DIVISION OF MOLECULAR GENETICS †

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The main interest of this division was characterizing various aspects of genetic and epigenetic gene regulations including the flower pigmentation of morning glories. In addition, we were undertaking reverse genetic approaches in order to elucidate the nature of dynamic genomes in rice, a model plant for cereals.

Because the activities of this group were terminated at the end of March, please consult the last year's annual report for most of our activities with the exception of the following knock-in targeting of a rice gene by homologous recombination.

I. Knock-in targeting of endogenous natural genes by homologous recombination in rice

Although the analysis of the expression of a reporter gene fused with an appropriate promoter segment in transgenic plants, often termed promoter-reporter gene fusion analysis, is widely used to characterize the cloned promoter sequences, inter-individual variation of the reporter gene expression among transgenic plants, which is attributed mainly to the insertion sites and/or copy number of the transgene and epigenetic gene silencing, hampers proper spatiotemporal evaluation of the promoter activity. To circumvent such problems, an approach known as a "promoter trap" is also employed; a transformant having a promoterless reporter gene, which is carried by appropriately modified T-DNA sequences or transposons, integrated into an exon of an endogenous target gene in the proper orientation and resulting in the reporter gene expression by transcriptional fusion, is to be isolated for promoter analysis among the transformants containing the randomly inserted transgenes. Because the promoter trap is based on random insertional mutagenesis, the isolation of an appropriate promoter-trapped mutant relies on a fortuitous integration of the transgene into the target gene of interest, even though the promoter trap is much more likely to accurately reflect the expression of a gene than promoter-reporter gene fusion. Moreover, simultaneous integration of additional transgene copies may cause a potential problem because multiple insertions may complicate the interpretation of spatiotemporal expression patterns. As is the case in mice, the homologous recombination-promoted knock-in-targeting strategy is expected to be a powerful tool to monitor promoter activity accurately in plants. Knock-in targeting appears to offer at least two advantages over the promoter trap; the junction sequence between a reporter gene and an endogenous target promoter can be designed properly, and transgenic plants carrying an identical and desired knock-in allele can be repeatedly obtained. We have succeeded in obtaining 15 independent and fertile transgenic knock-in rice plants, which have only one copy of the promoterless *GUS* reporter gene encoding β-glucuronidase fused with the endogenous promoter of *MET1a*, one of two rice *MET1* genes encoding a maintenance DNA methyltransferase. As Figure 1 shows, the spatiotemporal gene expression of *GUS* was reproducibly observed in a dosage-dependent manner among independently isolated plants.

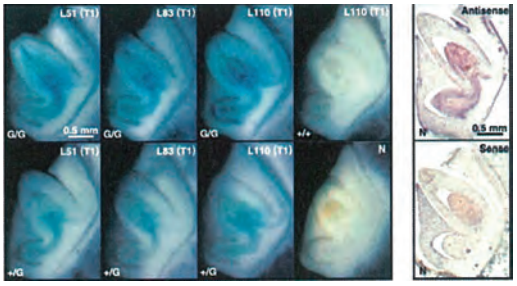

Figure 1. Reproducible and dosage-dependent expression of *GUS* in knock-in targeted plants. (**Left**) Embryos in T1 plants. (**Right**) *In situ* hybridization pattern of *MET1a* in embryos of Nipponbare. The symbols G/G, +/G, and +/+ indicate *GUS/GUS*, *MET1a/GUS*, and *MET1a/MET1a*, respectively.

Publication List

〔Original papers〕

- Hoshino, A., Park, K.I., and Iida, S. (2009). Identification of *r* mutations conferring white flowers in the Japanese morning glory, *Ipomoea nil*. J. Plant Res. *122*, 215-222.
- Ikeda-Kawakatsu, K., Yasuno, N., Oikawa, T., Iida, S., Nagato, Y., Maekawa., M., and Kyozuka, J. (2009). Expression level of *ABERRANT PANICLE ORGANIZATION* determines rice inflorescence form through control of cell proliferation in the meristem. Plant Physiol. *150*, 736-747.
- Shimatani, Z., Takagi, K., Eun, C.H., Maekawa, M., Takahara, H., Hoshino, A., Qian, Q., Terada, R., Johzuka-Hisatomi, Y., Iida, S., and Tsugane, K. (2009). Characterization of autonomous *Dart1* transposons belonging to the *hAT* superfamily in rice. Mol. Gen. Genomics *281*, 329-344.
- Yamauchi, T., Johzuka-Hisatomi, Y., Fukada-Tanaka, S., Terada, R., Nakamura, I., and Iida, S. (2009). Homologous recombination-mediated knock-in targeting of the *MET1a* gene for maintenance DNA methyltransferase reproducibly reveals dosage-dependent spatiotemporal gene expression in rice. Plant J. *60*, 386-396.

†: This laboratory was closed on 31 March, 2009.