# *Agrobacterium*, a bacterium full of surprises.

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Already more than 100 years ago it was discovered that the bacterium we now call *Agrobacterium tumefaciens* induces crown gall disease in plants. Many details about the molecular mechanism by which Agrobacterium causes disease are now known and this knowledge was used to develop Agrobacterium into an efficient vector for the genetic modification not only of plants, but also of yeasts and fungi.

Agrobacterium transforms plant cells by introducing a single stranded copy of a segment of its Ti plasmid, the T-DNA, into them through a Type4 (TFSS) Secretion System. T-DNA transfer is accompanied by the translocation of a set of Virulence proteins (effector proteins) into host cells, which are necessary for optimal transformation. The effector proteins which are secreted in the host cells are characterized by an arginine rich signal in their C-termini. Coupling of this signal to heterologous proteins can drive their transfer to host cells and this may be used for 'protein therapy' of eukaryotic cells. The T-DNA is transferred to plant cells as a nucleoprotein complex with the VirD2 relaxase covalently bound to its 5'end. The VirD2 protein has a special bipartite TFSS secretion signal, which is essential for transformation, but which can be replaced by the monopartite C-terminal translocation signal of the effector proteins. The transfer of DNA by the Agrobacterium TFSS therefore is a consequence of the covalent linkage of the T-strand to the relaxase with the translocation signal.

It has been observed that the T-DNA with the transgenes integrates at random positions and in variable copy numbers in the plant genome by non-homologous recombination (NHR). We have identified the genes (enzymes) that are responsible for (T-)DNA integration by using yeast as a model. In the yeast *Saccharomyces cerevisiae* DNA is preferably integrated by HR using Rad51 and Rad52, but, if homology is absent, by the Ku heterodimer and Lig4, which mediate non-homologous end-joining (NHEJ). Inactivation of NHEJ largely prevents non-homologous T-DNA integration in yeasts and fungi. However, in plants NHEJ-mutants still show non-homologous integration with high frequency indicating that other player(s) are at work. Recently, we found an enzyme that is essential for the first step of T-DNA integration in plants; in its absence no stable transformation was seen any more. This opens the way to develop systems for efficient targeted DNA integration in plants, which also employ site specific nucleases and/or site specific recombination enzymes, which may be delivered by the TFSS if coupled to the identified translocation signal.