学位論文要旨

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

（T-DNA 伝達と接合伝達の輸送機能に関する比較解析）

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Crown gall disease in plants causative agents are Gram-negative soil bacteria belonging to the family *Agrobacterium* (syn. *Rizobium*). The pathogenic *Agrobacterium* strains contain a tumor-inducing (Ti) plasmid and deliver a portion of the plasmid DNA (T-DNA) to the plant cells and transform the recipients to produce nutrients for the pathogenic bacteria. Under laboratory conditions, *Agrobacterium tumefaciens* is capable of genetically transforming a broad range of eukaryotic organisms including fungi and mammalian cells. T-DNA transfer system of *Agrobacterium* is a derivative of bacterial conjugal DNA transfer systems, which are originally used to exchange genetic materials between bacterial species.

Mechanism of T-DNA transfer processes have been approached from both donor and host cell sides. The pathogenic plasmid plays a primary role for the transfer in the donor cell side. Most virulence genes (*vir*) encode proteins that are essential for and involved directly either processing or transfer process. VirB/VirD4 type Ⅳ secretion system (T4SS) conveys T-DNA and effector proteins into the host cell. On the contrary, less effort was invested for host factor analysis. Further study is necessary for in detail mechanism and finding out new factors. *Arabidopsis thaliana* rat mutants, which are defective in *Agrobacterium*-mediated transformation (AMT) (Zhu et al., 2003), facilitated this line of study (Gelvin, 2010) in plants. Another type of approach employed yeast as a host in replace of plants.

In Chapter 1, I screened a yeast mutant collection using a yeast artificial chromosome (YAC) type T-DNA, which transfer is efficient and independent of integration into host chromosome to investigate the transfer factors in yeast. Four mutant strains (*srs2Δ*, *rad52Δ*, *smi1Δ* and *erg28Δ*) were found to show considerably low AMT efficiency. Structural analysis of T-DNA product replicons in AMT colonies of each one of the two DNA repair gene mutants, *srs2Δ* and *rad52Δ*, revealed that all of the T-DNA replicons maintained in the mutant cells are circularized. And, when a linear YAC DNA was introduced by lithium acetate transformation method, the two mutant strains showed significantly low transformation efficiencies compared with wild-type strain, whereas the mutants exhibited high efficiency comparable with the wild-type strain when using circular form of the YAC DNA. These results suggested that the two genes contribute to the stability of the linear replicons. In addition, the telomere-less plasmid pBYM3 also reduced the AMT efficiency of both mutant strains, demonstrating that the genes are involved in circularization of received T-DNA molecules. The scaffold gene for ergosterol synthesis enzymes, *ERG28*, probably contributes by sensing a congested environment, because growth of *erg28Δ* 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 their presence. Input recipient cell number at the start of the co-cultivation largely affected AMT with the YAC type T-DNA, largely affected AMT with the YAC type T-DNA, thus the continued growth of *erg28Δ* mutant cells is likely to attenuate AMT efficiency. Actually, *erg28Δ* mutant showed a wild-type level AMT efficiency in experiments that supplied a 4-fold larger number of input yeast cells. The high input cell number causes acute congestion also to the wild-type strain in the co-cultivation. The physiological role of *ERG28* gene in association with other

organism is unique and would attract attention as a good model. The cell wall synthesis regulator *SMI1* might have a role in the cell surface interaction between the donor and recipient cells, but the *smi1Δ* mutant exhibited pleiotropic effect, i.e. low effector protein transport as well as low AMT for the chromosomal T-DNA, but relatively high AMT for integrative T-DNAs. The mechanism for involvement of *SMI1* remains to be elucidated.

T-DNA transfer system is a derivative of the bacterial DNA transfer systems, which are originally used to exchange genetic materials between bacterial species. There are convincing similarities between T-DNA transfer and other bacterial transfer systems. *Agrobacterium* relaxase protein VirD2 makes a nick at two border sequences and covalently attaches to the 5′ end of the resulting single-stranded T-DNA. Essentially same reaction takes place in the bacterial conjugation. The fertility plasmid F contains *traI* and an origin of transfer (*oriT*) locus. TraI nickase binds to *oriT* site to nick there, bind covalently, and release a single-stranded DNA (ssDNA). The complex between nickase/relaxase protein and ssDNA is transported through type Ⅳ secretion apparatus into recipient cells. Until now, little is known about transfer of T-DNA between bacteria. What differences are there between the transfer processes of T-DNA transfer and bacterial conjugal transfer systems?

In Chapter 2, I investigated whether *Agrobacterium* can transfer T-DNA to Gram-negative bacteria. I challenged to transfer T-DNA to *Escherichia coli* with a focus and compared the genuine AMT by VirD2-driven T-DNA transfer and Mob relaxase-driven plasmid DNA transfer via VirB/D4 T4SS. I demonstrated that VirD2-driven transfer is able to spawn genetically modified *E. coli* cells, while the VirD2-driven AMT efficiency is lower than Mob-driven AMT efficiency. Structural investigation of plasmids transferred by VirD2 protein exhibited that these plasmids precisely retain the intact plasmid structure without any nucleotide sequence modification at the transfer border locus. I found mutations in several *E. coli* exonuclease genes increase the efficiency of AMT not only by VirD2 but also by Mob. Furthermore, similar effect provoked by exonuclease genes mutations was also exhibited in transfer of the self-transmissible plasmids, F′ and RP4. These results indicate that T-DNA transfer system retains characteristic feature of basic bacterial conjugal transfer, and suggest that recipient exonucleases play a suppressive role against invasion of exogenous DNA.

In conclusion, the transferred T-DNA molecules interact with DNA repair pathway proteins, and thereby the T-DNA molecules are maintained through the process such as stably replication or integration to recipient genome. The recipient genes, *ERG28* and *SMI1,* essential to arrange cell surface structures are also important for high-efficiency AMT. On the other hand, it became clear that T-DNA transfer system functionally retains characteristic feature of basic bacterial conjugal transfer. Recipient nucleases were found involving adversely to the transfer.