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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  $\theta$  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 dip 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.

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## 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.