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Host genes involved in *Agrobacterium*-mediated transformation

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General discussion

Summary and general discussion

Agrobacterium tumefaciens is a unique phytopathogen that by genetic transformation recruits the plant cell as a factory to produce specific nutrients on which it can grow. The virulence system of *Agrobacterium* which is encoded by the Tumor-inducing (Ti) plasmid plays a key role in transferring a piece of the Ti-plasmid, called T-DNA, to the plant cell. The *Agrobacterium* virulence genes involved in this process are relatively well-known. However, little is known about the molecular events that occur at later stages of transformation in the host cell. Previous studies indicate that host nuclear importins and recombination systems play key roles in *Agrobacterium*-mediated transformation (AMT). Many other candidate genes coding for products involved in AMT have been identified in searches for mutants resistant to *Agrobacterium* transformation in libraries of *Arabidopsis thaliana* mutants. As, besides plants, under laboratory conditions *A. tumefaciens* can transform a large number of non-plant species, I have set out to use the model eukaryotic yeast *Saccharomyces cerevisiae* for a genome-wide search for genes involved in AMT. **Chapter 1** outlines the flourishing application of *A. tumefaciens* for transformation of non-plant organisms, with a special focus on the yeast *S. cerevisiae*. During the last decade *S. cerevisiae* has served in our lab as an excellent model organism to understand the role of host genes involved in T-DNA integration. Indeed, by exploiting the genetic tools available for this model organism, the importance of the host DNA recombination machinery in T-DNA integration was discovered by our group. This discovery yielded an improved gene targeting system for filamentous fungi and plants. Hence, we are interested in identifying -in a systematic way- the complete set of genes/proteins of *S. cerevisiae* which are positively or negatively involved in *Agrobacterium*-mediated transformation.

Yeast *S. cerevisiae* has ~6200 genes of which ~4800 are non-essential. Because of the high efficiency of homologous recombination in yeast, deletion mutant collections of *S. cerevisiae*, both haploid and diploid, homozygous and heterozygous have been constructed and are maintained in 96-well microtiter plates. **Chapter 2** describes the development of a high throughput AMT system using the 96-well microtiter plates. This development enabled us to handle the yeast homozygous diploid deletion collection (~4800 strains) in three months. The basic difference of this protocol with the standard protocol is the streaking out of the bacterium-yeast

cocultivation mix on selection plates rather than the harvesting of the bacterium-yeast mix and spreading on selection and non selection plates (standard protocol). Indeed in the microtiter plate based protocol the output of yeast cells on non-selection plates is not regarded. Although this may make bias and may yield crude data, AMT of identified candidate AMT-mutants by the standard protocol confirmed the reproducibility of the high-throughput protocol for large-scale experiments.

As described in **Chapter 3**, using two strains of *A. tumefaciens* we recruited our 96-well microtiter-based protocol to perform a high-throughput AMT to screen the yeast homozygous diploid deletion collection for identification of the mutants that increase or decrease AMT efficiency. This resulted in the identification of 249 genes deletion of which either increased (141 genes) or decreased (108 genes) *Agrobacterium*-mediated transformation efficiency. The identified genes have different functions all over the cell indicating that the whole cellular system is challenged by AMT.

Since T-DNA mainly integrates into the host genome at DNA double-strand breaks, of special interest was the identification in our screen of host factors involved in chromatin modification and DNA repair, e.g. histone acetyltransferases (HATs) and histone deacetylases (HDACs). Hence, in **Chapter 4** we investigated the effect of deletion of yeast HATs and HDACs on AMT using the standard protocol. This study highlighted the effects of yeast *GCN5* histone acetyltransferase deletion of which increased AMT, and *HDA3* and *HDA2* histone deacetylases deletion of which decreased AMT.

After the initiation of T-DNA processing by the VirD1/VirD2 relaxase, VirD2 remains covalently bound to the 5'-end of the T-strand, and with cooperation of the type four secretion system pilots the whole T-strand to the host cell. Meantime several effector virulence proteins, i.e. VirD2, VirD5, VirE2, VirE3, and VirF are also translocated to host cells. When inside the host cell, the T-strand is covered by VirE2, thereafter called T-complex. VirE2 and VirD2 play key roles to translocate the T-complex into the host nucleus, and maybe to the sites of integration. In **Chapter 5**, Green Fluorescent Protein (GFP) and Yeast Two-Hybrid (Y2H) methodologies are recruited to investigate the localization of VirD2 in the yeast cell, and to identify the yeast proteins that interact with the VirD2 protein. GFP studies indicated that VirD2 localizes to the yeast nucleus. Y2H screening of *S. cerevisiae* genomic DNA libraries identified 12 potential candidates that may interact with the VirD2 protein.

In this thesis we have been able to show that a microtiter-based AMT protocol can be used for large scale *Agrobacterium*-mediated transformation of yeast strain collections. Moreover, we have identified 249 eukaryotic host genes whose deletion increases or decreases AMT efficiency and we further have shown the effect of host histone acetyltransferases and histone deacetylases on AMT. Such a broad range of host factors that affect AMT might be exploited as leads in biotechnology to optimize AMT, or in agriculture to prevent AMT.