T-DNA transfer from *Agrobacterium tumefaciens* to plant cells

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Agrobacterium tumefaciens strains have been engineered as vectors to transfer designed DNA segments into plant cells. In this presentation, I want to summarize some of the principles of this process but also indicate some of the many open questions.

Originally Zambryski and Stachel pointed out the similarity between the bacterial conjugation process and the inter-kingdom gene transfer process used by Agrobacterium. In both cases, an oriented transfer occurs from a nicked origin of transfer till a terminator. A displaced single strand piloted by a 5' covalently bound relaxase is transferred via a complex type IV secretion apparatus towards the acceptor cell (Christie, 2004) where it becomes double stranded via lagging strand synthesis. In this respect, it is still unclear how this is done in the plant nucleus, and it might differ according to the induced repair systems and the physiology of the acceptor cell. Intriguing is the finding of T-DNA circles after transient expression (Singer et al., 2012). However, it is unlikely that circles would be an intermediate in the integration process because most T-DNA/plant DNA junctions are found at the RB and LB positions, indicating that it inserts as a linear molecule. Rather, the T-circles may be the basic substrates for transient expression found early after transfer. Intriguing is the question on the control of the number of T-DNA copies found in transformed cells. Are several T-strands transferred occasionally? Are accessible cells transformed by several bacteria? Can extrachromosomal double-stranded DNA be replicated? Pros and cons will be discussed.
A milestone in our understanding of the random T-DNA integration was the observation that T-DNAs integrate at induced double-strand breaks (Salomon and Puchta, 1989), explaining the well-known fact that T-DNAs integrate randomly, but at the same time that T-DNAs transferred together from different bacteria integrate at the same locus (De Neve et al., 1987). The linkages between different T-DNAs resemble the T-DNA/plant DNA junctions, and suggest involvement of primarily the non-homologous end joining repair pathway.
The hope is to steer and optimize targeted T-DNA integration via the CRISPR-Cas system, either by inducing specific double-strand breaks or by increasing the homology-dependent repair pathway.