacterial and host-plant proteins) is followed by the targeting of the T-DNA molecule to the site of integration. The T-strand is then uncoated from its escorting proteins, which occurs inside the nucleus by targeted proteolysis, and the T-strand is converted into a double-stranded molecule that is integrated into the host genome. All of these events rely on the cooperation between bacterial factors and components of the host cellular and nuclear machineries (Gelvin 2000, 2003*b*; Tzfira and Citovsky 2002).

In this paper, we focus on the final steps of the infection process, i.e., those occurring inside the plant cell and in which the functions of the host nucleus are implicated. Specifically, some of the most important nucleus-related functions, e.g., protein nuclear import, intranuclear transport, intranuclear targeted proteolysis, DNA repair and recombination, and gene expression, are required to achieve expression of the T-DNA in the host cell.

Nuclear import of the T-DNA complex and associated proteins

Increasing evidence suggests that the VirD2-conjugated T-strand (immature T-complex) and VirE2, along with other effector proteins, are exported independently to the host plant cell's cytoplasm where the formation of mature T-complex occurs by association of the T-strand with bacterial proteins. First, T-DNA transfer from a *virE2* mutant *Agrobacterium* strain can be complemented by strains containing *virE2* but no T-DNA (Otten et al. 1984). Second, expression of VirE2 in plant cells can complement the virulence of an *Agrobacterium* strain lacking the *vir*E2 gene (Citovsky et al. 1992; Gelvin 1998). More recently, independent translocation of VirE2 from *Agrobacterium* to plant cell, as well as of other Vir proteins, through the VirB/VirD4 channel was demonstrated directly using a functional genetic assay (Vergunst et al. 2000). Subsequently, mature T-complex formation begins with the association of the VirD2-conjugated T-strand with the VirE2 molecules, in the plant-cell cytoplasm (Fig. 1, step 1). Indeed, VirE2 molecules bind cooperatively and nonspecifically to single-stranded DNA with high affinity (Citovsky et al. 1989). The structure of a bacteriophage M13 single-stranded DNA associated with purified VirE2 was examined by scanning transmission electron microscopy and single-particle image processing methods (Abu-Arish et al. 2004; Citovsky et al. 1997). The discovered structure was a rigid coiled filament, showing a hollow helical structure with an outer diameter of 12.8–15.7 nm (Abu-Arish et al. 2004; Citovsky et al. 1997). Maturation of the T-complex is likely to occur very quickly after the T-DNA enters the host-cell cytoplasm, because of the high affinity of VirE2 to single-stranded DNA (Citovsky et al. 1989) and consistent with T-DNA being protected against host-cell nucleases. Note that when the T-strand and VirE2 are produced inside the bacterial cell, their association is most likely prevented by VirE1, a VirE2 chaperone protein (Deng et al. 1999; Sundberg and Ream 1999). Whereas initially VirE1 was also thought to be implicated in export of VirE2 into the host cells (Sundberg et al. 1996), later studies argued against this idea (Vergunst et al. 2003). The outer diameter (12.8–15.7 nm) of the T-complex, and its extended length, which, as an example, is estimated at 3.6 μm for a 22-kb T-strand of the wild-type nopaline-specific strain of *A. tumefaciens* (Citovsky et al. 1997; Tzfira et al. 2005), is larger than the free diffusion limit of the nuclear pore (9 nm), but compatible with the size-exclusion limit of active transport through that nuclear pore, estimated to be 23 to 39 nm (Forbes 1992; Pante and Kann 2002). Therefore, the T-complex requires active transport to enter the nucleus, which is likely mediated by the VirD2 and VirE2 proteins and relies on interactions with the host cells' nuclear import machinery.

The VirD2 protein, as shown by its in-planta expression when fused with a reporter gene, is directed to the nucleus of plant cells (Citovsky et al. 1994; Herrera-Estrella et al. 1990; Howard et al. 1992; Ziemienowicz et al. 2001) as well as of yeast and animal cells (Guralnick et al. 1996; Relic et al. 1998; Rhee et al. 2000; Ziemienowicz et al. 1999). VirD2 is thus imported into the host-cell nucleus via a mechanism that is conserved between diverse organisms. The VirD2 sequence contains 2 distinct putative nuclear localization signals (NLSs): a bipartite NLS in its C-terminal portion and a monopartite NLS in its N-terminal part (Herrera-Estrella et al. 1990; Howard et al. 1992). Only the former may be essential for VirD2 and T-DNA nuclear import, as mutations in the C-terminal NLS reduce *Agrobacterium* virulence, whereas mutations in the N-terminal NLS have no significant effect (Howard et al. 1992). Consistently, mutations in the C-terminal, but not N-terminal, NLSs disrupt the nuclear localization of VirD2 in plant cells (Ziemienowicz et al. 2001). Yeast 2-hybrid experiments have shown that VirD2 interacts directly with karyopherin α of *Arabidopsis thaliana* (Ballas and Citovsky 1997). Karyopherin α (also termed importin α) is a component of the nuclear import machinery that recognizes and binds classical NLS sequences composed of basic amino acid residues. In most cases, karyopherin α functions in a heterodimer with

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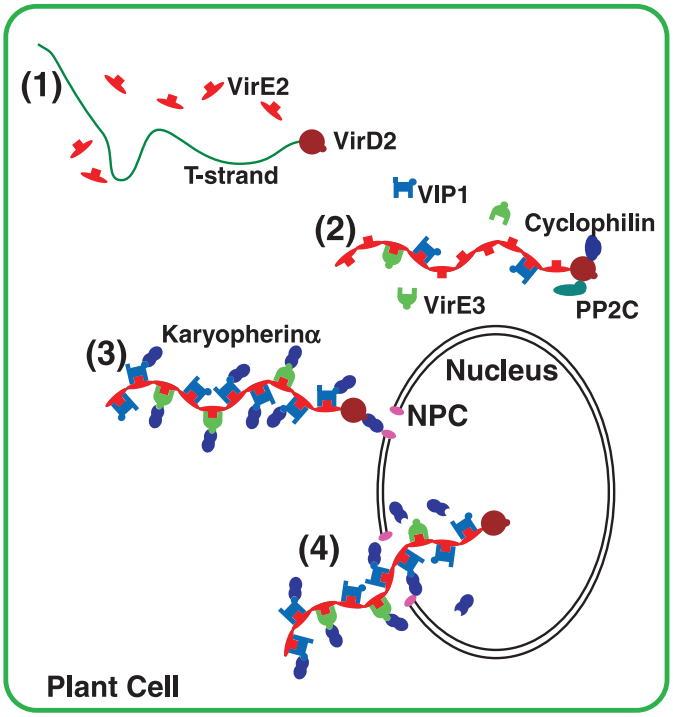
Fig. 1. Nuclear import of the T-complex. The mature T-complex is formed by association of the single-stranded T-DNA (T-strand) covalently linked at its 5′ end to VirD2 and cooperatively coated with VirE2 molecules (step 1). VIP1 and VirE3 molecules bind to VirE2, while plant cyclophilins and PP2C may interact with VirD2 (step 2). The entire T-complex then is directed to the nuclear pore via by plant karyopherins α that bind nuclear localization signals (NLSs) of VirD2, VIP1, and VirE3 (step 3). Finally, the T-complex is transported through the nuclear pore complex (NPC) into the karyoplasm (step 4).



karyopherin β (or importin β), which directs the NLS-containing protein-karyopherin α/β complex to the nuclear pore and into the cell nucleus (Chook and Blobel 2001; Goldfarb et al. 2004; Powers and Forbes 1994). The nuclear import of VirD2 may also be regulated by other plant factors; indeed, a protein from tomato, DIG3, a type 2C serine/threonine phosphatase (PP2C), was identified via its interaction with the C-terminal region of VirD2 in a yeast 2-hybrid assay, and its over-expression inhibited nuclear import of a GUS-VirD2 fusion in cultured tobacco cells (Tao et al. 2004). PP2C was suggested to negatively regulate VirD2 nuclear import, most probably by dephosphorylation of the VirD2 protein; indeed, an *Arabidopsis* mutant in the PP2C gene (*abi1*) showed higher susceptibility to *Agrobacterium* infection (Tao et al. 2004). Other plant proteins belonging to the family of plant cyclophilins, namely RocA, RocB and CypA, have been shown to interact with VirD2 (Deng et al. 1998). Plant cyclophilins represent a large family of proteins (Romano et al. 2004) generally implicated in protein maturation, but with diverse cellular functions. Cyclophilin role in T-DNA nuclear import and (or) integration is still unknown, but these proteins could act by maintaining VirD2 in a conformation compatible with its nuclear import.

VirE2 is also a nuclear protein in plant cells (Citovsky et al. 1992; Citovsky et al. 1994; Ziemienowicz et al. 2001); however, it fails to localize to the cell nucleus in non-plant systems such as yeast and mammalian cells (Citovsky et al. 2004; Guralnick et al. 1996; Rhee et al. 2000; Tzfira and Citovsky 2001; Tzfira et al. 2001). Further analysis has shown that VirE2 does not interact directly with host karyopherin α in a yeast 2-hybrid assay (Tzfira et al. 2001). Instead, VirE2 interacts with another plant protein, VIP1 (VirE2 interacting protein), a basic leucine zipper (bZIP) motif protein known to localize in the nucleus (Tzfira et al. 2001). VIP1 itself contains a conventional NLS, interacts with karyopherin α, and has been suggested to mediate the nuclear import of VirE2 (Citovsky et al. 2004; Tzfira et al. 2001). This latter function was first demonstrated in yeast and animal cells in which VIP1 expression induced nuclear import of GFP-VirE2, and then in plant cells in which lowered expression of VIP1 by antisense technology resulted in impaired nuclear targeting of GUS-VirE2. In the same VIP1-antisense plants, GUS-VirD2 was still nuclear, showing that VIP1 antisense expression did not interfere with the nuclear-import machinery in a non-specific fashion (Tzfira et al. 2001). The formation of ternary complexes in vitro, comprising VIP1, VirE2, and single-stranded DNA (Fig. 1, step 2) (Tzfira et al. 2001) and VirE2, VIP1, and karyopherin α (Fig. 1, step 3) (Citovsky et al. 2004) suggests that VIP1 molecules represent host-derived molecular adaptors between the T-complex and the karyopherin α-mediated nuclear import machinery of the host cell (Citovsky et al. 2004; Tzfira et al. 2001; Ward et al. 2002). The activity of VIP1 was later shown to be critical for VirE2 and T-complex nuclear import, because its over-expression or inhibition modulated the efficiency of T-DNA transfer from bacterial to plant genomes (Tzfira et al. 2002). It was thus suggested that VIP1, and maybe other plant proteins, may represent limiting factors for *Agrobacterium*-mediated transformation of less susceptible plant species (Gelvin 2003*a*).

VirE2 and VirD2 may have partially redundant functions for the nuclear import of T-DNA, as VirE2 was able to mediate nuclear import of single-stranded DNA independently of VirD2 (Gelvin 1998; Zupan et al. 1996), and VirD2 was also able to mediate nuclear import of short-length single-stranded DNA segments in the absence of VirE2 (Ziemienowicz et al. 2001). But, under natural conditions, it is more likely that both VirD2 and VirE2 contribute to the nuclear import of T-DNA. Potentially, VirD2 is sufficient to direct the T-DNA to the nuclear pore (Fig. 1, step 3), whereas VirE2 is required for its passage through the pore (Fig. 1, step 4), by presenting the T-DNA in a structure compatible with entry into the nucleus (Ziemienowicz et al. 2001) and interacting with the host nuclear import machinery to drive the long and rigid molecule of the T-complex (Abu-Arish et al. 2004; Citovsky et al. 1997) through the nuclear pore in a polar manner (Citovsky et al. 1997; Sheng and Citovsky 1996; Tzfira et al. 2000). Thus, the molecular composition of the T-complex may help determine its polarity: its 5′ end carries a molecule of VirD2, whereas the rest of the T-strand length is coated by VirE2 molecules (Sheng and Citovsky 1996). Polarity may represent a common feature of nucleic acid transport through the nuclear pore (Citovsky and Zambryski 1993); for example, nuclear export of a 75S premessenger ribonucleoprotein particle in *Chironomus tentans*

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initiates exclusively at the 5′ end of the RNA (Mehlin et al. 1992).

The rigid 3-dimensional conformation of the T-complex (Abu-Arish et al. 2004; Citovsky et al. 1997) is also thought to be an important structural feature for its transit through the nuclear pore, which would most likely be impractical for a free T-strand in random-coiled form. VirD2, the VIP1/VirE2 complex and, by implication, the entire T-complex are imported into the host nucleus via the karyopherin α-dependent pathway, in which NLS-containing proteins are recognized by karyopherins α (Jans et al. 2000). This pathway, which is widely conserved in eukaryotic organisms, also involves karyopherin β, which binds to the NLS-containing protein-karyopherin α complex and targets it to the nuclear pore (Görlich et al. 1995; Merkle 2001). However, in plants, nuclear import may also occur by a karyopherin β-independent pathway (Hubner et al. 1999); the role of karyopherin β in T-DNA nuclear import has not been examined as yet. Inside the nucleus, release of the imported protein is mediated by Ran, a GTP-binding protein; nonhydrolyzable GTP analogs inhibit this process. Consistent with this conserved model, GTPγS inhibited VirD2 and VirE2 nuclear import in plant cells (Ziemienowicz et al. 2001; Zupan et al. 1996).

A recent report has shown that another translocated *Agrobacterium* protein, VirE3, is able to partially mimic VIP1 activity (Lacroix et al. 2005). Like VIP1, the VirE3 protein interacted with VirE2 and karyopherin α (Fig. 1, step 2), and was able to assist in the nuclear import of VirE2 in animal cells and in VIP1-antisense plant cells. VirE3 itself localized to the nucleus of mammalian and plant cells, as shown by its tagging with green fluorescent protein (GFP); nuclear import was mediated by 2 functional NLSs located in the N-terminal region of the VirE3 protein, which were independently able to mediate import of the fused β-glucuronidase (GUS) reporter protein into the plant-cell nucleus. A mutant of VirE3, impaired in both NLSs, exhibited mainly cytoplasmic localization and was unable to assist in the nuclear import of VirE2 in mammalian cells, while it still interacted with VirE2. Whereas VirE3 is not essential for the transformation of tobacco and *Kalanchoe* leaves in vitro (Kalogeraki et al. 2000; Winans et al. 1987), both of which are highly susceptible to *Agrobacterium* (Hirooka and Kado 1986), it may play a role in transformation of other plant species in which there is no active form of VIP1. This idea is supported by the observation that, in transgenic plants in which *vip1* expression has been reduced by antisense technology and that show reduced susceptibility to *Agrobacterium* (Tzfira et al. 2001), VirE3 over-expression partially restored VirE2 nuclear import and susceptibility to *Agrobacterium*-mediated genetic transformation (Lacroix et al. 2005).

Certain strains of *A. rhizogenes*, which do not encode VirE1 or VirE2, are still able to infect plants and thus to transfer and integrate DNA into the host genome. However, those strains contain a gene encoding the GALLS protein, which has been suggested to fulfill a function similar to VirE2 (Hodges et al. 2004). Indeed, in experiments of mixed infections, the pathogenicity of an *A. tumefaciens* strain mutated in the *vir*E1 and *vir*E2 genes was restored by expression the *GALLS* gene. Although GALLS shows some similarity with TraA, a bacterial strand transferase conjugation protein (Hodges et al. 2004), and also contains NLS sequences, it does not show obvious homology with VirE2, which implies a potentially different mechanism of action for GALLS and VirE2. This example suggests that different species of *Agrobacterium* may have evolved different strategies to ensure T-DNA nuclear import and further integration in the host plant cell.

Nuclear import of *Agrobacterium* T-strand and its associated proteins is not the only example of macromolecules of bacterial origin being translocated from phytopathogenic bacteria to their host plant cells where they are imported to the nucleus by means of the host plant's nuclear-import machinery. For example, members of the AvrBs3 protein family, avirulence proteins encoded by different *Xanthomonas* species and translocated to their host plant cells via a type III secretion system (T3SS), all contain functional NLSs and are likely to function in the nucleus of host plants (Yang and Gabriel 1995). Interaction with karyopherin α and nuclear localization were demonstrated for AvrBs3 from *Xanthomonas campestris* (Szurek et al. 2001), and for AvrXa7 from *X. oryzae* (Yang et al. 2000). Similarly, PopP2, an effector protein exported from *Ralstonia solanacearum* to plant cells, is directed to the host nucleus (Deslandes et al. 2003); moreover, PopP2 is able to mediate nuclear import of a plant protein, RRS1-R. In the case of virus–host cell interactions, it is also common for the virus genome and proteins to be imported into the host-cell nucleus in different structural forms (i.e., as a complete virus or as a nucleic acid molecule partially or completely depleted of its cognate proteins), which generally interact with host factors of the nuclear transport pathways (Whittaker and Helenius 1998; Whittaker et al. 2000).

Uncoating of the T-complex in the host-cell nucleus

After import of the T-complex into the nucleus, several steps are necessary before the event of T-DNA integration into the host genome itself can occur: the coating proteins of the T-complex have to be at least partially removed and the T-complex should be targeted to its site of future integration in the host chromatin. It is still unknown whether T-DNA is directed to its integration site as a whole T-complex or after, at least partial, removal of its protein components.

Recently, the VirF protein of *Agrobacterium* was proposed to play a key role in the T-DNA uncoating (Tzfira et al. 2004*b*). VirF is one of the *Agrobacterium* virulence proteins exported to the plant cell (Vergunst et al. 2000). It was first identified as a host-range factor because it is only necessary for the *Agrobacterium*-mediated genetic transformation of certain host species; moreover, the *vir*F gene is not present in all *Agrobacterium* strains. Specifically, octopine strains of *A. tumefaciens* were virulent on *Nicotiana glauca*, whereas nopaline strains showed a strongly attenuated virulence (Melchers et al. 1990). This difference was due to the absence of a functional VirF in the latter strains, which could be complemented by co-infection with a VirF-containing strain. It was later shown that over-expression of VirF in *N. glauca* transgenic plants restores the virulence of the nopaline strain (which does not possess *vir*F) and an oc-

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topine mutant deficient in VirF (Regensburg-Tuink and Hooykaas 1993). In contrast, VirF appears to be an inhibitor of *Agrobacterium* T-DNA transfer to corn (*Zea mays*), a plant species that is difficult to transform by *Agrobacterium* (Jarchow et al. 1991).

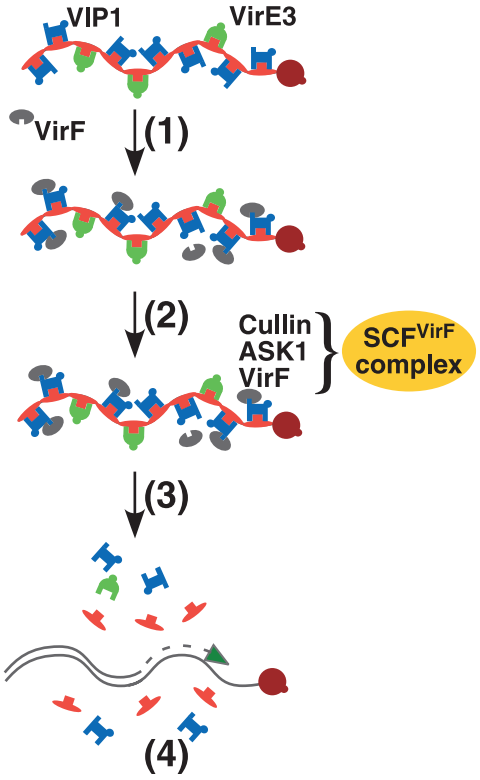
The first step in understanding VirF molecular function was the discovery of its interaction with ASK1 (*Arabidopsis* Skp1-like) proteins, which are plant homologues of the yeast Skp1 protein (Schrammeijer et al. 2001). Skp1 is part of a class of E3 ubiquitin ligases belonging to the SCF (Skp1-Cdc53-F-box protein) complexes, which mediate targeted proteolysis (Cardozo and Pagano 2004). VirF contains an F-box domain found in F-box proteins known as receptors that direct specific target proteins to the SCF complex and ultimately to proteasomal degradation (Kipreos and Pagano 2000). This suggests a role for VirF, as a part of the VirF-containing SCF (SCFVirF) complex, in activating the host pathway for targeted proteolysis during the transformation process. Consistent with the functional role of the F-box domain of VirF in formation of the SCFVirF complex, VirF lacking its N-terminal portion that contains the F-box motif did not interact with ASK1.

The function of VirF in targeting the host and bacterial protein components of the T-complex to degradation was demonstrated directly (Tzfira et al. 2004*b*). VirF interacted with VIP1 (Fig. 2, step 1), but did not interact with VirE2 or VirD2; moreover, VirF, VIP1, VirE2, and ASK1 colocalized in the nucleus of plant cells. VirF-dependent VIP1 degradation in yeast and plant cells was shown by the observation that GFP-tagged VIP1 is destabilized by the over-expression of VirF, most likely via the SCFVirF pathway (Fig. 2, step 2). Furthermore, although expression of VirF itself did not affect the stability of GFP-tagged VirE2, co-expression of VirF and VIP1 destabilized GFP-VirE2, showing a role for VIP1 in bridging between VirE2 and VirF to target VirE2 to degradation by the SCFVirF complex (Fig. 2, step 3) (Tzfira et al. 2004*b*). Ultimately, the uncoated T-strand is converted to a double-stranded form (Fig. 2, step 4) and enters the integration pathway (see below).

That VirF-mediated destabilization of VIP1 and VirE2 indeed occurred by the SCF-dependent mechanism was indicated by the inability of a yeast Skp1 temperature-sensitive mutant, s*kp1-4* (Connelly and Hieter 1996), to support the VirF-mediated destabilization of VIP1 and VirE2 at the restrictive temperature (Tzfira et al. 2004*b*). Moreover, a proteasome inhibitor applied onto *Agrobacterium*-inoculated plant tissues significantly reduced expression of T-DNA, but not of the host cell genes (Tzfira et al. 2004*b*), demonstrating the critical role of proteasomal degradation in *Agrobacterium*-mediated genetic transformation. That VirF is a host-range factor that is not required for genetic transformation of all plant species also suggests that the cellular F-box proteins may fulfill the VirF-like function during the transformation process; indeed, some mutants in F-box protein-encoding genes show decreased susceptibility to *Agrobacterium* infection (Zhu et al. 2003).

Interestingly, VirE2, VirE3, VirD2, and VirD5, but not VirF, contain PEST motifs (i.e., protein sequences enriched in proline, glutamic acid, serine, and threonine residues) that are often present in proteins destined for proteolysis and

Fig. 2. Uncoating of the T-complex in the plant-cell nucleus. Inside the nucleus, VirF associates with the T-complex via its binding to VIP1 (step 1), and recruits the host cell ASK1 and cullin proteins, forming the SCFVirF complex (step 2), which activates proteasomal degradation of VIP1 and VirE2, leading to uncoating of the T-strand (step 3), a step that might be coupled with the synthesis of a second strand on the T-DNA (step 4) and the binding of NHEJ proteins.



suggested to act as proteolytic signals (Rechsteiner and Rogers 1996). The presence of these sequence motifs in most of the exported Vir proteins supports the importance of targeted proteolysis during the *Agrobacterium*-host cell interaction.

T-DNA integration and expression

Little is known about the targeting of the T-DNA to its site of integration within the host chromatin. Although the T-DNA integration is not sequence-specific and overall random (Alonso et al. 2003; Tinland 1996), recent data suggest certain bias for integration within active areas of the chromatin and into the regulatory regions of genes, at least in some plant species (Alonso et al. 2003; Barakat et al. 2000; Chen et al. 2003; Schneeberger et al. 2005). Regardless of the integration preference, the T-DNA must be directed to a site within the host chromatin that is suitable for integration, which likely requires interaction with chromatin or chromatin-associated proteins, and is influenced by the molecular structure of the chromatin. Indeed, the importance of histones and histone-modifying enzymes in T-DNA integration was recently demonstrated (Yi et al. 2002; Zhu et al. 2003). Nevertheless, at precisely which stage of the integration process histones are involved remains unknown (i.e., are they involved in the initial targeting of T-DNA to its po-

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tential site of integration and (or) later, during the integration process itself?). Several *Arabidopsis* mutants in histone and histone deacetylase genes have been identified as rat (resistant to *Agrobacterium*) mutants (Zhu et al. 2003). Specifically, a histone H2A mutant was deficient in T-DNA stable integration but not in its transient expression (Mysore et al. 2000); moreover, the susceptibility of *Arabidopsis* root cells to *Agrobacterium* transformation correlated with the level of expression of the *H2A-1* gene (Yi et al. 2002). As mentioned above, it remains unknown whether the targeting of the T-complex to its site of integration in the host genome occurs before or after the uncoating and conversion of the T-strand to a double-stranded form, and further work is needed to address this question. On the one hand, the finding that VIP1, as a multifunctional protein, may be involved not only in T-DNA nuclear import (Tzfira et al. 2001, 2002; Ward et al. 2002) and T-DNA uncoating (Tzfira et al. 2004*b*), but also in its intranuclear targeting and (or) integration (e.g., by interaction with chromatin components such as core histones (Li et al. 2005; Loyter et al. 2005)) suggests that the uncoating occurs after the T-DNA-host chromatin interaction and prior to integration. On the other hand, recent results strongly suggest that the T-strand is converted to a double-stranded form before its integration (Chilton and Que 2003; Tzfira et al. 2003), which requires prior uncoating of the T-DNA. Thus, uncoating of the T-strand and its second strand synthesis may be carried out by reactions that are coupled to each other and occur near the potential site of integration in the host chromatin.

Integration of foreign DNA generally occurs in double strand breaks (DSBs) within the target DNA of eukaryotic genomes in 2 ways: via illegitimate or non-homologous recombination (NHR) or via homologous recombination (HR), which requires homology between the integrating DNA and the target DNA. The foreign DNA substrate of NHR is generally double-stranded, and its integration involves nonhomologous end-joining (NHEJ) enzymes, whereas HR requires a foreign DNA substrate that is, at least partially, single-stranded and generally involves a mechanism of single-strand gap repair (SSGR) (Critchlow and Jackson 1998; Gorbunova and Levy 1999; Pastink et al. 2001; Valerie and Povirk 2003). Initial studies of T-DNA integration relied on sequence analyses of a few integration sites in plants (Gheysen et al. 1991; Mayerhofer et al. 1991). These studies showed the presence of small deletions in the plant genome DNA and T-DNA and of microhomologies between the T-DNA borders and integration sites in the host genome. The existence of microhomologies, combined with the fact that VirD2 had been reported to possess DNA ligase activity in vivo (Pansegrau et al. 1993), favored a model of SSGR, the mechanism of which requires these features (Tinland 1996). However, mutations in the conserved H-R-Y motif of VirD2, characteristic of DNA ligase, resulted in loss of precision of the T-DNA integration, but not in a decrease in the overall integration efficiency (Tinland et al. 1995). Moreover, another study questioned the ability of VirD2 to act as a DNA ligase (Ziemienowicz et al. 2000). Nevertheless, the possibility that VirD2 is required for the activity of a plant ligase or for the recruitment of a plant ligase to the site of integration cannot be ruled out. The SSGR model was also weakened by later high-throughput analyses of very large numbers of integration junctions, showing that microhomologies are not observed in a consistent and statistically significant manner (Alonso et al. 2003). Finally, several studies revealed the existence of complex patterns of T-DNA integration (De Buck et al. 1999; De Neve et al. 1997). Specifically, multiple T-DNAs could be integrated in direct or reverse orientation relative to each other, with or without filler DNA. The particular case of 2 T-DNA molecules integrated in a head-to-head orientation is not compatible with the SSGR model because head-to-head recombination is not possible for single-stranded DNA, except if it is assumed that the 2 T-DNAs are integrated sequentially and at precisely the same spot into the host genome. However, this possibility does not explain the presence of filler DNA that is frequently observed at the T-DNA integration sites. Filler DNA has also been observed in direct transformation of tobacco plants with plasmid DNA, and the DSB repair mechanism along with the formation of deletions and filler DNA were implicated in this transformation process (Gorbunova and Levy 1997). The induction of DSBs in the plant genome with a rare-cutting DNA endonuclease resulted in the more frequent integration of foreign DNA into these artificially created DSBs (Salomon and Puchta 1998), which was later shown for the specific cases of T-DNA integration (Windels et al. 2003). Interestingly, smaller deletions were observed for T-DNA integration relative to typical DSB repair (Kirik et al. 2000; Orel et al. 2003; Windels et al. 2003), suggesting that T-complex proteins may augment the activity of host DSB repair enzymes and enhance their precision. Direct evidence for T-DNA conversion to a double-stranded form before integration was provided by experiments that used rare-cutting endonuclease sites present in both the host DNA and T-DNA (Chilton and Que 2003; Tzfira et al. 2003). These studies observed integration of truncated T-DNA molecules digested in vivo by the rare-cutting enzyme, and even some instances of precise ligation, reconstituting the endonuclease recognition site. As the restriction endonucleases employed in these studies cut only double-stranded DNA, these results implied that T-DNA had been converted to a double-stranded form before digestion and, thus, before integration. Analysis of the relative frequency of integration also revealed that the DSBs represent the preferred locations for T-DNA integration (Salomon and Puchta 1998; Windels et al. 2003). The latter observation is consistent with the fact that exposing plants to X-rays (known to create DSBs in the genome) enhances the transgene integration (Leskov et al. 2001).

All these results strongly support the hypothesis that double-stranded T-DNA integration at DSB sites present in the host genome is the native mode of T-DNA integration (Chilton and Que 2003; Tzfira et al. 2003), and that DSBs and (or) DSB repair enzymes may act as "baits" to attract the invading T-DNA molecules to the sites of their integration. Indeed, in mammalian cells, proteins involved in DSB repair are attracted to artificially created DSBs, both in the case of the Mre11 complexes implicated in HR (Mirzoeva and Petrini 2001) and in the case of the XRCC4 and DNA ligase IV complexes implicated in NHR (Drouet et al. 2005). GFP fusions with Ku70 and Ku80, both involved in NHR, showed their high mobility within the nucleus, suggesting a rapid flux of these proteins between their nuclear

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substrates and a transient association with the nuclear matrix (Rodgers et al. 2002). The mobilization of DSB repair machinery may be mediated by the histone modifications (particularly phosphorylation of H2A) generally occurring at the DSB sites (Pilch et al. 2003; Shroff et al. 2004; Unal et al. 2004; van Attikum et al. 2004). This transport of the DSB repair enzymes most likely represents a general process of intranuclear protein traffic (Phair and Misteli 2000) that also allows transcription factors and other transcription-machinery proteins to reach their target promoters (Zaidi et al. 2004, 2005).

The involvement of DSB repair enzymes, whether they be the NHEJ enzymes involved in NHR or the enzymes involved in HR, in the process of T-DNA integration was further examined by taking advantage of *Saccharomyces cerevisiae* as a host for *Agrobacterium* T-DNA integration (Bundock et al. 1995). This system is unique because, in yeast, T-DNA integration may occur by HR or illegitimately by NHR, depending upon the presence or absence, respectively, of sequences in the T-DNA that share homology with the host genomic DNA (Bundock and Hooykaas 1996; Bundock et al. 1999). Moreover, numerous mutants in genes coding for proteins involved in these 2 DNA recombination pathways are available in yeast. The studies of T-DNA integration in yeast cells allowed the identification of proteins involved in both integration pathways. Two key enzymes were identified: Rad52, a single-stranded DNA-binding protein necessary for HR (van Attikum et al. 2001), and Ku70, a double-stranded DNA-binding protein that functions in the form of a heterodimer complex with Ku80, required for NHR (van Attikum and Hooykaas 2003). Mutants in each of these genes were impaired in T-DNA integration by HR and NHR, respectively, whereas a double mutant showed no T-DNA integration at all. Involvement of other cellular proteins in each of these pathways was also demonstrated (van Attikum et al. 2001; van Attikum and Hooykaas 2003).

In higher plants, the main pathway of foreign DNA integration is by illegitimate recombination (i.e., NHR), whereas HR occurs only at an extremely low rate (Gheysen et al. 1991; Mayerhofer et al. 1991; Terada et al. 2002). When several available *Arabidopsis* mutants in DSB repair enzymes were analyzed for T-DNA integration, the *Arabidopsis* ligase AtLig4 was not required (Friesner and Britt 2003; van Attikum et al. 2003), whereas AtKu80 was reported to be either required or dispensable for T-DNA integration, depending upon the *Agrobacterium* inoculation method employed (Friesner and Britt 2003; Gallego et al. 2003). These recent insights into the mechanism of T-DNA integration have led to a new model for this process in the plant hosts (Tzfira et al. 2004*a*), described in Fig. 3. Recent results, obtained in our laboratory (J.L, unpublished data), tend to confirm the importance of the Ku80 complex in T-DNA integration. Indeed, in a root-transformation assay, an *Arabidopsis* Ku80 mutant was resistant to stable T-DNA expression, occurring from the integrated T-DNA (Janssen and Gardner 1990), whereas transient T-DNA expression that resulted from T-DNA nuclear import and expression, but without integration into the host genome (Janssen and Gardner 1990; Nam et al. 1999) was not affected in these plants. Moreover, Ku80 over-expression in *Arabidopsis* increased T-DNA integration efficiency. Chromatin immunoprecipitation directly confirmed binding of Ku80 to double-stranded T-DNA (J.L, unpublished data). Taken together, these studies of the involvement of NHEJ enzymes in the T-DNA integration process suggest a mechanism that is more complicated in plants than in yeast and the likely involvement of other, as yet unidentified, plant proteins in the integration process. Interestingly, the presence of a "backup", independent of Ku70/Ku80, NHEJ pathway was recently demonstrated in human cells (Wang et al. 2003) and could exist in plants as well. As described above, recent reports in which large numbers of T-DNA integration sites were analyzed, indicated a bias in T-DNA integration sites toward intergenic regions and regulatory regions of genes in some

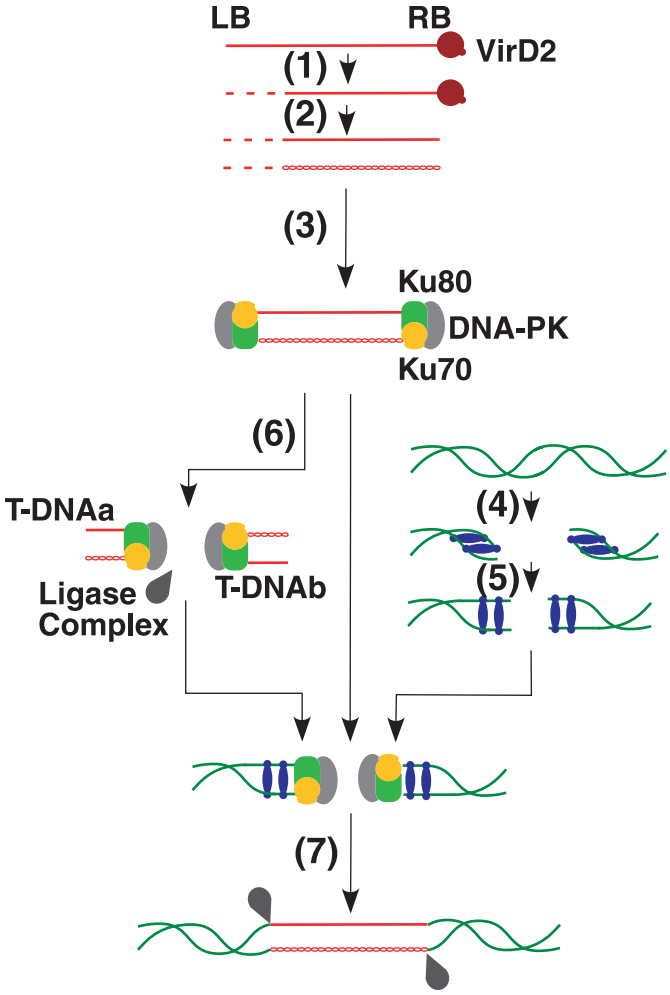

Fig. 3. Integration of the T-DNA into the host-plant genome. The uncoated T-strand can undergo partial degradation in the nucleus (step 1), and then becomes converted to a double-stranded form (step 2). In the NHR pathway, the DNA–protein kinase (PK) complex, composed of the Ku70-Ku80 heterodimer associated with DNA-PK, binds to the free double-stranded ends of the T-DNA (step 3), and is directed to DSBs formed in the host genome (step 4, step 5), often after several T-DNA molecules have been ligated to each other in different orientations (step 6). The integrating double-stranded T-DNA molecule(s) are ligated to the ends of the DSB via the activity of plant DNA ligases (step 7). (From Tzfira et al. (2004*a*), reproduced and modified with permission of Trends Genet., Vol. 20, pp. 375–383, © 2004 Elsevier.).

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plant hosts (Alonso et al. 2003; Barakat et al. 2000; Chen et al. 2003; Schneeberger et al. 2005), which represent areas of active transcription and chromatin decondensation. In mammalian cells, decondensed chromatin has been shown to be more susceptible to DSB-inducing agents (Oleinick et al. 1994); by analogy, plant DNA may also have higher frequency of DSBs in these areas of active, decondensed chromatin, which would explain their higher susceptibility to T-DNA integration.

Genes encoded by the T-DNA are expressed in the plant cell either transiently if the T-DNA is not integrated or stably after T-DNA integration (Janssen and Gardner 1990; Nam et al. 1999). This implies that genes of a bacterial origin contain regulatory sequences recognized by the eukaryotic transcription/translation machinery of the host plant. Indeed, typical eukaryotic regulatory sequences such as TATA box, CAAT box, and polyadenylation signal sequences are found in the flanking regions of genes contained within the T-DNA (Barker et al. 1983). Another type of regulatory sequences found both in the bacterial genes of the T-DNA (Ellis et al. 1987) and in the plant genes (Ellis et al. 1993) as well as in the genes of several plant viruses (Bouchez et al. 1989) are the ocs-elements that comprise a family of 20-bp sequences located less than 200 bp from the TATA box (Bouchez et al. 1989; Ellis et al. 1987). Moreover, T-DNA transcription is mediated by plant RNA polymerase II (Willmitzer et al. 1981), and the resulting mRNA is polyadenylated (Gelvin et al. 1982). On the other hand, regulatory sequences of T-DNA genes are able to respond to eukaryotic regulatory signals from plant cells; for example, nopaline synthase promoter is induced by host cell wounding and plant hormone auxin (An et al. 1990).

Conclusions

Similar to many other pathogens, *Agrobacterium* uses host cellular mechanisms and pathways for infection. More specifically, the transfer and integration of T-DNA into the host genome requires interaction with the host nucleus at different levels. The bacterial effector proteins involved in this process contain some typical eukaryotic domains involved in their interactions with host factors such as NLSs recognized by plant importins and F-box and PEST domains recognized by the targeted proteolysis machinery of the host cell. These domains of the bacterial effectors may have evolved by a mechanism of convergent evolution that renders their homology-based functional annotation difficult (Nagai and Roy 2003).

Despite several decades of intensive studies, many key steps of the T-DNA transfer and integration remain poorly understood and require further investigation. Most notably is the targeting of T-DNA to its integration site and the mechanism of integration as well as the biological function of some *Agrobacterium* effector proteins translocated to the host cell. For example, the VirD5 protein that is exported into the host cell (Vergunst et al. 2005) and accumulates in the plant cell nucleus (B.L. and V.C., unpublished data) function remains completely unknown. Furthermore, it is possible that *Agrobacterium* encodes many other, as yet unidentified, effector proteins that are transported into the host cell to participate in cellular processes required for infection and genetic transformation. Future experiments will provide critical insights into these questions. Besides providing new and exciting insights into the fundamental mechanisms of genetic transformation of eukaryotic cells, a better understanding of the T-DNA transfer from *Agrobacterium* to the host genome is essential for improving the biotechnological applications of this microorganism as a gene vector for genetic modification of plant and non-plant species.

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References

Abu-Arish, A., Frenkiel-Krispin, D., Fricke, T., Tzfira, T., Citovsky, V., Grayer Wolf, S., and Elbaum, M. 2004. Three-dimensional reconstruction of *Agrobacterium* VirE2 protein with single-stranded DNA. J. Biol. Chem. **279**: 25359–25363. doi:10.1074/jbc.M401804200. PMID: 15054095.

Alonso, J.M., Stepanova, A.N., Leisse, T.J., Kim, C.J., Chen, H., Shinn, P., et al. 2003. Genome-wide insertional mutagenesis of *Arabidopsis thaliana.* Science, **301**: 653–657. doi:10.1126/science.1086391. PMID: 12893945.

An, G., Costa, M.A., and Ha, S.B. 1990. Nopaline synthase promoter is wound inducible and auxin inducible. Plant Cell, **2**: 225–233. doi:10.1105/tpc.2.3.225. PMID: 2152114.

Ballas, N., and Citovsky, V. 1997. Nuclear localization signal binding protein from *Arabidopsis* mediates nuclear import of *Agrobacterium* VirD2 protein. Proc. Natl. Acad. Sci. U.S.A. **94**: 10723–10728. doi:10.1073/pnas.94.20.10723. PMID: 9380702.

Barakat, A., Gallois, P., Raynal, M., Mestre-Ortega, D., Sallaud, C., Guiderdoni, E., Delseny, M., and Bernardi, G. 2000. The distribution of T-DNA in the genomes of transgenic *Arabidopsis* and rice. FEBS Lett. **471**: 161–164. doi:10.1016/S0014-5793(00)01393-4. PMID: 10767414.

Barker, R.F., Idler, K.B., Thompson, D.V., and Kemp, J.D. 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi 15955. Plant Mol. Biol. **2**: 335–350. doi:10.1007/BF01578595.

Bouchez, D., Tokuhisa, J.G., Llewellyn, D.J., Dennis, E.S., and Ellis, J.G. 1989. The ocs-element is a component of the promoters of several T-DNA and plant viral genes. EMBO J. **8**: 4197–4204. PMID: 2591372.

Bundock, P., and Hooykaas, P.J.J. 1996. Integration of *Agrobacterium tumefaciens* T-DNA in the *Saccharomyces cerevisiae* genome by illegitimate recombination. Proc. Natl. Acad. Sci. U.S.A. **93**: 15272–15275. doi:10.1073/pnas.93.26.15272. PMID: 8986800.

Bundock, P., den Dulk-Ras, A., Beijersbergen, A., and Hooykaas, P.J.J. 1995. *Trans*-kingdom T-DNA transfer from *Agrobacterium tumefaciens* to *Saccharomyces cerevisiae.* EMBO J. **14**: 3206–3214. PMID: 7621833.

Bundock, P., Mroczek, K., Winkler, A.A., Steensma, H.Y., and Hooykaas, P.J. 1999. T-DNA from *Agrobacterium tumefaciens* as an efficient tool for gene targeting in *Kluyveromyces lactis.* Mol. Gen. Genet. **261**: 115–121. PMID: 10071217.

© 2006 NRC Canada

Cardozo, T., and Pagano, M. 2004. The SCF ubiquitin ligase: insights into a molecular machine. Nat. Rev. Mol. Cell Biol. **5**: 739–751. doi:10.1038/nrm1471. PMID: 15340381.

Chen, S., Jin, W., Wang, M., Zhang, F., Zhou, J., Jia, Q., et al. 2003. Distribution and characterization of over 1000 T-DNA tags in rice genome. Plant J. **36**: 105–113. doi:10.1046/j.1365-313X.2003.01860.x. PMID: 12974815.

Chilton, M.-D.M., and 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. doi:10.1104/pp.103.026104. PMID: 14551336.

Chook, Y.M., and Blobel, G. 2001. Karyopherins and nuclear import. Curr. Opin. Struct. Biol. **11**: 703–715. doi:10.1016/S0959-440X(01)00264-0. PMID: 11751052.

Christie, P.J. 2004. Type IV secretion: the *Agrobacterium* VirB/D4 and related conjugation systems. Biochim. Biophys. Acta, **1694**: 219–234. PMID: 15546668.

Citovsky, V., and Zambryski, P.C. 1993. Transport of nucleic acids through membrane channels: snaking through small holes. Annu. Rev. Microbiol. **47**: 167–197. doi:10.1146/annurev.mi.47.100193.001123. PMID: 8257097.

Citovsky, V., Wong, M.L., and Zambryski, P.C. 1989. Cooperative interaction of *Agrobacterium* VirE2 protein with single stranded DNA: implications for the T-DNA transfer process. Proc. Natl. Acad. Sci. U.S.A. **86**: 1193–1197. PMID: 2919168.

Citovsky, V., Zupan, J., Warnick, D., and Zambryski, P.C. 1992. Nuclear localization of *Agrobacterium* VirE2 protein in plant cells. Science, **256**: 1802–1805. PMID: 1615325.

Citovsky, V., Warnick, D., and Zambryski, P.C. 1994. Nuclear import of *Agrobacterium* VirD2 and VirE2 proteins in maize and tobacco. Proc. Natl. Acad. Sci. U.S.A. **91**: 3210–3214. PMID: 8159726.

Citovsky, V., Guralnick, B., Simon, M.N., and Wall, J.S. 1997. The molecular structure of *Agrobacterium* VirE2-single stranded DNA complexes involved in nuclear import. J. Mol. Biol. **271**: 718–727. doi:10.1006/jmbi.1997.1230. PMID: 9299322.

Citovsky, V., Kapelnikov, A., Oliel, S., Zakai, N., Rojas, M.R., Gilbertson, R.L., Tzfira, T., and 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. doi:10.1074/jbc.M403159200. PMID: 15123622.

Connelly, C., and Hieter, P. 1996. Budding yeast *SKP1* encodes an evolutionarily conserved kinetochore protein required for cell cycle progression. Cell, **86**: 275–285. doi:10.1016/S0092-8674(00)80099-9. PMID: 8706132.

Critchlow, S.E., and Jackson, S.P. 1998. DNA end-joining: from yeast to man. Trends Biochem. Sci. **23**: 394–398. doi:10.1016/S0968-0004(98)01284-5. PMID: 9810228.

De Buck, S., Jacobs, A., Van Montagu, M., and Depicker, A. 1999. The DNA sequences of T-DNA junctions suggest that complex T-DNA loci are formed by a recombination process resembling T-DNA integration. Plant J. **20**: 295–304. PMID: 10571890.

de Groot, M.J., Bundock, P., Hooykaas, P.J.J., and Beijersbergen, A.G. 1998. *Agrobacterium tumefaciens*-mediated transformation of filamentous fungi. Nat. Biotechnol. **16**: 839–842[published erratum appears in Nat. Biotechnol. 16, 1074 (1998)]. doi:10.1038/nbt0998-839. PMID: 9743116.

De Neve, M., De Buck, S., Jacobs, A., Van Montagu, M., and 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. PMID: 9025300.

Deng, W., Chen, L., Wood, D.W., Metcalfe, T., Liang, X., Gordon, M.P., Comai, L., and Nester, E.W. 1998. *Agrobacterium* VirD2 protein interacts with plant host cyclophilins. Proc. Natl. Acad. Sci. U.S.A. **95**: 7040–7045. doi:10.1073/pnas.95.12.7040. PMID: 9618535.

Deng, W., Chen, L., Peng, W.T., Liang, X., Sekiguchi, S., Gordon, M.P., Comai, L., and Nester, E.W. 1999. VirE1 is a specific molecular chaperone for the exported single-stranded-DNA-binding protein VirE2 in *Agrobacterium.* Mol. Microbiol. **31**: 1795–1807. doi:10.1046/j.1365-2958.1999.01316.x. PMID: 10209751.

Deslandes, L., Olivier, J., Peeters, N., Feng, D.X., Khounlotham, M., Boucher, C., et al. 2003. Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc. Natl. Acad. Sci. U.S.A. **100**: 8024–8029. doi:10.1073/pnas.1230660100. PMID: 12788974.

Drouet, J., Delteil, C., Lefrancois, J., Concannon, P., Salles, B., and Calsou, P. 2005. DNA-dependent protein kinase and XRCC4-DNA ligase IV mobilization in the cell in response to DNA double strand breaks. J. Biol. Chem. **280**: 7060–7069. doi:10.1074/jbc.M410746200. PMID: 15520013.

Ellis, J.G., Llewellyn, D.J., Walker, J.C., Dennis, E.S., and Peacock, W.J. 1987. The ocs element: A 16 base pair palindrome essential for activity of the octopine synthase enhancer. EMBO J. **6**: 3203–3208. PMID: 16453801.

Ellis, J.G., Tokuhisa, J.G., Llewellyn, D.J., Bouchez, D., Singh, K., Dennis, E.S., and Peacock, W.J. 1993. Does the ocs-element occur as a functional component of the promoters of plant genes? Plant J. **4**: 433–443. doi:10.1046/j.1365-313X.1993.04030433.x. PMID: 8220489.

Escobar, M.A., and Dandekar, A.M. 2003. *Agrobacterium tumefaciens* as an agent of disease. Trends Plant Sci. **8**: 380–386. doi:10.1016/S1360-1385(03)00162-6. PMID: 12927971.

Forbes, D.J. 1992. Structure and function of the nuclear pore complex. Annu. Rev. Cell Biol. **8**: 495–527. doi:10.1146/annurev.cb.08.110192.002431. PMID: 1282353.

Friesner, J., and Britt, A.B. 2003. Ku80- and DNA ligase IV-deficient plants are sensitive to ionizing radiation and defective in T-DNA integration. Plant J. **34**: 427–440. doi:10.1046/j.1365-313X.2003.01738.x. PMID: 12753583.

Gallego, M.E., Bleuyard, J.Y., Daoudal-Cotterell, S., Jallut, N., and White, C.I. 2003. Ku80 plays a role in non-homologous recombination but is not required for T-DNA integration in *Arabidopsis.* Plant J. **35**: 557–565. doi:10.1046/j.1365-313X.2003.01827.x. PMID: 12940949.

Gelvin, S.B. 1998. *Agrobacterium* VirE2 proteins can form a complex with T strands in the plant cytoplasm. J. Bacteriol. **180**: 4300–4302. PMID: 9696783.

Gelvin, S.B. 2000. *Agrobacterium* and plant genes involved in T-DNA transfer and integration. Annu. Rev. Plant Physiol. Plant Mol. Biol. **51**: 223–256. doi:10.1146/annurev.arplant.51.1.223. PMID: 15012192.

Gelvin, S.B. 2003*a*. Improving plant genetic engineering by manipulating the host. Trends Biotechnol. **21**: 95–98. doi:10.1016/S0167-7799(03)00005-2. PMID: 12628361.

Gelvin, S.B. 2003*b*. *Agrobacterium*-mediated plant transformation: the biology behind the "gene-jockeying" tool. Microbiol. Mol. Biol. Rev. **67**: 16–37. PMID: 12626681.

Gelvin, S.B., Thomashow, M.F., McPherson, J.C., Gordon, M.P., and Nester, E.W. 1982. Sizes and map positions of several plasmid-DNA-encoded transcripts in octopine-type crown gall tumors. Proc. Natl. Acad. Sci. U.S.A. **79**: 76–80. PMID: 6275392.

Gheysen, G., Villarroel, R., and Van Montagu, M. 1991. Illegitimate recombination in plants: a model for T-DNA integration. Genes Dev. **5**: 287–297. PMID: 1995418.

© 2006 NRC Canada

Goldfarb, D.S., Corbett, A.H., Mason, D.A., Harreman, M.T., and Adam, S.A. 2004. Importin alpha: a multipurpose nuclear-transport receptor. Trends Cell Biol. **14**: 505–514. doi:10.1016/j.tcb.2004.07.016. PMID: 15350979.

Gorbunova, V., and Levy, A.A. 1997. Non-homologous DNA end joining in plant cells is associated with deletions and filler DNA insertions. Nucleic Acids Res. **25**: 4650–4657. doi:10.1093/nar/25.22.4650. PMID: 9358178.

Gorbunova, V., and Levy, A.A. 1999. How plants make ends meet: DNA double-strand break repair. Trends Plant Sci. **4**: 263–269. doi:10.1016/S1360-1385(99)01430-2. PMID: 10407442.

Görlich, D., Vogel, F., Mills, A.D., Hartmann, E., and Laskey, R.A. 1995. Distinct functions for the two importin subunits in nuclear protein import. Nature, **377**: 246–248. PMID: 7675110.

Guralnick, B., Thomsen, G., and Citovsky, V. 1996. Transport of DNA into the nuclei of *Xenopus* oocytes by a modified VirE2 protein of *Agrobacterium.* Plant Cell, **8**: 363–373. doi:10.1105/tpc.8.3.363. PMID: 8721747.

Herrera-Estrella, A., Van Montagu, M., and Wang, K. 1990. A bacterial peptide acting as a plant nuclear targeting signal: the amino-terminal portion of *Agrobacterium* VirD2 protein directs a beta-galactosidase fusion protein into tobacco nuclei. Proc. Natl. Acad. Sci. U.S.A. **87**: 9534–9537. PMID: 2124696.

Hirooka, T., and Kado, C.I. 1986. Location of the right boundary of the virulence region on *Agrobacterium tumefaciens* plasmid pTiC58 and a host specifying gene next to the boundary. J. Bacteriol. **168**: 237–243. PMID: 3019998.

Hodges, L.D., Cuperus, J., and Ream, W. 2004. *Agrobacterium rhizogenes* GALLS protein substitutes for *Agrobacterium tumefaciens* single-stranded DNA-binding protein VirE2. J. Bacteriol. **186**: 3065–3077. doi:10.1128/JB.186.10.3065-3077.2004. PMID: 15126468.

Howard, E.A., Zupan, J.R., Citovsky, V., and Zambryski, P.C. 1992. The VirD2 protein of *A. tumefaciens* contains a C-terminal bipartite nuclear localization signal: implications for nuclear uptake of DNA in plant cells. Cell, **68**: 109–118. doi:10.1016/0092-8674(92)90210-4. PMID: 1732061.

Hubner, S., Smith, H.M.S., Hu, W., Chan, C.K., Rihs, H.P., Paschal, B.M., Raikhel, N.V., and Jans, D.A. 1999. Plant importin alpha binds nuclear localization sequences with high affinity and can mediate nuclear import independent of importin beta. J. Biol. Chem. **274**: 22610–22617. doi:10.1074/jbc.274.32.22610. PMID: 10428841.

Jans, D.A., Xiao, C.Y., and Lam, M.H. 2000. Nuclear targeting signal recognition: a key control point in nuclear transport? Bioessays, **22**: 532–544. doi:10.1002/(SICI)1521-1878(200006)22:6<532::AID-BIES6>3.0.CO;2-O. PMID: 10842307.

Janssen, B.J., and Gardner, R.C. 1990. Localized transient expression of GUS in leaf discs following cocultivation with *Agrobacterium.* Plant Mol. Biol. **14**: 61–72. doi:10.1007/BF00015655. PMID: 2101312.

Jarchow, E., Grimsley, N.H., and Hohn, B. 1991. *virF*, the host range-determining virulence gene of *Agrobacterium tumefaciens*, affects T-DNA transfer to *Zea mays.* Proc. Natl. Acad. Sci. U.S.A. **88**: 10426–10430. PMID: 11607242.

Kalogeraki, V.S., Zhu, J., Stryker, J.L., and Winans, S.C. 2000. The right end of the *vir* region of an octopine-type Ti plasmid contains four new members of the *vir* regulon that are not essential for pathogenesis. J. Bacteriol. **182**: 1774–1778. doi:10.1128/JB.182.6.1774-1778.2000. PMID: 10692388.

Kipreos, E.T., and Pagano, M. 2000. The F-box protein family. Genome Biol. **1**: REVIEWS3002. doi:10.1186/gb-2000-1-5-reviews3002. PMID: 11178263.

Kirik, A., Salomon, S., and Puchta, H. 2000. Species-specific double-strand break repair and genome evolution in plants. EMBO J. **19**: 5562–5566. doi:10.1093/emboj/19.20.5562. PMID: 11032823.

Kunik, T., Tzfira, T., Kapulnik, Y., Gafni, Y., Dingwall, C., and Citovsky, V. 2001. Genetic transformation of HeLa cells by *Agrobacterium.* Proc. Natl. Acad. Sci. U.S.A. **98**: 1871–1876. doi:10.1073/pnas.041327598. PMID: 11172043.

Lacroix, B., Vaidya, M., Tzfira, T., and Citovsky, V. 2005. The VirE3 protein of *Agrobacterium* mimics a host cell function required for plant genetic transformation. EMBO J. **24**: 428–437. doi:10.1038/sj.emboj.7600524. PMID: 15616576.

Leskov, K.S., Criswell, T., Antonio, S., Li, J., Yang, C.R., Kinsella, T.J., and Boothman, D.A. 2001. When X-ray-inducible proteins meet DNA double strand break repair. Semin. Radiat. Oncol. **11**: 352–372. doi:10.1053/srao.2001.26912. PMID: 11677660.

Li, J., Krichevsky, A., Vaidya, M., Tzfira, T., and 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. U.S.A. **102**: 5733–5738. doi:10.1073/pnas.0404118102. PMID: 15824315.

Loyter, A., Rosenbluh, J., Zakai, N., Li, J., Kozlovsky, S.V., Tzfira, T., and 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. doi:10.1104/pp.105.062547. PMID: 16010006.

Mayerhofer, R., Koncz-Kalman, Z., Nawrath, C., Bakkeren, G., Crameri, A., Angelis, K., et al. 1991. T-DNA integration: a mode of illegitimate recombination in plants. EMBO J. **10**: 697–704. PMID: 2001683.

Mehlin, H., Daneholt, B., and Skoglund, U. 1992. Translocation of a specific premessenger ribonucleoprotein particle through the nuclear pore studied with electron microscope tomography. Cell, **69**: 605–613. doi:10.1016/0092-8674(92)90224-Z. PMID: 1586943.

Melchers, L.S., Maroney, M.J., den Dulk-Ras, A., Thompson, D.V., van Vuuren, H.A., Schilperoort, R.A., and Hooykaas, P.J.J. 1990. Octopine and nopaline strains of *Agrobacterium tumefaciens* differ in virulence; molecular characterization of the *virF* locus. Plant Mol. Biol. **14**: 249–259. doi:10.1007/BF00018565. PMID: 2101693.

Merkle, T. 2001. Nuclear import and export of proteins in plants: a tool for the regulation of signalling. Planta, **213**: 499–517. doi:10.1007/s004250100621. PMID: 11556782.

Michielse, C.B., Hooykaas, P.J., van den Hondel, C.A., and Ram, A.F. 2005. *Agrobacterium*-mediated transformation as a tool for functional genomics in fungi. Curr. Genet. **48**: 1–17. doi:10.1007/s00294-005-0578-0. PMID: 15889258.

Mirzoeva, O.K., and Petrini, J.H. 2001. DNA damage-dependent nuclear dynamics of the Mre11 complex. Mol. Cell. Biol. **21**: 281–288. doi:10.1128/MCB.21.1.281-288.2001. PMID: 11113202.

Mysore, K.S., Nam, J., and Gelvin, S.B. 2000. An Arabidopsis histone H2A mutant is deficient in Agrobacterium T-DNA integration. Proc. Natl. Acad. Sci. U.S.A. **97**: 948–953. doi:10.1073/pnas.97.2.948. PMID: 10639185.

Nagai, H., and Roy, C.R. 2003. Show me the substrates: modulation of host cell function by type IV secretion systems. Cell. Microbiol. **5**: 373–383. doi:10.1046/j.1462-5822.2003.00285.x. PMID: 12780775.

Nam, J., Mysore, K.S., Zheng, C., Knue, M.K., Matthysse, A.G., and Gelvin, S.B. 1999. Identification of T-DNA tagged *Arabidopsis* mutants that are resistant to transformation by *Agrobacterium.* Mol. Gen. Genet. **261**: 429–438. PMID: 10323222.

Newell, C.A. 2000. Plant transformation technology. Developments and applications. Mol. Biotechnol. **16**: 53–65. doi:10.1385/MB:16:1:53. PMID: 11098468.

© 2006 NRC Canada

Nilsson, O., and Olsson, O. 1997. Getting tothe root: the role of the *Agrobacterium rhizogenes rol* genes in the formation of hairy roots. Physiol. Plant. **100**: 463–473. doi:10.1034/j.1399-3054.1997.1000307.x.

Oleinick, N.L., Balasubramaniam, U., Xue, L., and Chiu, S. 1994. Nuclear structure and the microdistribution of radiation damage in DNA. Int. J. Radiat. Biol. **66**: 523–529. PMID: 7983440.

Orel, N., Kyryk, A., and Puchta, H. 2003. Different pathways of homologous recombination are used for the repair of double-strand breaks within tandemly arranged sequences in the plant genome. Plant J. **35**: 604–612. doi:10.1046/j.1365-313X.2003.01832.x. PMID: 12940953.

Otten, L., DeGreve, H., Leemans, J., Hain, R., Hooykass, P., and Schell, J. 1984. Restoration of virulence of *vir* region mutants of *A. tumefaciens* strain B6S3 by coinfection with normal and mutant *Agrobacterium* strains. Mol. Gen. Genet. **195**: 159–163. doi:10.1007/BF00332739.

Pansegrau, W., Schoumacher, F., Hohn, B., and Lanka, E. 1993. Site-specific cleavage and joining of single-stranded DNA by VirD2 protein of *Agrobacterium tumefaciens* Ti plasmids: analogy to bacterial conjugation. Proc. Natl. Acad. Sci. U.S.A. **90**: 11538–11542. PMID: 8265585.

Pante, N., and Kann, M. 2002. Nuclear pore complex is able to transport macromolecules with diameters of about 39 nm. Mol. Biol. Cell, **13**: 425–434. doi:10.1091/mbc.01-06-0308. PMID: 11854401.

Pastink, A., Eeken, J.C., and Lohman, P.H. 2001. Genomic integrity and the repair of double-strand DNA breaks. Mutat. Res. **480–481**: 37–50. PMID: 11506797.

Phair, R.D., and Misteli, T. 2000. High mobility of proteins in the mammalian cell nucleus. Nature, **404**: 604–609. PMID: 10766243.

Piers, K.L., Heath, J.D., Liang, X., Stephens, K.M., and Nester, E.W. 1996. *Agrobacterium tumefaciens*-mediated transformation of yeast. Proc. Natl. Acad. Sci. U.S.A. **93**: 1613–1618. doi:10.1073/pnas.93.4.1613. PMID: 8643679.

Pilch, D.R., Sedelnikova, O.A., Redon, C., Celeste, A., Nussenzweig, A., and Bonner, W.M. 2003. Characteristics of gamma-H2AX foci at DNA double-strand breaks sites. Biochem. Cell Biol. **81**: 123–129. doi:10.1139/o03-042. PMID: 12897845.

Powers, M.A., and Forbes, D.J. 1994. Cytosolic factors in nuclear import: what's importin? Cell, **79**: 931–934. doi:10.1016/0092-8674(94)90024-8. PMID: 8001141.

Rechsteiner, M., and Rogers, S.W. 1996. PEST sequences and regulation by proteolysis. Trends Biochem. Sci. **21**: 267–271. doi:10.1016/0968-0004(96)10031-1. PMID: 8755249.

Regensburg-Tuink, A.J., and Hooykaas, P.J.J. 1993. Transgenic *N. glauca* plants expressing bacterial virulence gene virF are converted into hosts for nopaline strains of *A. tumefaciens.* Nature, **363**: 69–71. PMID: 8479538.

Relic, B., Andjelkovic, M., Rossi, L., Nagamine, Y., and Hohn, B. 1998. Interaction of the DNA modifying proteins VirD1 and VirD2 of *Agrobacterium tumefaciens*: analysis by subcellular localization in mammalian cells. Proc. Natl. Acad. Sci. U.S.A. **95**: 9105–9110. PMID: 9689041.

Rhee, Y., Gurel, F., Gafni, Y., Dingwall, C., and Citovsky, V. 2000. A genetic system for detection of protein nuclear import and export. Nat. Biotechnol. **18**: 433–437. PMID: 10748526.

Rodgers, W., Jordan, S.J., and Capra, J.D. 2002. Transient association of Ku with nuclear substrates characterized using fluorescence photobleaching. J. Immunol. **168**: 2348–2355. PMID: 11859125.

Romano, P.G., Horton, P., and Gray, J.E. 2004. The *Arabidopsis* cyclophilin gene family. Plant Physiol. **134**: 1268–1282. doi:10.1104/pp.103.022160. PMID: 15051864.

Salomon, S., and Puchta, H. 1998. Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. EMBO J. **17**: 6086–6095. doi:10.1093/emboj/17.20.6086. PMID: 9774352.

Schneeberger, R.G., Zhang, K., Taratinova, T., Troukhan, M., Kwok, S.F., Drais, J., et al. 2005. *Agrobacterium* T-DNA integration in *Arabidopsis* is correlated with DNA sequence compositions that occur frequently in gene promoter regions. Funct. Integr. Genomics, **5**: 240–253. doi:10.1007/s10142-005-0138-1. PMID: 15744539.

Schrammeijer, B., Risseeuw, E., Pansegrau, W., Regensburg-Tuïnk, T.J.G., Crosby, W.L., and Hooykaas, P.J.J. 2001. Interaction of the virulence protein VirF of *Agrobacterium tumefaciens* with plant homologs of the yeast Skp1 protein. Curr. Biol. **11**: 258–262. doi:10.1016/S0960-9822(01)00069-0. PMID: 11250154.

Sheng, J., and Citovsky, V. 1996. *Agrobacterium*-plant cell interaction: have virulence proteins - will travel. Plant Cell, **8**: 1699–1710. doi:10.1105/tpc.8.10.1699. PMID: 8914322.

Shroff, R., Arbel-Eden, A., Pilch, D., Ira, G., Bonner, W.M., Petrini, J.H., Haber, J.E., and Lichten, M. 2004. Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. Curr. Biol. **14**: 1703–1711. doi:10.1016/j.cub.2004.09.047. PMID: 15458641.

Sundberg, C.D., and Ream, L.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. PMID: 10542192.

Sundberg, C., Meek, L., Carrol, K., Das, A., and Ream, L.W. 1996. VirE1 protein mediates export of single-stranded DNA binding protein VirE2 from *Agrobacterium tumefaciens* into plant cells. J. Bacteriol. **178**: 1207–1212. PMID: 8576060.

Szurek, B., Marois, E., Bonas, U., and Van den Ackerveken, G. 2001. Eukaryotic features of the *Xanthomonas* type III effector AvrBs3: protein domains involved in transcriptional activation and the interaction with nuclear import receptors from pepper. Plant J. **26**: 523–534. doi:10.1046/j.0960-7412.2001.01046.x. PMID: 11439138.

Tao, Y., Rao, P.K., Bhattacharjee, S., and Gelvin, S.B. 2004. Expression of plant protein phosphatase 2C interferes with nuclear import of the *Agrobacterium* T-complex protein VirD2. Proc. Natl. Acad. Sci. U.S.A. **101**: 5164–5169. doi:10.1073/pnas.0300084101. PMID: 15047887.

Terada, R., Urawa, H., Inagaki, Y., Tsugane, K., and Iida, S. 2002. Efficient gene targeting by homologous recombination in rice. Nat. Biotechnol. **20**: 1030–1034. doi:10.1038/nbt737. PMID: 12219079.

Tinland, B. 1996. The integration of T-DNA into plant genomes. Trends Plant Sci. **1**: 178–184. doi:10.1016/1360-1385(96)10020-0.

Tinland, B., Schoumacher, F., Gloeckler, V., Bravo-Angel, A.M., and 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. PMID: 7628458.

Tzfira, T., and Citovsky, V. 2001. Comparison between nuclear import of nopaline- and octopine-specific VirE2 protein of *Agrobacterium* in plant and animal cells. Mol. Plant Pathol. **2**: 171–176. doi:10.1046/j.1364-3703.2001.00065.x.

Tzfira, T., and Citovsky, V. 2002. Partners-in-infection: host proteins involved in the transformation of plant cells by *Agrobacterium.* Trends Cell Biol. **12**: 121–129. doi:10.1016/S0962-8924(01)02229-2. PMID: 11859024.

Tzfira, T., Rhee, Y., Chen, M.-H., and Citovsky, V. 2000. Nucleic acid transport in plant-microbe interactions: the molecules that walk through the walls. Annu. Rev. Microbiol. **54**: 187–219. doi:10.1146/annurev.micro.54.1.187. PMID: 11018128.

© 2006 NRC Canada

Tzfira, T., Vaidya, M., and 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. doi:10.1093/emboj/20.13.3596. PMID: 11432846.

Tzfira, T., Vaidya, M., and Citovsky, V. 2002. Increasing plant susceptibility to *Agrobacterium* infection by overexpression of the *Arabidopsis VIP1* gene. Proc. Natl. Acad. Sci. U.S.A. **99**: 10435–10440. doi:10.1073/pnas.162304099. PMID: 12124400.

Tzfira, T., Frankmen, L., Vaidya, M., and Citovsky, V. 2003. Site-specific integration of *Agrobacterium* T-DNA via double-stranded intermediates. Plant Physiol. **133**: 1011–1023. doi:10.1104/pp.103.032128. PMID: 14551323.

Tzfira, T., Li, J., Lacroix, B., and Citovsky, V. 2004*a*. *Agrobacterium* T-DNA integration: molecules and models. Trends Genet. **20**: 375–383. doi:10.1016/j.tig.2004.06.004. PMID: 15262410.

Tzfira, T., Vaidya, M., and Citovsky, V. 2004*b*. Involvement of targeted proteolysis in plant genetic transformation by *Agrobacterium.* Nature, **431**: 87–92. doi:10.1038/nature02857. PMID: 15343337.

Tzfira, T., Li, J., Lacroix, B., and Citovsky, V. 2005. Nuclear import of *Agrobacterium* T-DNA. *In* Nuclear import and export in plants and animals. *Edited by* T. Tzfira, and V. Citovsky. Eurekah.com and Kluwer Academic/Plenum. pp. 83–99.

Unal, E., Arbel-Eden, A., Sattler, U., Shroff, R., Lichten, M., Haber, J.E., and Koshland, D. 2004. DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. Mol. Cell, **16**: 991–1002. doi:10.1016/j.molcel.2004.11.027. PMID: 15610741.

Valerie, K., and Povirk, L.F. 2003. Regulation and mechanisms of mammalian double-strand break repair. Oncogene, **22**: 5792–5812. doi:10.1038/sj.onc.1206679. PMID: 12947387.

van Attikum, H., and Hooykaas, P.J.J. 2003. Genetic requirements for the targeted integration of *Agrobacterium* T-DNA in *Saccharomyces cerevisiae.* Nucleic Acids Res. **31**: 826–832. doi:10.1093/nar/gkg183. PMID: 12560477.

van Attikum, H., Bundock, P., and Hooykaas, P.J.J. 2001. Non-homologous end-joining proteins are required for *Agrobacterium* T-DNA integration. EMBO J. **20**: 6550–6558. doi:10.1093/emboj/20.22.6550. PMID: 11707425.

van Attikum, H., Bundock, P., Overmeer, R.M., Lee, L.Y., Gelvin, S.B., and Hooykaas, P.J.J. 2003. The *Arabidopsis AtLIG4* gene is required for the repair of DNA damage, but not for the integration of *Agrobacterium* T-DNA. Nucleic Acids Res. **31**: 4247–4255. doi:10.1093/nar/gkg458. PMID: 12853643.

van Attikum, H., Fritsch, O., Hohn, B., and Gasser, S.M. 2004. Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. Cell, **119**: 777–788. PMID: 15607975.

Vergunst, A.C., Schrammeijer, B., den Dulk-Ras, A., de Vlaam, C.M.T., Regensburg-Tuink, T.J., and Hooykaas, P.J.J. 2000. VirB/D4-dependent protein translocation from *Agrobacterium* into plant cells. Science, **290**: 979–982. doi:10.1126/science.290.5493.979. PMID: 11062129.

Vergunst, A.C., van Lier, M.C.M., den Dulk-Ras, A., and Hooykaas, P.J.J. 2003. Recognition of the *Agrobacterium* VirE2 translocation signal by the VirB/D4 transport system does not require VirE1. Plant Physiol. **133**: 978–988. doi:10.1104/pp.103.029223. PMID: 14551327.

Vergunst, A.C., van Lier, M.C.M., den Dulk-Ras, A., Stüve, T.A., Ouwehand, A., and Hooykaas, P.J.J. 2005. Positive charge is an important feature of the C-terminal transport signal of the VirB/D4-translocated proteins of *Agrobacterium.* Proc. Natl. Acad. Sci. U.S.A. **102**: 832–837. doi:10.1073/pnas.0406241102. PMID: 15644442.

Wang, H., Perrault, A.R., Takeda, Y., Qin, W., and Iliakis, G. 2003. Biochemical evidence for Ku-independent backup pathways of NHEJ. Nucleic Acids Res. **31**: 5377–5388. doi:10.1093/nar/gkg728. PMID: 12954774.

Ward, D.V., Zupan, J.R., and Zambryski, P.C. 2002. *Agrobacterium* VirE2 gets the VIP1 treatment in plant nuclear import. Trends Plant Sci. **7**: 1–3. doi:10.1016/S1360-1385(01)02175-6. PMID: 11804814.

Whittaker, G.R., and Helenius, A. 1998. Nuclear import and export of viruses and virus genomes. Virology, **246**: 1–23. doi:10.1006/viro.1998.9165. PMID: 9656989.

Whittaker, G.R., Kann, M., and Helenius, A. 2000. Viral entry into the nucleus. Annu. Rev. Cell Dev. Biol. **16**: 627–651. doi:10.1146/annurev.cellbio.16.1.627. PMID: 11031249.

Willmitzer, L., Schmalenbach, W., and Schell, J. 1981. Transcription of T-DNA in octopine and nopaline crown gall tumours is inhibited by low concentrations of alpha-amanitin. Nucleic Acids Res. **9**: 4801–4812. PMID: 6171771.

Winans, S.C., Allenza, P., Stachel, S.E., McBride, K.E., and Nester, E.W. 1987. Characterization of the *virE* operon of the *Agrobacterium* Ti plasmid pTiA6. Nucleic Acids Res. **15**: 825–837. PMID: 3547330.

Windels, P., De Buck, S., Van Bockstaele, E., De Loose, M., and Depicker, A. 2003. T-DNA integration in *Arabidopsis* chromosomes. Presence and origin of filler DNA sequences. Plant Physiol. **133**: 2061–2068. doi:10.1104/pp.103.027532. PMID: 14645727.

Yang, Y., and Gabriel, D.W. 1995. *Xanthomonas* avirulence/pathogenicity gene family encodes functional plant nuclear targeting signals. Mol. Plant Microbe Interact. **8**: 627–631. PMID: 8589417.

Yang, B., Zhu, W., Johnson, L.B., and White, F.F. 2000. The virulence factor AvrXa7 of *Xanthomonas oryzae* pv. *oryzae* is a type III secretion pathway-dependent nuclear-localized double-stranded DNA-binding protein. Proc. Natl. Acad. Sci. U.S.A. **97**: 9807–9812. doi:10.1073/pnas.170286897. PMID: 10931960.

Yi, H., Mysore, K.S., and Gelvin, S.B. 2002. Expression of the *Arabidopsis* histone *H2A–1* gene correlates with susceptibility to *Agrobacterium* transformation. Plant J. **32**: 285–298. doi:10.1046/j.1365-313X.2002.01425.x. PMID: 12410808.

Zaidi, S.K., Young, D.W., Choi, J.Y., Pratap, J., Javed, A., Montecino, M., et al. 2004. Intranuclear trafficking: organization and assembly of regulatory machinery for combinatorial biological control. J. Biol. Chem. **279**: 43363–43366. doi:10.1074/jbc.R400020200. PMID: 15277516.

Zaidi, S.K., Young, D.W., Choi, J.Y., Pratap, J., Javed, A., Montecino, M., et al. 2005. The dynamic organization of gene-regulatory machinery in nuclear microenvironments. EMBO Rep. **6**: 128–133. doi:10.1038/sj.embor.7400337. PMID: 15689940.

Zhu, Y., Nam, J., Humara, J.M., Mysore, K., Lee, L.Y., Cao, H., et al. 2003. Identification of *Arabidopsis rat* mutants. Plant Physiol. **132**: 494–505. doi:10.1104/pp.103.020420. PMID: 12805582.

Ziemienowicz, A., Görlich, D., Lanka, E., Hohn, B., and Rossi, L. 1999. Import of DNA into mammalian nuclei by proteins originating from a plant pathogenic bacterium. Proc. Natl. Acad. Sci. U.S.A. **96**: 3729–3733. doi:10.1073/pnas.96.7.3729. PMID: 10097105.

Ziemienowicz, A., Tinland, B., Bryant, J., Gloeckler, V., and Hohn, B. 2000. Plant enzymes but not *Agrobacterium* VirD2 mediate T-DNA ligation *in vitro.* Mol. Cell. Biol. **20**: 6317–6322. doi:10.1128/MCB.20.17.6317-6322.2000. PMID: 10938108.

Ziemienowicz, A., Merkle, T., Schoumacher, F., Hohn, B., and Rossi, L. 2001. Import of *Agrobacterium* T-DNA into plant nu-

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clei: two distinct functions of VirD2 and VirE2 proteins. Plant Cell, **13**: 369–384. doi:10.1105/tpc.13.2.369. PMID: 11226191.

Zupan, J.R., Citovsky, V., and Zambryski, P. 1996. *Agrobacterium* VirE2 protein mediates nuclear uptake of ssDNA in plant cells. Proc. Natl. Acad. Sci. U.S.A. **93**: 2392–2397. doi:10.1073/pnas.93.6.2392. PMID: 8637884.

Zupan, J., Muth, T.R., Draper, O., and Zambryski, P.C. 2000. The transfer of DNA from *Agrobacterium tumefaciens* into plants: a feast of fundamental insights. Plant J. **23**: 11–28. doi:10.1046/j.1365-313x.2000.00808.x. PMID: 10929098.

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