Trends in Microbiology

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Spotlight

The VirE-asy Way to Genetically Transform Plants

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***Agrobacterium* transfers T-DNA and several virulence effector proteins to plant cells. It is not known how and where T-complexes containing these components are assembled. A new study suggests that T-complexes form on the plant plasma membrane, mediated by the effector protein VirE3.**

Agrobacterium-mediated plant genetic transformation is the best extant example of interkingdom horizontal gene transfer. Virulent *Agrobacterium* species can transfer T-(transfer) DNA to plants and, under defined laboratory conditions, to yeast, fungi, and even animal cells. In nature, the resulting infection generates crown gall or hairy root tumors due to expression of oncogenes encoded by T-DNA. Scientists have learned to 'disarm' T-DNA by deleting the oncogenes and replacing them with genes of interest, thus generating transgenic plants with enhanced agronomic characteristics.

T-DNA derives from a bacterial Ti-(tumor inducing) or Ri-(rhizogenic) plasmid, from which it is processed by the VirD1/VirD2 endonuclease. During processing, the VirD2 relaxase covalently binds to the 5′-end of T-DNA; single-strand molecules (T-strands) are released from the Ti-plasmid and, led by VirD2, exit the bacterium through a dedicated type IV protein secretion system (T4SS) [1]. In addition to VirD2/T-strands, several other bacterial virulence effector proteins (VirD5, VirE2, VirE3, VirF, and – in certain *Agrobacterium rhizogenes* strains – GALLS) are transferred through the T4SS into the plant cytoplasm [2]. These effector proteins aid in the transformation process, most likely by interacting with VirD2/T-strands either directly or indirectly by mediating interactions with host proteins that associate with VirD2/T-strands [3]. Among these additional effector proteins, VirE2 plays a crucial role in transformation. VirE2 has single-strand DNA binding activity and is thought to coat T-strands in the host plant cell, protecting it from nucleases. Although the VirD2/T-strand/VirE2 'T-complex' has never directly been identified in *Agrobacterium*-infected plant cells, strong circumstantial evidence for this complex exists: *virE2*$^{-}$ mutant *Agrobacterium* strains are almost avirulent, and when such strains are used to transform plants much less T-DNA can be recovered from the infected cells. In addition, integrated T-DNA delivered from *virE2*$^{-}$ mutant *Agrobacterium* strains is often severely truncated [4], suggesting lack of protection of T-strands as they traverse the plant cytoplasm and enter the nucleus.

VirE2 protein is transferred to plants independently of VirD2/T-strands [5]. How, then, does VirE2 meet T-strands in the plant cell? A hint to this answer came from the observation that VirE2 could form voltage-gated channels to facilitate single-strand DNA transfer through membranes [6]. A model derived from these studies postulates that VirE2 is transferred through the *Agrobacterium* T4SS and remains in the plant plasma membrane to 'pick up' VirD2/T-strands as they exit the bacterium. The resulting T-complexes would then be released into the plant and target the nucleus. This attractive model still does not explain how VirE2 finds T-strands in the plant membrane. In a recent issue of *Cell Reports*, Li *et al*. show that a third secreted virulence effector protein, VirE3, localizes to the plant plasma membrane at *Agrobacterium* attachment points, interacts with and anchors VirE2 to the membrane, and may serve to assemble VirE2 with VirD2/T-strands exiting the bacterium [7].

Many studies have expressed fluorescently tagged *Agrobacterium* virulence proteins *in planta* and determined their subcellular localization (e.g., [8]). However, expression *in planta* bypasses the normal route of entry of these proteins into the plant cell, and may therefore obscure the natural site of localization. Li and Pan [9] previously used a split green fluorescent protein (GFP) approach, in which a virulence protein is tagged with domain 11 of GFP, and domains 1–10 are expressed in plants, to show that VirE2 initially localizes to the plant plasma membrane. Soon thereafter, VirE2 is internalized via clathrin-coated vesicles which subsequently track the endoplasmic reticulum to the nucleus. In their present study, Li *et al*. [7] used a similar approach to localize VirE3 protein as punctate aggregates in the plant plasma membrane after it is secreted from *Agrobacterium*. If, however, VirE2 is delivered from an *Agrobacterium* strain lacking VirE3, VirE2 directly localizes to the plant cytoplasm, not the plasma membrane. These authors speculated that VirE3, localized in the membrane, interacts with VirE2 and retains it at the site of *Agrobacterium* attachment and, presumably, VirD2/T-strand entry. Indeed, VirE3 localized to the sites of bacterial attachment. Further analysis demonstrated that a transmembrane domain within VirE3 is responsible for plant plasma membrane localization, and a second domain mediates VirE3–VirE3 interactions to form punctate structures in the membrane. Lacroix *et al*. [10] had previously shown that VirE2 interacts with VirE3, and Li *et al*. determined that the C-terminal region of VirE3 constitutes a third domain that is responsible for VirE2 interaction. Extracellular complementation assays, in which one *Agrobacterium* strain that serves as a T-DNA and VirE2 donor was mixed with another strain that serves as a VirE3 donor, failed to achieve transient plant transformation. However, when one

Agrobacterium strain that could transfer T-DNA (but not VirE3) donor was mixed with another that could transfer both VirE2 and VirE3, partial complementation of transformation was observed. These results suggest that VirE2 and VirE3 need to be secreted from the same cell to colocalize in the plant plasma membrane and mediate transformation.

Because VirE2 was shown to be important for protecting T-strands in the plant [4], Li *et al*. investigated the integrity of T-DNA integrated into the *Arabidopsis* genome following transformation by *VirE3* proficient or *virE3*$^{-}$ mutant *Agrobacterium* strains. Integrated T-DNA molecules delivered from the *virE3*$^{-}$ mutant *Agrobacterium* strain had more extensive deletions at their 3′ ends, implying that a lack of VirE3 protein resulted in less protection of T-strands by VirE2.

Taken together, these results suggest that VirE3 protein, localized in the plant plasma membrane, serves as an anchor for secreted VirE2 protein that, in turn, can bind incoming VirD2/T-strands. Thus, VirE3 may serve as a nucleation site to

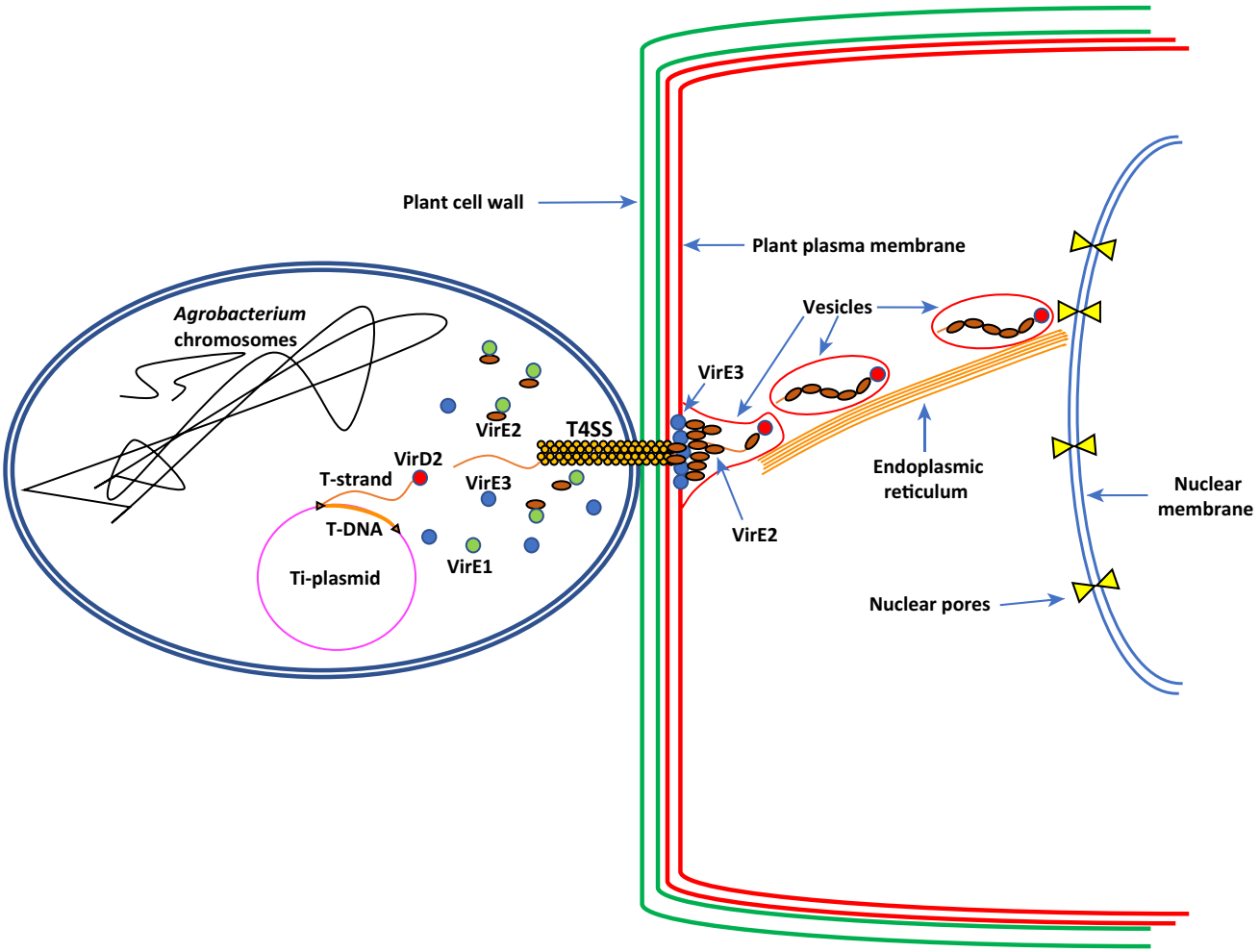

Figure 1. Model for Assembly of the T-Complex on the Plant Plasma Membrane and Its Entry into Plant Cells. Within *Agrobacterium* cells, T-DNA is processed from the Ti-plasmid by the VirD2 endonuclease (in collaboration with VirD1, not pictured). VirD2/T-strands are exported through a type IV secretion system (T4SS) into the plant cell. Within *Agrobacterium*, the VirE1 chaperone interacts with VirE2, keeps it from interacting with T-strands within the bacterium, and helps to keep it from aggregating. Vir effector proteins, including VirE2 and VirE3, are separately transferred to plant cells. VirE3 remains in the plant plasma membrane where it associates with VirE2. VirE2 forms a channel for passage of VirD2/T-strands into the plant cell. VirE2 associates with VirD2/T-strands, forming the T-complex which enters the cytoplasm in clathrin-coated vesicles. These vesicles track the endoplasmic reticulum to the nuclear pores, following which T-strands are released to enter the nucleus. It is not clear how the T4SS traverses the plant cell wall, or whether it needs to. Not pictured are other Vir effector proteins, including VirD5, VirF, and (in *Agrobacterium rhizogenes*), GALLS. ΔT-DNA right and left borders.

bring together the single-strand DNA binding protein VirE2 with the incoming single-strand T-DNA (Figure 1). As the authors point out, it is not clear how VirD2/T-DNA/VirE2 complexes, once formed on the plant plasma membrane, are released into the cytoplasm. Li *et al*. [7] suggest that the plant AP-2 complex may interact with VirE2, releasing VirE2 (and by implication, VirD2/T-strand/VirE2 complexes) into clathrin coated vesicles via endocytosis. The AP-2 complex may thus compete with VirE3 for VirE2 interaction, releasing T-complexes into the cell for their journey to the nucleus.

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