Modification of an endogenous gene by homologous recombination-promoted gene targeting in rice

Shigeru Iida

Global COE Program, Laboratory of Molecular Genetics, Graduate School of Nutritional and Environmental Sciences and Graduate School of Pharmaceutical Sciences, University of Shizuoka

Rice is an important staple food for more than one half of the population of the world, and it has become the first crop plant to have its 389-Mb genome sequenced. Even though various functional genomic tools for elucidating the function of rice genes are available, developing new methods for characterizing genes of interest by reverse genetic approach has become particularly important.

Gene targeting refers to the alteration of a specific DNA sequence in an endogenous gene at its original locus in the genome and, often, to the conversion of the endogenous gene into a designed sequence. While various targeted modifications of endogenous genes, including knock-in to monitor the expression of a gene by fusing a reporter gene with its endogenous promoter, are routine practice in mice, gene targeting by homologous recombination in flowering plants remains in its infancy. We have developed a reproducible gene-targeting procedure with positive-negative selection and succeeded in obtaining fertile transgenic knock-out or knock-in rice plants. Moreover, base changes within the homologous segments carried by the vector employed were found to be efficiently transferred into the corresponding genomic sequences of rice recombinants. Based on these results, implications for the modification of endogenous genes for functional genomic analysis by gene targeting are discussed.

References

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