Functional characterization of T-DNA transfer via VirB/D4 type IV secretion system in reference to conjugational DNA transfer

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Content of the thesis

Chapter 1. DNA repair genes *RAD52* and *SRS2*, a cell wall synthesis regulator gene *SMI1*, and the membrane sterol synthesis scaffold gene *ERG28* are important in efficient *Agrobacterium*-mediated yeast transformation with chromosomal T-DNA

Abstract: Plant pathogenic *Agrobacterium* strains can transfer T-DNA regions of their Ti plasmids to a broad range of eukaryotic hosts including fungi in *in vitro* environments. In the recent decade, the yeast *Saccharomyces cerevisiae* is used as a model host to reveal important host proteins for the *Agrobacterium*-mediated transformation (AMT), because the transfer occurs by simple co-cultivation and output AMT transformant yeast cells are easily countable after several days of cultivation. Further investigation is required to understand the fundamental mechanism of AMT, including interaction at the cell surface, to expand the host range, and to develop new tools. In this study, I screened a yeast mutant library for low AMT mutant strains by advantage of a chromosome type T-DNA, which transfer is efficient and independent on integration into host chromosome. By the mutant screening, I identified four mutant strains (*srs2Δ*, *rad52Δ*, *smi1Δ* and *erg28Δ*), which showed considerably low AMT efficiency. Physiological experiments indicated that growth of *erg28Δ* mutant strain was unaffected by the presence of donor bacterial cells, while the growth of the wild-type and other mutant yeast strains was suppressed by the bacterial presence. Excess quantities of the donor cells severely decreased AMT efficiency of the wild type strain. Therefore, the ergosterol synthesis regulator and synthesis enzyme-scaffold gene *ERG28* probably contributes to AMT by sensing the presence of donor bacteria and a congested environment and subsequently suppressing cell division. Structural analysis of T-DNA product replicons in AMT colonies suggested that the two DNA repair genes, *SRS2* and *RAD52* act soon after T-DNA entry for modification of the chromosomal T-DNA to stably maintain them as linear replicons and to circularize certain

T-DNA simultaneously. The cell wall synthesis regulator SMI1 is necessary for maintenance of the rigid cell surface structure and is supposed to be important for interaction between the donor and recipient cells. The *smi1Δ* mutant exhibited pleiotropic effects, i.e. low effector protein transport as well as low AMT for the chromosomal T-DNA, but unexpectedly high AMT for integrative T-DNAs. The involvement of the cell wall synthesis regulator *SMI1* remains to be elucidated.

Chapter 2. Comparison of *Agrobacterium*-mediated bacterial transformation between VirD2- and Mob-driven transfers

Abstract: *Agrobacterium* T-DNA transfer system is capable of genetically transforming a broad range of eukaryotic organisms including fungi and mammalian cells under laboratory conditions in addition to the transformation of plant species in nature. Thereby, it is an interesting subject to reveal how much broadly the system's host range is widened further. This inter-domain transfer system has its origin from conjugal plasmid transfer systems among bacterial. There are convincing similarities between T-DNA transfer and bacterial conjugal transfer systems. In fact, a literature reports that T-DNA was delivered to a Gram-positive bacterium *Streptomyces lividans*. I report for the first time transfer of a model T-DNA plasmid to Gram-negative bacterium, namely *Escherichia coli.* The model T-DNA plasmid in my experiments contains the T-DNA right border RB and its transfer is mediated by the genuine nickase/relaxase VirD2, and another plasmids for comparative purpose are mobilizable plasmids having an origin of transfer (*oriT*) and its cognate nickase/relaxase gene *mob*. Transfer of the plasmids was carried out using the *Agrobacterium* VirB/D4 type IV secretion system. The VirD2-driven transfer to *E. coli* strain BW2511 established AMT colonies at an efficiency much less than those of Mob-driven transfer. The plasmid DNAs extracted from the *E. coli* colonies retained its intact plasmid structure without any nucleotide modification at the border sequence RB. VirD2-driven transfer was superior to Mob-driven transfer when the two plasmids were transferred to yeast cells. The inverse preference suggests adaptation of VirD2 to eukaryote cells during the evolution from the bacterial conjugation system. Deletion mutations in several *E. coli* exonuclease genes increased the efficiency of not only VirD2-driven but also Mob-driven AMT. Similar enhancement was also provoked by exonuclease mutations in transfer of the self-transmissible plasmids, F′ and RP4. These results indicate that T-DNA transfer system retains characteristics of basic bacterial conjugal transfer, and suggest that exonucleases in recipient cells play a role to suppress invasion of exogenous DNA *via* type IV secretion systems.