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Summary

Agrobacterium tumefaciens, a gram-negative plant pathogen belonging to the family *Rhizobiaceae*, is the causative agent of crown gall disease and plays critical roles in worldwide agriculture. It induces tumor formation in plants by transferring a segment of its tumor-inducing plasmid (Ti-plasmid) to the plant cell. This transferred DNA (T-DNA) contains genes involved in the synthesis of auxin, cytokinin and opines resulting in uncontrolled cell proliferation and production of opines. Under laboratory conditions it is also able to transform other eukaryotes such as the yeast *Saccharomyces cerevisiae* and other fungi (Bundock et al., 1995; de Groot et al., 1998). Hence, the many experimental tools available for research on *S. cerevisiae* can be applied to obtain new insights into the mechanism of *Agrobacterium*-mediated transformation (AMT). These insights can contribute to the development of more efficient transformation methods for use in the plant field. In this respect more insight in the role of host factors in AMT is important and useful.

The yeast *S. cerevisiae* has emerged as an excellent host model to study the mechanism of AMT (Bundock et al., 1995; van Attikum et al., 2001, 2003; Ohmine et al., 2016; Hooykaas et al., 2018). The bacterial side of AMT has been investigated rather well but the host factors involved in this process remain obscure. Previous research in our group revealed that the efficiency of AMT of the yeast *ada2Δ* deletion mutant is higher than that of the wild type strain when the T-DNA is able to integrate by homologous recombination (Soltani, 2009). In Chapter 2, it was confirmed that the efficiency of AMT of yeast cells lacking *ADA2* was increased when the T-DNA can be integrated by homologous recombination (HR). In addition, we showed that the AMT efficiency of the *ada2Δ* mutant was also highly increased when the T-DNA has to be integrated by non-homologous end joining (NHEJ). Deficiency of *ADA2* confers hypersensitivity to DNA damaging agents such as methyl methanesulfonate (MMS) and hydroxyurea (HU). It has been reported that the homologous protein (*ADA2b*) from *Drosophila* and *Arabidopsis* is involved in the DNA damage response (Qi et al., 2004 and Lai et al., 2018). Even though the link between the increased AMT efficiency and impaired double strand break (DSB) repair in *ada2Δ* deletion mutant remains obscure, one hypothesis is that the enhanced sensitivity to DNA damage leads to the formation of more DSBs as a landing pad for T-DNA integration. In general, DNA damage events may occur at random chromosomal sites. Consistent with this, AMT efficiency by NHEJ increased 50 fold while that by HR was only 2 fold higher in the absence of *ADA2* compared to wild-type strain. A genome-wide rescue screen indicated that overexpression of *SFP1* in the *ada2Δ* background enhances the resistance to DNA damaging agents and attenuate the increased AMT efficiency to wildtype levels in the *ada2Δ* deletion mutant. That the increased AMT efficiency of the *ada2Δ* deletion mutant is the consequence of incorrect regulation of unknown genes involved in the integration process, however, could not be ruled out. Furthermore, considering the role of *ADA2* in defining the boundary of heterochromatin regions, the increased AMT efficiency of the *ada2Δ* deletion mutant may be the result of expression of the selection gene at integration sites which may otherwise be silenced and could therefore not be detected.

Upon AMT a considerable amount of T-DNA is present as circular extrachromosomal structures rather than integrated into chromosomal DNA (Singer et al., 2012). Such structures are present at a higher frequency in the *ada2Δ* deletion mutant than in the wild type strain. In Chapter 3, by analysis of the junctions formed between the LB and RB, T-circles isolated from *Nicotiana benthamiana* or the yeast *ada2Δ* deletion mutant were characterized. These

extrachromosomal T-DNA structures contained various formats such as perfect fusions of LB and RB, binary plasmid sequences, filler host DNA sequences and T-DNA truncations. The sequence of the LB- RB junctions resembled that of repaired DNA double-strand breaks sites and therefore the formation of extrachromosomal T-circles was proposed to exploit the same DNA repair mechanisms as those used to explain T-DNA integration. Collectively, our results lead to a conceptually simple mechanism for T-DNA junction formation: HR in yeast and TMEJ mediated by *Pol θ* in plants. The distinct ability of *Pol θ* to extend minimally paired 3' ends may provide a possible explanation for the existence of filler sequences to a large extent.

The *Agrobacterium* virulence protein VirD2 is potentially involved in the T-DNA integration process. To expand our knowledge about the function of VirD2 during AMT, in Chapter 4 we exploited the auxin-inducible degron (AID) system (Morawska and Ulrich, 2013) to specifically degrade VirD2 after translocation into the host nucleus. The efficiency of AMT of yeast was strongly reduced upon auxin-induced degradation of VirD2. With respect to plant cells, the size of the tumors was reduced and the number of calli decreased strikingly, thus indicating T-DNA integration was greatly diminished. Combined with the results that T-DNA could be delivered into the plant nucleus successfully with the guide of AID-tagged VirD2, we can conclude that VirD2 plays a role inside the host nucleus, possibly in the T-DNA integration process. Previously it has been shown that when the VirD2 omega domain was mutated, the efficiency of transient transformation was reduced to 20-25% and stable transformation was almost eliminated (only 1-3% of that of wild type) (Narasimhulu et al., 1996). Besides, VirD2 was reported to interact with yeast histones (Wolterink-van Loo et al., 2015) and probably plant histone proteins or other chromatin structural proteins (Gelvin and Kim, 2007). In this scenario, T-DNA may target DSB sites with the help of VirD2 and VirD2 could interact with host DNA repair proteins to serve as a prelude to guide T-DNA to chromatin and facilitate the integration process.

Many different *Agrobacterium* strains have been isolated from nature and they may differ in their virulence on specific plants. They are often classified on the basis of the opines, tumor-specific metabolites, that are found in the tumors and serve as a nitrogen and carbon source for the bacterium (Dessaux et al., 1993). Several Ti-plasmids have been completely sequenced, such as those from both nopaline and octopine-*Agrobacterium* strains. On the other hand, until now Ti-plasmids from chrysopine and succinomopine *Agrobacterium* strains have not been sequenced.

In Chapter 5, we report the complete nucleotide sequence of pTiChry5, a Ti-plasmid isolated from a chrysopine *Agrobacterium* strain. A detailed alignment between pTiChry5 and agropine plasmid pTiBo542 was made in order to more comprehensively understand their evolutionary relationship. In general, these two plasmids have a similar order of functional areas with different interruptions by transposable elements and losses or gains of small sets of genes. Based on similarity, unexpectedly, only roughly one half of the plasmid containing *rep*, *vir*, *tra*, *acc*, TL-DNA is very similar in the two plasmids, whereas the other half (*trb*, Amadori opine catabolism up to the chrysopine synthase gene), is much more different suggesting that these plasmids are chimeric due to recombination with other opine-related plasmids. The presence of two *repABC* units in both these plasmids is in line with this hypothesis. Especially, the development of new chrysopine profiles may have conferred evolutionary advantage on their host bacteria in some specific environments.

Based on phylogenetic analysis, the evolutionary relatedness of nopaline-type Ti-plasmids was elucidated in Chapter 6. We elucidated the sequence of the succinamopine Ti plasmid pTiEU6. Analysis of the sequence confirms its close relatedness to the nopaline Ti-plasmids in line with the fact that succinamopine has a similar chemical structure as nopaline. By a detailed comparison to the sequences of 8 nopaline Ti plasmids (of which 6 were newly sequenced) we could infer that the succinamopine Ti plasmid is evolutionary derived from the group A nopaline Ti plasmids (pTiSAKURA, pTiT37), which is characterized by the presence of a copy of IS1182 at a specific location and a set of *tra* genes that has only 77% similarity to that of other nopaline Ti plasmids. In pTiEU6 the area occupying the right part of the T-region (including gene *6b* and *nos*) and the cluster of genes involved in nopaline import and catabolism up to the conserved *trb* cluster involved in conjugation was replaced by a new set of genes that is overall more similar to that present in chrysopine and agropine Ti plasmids and involved in succinamopine/leucinopine synthesis, import and catabolism. It is likely that this was brought about by a recombination event between a group A nopaline Ti plasmid and another type of Ti plasmid.

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