

Gene targeting by homologous recombination as a biotechnological tool for rice functional genomics

Yasuyo JOHZUKA-HISATOMI

Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences

Through a gene-targeting procedure with positive-negative selection, we previously reported the generation of fertile transgenic rice plants with a positive marker inserted into the *Adh2* gene by using an *Agrobacterium*-mediated transformation vector containing the positive marker flanked by two 6-kb homologous segments for recombination (1). We also reported that base changes within the homologous segments in the vector were efficiently transferred into the endogenous *Adh2* gene region of rice recombinants (2).

To introduce only point mutations into rice endogenous gene through gene-targeting by homologous recombination, I tried to remove a selective-marker gene by site-specific recombination systems such as the Cre/*lox* system. To eliminate *hpt* selective-marker gene flanked by directly repeated *loxP* sites from *Adh2* gene with base changes, I have employed the estrogen receptor-based XVE system to induce the Cre recombinase. I attempted to remove *hpt* from *adh2::hpt* by introducing T-DNA carrying the inducible Cre system (pXVE-Cre) into calli derived from seeds of the targeted plants (*adh2::hpt/adh2::hpt*). After selection of calli having pXVE-Cre, I succeeded to obtain several calli without *hpt* gene screened by PCR amplification.

As for the genome sequence in *Adh2* gene region of marker-free plants, it must be identical to wild type plant except for the base changes introduced by gene-targeting and 34 bp *loxP* sequences. The modifications of genomic sequences not only introduction of point mutations but also large deletion into specific target gene using gene-targeting by homologous recombination combined with the Cre/*lox* site-specific recombination system have potential application to functional genomic analysis in rice.

References

- 1) Plant Physiology (2007) 144: 846-856.
- 2) Nucleic Acids Res (2008) 36, 4727-4735.