Is there a unique integration mechanism of *Agrobacterium* T-DNA into a plant genome?

Denis Faure

Is there a unique integration mechanism of *Agrobacterium* T-DNA into plant genome?

"Nishizawa-Yokoi et al. suggest that host factors controlling T-DNA integration are different in somatic and germinal plant cells"

Agrobacterium evolved the capability to transfer a DNA fragment (T-DNA) of their own genome into the nuclear genome of plants (Gelvin, 2017). This natural process of inter-kingdom horizontal gene transfer (HGT) contributes to exploitation of the plant host by *Agrobacterium* bacterial pathogens (Gonzalez-Mula *et al*., 2018 and 2019). This unique HGT process also inspired the development of a wide spectrum of tools and technologies dedicated to genetic engineering of plants and some other eukaryotes (Sardesai & Subramanyam, 2018). In spite of a popular usage of these tools in basic science and genetic engineering, the ultimate step of the HGT process, namely integration of the T-DNA into nuclear genome of plants remains in a grey zone of knowledge (Singer, 2018). In this issue of *New phytologist*, Nishizawa-Yokoi *et al*. (pp. 000-000) suggest that host factors controlling T-DNA integration are different in somatic and germinal plant cells. This article invites the scientific community to debate about the existence of different molecular machineries allowing T-DNA integration in plant genome.

The successive steps of T-DNA transfer from *Agrobacterium tumefaciens* into plant nucleus involve bacterial and plant molecular factors (Gelvin, 2017; Figure 1). Upon activation of

virulence (*vir*) genes by plant phenolics, a single-strand DNA (T-strand) of T-DNA is excised from the tumor-inducing (Ti) plasmid of *A. tumefaciens* (Step-1 in the Figure 1) and then, transferred along with virulence proteins such as VirD2 and VirE2 (VirD2 being covalently attached to 5' end of T-strand) into plant cytoplasm using a dedicated type IV secretion system (Step-2). The complex associating T-strand and VirD2 and VirE2 proteins (called as T-complex) moves in cytoplasm and enters into the nucleus via nucleus pores (Step-3). In the nucleus, VirE2 proteins are through to be removed. T-DNA can become double-stranded (in linear and circular form) and can express transiently the encoded genes (Step-4). T-DNA can also integrate into plant genome and then can be expressed (Step-5). In the case of a stable transformation, the T-DNA together with plant genome is transmitted to the daughter cells along cell divisions that may lead to generation of a stably transformed plant. A few years ago, a milestone paper revealed the importance of the DNA polymerase theta (Pol θ also known as POLQ) encoded by *TEBICHI* (*polQ*) gene in stable integration of T-DNA in *Arabidopsis thaliana* (van Kreten *et al*., 2016). In eukaryotes, POLQ is a low fidelity DNA polymerase, which has been associated to several DNA modification processes, including repair of DNA double-stranded breaks that happen naturally during cell cycle and upon external stresses, such as infection by pathogens, radiations or exposure to toxic compounds (Inagaki *et al*., 2006 & 2009; Mateos-Gomez *et al*., 2015; van Schendel *et al*., 2015; Mara *et al*., 2019; Feng *et al*., 2019; Davis *et al*., 2020).

Nishizawa-Yokoi *et al*. investigated the contribution of POLQ in stable and transient T-DNA transformation in *A. thaliana* (ecotype Columbia) and *Oryza sativa* (cultivar Nipponbare) using *polQ* defective mutants. A strength of this work is to compare different conditions in which the T-DNA transfer succeeds (Figure 2). They used a disarmed T-DNA, in which the

oncogenes were removed, to evaluate T-DNA transfer in *A. thaliana* germ cells by the floral dip method and in somatic cells by infecting *A. thaliana* roots and *O. sativa* calli. They also used a virulent *A. tumefaciens* to investigate T-DNA transfer in *A. thaliana* root segments in a pathogenic context leading to the formation of galls.

In the case of germ cells, Nishizawa-Yokoi *et al*. reported that stable transformation by floral deep method was clearly impaired in *A. thaliana polQ* mutant: none stable transformants were obtained. According to the number of analyzed seeds, the percentage of T-DNA transfer was estimated below 0.003% of the collected seeds. In contrast, this percentage reached 0.17% in *A. thaliana* wild-type and up to 0.13% in a complemented *polQ* mutant. These results are in agreement with those published by van Kreten *et al*. (2016).

In the case of somatic cells, Nishizawa-Yokoi *et al.* reported a decreased of transient transformation (~ 11-65% efficiency of wild-type) and stable transformation (~ 55% efficiency of wild-type) by infecting roots of *A. thaliana polQ* mutants with *A. tumefaciens* carrying a disarmed T-DNA. Using a virulent *A. tumefaciens*, the *A. thaliana polQ* mutants were transformed to produce galls at 25% the efficient of wild-type roots. Presence of T-DNA insertions was confirmed by amplifying and sequencing T-DNA/plant genome junctions that resemble in *polQ* mutants and wild-type *A. thaliana*. These results evidence a measurable but moderate contribution of POLQ in stable transformation; therefore suggest the lack of absolute requirement of POLQ in the transformation of *A. arabidopsis* somatic cells. These observations were less dramatic than those reported by van Kreten *et al.* (2016): they obtained none stable transformation among 853 root segments of *polQ* mutants (< 0.12% of

the analyzed root segments) while stable transformation succeeded in 80% wild-type root segments.

In addition to *A. thaliana*, Nishizawa-Yokoi *et al.* explored what happened in rice. Using CRISPR mutagenesis and appropriate selective steps, they constructed homozygous *polQ* mutant lines in *O. sativa*. They evidenced that stable transformation occurred in *polQ* mutant calli at 15-20% the level of that of wild-type calli. DNA junction analysis also confirmed T-DNA integration into the plant genome. This stable transformation level is in the same range of that observed in *A. thaliana*, supporting a moderate contribution of POLQ in T-DNA stable transformation in somatic cells.

Overall, the study of Nishizawa-Yokoi *et al.* pinpoints a different contribution of POLQ in T-DNA integration into germ and somatic cells. Until now, only two papers investigated the role of POLQ in T-DNA integration with some consistent results but some discrepancies (van Kreten *et al.*, 2016; Nishizawa-Yokoi *et al.,* 2020): the progressive validation of knowledge is unexceptional in experimental sciences. What next? We need to know more about the role of POLQ along the T-DNA integration process. To exemplify some unsolved issues, we do not know gene expression of polQ and that of alternative DNA repair systems in somatic and germ cells in the presence and absence of Agrobacterium. Moreover, because A. thaliana polQ KO-mutants exhibit a pleiotropic phenotype (Inagaki et al., 2006 and 2009), we need additional tools and constructions to modulate with more precision POLQ activity and then examine its contribution to DNA repair and to T-DNA transfer in somatic and germ cells. Clearly, the paper of Nishizawa-Yokoi et al. invites the scientific community to investigate deeper how the Agrobacterium-mediated T-DNA integration succeeds in plant genomes.

ORCID http://orcid.org/0000-0002-5379-8867

Denis Faure

Paris-Saclay University, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France

denis.faure@i2bc.paris-saclay.fr

References

Davis L, Khoo KJ, Zhang Y, Maizels N. 2020. POLQ suppresses interhomolog recombination and loss of heterozygosity at targeted DNA breaks. *Proceedings of the National Academy of Sciences U.S.A*. **117**(37):22900-22909.

Feng W, Simpson DA, Carvajal-Garcia J, Price BA, Kumar RJ, Mose LE, Wood RD, Rashid N, Purvis JE, Parker JS, Ramsden DA, Gupta GP. 2019. Genetic determinants of cellular addiction to DNA polymerase theta. *Nature Communications* **10**(1):4286.

Gelvin SB. 2017. Integration of *Agrobacterium* T-DNA into the plant genome. *Annual Review of Genetics* **51**:195-217.

Gonzalez-Mula A, Lachat J, Mathias L, Naquin D, Lamouche F, Mergaert P, Faure D. 2019. The biotroph *Agrobacterium tumefaciens* thrives in tumors by exploiting a wide spectrum of plant host metabolites. *New Phytologist* **222**(1):455-467.

González-Mula A, Lang J, Grandclément C, Naquin D, Ahmar M, Soulère L, Queneau Y, Dessaux Y, Faure D. 2018. Lifestyle of the biotroph *Agrobacterium tumefaciens* in the ecological niche constructed on its host plant. *New Phytologist* **219**(1):350-362.

Inagaki S, Nakamura K, Morikami A. 2009. A link among DNA replication, recombination, and gene expression revealed by genetic and genomic analysis of TEBICHI gene of *Arabidopsis thaliana*. *PLoS Genetics* **5**(8):e1000613.

Inagaki S, Suzuki T, Ohto MA, Urawa H, Horiuchi T, Nakamura K, Morikami A. 2006. *Arabidopsis* TEBICHI, with helicase and DNA polymerase domains, is required for regulated cell division and differentiation in meristems. *Plant Cell* **18**(4):879-892.

Mara K, Charlot F, Guyon-Debast A, Schaefer DG, Collonnier C, Grelon M, Nogué F. 2019. POLQ plays a key role in the repair of CRISPR/Cas9-induced double-stranded breaks in the moss *Physcomitrella patens*. *New Phytologist* **222**:1380-1391.

Mateos-Gomez PA, Gong F, Nair N, Miller KM, Lazzerini-Denchi E, Sfeir A. 2015. Mammalian polymerase teta promotes alternative NHEJ and suppresses recombination. *Nature* **518**(7538):254-257.

Nishizawa-Yokoi A, Saika H, Hara N, Lee LY, Toki S, Gelvin SB. 2020. *Agrobacterium* T-DNA integration in somatic cells does not require the activity of DNA polymerase theta. *New Phytologist* doi: 10.1111/nph.17032.

Sardesai N, Subramanyam S. 2018. *Agrobacterium*: a genome-editing tool-delivery system. *Current Topics in Microbiology and Immunology* **418**:463-488.

Singer K. 2018. The mechanism of T-DNA integration: some major unresolved questions. *Current Topics in Microbiology and Immunology* **418**:287-317.

van Kregten M, de Pater S, Romeijn R, van Schendel R, Hooykaas PJ, Tijsterman M. 2016. T-DNA integration in plants results from polymerase-teta-mediated DNA repair. *Nature Plants* **2**(11):16164.

van Schendel R, Roerink SF, Portegijs V, van den Heuvel S, Tijsterman M. 2015. Polymerase teta is a key driver of genome evolution and of CRISPR/Cas9-mediated mutagenesis. *Nature Communications* **6**:7394.

Figure legends

Figure 1. *Agrobacterium*-mediated T-DNA transformation in plants.

A schematic representation of the main steps of *Agrobacterium*-mediated T-DNA transformation in plants: (1) T-strand excision; (2) T-strand transfer into plant cytoplasm; (3) T-complex transfer into nucleus; (4) transient expression of T-DNA; (5) stable integration and expression of T-DNA. More details are given in the text.

Figure 2. *Agrobacterium*-mediated T-DNA transformation in germinal and somatic cells.

In plants, Arabidopsis thaliana for instance, disarmed T-DNA may be transferred into germ cells to produce some stably transformed seeds, which may germinate and develop to transformed plants; disarmed T-DNA may be transferred into somatic cells (in roots for instance) to generate calli, which may regenerate transformed plants; T-DNA may be transferred into somatic cells to provoke galls in a pathogenic context.