Partners-in-infection: host proteins involved in the transformation of plant cells by *Agrobacterium*

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Genetic modification of plant cells by *Agrobacterium* is the only known natural example of DNA transport between kingdoms. While the bacterial factors involved in *Agrobacterium* infection have been relatively well characterized, studies of their host cellular partners are just beginning. Here, we describe the plant cell factors that might participate in *Agrobacterium*-mediated genetic transformation and discuss their possible roles in this process. Because *Agrobacterium* probably adapts existing cellular processes for its life cycle, identifying the host factors participating in *Agrobacterium* infection might contribute to a better understanding of such basic biological processes as cell communication, intracellular transport and DNA repair and recombination as well as help expand the host range of *Agrobacterium* as a genetic engineering tool.

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Agrobacterium is the only known organism capable of *trans*-kingdom DNA transfer, transforming mainly plants but also other eukaryotic species, from fungi [1,2] to human cells [3]. Because *Agrobacterium* represents a major tool for plant molecular breeding, the molecular mechanism by which it genetically transforms the host cells has been intensively studied for the past three decades (for recent reviews, see [4–7]).

Genetic transformation by *Agrobacterium*, which in nature causes neoplastic growths called 'crown galls', results from the transfer of a specific DNA fragment(s) ('transferred DNA' or 'T-DNA') from the bacterial Ti (tumor-inducing) plasmid to the plant cell, followed by T-DNA integration into the host cell genome and expression of the introduced genes in the transformed host cell (reviewed in Ref. 8). Several *Agrobacterium* chromosomal virulence (*chv*) genes and a series of Ti plasmid-encoded virulence (*vir*) genes have been identified as participants in the different stages of the *Agrobacterium*–plant interaction process (reviewed in [4]). The biological functions of most of these bacterial virulence proteins have been well characterized [4,7], but the roles that host proteins might play in the *Agrobacterium* infection are mostly unknown. In recent years, the hunt for specific host proteins involved in the *Agrobacterium*–plant interaction has begun, leading to several important insights into how *Agrobacterium* hijacks various cellular processes [e.g. nuclear import and DNA repair, Fig. 1(e)] for genetic transformation of plant cells.

Agrobacterium-mediated genetic transformation – a brief overview

The T-DNA element is a specific DNA fragment located on the *Agrobacterium* Ti plasmid and delimited by two 25-bp direct repeats, termed left and right T-DNA borders (reviewed in [9]) (Fig. 2). Following induction of the *Agrobacterium* Vir protein machinery by specific host signals, the VirD1 and VirD2 proteins nick both borders at the bottom strand of the T-DNA, resulting in a single-stranded (ss) T-DNA molecule (T-strand), which, together with several Vir proteins, is exported into the host cell cytoplasm through a channel formed by *Agrobacterium* VirD4 and VirB proteins (reviewed in [4–7,10]). The T-strand with one VirD2 molecule covalently attached to its 5′ end and coated with many VirE2 molecules forms a T-DNA transport complex (T-complex) [11] (Fig. 2). This complex is then imported into the host cell nucleus with the help of VirD2 and VirE2 (reviewed in [4,5]), which might also facilitate, directly or indirectly, the subsequent integration of the T-strand into the host genome [12–16]. The entire transformation process can be considered in eight distinct steps (Fig. 2), most of which require not only the bacterial Vir proteins, but also specific host factors (summarized in Table 1). Below, we discuss our current knowledge about such plant factors and their roles in the *Agrobacterium*-mediated genetic transformation of plant cells.

Plant factors required for *Agrobacterium* chemotaxis, attachment and signal transduction

In nature, *Agrobacterium* mainly attacks wounded plant tissues. Such wounds secrete a wide range of phenolic and sugar compounds, which are thought to elicit chemotaxis of *Agrobacterium* cells [17] towards the wounded host tissue. *Agrobacterium* cells anchor to the host cell wall [Fig. 1(a), cell–cell recognition step] at the wound site with the assistance of their binding and attachment proteins (encoded by ChvA, ChvB, PscA and Att) (Fig. 2, step 1). The host cell-surface receptors remain largely unknown, yet two possible receptors: a vitronectin-like protein [18] and a rhicadhesin-binding protein [19] have been described (Fig. 2, step 1). In animals, vitronectin is utilized as a specific receptor by different pathogenic bacteria [20]. Potentially, plant vitronectin-like molecules [21] might also play a role in *Agrobacterium* attachment to its host cells. Indeed, attachment of *Agrobacterium* cells to plant tissues was inhibited by human vitronectin or antibodies

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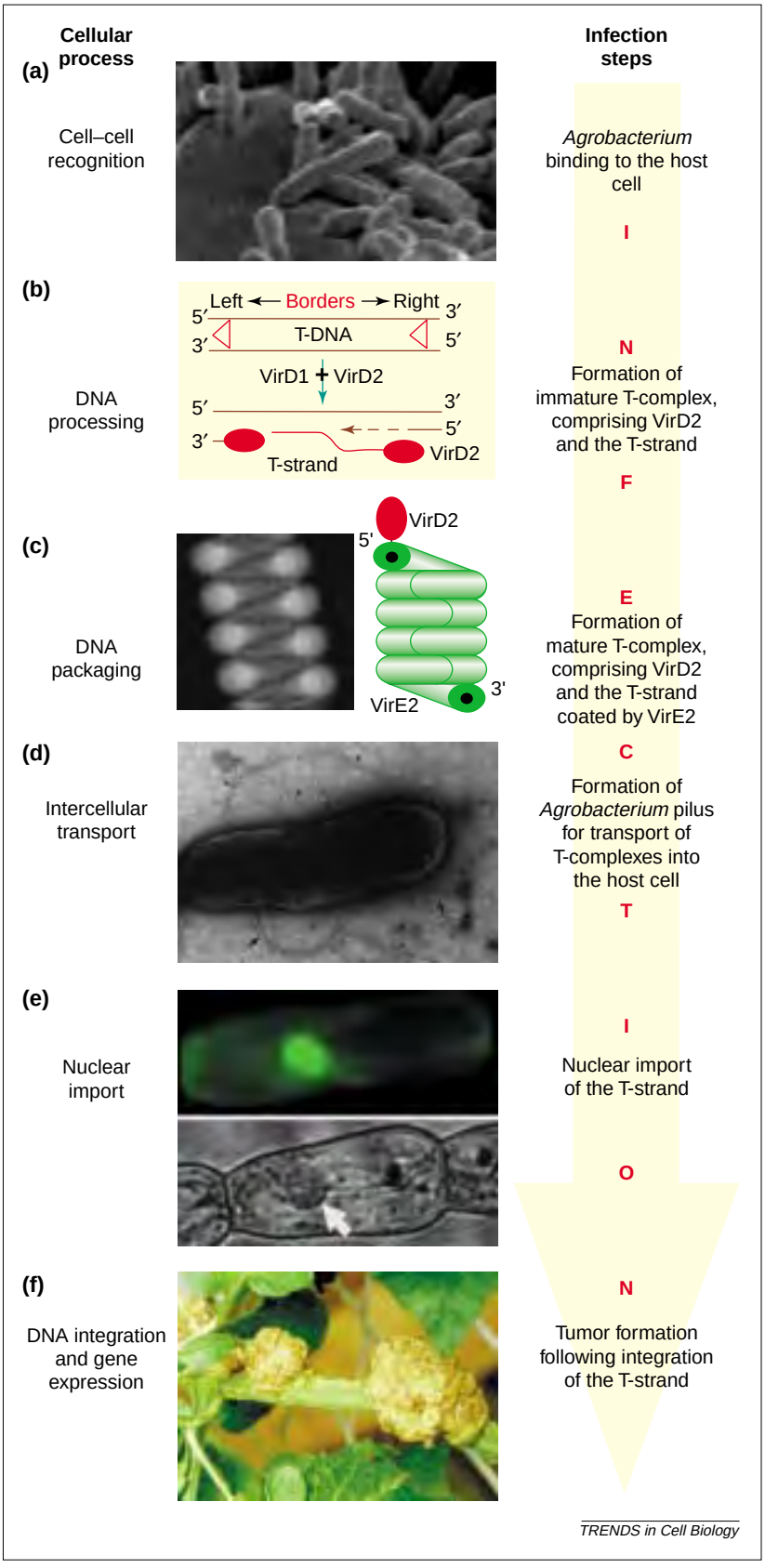

Fig. 1. Cellular processes involved in major steps of the *Agrobacterium*–host interaction. (a) Cell–cell recognition – *Agrobacterium* cells colonizing plant roots [4]; (b) DNA processing – a model for T-strand formation in *Agrobacterium* cells [74]; (c) DNA packaging – scanning transmission electron microscopy computer imaging of VirE2 bound to single-stranded (ss) DNA (left) [31], and structural model of the resulting mature T-complex (right); (d) intercellular transport – *Agrobacterium* cell producing pili (arrowheads, pili; arrows, the flagella) [37]; (e) nuclear import – accumulation of fluorescently labeled ssDNA packaged with VirE2 in the plant cell nucleus {top, fluorescent image; bottom, phase image (nucleus indicated by arrow) [48]}; (f) DNA integration and gene expression – *Agrobacterium*-induced tumors on infected plant [4]. All images are reproduced with permission.

against vitronectin, and *chvB*, *pscA* and *att* *Agrobacterium* mutants, which are unable to bind to plant cells, exhibited reduced binding to vitronectin [18]. In addition, a putative plant receptor for rhicadhesin, an adhesion protein encoded by *Agrobacterium* and a related phytobacterium *Rhizobium*, has been purified from cell walls of pea roots [19], although its role in *Agrobacterium* infection has not been demonstrated.

Recent genetic data indicate that additional plant cell-surface molecules might play a role in *Agrobacterium* binding. For example, two *Arabidopsis* ecotypes, Bl-1 and Petergof, and two T-DNA-insertion mutants of the WS ecotype, designated *rat1* and *rat3* (resistant to *Agrobacterium* transformation), are deficient in *Agrobacterium* binding to their root explants [22,23]. While the specific genes responsible for the decrease in *Agrobacterium* binding in Bl-1 and Petergof remain unknown, the *rat1* and *rat3* mutations were found to affect an arabinogalactan protein (AGP) and a potential cell-wall protein, respectively. However, while root explants of *rat1*, *rat3*, B1-1 and Petergof were highly recalcitrant to *Agrobacterium* infection, their female gametophytes remained transformable [24], suggesting that different surface proteins are involved in *Agrobacterium* attachment to different plant tissues.

Plant exudates are required for the transcriptional activation of the *Agrobacterium* virulence machinery as well as for *Agrobacterium* attachment. Specifically, monosaccharides and small phenolic compounds [25,26] released from plant wounds are recognized by the *Agrobacterium* 'two-component' signal-transduction system (Fig. 2, step 2). In this system, the membrane-bound sensor VirA directly interacts with the plant exudates [27] and undergoes autophosphorylation, which leads to transphosphorylation of VirG, a transcriptional regulator, which in turn activates the *vir* gene promoters (Fig. 2, step 3).

Plant factors can also act as inhibitors of the *Agrobacterium* sensory machinery. Recent data indicate that 2-hydroxy-4,7-dimethoxybenzoxazin-3-one (MDIBOA), the major organic exudate of maize seedling roots, specifically inhibits induction of *vir* gene expression by an as-yet-unknown mechanism [28]. Thus, the relative resistance of many agronomically important plant species, such as maize, to *Agrobacterium*-mediated genetic transformation might be due to the presence of such inhibitors, rather than to insufficient activation of the *Agrobacterium* virulence machinery by host cell exudates.

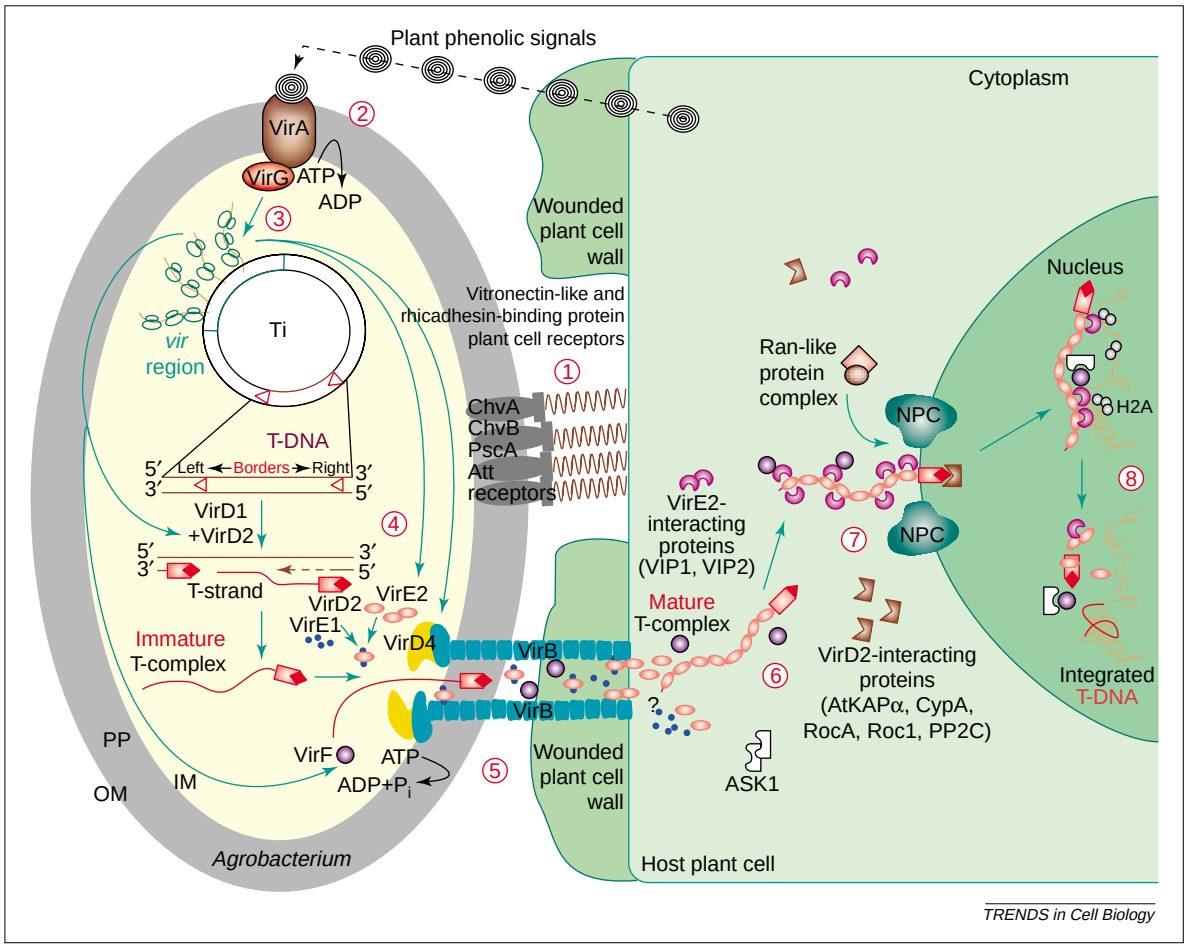

Fig. 2. A model for molecular interactions during *Agrobacterium*-mediated genetic transformation of plant cells. The transformation process comprises eight distinct steps: (1) *Agrobacterium* recognition of and attachment to the host cell, mediated by *Agrobacterium* chromosome-encoded proteins and specific host receptors; (2) sensing of specific plant signals by the *Agrobacterium* two-component (VirA–VirG) signal-transduction system machinery; (3) VirG-mediated signal transduction and *vir* gene activation; (4) generation of mobile copy of T-DNA, the T-strand; (5) generation of the VirB–VirD4 transporter complex, and transport of T-strands and Vir proteins into the host cell cytoplasm; (6) formation of the mature T-complex; (7) T-complex nuclear import facilitated by the AtKAPα, VIP1 and Ran proteins of the host cell; (8) intranuclear transport of the T-complex to the host chromosome, and T-DNA integration into the host cell genome mediated by VirD2 and/or VirE2 and by host factors. Abbreviations: IM, bacterial inner membrane; NPC, nuclear pore complex; OM, bacterial outer membrane; PP, bacterial periplasm. (Figure adapted from Ref. [74] with permission from the American Society of Plant Biologists.)

Processing of T-DNA and plant factors involved in its export

Expression of the *vir* genes leads to the production of T-strands, the formation of a bacterium-to-host cell channel and the export of the T-strands and several Vir proteins into the plant cell. T-strand production and formation of the export channel are not directly affected by the host cell factors. T-strand production is initiated by VirD2 and VirD1, which are known to interact with each other [29] and to function as a strand- and site-specific endonuclease [30]. Following nicking of the T-DNA, VirD2 covalently attaches to the 5′ end of the nicked T-DNA strand, and the resulting VirD2–T-strand complex [i.e. an immature T-complex comprising VirD2 and the T-strand but lacking VirE2; see Fig. 1(b), DNA processing step] is released, presumably by bacterial helicase functions. The remaining gap in the bottom strand of the T-DNA region of the Ti plasmid might be repaired by the bacterial DNA synthesis and repair machinery (Fig. 2, step 4).

Ultimately, the mobilized T-strand is thought to be packaged by VirE2 into semirigid, hollow, cylindrical filaments with a coiled structure [31] [Fig. 1(c), DNA packaging step], protecting it from cellular nucleases [32] and facilitating its nuclear import within the host cell (Fig. 2, steps 6 and 7). The cellular location where VirE2 binds to the T-strand and forms the mature T-complexes remains contentious. This event might occur within the bacterial cell [33]; alternatively, it could take place in the host cell cytoplasm, following independent export of VirE2 and the T-strand–VirD2 complexes [34,35]. In the latter scenario, the T-strand would be exported into the plant cell cytoplasm either immediately following its mobilization from the Ti plasmid (Fig. 2, step 5) or, by analogy to prokaryotic DNA replication systems, concomitantly with its unwinding. VirE2 would then be exported in a complex with another *Agrobacterium* protein, VirE1, which prevents VirE2 binding to T-strands [36] (Fig. 2, step 5) until the complex disassociates in the host cytoplasm (Fig. 2, step 6).

Transport of the T-strands and Vir proteins into the host cell most likely occurs through a type IV secretion

Table 1. Summary of host factors, genes, mutants and ecotypes postulated to be involved in the *Agrobacterium*–plant interaction

Plant factor/mutant	Possible function	Refs
***Agrobacterium*-host cell recognition and attachment**		
Phenolic and sugar compounds	Positive chemotaxis	[17]
Vitronectin, putative host cell-wall receptor	Binding of *Agrobacterium* to host cells	[18]
Rhicadhesin-binding protein	Binding to the *Agrobacterium* adhesion protein	[19]
Arabidopsis rat1 mutant (arabinogalactan protein)	Mutant does not bind to *Agrobacterium*	[22,23]
Arabidopsis rat3 mutant (putative cell-wall protein)	Mutant does not bind to *Agrobacterium*	[22,23]
Arabidopsis ecotypes Bl-1 and Petergof (unknown factors)	Ecotypes do not bind to *Agrobacterium*	[22,23]
Nodulin-like protein	Might be involved in cell-to-cell recognition	[22,23]
Lectin-like protein kinase	Might be involved in cell-to-cell recognition	[22,23]
Sensing plant signals by *Agrobacterium*		
Acetosyringone, acetovanillone, hydroxyacetosyringone, phenylpropanoid glucoside coniferin, syringaldehyde	VirA activators	[25,26]
Monosaccharides	VirA activators and coactivators with acetosyringone	[25]
Synthetic amide derivatives of syringic acid	Synthetically produced VirA activators	[26]
MDIBOA, maize seeding root exudate	Inhibit *vir* induction	[28]
Signal transduction and vir gene induction		
None	N/A	
Generation of transported T-DNA molecule		
None	N/A	
T-DNA transport into the host cytoplasm		
VirB1* interactor, still unknown	Establishing cell–cell contact	[38]
VirB2 and VirB5 interactors, still unknown	Recognition of the *Agrobacterium* pilus, activation of the transporter	[38]
Mature T-complex assembly in plant cells		
RocA, Roc4, CypA	Chaperones, possibly involved in maintaining VirD2 conformation	[54]
T-complex nuclear import		
AtKAPα	Binds to VirD2, facilitates VirD2 nuclear import	[55]
Abi1 mutant (type 2C serine/threonine protein phosphatase, PP2C)	Mutant has increased susceptibility to *Agrobacterium* infection; overexpression of PP2C enhances activity of the VirD2 nuclear-localization sequence (NLS)	[6]
Protein kinase, still unknown	Downregulates VirD2 nuclear import by phosphorylating its NLS region	[6]
Ran	Facilitates nuclear import of VirD2 and VirE2	[59]
VIP1	Binds to VirE2, facilitates VirE2 nuclear import; might also assist subsequent intranuclear transport of T-complexes	[57]
Intranuclear transport of T-complexes and T-DNA integration		
VIP2	Binds to VirE2 and VIP1, might participate in intranuclear transport of VirE2 and T-complexes and/or in T-DNA integration; VIP1 might also assist in these processes	[4]
ASK1 and other components of the SCF complex	Might participate in targeted proteolysis during uncoating of T-complexes and/or exposing the host cell genomic DNA prior to or during integration	[68]
DNA ligase	Ligation of the integrating T-DNA into the plant genomic DNA	[61]
DNA polymerase	T-strand conversion to double-stranded DNA	
Arabidopsis rat5 mutant (H2A histone)	Mutant deficient in T-DNA integration, H2A histone might specify chromatin conformation at the integration site	[64]
Arabidopsis ecotype UE-1(unknown factor)	Ecotype deficient in T-DNA integration	[23]

system [10] (Fig. 2, step 5). Such systems usually constitute up to 12 proteins that form two functional components: a filamentous virulence pilus [37] [Fig. 1(d), intercellular transport step] and a transporter complex, which translocates substrates through the cell membrane. In *Agrobacterium*, the type IV transporter comprises proteins encoded by the *virD4* gene and by 11 open reading frames of the *virB* operon [10,38]. Of these proteins, VirB1* (a processed form of VirB1), VirB2 and VirB5 are most likely to interact with the putative host cell receptors because these three bacterial proteins are thought to reside in the exterior portions of the channel [39,40]. Specifically, VirB1* is secreted to the *Agrobacterium* cell exterior [41] and was proposed to establish the cell-to-cell contact between *Agrobacterium* and the host cell wall [39], whereas VirB2 [42], VirB5 [43] and VirB7 [44] are structural components of the *Agrobacterium* pilus, which directly interacts with the host cell. Although the involvement of the VirD4–VirB transporter in the export of the T-strand and its associated proteins is widely accepted, it is contradicted by the recent

report that VirD2, VirE2 and VirF can be exported independently of the *virB* operon [45].

Assembly of the virulence pilus and the transporter complex might not be sufficient to initiate T-complex export, and physical contact with the recipient plant cell might be required for activating the transport machinery. Indeed, T-strands are known to accumulate in *vir*-induced *Agrobacterium* cells in the absence of the recipient plant cells [46]. Thus, although the virulence pilus and the transporter complex are formed by bacterial proteins, this export channel might become operational only following interaction with a putative cell-surface receptor(s) [38], suggesting the role of as-yet-unknown host cell factors during export of T-strands and Vir proteins.

Plant cell components of the T-complex nuclear import pathway

Once inside the host cell cytoplasm, the T-complex must enter the cell nucleus in order to integrate into the plant genome. The probable large size of the T-complex (~13 nm in diameter [31]) requires active nuclear import, which is presumably mediated by the T-complex protein components and their specific cellular partners (Fig. 2, step 7). Indeed, both VirE2 and VirD2 have nuclear-localizing activities. The roles of VirD2 and VirE2 in T-complex nuclear import were demonstrated by reduced T-DNA expression and tumorigenicity of *Agrobacterium* strains that carry VirD2 mutants lacking their nuclear-localization signal (NLS) [15,47] and by nuclear import of *in vitro*-assembled VirE2-ssDNA complexes, but not ssDNA alone, microinjected into living plant cells [48] [Fig. 1(e), nuclear import step].

Interestingly, while VirD2 is imported into the cell nucleus by a mechanism conserved between animal, yeast and plant cells [49], nuclear import of VirE2 might be by a plant-specific mechanism as VirE2 fails to localize to the cell nucleus of yeast or animal cells [49–51]. These observations in living cells contrast with data from *in vitro* nuclear import assays, in which VirE2 was reported to enter plant as well as mammalian cell nuclei but was unable to mediate nuclear import of ssDNA in these systems [52,53]. Thus, nuclear import of VirE2 itself and its ability to facilitate nuclear import of ssDNA might have different manifestations in living cells compared with cell-free systems. Despite these differences, however, both VirD2 and VirE2 were required for optimal nuclear uptake of ssDNA even in *in vitro* [52,53], further supporting the notion that both of these proteins function during nuclear import of the T-complexes.

In host plant cells, VirD2 and VirE2 likely cooperate with cellular factors to mediate T-complex nuclear import and integration into the host genome (Fig. 2, steps 7 and 8). Several plant proteins that interact with VirD2 and VirE2 have been identified using the yeast two-hybrid protein–protein interaction screen. VirD2 was found to bind to three members of the *Arabidopsis* cyclophilin chaperone family – RocA, Roc4 and

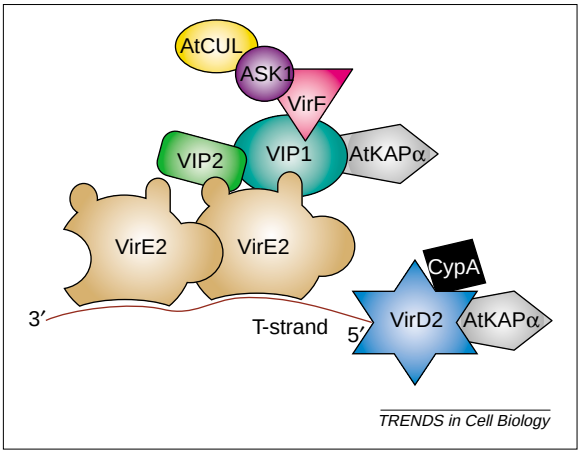

Fig. 3. Possible interactions between host cell proteins and the molecular components of the mature *Agrobacterium* T-complex. The mature T-complex is thought to comprise multiple VirE2 molecules bound along the length of the T-strand and interacting with each other for binding cooperativity, and a single molecule of VirD2 covalently attached to the 5′ end of the T-strand. This T-complex interacts with the following host cell proteins: to preserve its proper conformation within the plant cell, VirD2 might bind to the CypA chaperone; for nuclear import, VirD2 interacts directly with AtKAPα, whereas VirE2 interacts with AtKAPα via VIP1; for intranuclear transport to the integration site, VirE2 might interact with VIP2 (VIP1, which also binds to VIP2, might also play a role in this process); for uncoating of the T-complex and/or removal of its cellular interactors, VirF might bind to VIP1 and bridge it with ASK1 and AtCUL components of the targeted proteolysis machinery.

CypA [54] (Fig. 3). Inhibition of the VirD2–CypA interaction abolished *Agrobacterium* tumorigenicity. While the exact roles of RocA, Roc4 and CypA in *Agrobacterium* infection is unknown, these cyclophilins might maintain the proper conformation of VirD2 in the host cell cytoplasm and/or nucleus during T-DNA nuclear import and/or integration [54].

VirD2 was also found to interact with a type 2C serine/threonine protein phosphatase (PP2C) [6]. Coexpression of PP2C and the VirD2 NLS fused to the β-glucuronidase (GUS) reporter (in tobacco protoplasts) resulted in a dramatic decrease in GUS nuclear accumulation, suggesting a negative effect of dephosphorylation on VirD2 NLS activity. Consistent with this observation, an *Arabidopsis* mutant in the PP2C gene (*abi1*) exhibited an increased susceptibility to *Agrobacterium* infection. Based on these results, phosphorylation of the VirD2 NLS region (by an as-yet-unknown protein kinase) is proposed to potentiate VirD2 nuclear import, whereas NLS dephosphorylation by PP2C is suggested to negatively regulate this process [6].

Finally, VirD2 was also found to interact with a member of the *Arabidopsis* karyopherin α family, AtKAPα [55] (Fig. 3). Members of this protein family mediate nuclear import of NLS-containing proteins [56], suggesting the involvement of AtKAPα in nuclear import of VirD2 and, by implication, the T-complex in *Agrobacterium*-infected plant cells. Indeed, AtKAPα potentiated nuclear import of VirD2 in permeabilized yeast cells [55].

Unlike VirD2, VirE2 does not interact with AtKAPα, but it does specifically interact with two

other *Arabidopsis* proteins – VIP1 [57] and VIP2 [4] (Fig. 3). In a functional genetic assay as well as in confocal microscopy studies, VIP1 facilitated VirE2 nuclear import in yeast and mammalian cells [57]. Because VIP1, a basic leucine zipper (bZIP) motif protein, shows no significant homology to known animal or yeast proteins, it was suggested to be a cellular factor at least in part responsible for plant-specific VirE2 nuclear import. Exactly how VIP1 facilitates nuclear import of VirE2 is still unknown, but, because VIP1 itself localizes to the nucleus of animal, yeast and plant cells, it was proposed to bind to VirE2 and target it to the nucleus by a 'piggy-back' mechanism. In this process, VIP1 (with the attached VirE2 or, by implication, the entire T-complex) presumably interacts with a cellular karyopherin α – e.g. AtKAPα (Fig. 3) – which mediates its nuclear import. Thus, VIP1, which carries a conventional NLS [57], might function as an adaptor between VirE2 and the conventional nuclear import machinery of the host cell [58].

The role of VIP1 in nuclear import was further studied using transgenic plants that express *VIP1* cDNA in antisense orientation (VIP1-antisense plants). These plants exhibit significantly reduced nuclear import of GUS–VirE2 but not of GUS–VirD2, confirming that, first, VIP1 is involved in VirE2 nuclear import, second, VirE2 and VirD2 are imported into the host cell nucleus by different mechanisms and, third, antisense expression of VIP1 does not nonspecifically interfere with the nuclear import reactions of the cell [57]. The function of VIP1 is presently unknown; however, it might represent one of the cellular chromatin-associated proteins. The role of VIP1 in the nuclear import of the T-complexes is also consistent with observations that VIP1, which by itself is unable to associate with ssDNA, is able to interact with VirE2 while the latter is bound to the ssDNA, forming ternary VIP1–VirE2–ssDNA complexes *in vitro* [57].

Besides AtKAPα and VIP1, the small GTPase Ran, known to be required for nuclear import in other systems (reviewed in Ref. [59]), most likely also participates in the nuclear import of T-complexes (Fig. 2, step 7). Indeed, nonhydrolyzable analogs of GTP, which block nuclear import by inhibiting Ran, also blocked nuclear import of VirE2 and VirD2 [48,51,53].

Plant factors participating in T-DNA integration

T-DNA integration into the host cell genome is the final [Fig. 1(f), DNA integration step leading to tumor formation] and most crucial step of the transformation process. Although the T-strands are known to be converted into double-stranded DNA in the host cell nucleus, it remains unresolved whether the T-DNA integrates as a double-stranded [60] or a single-stranded molecule [12]. Interestingly, the majority of the invading T-DNA molecules do not integrate into the host genome. Nevertheless, the efficiency of the T-DNA integration is significantly higher than that of DNA molecules introduced by *Agrobacterium*-independent methods such as particle bombardment transformation. This might be due to the activity of the T-complex protein components VirD2 and VirE2, which, in concert with the host cell nuclear import and DNA repair machinery, facilitate the T-DNA nuclear import and integration, respectively.

VirD2 might play a dual role in the integration process, ensuring both its fidelity [12,13] and efficiency [16]. However, VirD2 is not a *bona fide* ligase [61], indicating involvement of plant DNA ligases in T-DNA integration [61,62]. Also, there must be host cell DNA polymerases that convert the T-strand to the double-stranded T-DNA molecule, either before or during the integration event itself.

Besides DNA ligases and polymerases, additional cellular functions must be involved in the integration process. For example, there might be plant proteins that direct the T-DNA to the integration site and prepare it for integration by relaxing the chromatin structure and nicking and/or cleaving the host DNA genome. One such possible host protein is VIP2, an *Arabidopsis* protein that binds to VirE2 [4] and shares homology with the *Drosophila* Rga protein that is thought to mediate interaction between chromatin proteins and transcriptional complexes [63]. Unlike VIP1, VIP2 was unable to mediate VirE2 into the yeast cell nucleus. However, VIP2 and VIP1 interacted with each other in the yeast two-hybrid system [4]. In uninfected cells, VIP1 and VIP2 might be involved in transcription, associating with the chromosomal DNA either directly or through other components of transcription complexes. Thus, VIP1, VIP2 and VirE2 might function in a multiprotein complex (Fig. 3) that performs a dual function: it first facilitates nuclear targeting of VirE2 and then mediates intranuclear transport of VirE2 and its cognate T-strand to chromosomal regions where the host DNA is more exposed (Fig. 2, step 8) and thus better suited for T-DNA integration.

Identification of additional cellular factors involved in the T-DNA interaction might come from genetic approaches. For example, several *rat* mutants of *Arabidopsis*, when inoculated with *Agrobacterium*, exhibited a phenotype whereby wild-type levels of T-DNA-encoded reporter gene were expressed – but the rates of stable transformation were low, suggesting that these mutations affected the T-DNA integration step of the infection process. Of these integration-deficient *rat* mutants, *rat5* was characterized in detail; its mutation affected histone H2A [64]. The exact mechanism by which H2A affects stable transformation is still unknown; potentially, it might specify chromatin conformation at the T-DNA integration site (Fig. 2, step 8). This function of RAT5 is probably tissue specific because female gametophytes of *rat5* plants remain susceptible to *Agrobacterium* transformation [24]. Recent studies lend support to this idea by demonstrating a positive correlation between *RAT5* gene expression in various root tissues and their receptiveness to *Agrobacterium* infection;

specifically, the highest levels of *RAT5* expression were detected in the elongating zone of the root, a region that is most susceptible to *Agrobacterium* (S. Gelvin, pers. commun.).

In addition to screening for *rat* mutants, almost 40 different *Arabidopsis* ecotypes were examined for their susceptibility to T-DNA integration. These experiments identified one ecotype, UE-1, with high levels of T-DNA-encoded reporter gene transient expression but low levels of stable T-DNA integration [23]. Because UE-1 plants are slightly radiation-sensitive, it is possible that their recalcitrance to T-DNA integration results from deficiencies in DNA repair and/or recombination.

VirF, an *Agrobacterium* host range factor and its plant cell partners

Expression of the wild-type *Agrobacterium* T-DNA in transformed plant cells leads to the formation of tumors and the production and secretion of specific amino acid and sugar phosphate derivatives – opines. These compounds are utilized by the bacterium, but not the plant, as a carbon/nitrogen source and are used to classify *Agrobacterium* strains [65]. The *Agrobacterium* strains octopine and nopaline share a range of hosts but differ in their virulence towards other hosts. For example, tomato and *Nicotiana glauca* can be infected by the octopine-type but not by nopaline-type *Agrobacterium* [66]. The molecular basis for this host-range difference is that the nopaline-specific Ti plasmid lacks a *virF* locus found in the octopine-specific Ti plasmid. Thus, VirF is thought to be a host-range factor of *Agrobacterium*.

Transgenic expression of VirF in *N. glauca* plants allowed infection by a VirF-deficient octopine-specific *Agrobacterium* strain, which does not transform wild-type *N. glauca*, suggesting that VirF functions within the host plant cell [66]. Indeed, genetic experiments demonstrated that VirF is secreted into plant cells through the VirB–VirD4 transport system [67] (Fig. 2, step 5), where it most likely requires cellular proteins for its function. One class of such cellular factors might be the *Arabidopsis* Skp1-like (ASK) proteins, which bind to VirF in the yeast two-hybrid system [68]. Yeast Skp1 and its animal and plant homologs act as subunits of E3 ubiquitin ligases, termed SCF (Skp1/Cdc53-cullin/F-box) complexes. SCF complexes target specific proteins for proteolysis by the ubiquitin-dependent degradation pathway. Skp1 associates with the so-called F-box domain of a variety of F-box proteins and recruits their substrate proteins to the SCF complex [69]. In the case of VirF, its F-box motif was identified and shown to be involved in binding to ASK1, ASK2 and ASK10. In addition, when a mutated VirF protein unable to bind to ASK1 was introduced into an *Agrobacterium virF*-minus strain, it could not restore virulence toward *N. glauca* [68]. These findings indicate that VirF, together with ASK1 and a plant cell cullin [69,70], functions in a plant SCF complex (Fig. 3), specifying targeted proteolysis during *Agrobacterium* infection. The target substrate of this proteolysis, however, remains elusive.

One potential candidate for such a VirF-specific proteolysis substrate is VIP1, which was also found to interact with VirF in the yeast two-hybrid system. It is tempting to speculate that VirF might specifically recognize VIP1 within the T-complex (Fig. 3) and target it, and possibly its cognate VirE2, for proteolysis. Such proteolysis might be essential for dissociation of the T-complex before integration. Consistent with this idea, VirF and other components of the SCF complex were found in the plant cell nucleus where T-complex uncoating must take place. In plant species that do not require VirF for *Agrobacterium* infection, this function is likely performed by endogenous F-box proteins that can recognize the protein components of the T-complex.

The plant gene expression response to *Agrobacterium*

Agrobacterium infection represents a major physiological, biochemical and genetic challenge to the host plant. Most likely, this event triggers changes in host cell gene expression patterns, inducing or repressing specific sets of plant genes. The cDNA-amplified fragment length polymorphism (AFLP) technique was used to examine gene expression patterns in *Agrobacterium*-infected cells of a highly transformable *Ageratum conyzoides* suspension cell culture [71]. From 16 000 AFLP cDNA fragments analyzed in this study, 251 were differentially regulated – that is, induced or repressed – 48 hours after infection, but only four genes were specifically induced by *Agrobacterium* infection [71], whereas the others were likely involved in general plant cell responses to the presence of bacteria in the cell culture. One of these four *Agrobacterium*-induced genes encoded a nodulin-like protein belonging to a class of proteins induced in root nodules of *Rhizobium*-infected plants and thought to play a role in cell division/differentiation [72]. Another *Agrobacterium*-induced host gene encoded a lectin-like protein kinase, which might be involved in cell-to-cell recognition by responding to oligosaccharides signals [73], such as those involved in *Rhizobium* infection. Thus, two related phytobacteria, *Agrobacterium* and *Rhizobium*, might elicit similar changes in gene expression in their host plant cells.

Although this AFLP analysis is an important step towards identifying global patterns of host gene regulation during *Agrobacterium* infection, additional studies employing techniques such as cDNA microarrays, mRNA differential display and subtraction library approaches will be required. Furthermore, the wealth of available *Agrobacterium* mutants affecting virtually all infection-related bacterial functions will allow us to further define the infection steps responsible for regulating each subset of the identified differentially-expressed host genes.

Future prospects

Different plants species, cultivars and even specific plant tissues vary greatly in their response and susceptibility to *Agrobacterium* infection. Unraveling the molecular basis for these differences might help to expand the host range of *Agrobacterium* as a genetic engineering tool as well as define the plant cellular functions involved in the transformation process. Furthermore, *Agrobacterium* most likely utilizes existing cellular processes and adapts them for its life cycle. Thus, identifying host factors participating in *Agrobacterium* infection might contribute to a better understanding of basic biological processes, such as cell communication, intracellular transport, DNA repair and recombination. Questions for the future include:

- Why are some plant species infected by *Agrobacterium* and others not?
- Do *Agrobacterium* VirD4–VirB transporter channels dock to specific receptors or membrane channels of the host cells?
- How does the T-complex reach and recognize the integration site?
- What are the host proteins that uncoat the T-complex, and how do they function?
- And, importantly, how does the bacterial T-DNA integrate into the host genome?

With further development of genetic, biochemical and biological tools to identify the cellular participants in the interaction between *Agrobacterium* and its host cells, crucial insights into the detailed mechanisms of this process will follow.

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