# A conduit for T-DNA through the plant cell membrane: dig it yourself!

*Agrobacterium tumefaciens* is a plant-pathogenic bacterium notorious for its ability to redirect the plant metabolism to meet the pathogen's gourmandise. This is achieved by the stable genetic modification of the host plant after integration of the bacterial T-DNA into the plant genome, a unique example of inter-kingdom DNA transfer. The T-DNA stems from the bacterial tumor-inducing (Ti) plasmid and travels as a single-stranded molecule from the bacterial to the plant cell, tipped with a covalently linked single molecule of VirD2 protein. Once inside the plant cell, many, co-operatively bound VirE2 protein molecules coat the T-DNA. Laboratory strains of *Agrobacterium* have had to endure significant disarmament of their pathogenicity and subsequent widespread exploitation as a tool in plant transformation. The genes on the Ti plasmid can be reduced to those that are essential for a successful T-DNA transfer, and sequences intended for plant genome integration are routinely inserted into the T-DNA as 'stowaways'.

*En route* from the bacterium to the plant nucleus, the T-DNA complex has to overcome numerous obstacles including several membranes. Although there is good evidence that leaving home through the inner and outer bacterial membranes is achieved by a bacterial secretion system, the means of entering the less familiar territory of the plant cell remains unknown. Now, Fabrice Dumas and colleagues[1] provide evidence for a novel and surprising function of the VirE2 protein: so far known to wrap the single-stranded T-DNA inside the plant cell, it can also operate as a transmembrane DNA transporter. Combining methods of molecular biology and biophysics, the authors show that recombinant VirE2 interacts with lipid layers and forms transmembrane channels *in vitro*. These channels are anion-specific and interact with single-stranded (ss) DNA of different composition. Because VirE2-containing proteoliposomes show osmotic swelling after the addition of ssDNA and incorporate radiolabeled oligonucleotides, VirE2 pores are indeed able to facilitate the transfer of ssDNA through lipid bilayers. The voltage-gating property of the channels suggests that the pores would be closed *in planta*, therefore not necessarily detrimental to the plant cell.

Although it is premature to conclude that T-DNA crosses the plant plasma membrane through VirE2-formed channels *in vivo*, the concept of *Agrobacterium* approaching the plant cell with its own, specific drill is stimulating and raises many new questions. Does VirE2 discriminate between bacterial and plant membranes, and if so, how? How does VirE2 switch from soluble to membrane-bound conformation? How are the putatively locked VirE2 channels opened upon arrival of the T-DNA complex, and do they have an active role during import of the huge complexes? The data described by Dumas *et al.*[1] open the way for a new exciting direction in *Agrobacterium* research and for possible applications to achieve transmembrane-trafficking of DNA-protein complexes.

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**1** Dumas, F. *et al.* An *Agrobacterium* VirE2 channel for T-DNA transport into plant cells. *Proc. Natl. Acad. Sci. U. S. A.* (in press)

# Post-transcriptional gene silencing: conservation and sequences

Gene expression is controlled by a variety of mechanisms. Post-transcriptional gene silencing (PTGS) is one mechanism that degrades specific messenger RNAs and thereby reduces the expression of a specific gene. PTGS has many names: cosuppression in plants, quelling in fungi and RNA interference in animals, but in all cases, degraded mRNA decreases gene expression. It is becoming clear that plants and animals share molecular components of PTGS. For example, two genes necessary for PTGS, SGS2 in *Arabidopsis* and QDE1 in *Neurospora*, encode gene products that are similar to an RNA-dependent RNA polymerase. As most studies of PTGS in plants involve silenced transgenes or silencing of endogenous genes to generate mutants, the scope of post-transcriptional endogenous gene silencing during plant development is unclear. At least one class of genes in *Arabidopsis* (the SAURs) is regulated at the post-transcriptional level, but it is unclear how commonly plants use PTGS as a mechanism to control gene expression throughout development.

Now, Mathilde Fagard *et al.*[1] strengthen the idea that PTGS is molecularly conserved. The authors generated loss-of-function mutants in *Arabidopsis* that relieve PTGS of a GUS reporter transgene. The silencing mutants phenotypically resemble previously identified mutants in the *ARGONAUTE1* (*AGO1*) gene. *AGO1* loss of function results in plants with pleiotropic developmental abnormalities, such as pin-like leaves and petals, male sterility and dwarfing. Fagard *et al.*[1] show that the PTGS mutants from their screen contain mutations in the *AGO1* gene. Further, a previously isolated null *ago1* allele also reduces PTGS, demonstrating that *AGO1* is necessary for PTGS of transgenes and homologous endogenous genes. *AGO1* is similar to *QDE-2* from *Neurospora* and *RDE-1* from *Caenorhabditis elegans*. *QDE-2* and *RDE-1* are known to function in the PTGS pathway of their respective organisms. Thus, *AGO1*/*QDE-2*/*RDE-1* represents another component of PTGS that is highly conserved between plant and animal kingdoms.

The report from Fagard *et al.*[1] also links PTGS and plant development. PTGS could be a mechanism plants use to regulate gene expression and *ago1* mutants might alter PTGS of genes expressed at specific times or locations during plant development. For example, another group reported that one cytochrome P450 (CYP78A5) shows constitutive expression in an *ago1* mutant background, consistent with the hypothesis that *AGO1* functions to maintain normal expression patterns of endogenous genes. It will be of interest to determine how many genes are misexpressed in *ago1* mutants and whether the misexpression is because of alterations in PTGS. The *ago1* mutation also causes a decrease in DNA methylation of the GUS reporter transgene, possibly enforcing silencing at the transcriptional level. It will be of interest to examine *AGO1*-dependent methylation changes of endogenous genes. The pleiotropic phenotype of *ago1* mutants might be the result of genes with altered PTGS. It will be important to determine how commonly PTGS controls endogenous gene expression in plants.

AGO1, QDE-2 and RDE-1 are related proteins required for post-transcriptional gene silencing in plants, quelling in fungi and RNA interference in animals.

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**Reference**

**1** Fagard, M. *et al.* (2000) AGO1, QDE-2, and RDE-1 are related proteins required for post-transcriptional gene silencing in plants, quelling in fungi, and RNA interference in animals. *Proc. Natl. Acad. Sci. U. S. A.* 97, 11650–11654