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The main interest of this division is understanding the biology of the dynamic genome, namely, genome organization and reorganization and its impact on gene expression and regulation. We are also characterizing various aspects of genetic and epigenetic gene regulations, particularly the flower pigmentation of morning glories. In addition, we are undertaking reverse genetic approaches in order to elucidate the nature of dynamic genome in rice, a model plant for cereals.

Ⅰ. Spontaneous mutants in morning glories

The genus *Ipomoea* includes about 600 species, distributed on a worldwide scale, that exhibit various flower morphologies and pigmentation patterns. Among them, three morning glories - *Ipomoea nil* (the Japanese morning glory), *Ipomoea purpurea* (the common morning glory), and *Ipomoea tricolor* - have been domesticated as floricultural plants. Of these, the Japanese morning glory has an extensive history of genetic studies, and about one-third of more than 200 spontaneous mutants described exhibit mainly altered flower pigmentation. The wild-type *I. nil* displays blue flowers with pigmented red stems and dark-brown seeds, and its spontaneous mutants with various flower colors have been isolated and cultivated in Japan since the 17th century. According to classical genetic studies, mutations conferring white flowers can be classified into four groups: *a*, *c*, *ca*, and *r*. The recessive *a* and *r* mutations confer white flowers with green stems and normal colored seeds. The first such mutation was originally designated *r*, and other recessive mutations that can complement the *r* mutation were named *a*. One of the *a* mutations was found to be caused by the integration of a DNA transposon *Tpn1* belonging to the CACTA superfamily into the *DFR-B* gene encoding dihydroflavonol 4-reductase. The *c* mutants exhibit white flowers with red stems and colored seeds, whereas the *ca* mutants display white flowers with green stems and ivory seeds. The *c* and *ca* mutations were identified as frameshift mutations in the genes encoding transcriptional regulators containing R2R3-MYB domains and those carrying conserved WD40 repeats (WDR), designated as InMYB1 and InWDR1, respectively. We found that the *r* mutants carry either 5.6-kb *Tpn3* or 4.7-kb *Tpn6*, DNA transposons of the *Tpn1* family, integrated into the *CHS-D* gene for chalcone synthase in the anthocyanin biosynthesis pathway (Figure 1). We designate these two mutant alleles as *r1-1* and *r1-2*. Because excision of *Tpn3* from *CHS-D* scarcely ever occurs, the *r1* mutant shows an apparent stable white flower phenotype. These findings further strengthen the assumption that the *Tpn1* family of elements act as major mutagens for generating various spontaneous mutations for floriculturally important traits in *I. nil*.

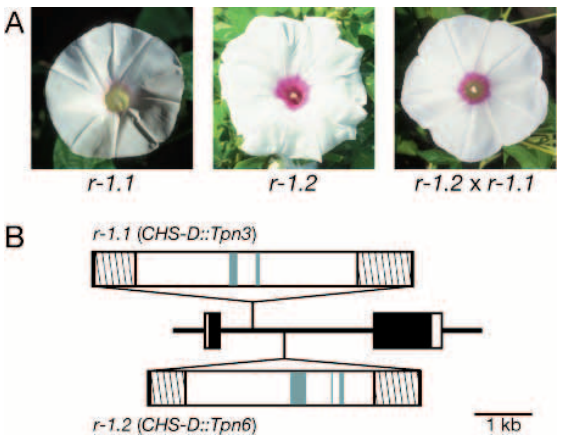

Figure 1. Flower phenotypes of the *r1* mutants and their *CHS-D* structures. (**A**) Flower phenotypes. (**B**) Mutant *CHS-D* genes. Boxes on the central horizontal bar indicate exons, with their coding regions shaded black. The transposons are shown above and under the horizontal bar.

Ⅱ. Modification of endogenous natural genes by homologous recombination in rice

Rice (*Oryza sativa*), with the sequenced genome of 389-Mb, is an important staple food for more than half of the world's population and is a model plant for other cereal species. Through a gene-targeting procedure with positive-negative selection, we have 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. The vector used also contained the two constitutively expressed *DT-A* genes at both ends of the T-DNA segment adjacent to its border sequences, which acted effectively to eliminate transformants carrying entire T-DNA segment(s) integrated randomly and to enrich targeted transformants among the

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

surviving calli. We found that base changes within the homologous segments in the vector could be efficiently transferred into the corresponding genomic sequences of rice recombinants. Interestingly, a few sequences from the host genome were flanked by the changed sequences derived from the vector in most of the recombinants (Figure 2). A likely explanation for the observation would be a result of the mismatch correction of heteroduplex molecules formed between the genomic and introduced T-DNA sequences during homologous recombination. These results offer new insights into the homologous recombination processes of gene targeting with positive-negative selection.

Because a single-stranded T-DNA molecule in *Agrobacterium*-mediated transformation is imported into the plant nucleus and becomes double-stranded, both single-stranded and double-stranded T-DNA intermediates can serve in gene-targeting processes. Since the *DT-A* gene is a cell-autonomous, nonconditional, and lethal negative selection marker, the *DT-A* gene region of a single-stranded T-DNA intermediate in the plant nucleus would neither become double stranded nor express transiently in the successfully targeted transformants because the transient expression of the double-stranded *DT-A* gene before integration is thought to kill host plant cells.

Several alternative models, including the occurrence of the mismatch correction of heteroduplex molecules formed between the genomic DNA and either a single-stranded or double-stranded T-DNA intermediate, are compared in order to explain the observation (Figure 3). Since the introduction of a double-strand break (DSB) in the plant genome greatly stimulates the integration of transgenes flanked by homologous sequences on T-DNA introduced into the DSB site, we considered (A) chromosomal DSB-initiated gene-targeting processes, and (B) chromosomal DSB-independent gene-targeting processes separately. According to the models illustrated, no heteroduplex would form in the

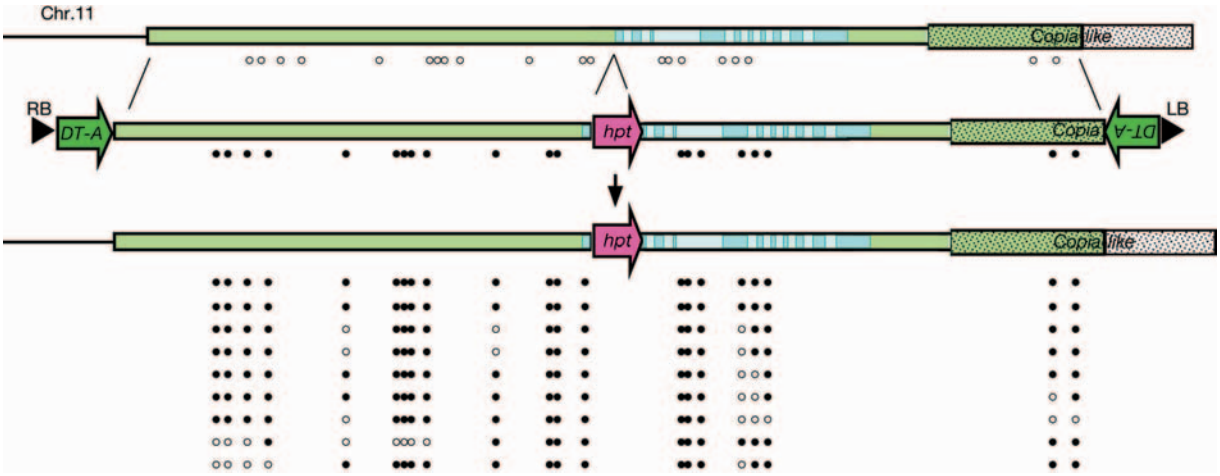

Figure 2. Transfer of base changes from a vector to the rice genome. The rice *Adh2* locus on chromosome 11 bears a *Copia*-like retroelement, and the vector used contains the 6.2-kb *Adh2* promoter sequence including a 0.1-kb 5'-untranslated region, *hpt*, 4.0-kb *Adh2* region, and 2.0-kb 3' part of a *Copia*-like retroelement. The large black arrowheads with RB and LB indicate the right and left borders of T-DNA, respectively. The blue boxes indicate *Adh2* exons, and *hpt* and *DT-A* are not drawn to scale. The filled and open circles indicate the base changes in the vector and the corresponding genomic sequences, respectively.

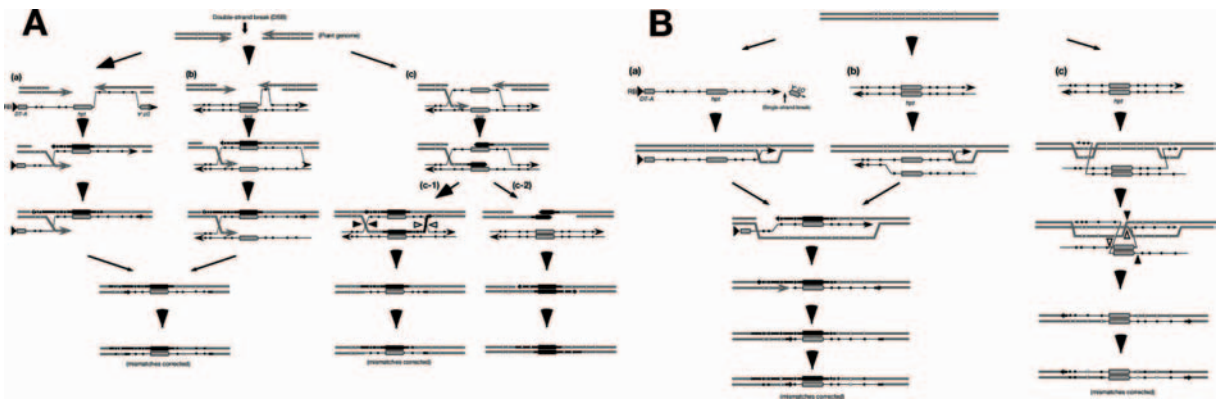

Figure 3. Models for gene targeting. (**A**) DSB-induced gene targeting processes with a single-stranded T-DNA intermediate (**a**) and a truncated double-stranded T-DNA intermediate (**b** and **c**). (**B**) DSB-independent gene targeting processes with a single-stranded T-DNA intermediate (**a**) and with a truncated double-stranded T-DNA intermediate (**b** and **c**).

simple DSB-induced synthesis-dependent strand annealing (SDSA) model involved in the annealing of the newly synthesized DNA strands (Figure 3A c-2), whereas a long heteroduplex would likely be produced in only one of the two homologous regions flanking the *hpt* gene in the formal double-strand break repair (DSBR) model. As the gene conversion was observed over the entire region examined, the observation appears to be in contrast to the fact that major DSB-induced conversion tracts were reported to be bidirectional and usually shorter than 100 - 300 bp when double-stranded DNA molecules served in homologous recombination with the genomic homologous sequences in mouse ES cells. We are condidering the possibility that a single-stranded T-DNA intermediate plays an important role in the major pathways leading to our successful GT events.

Ⅲ. Characterization of mutable *virescent* allele in rice

We have identified an active nonautonomous rice transposon *nDart1* of about 0.6 kb, belonging to the *hAT* superfamily, as a causative transposon of a mutable *virescent* allele *pyl-v* (*pyl-variegated*) conferring pale yellow leaves with dark green sectors in its seedlings. The transposition of *nDart1* can be controlled under natural growth conditions, and can be induced by crossing with a line containing an active autonomous element *aDart* and stabilized by segregating *aDart*. In the sequenced Nipponbare genome containing no active *aDart* elements, the *nDart*-related elements can be classified into three subgroups of about 0.6-kb nonautonomous elements and four subgroups of elements longer than 2 kb, which comprise epigenetically silenced inactive *iDart* and genetically defective *dDart* elements including 53 copies of *iDart1/dDart1* elements on the basis of their lengths and sequence characteristics. Among the 53 *iDart1/dDart1* elements, 38 *iDart1* elements are putative autonomous elements silenced epigenetically because their putative transposase genes carry no apparent nonsense or frameshift mutations. Because the excision of a fraction of *nDart1* elements can be induced by treating Nipponbare with 5-azacytidine (5-azaC), Nipponbare must contain an epigenetically silenced autonomous element or elements that can become *aDart* by 5-azaC treatment.

Mapping data indicated that *aDart* in the mutable pyl-v plants resides within 170-kb region on chromosome 6, and the 170-kb region contains a *Dart1* element identical to *Dart1-27* in Nipponbare. We chose six representative *Dart1* elements (*Dart1-1*, *Dart1-20*, *Dart1-27*, *Dart1-28*, *Dart1-44*, and *Dart1-52*) from Nipponbare to examine whether they are able to excise from the *GUS* gene in the vectors to be introduced into Arabidopsis by *Agrobacterium*-mediated transformation (Figure 4). Of these *Dart1* elements, only *Dart1-44* is likely to be *dDart1*, because its putative transposase gene contains a premature TAA stop codon. Transposition of all of the *Dart1* elements except for *Dart1-44* can be detected and expected footprints are generated, indicating that they are potential autonomous elements silenced epigenetically in Nipponbare and that autonomous *Dart1* elements bear a considerable sequence divergence of transposases. We also showed that *Dart1-27* can mobilize *nDart1-0* in Arabidopsis. These findings should facilitate the development of an efficient gene-tagging system in rice and shed light on epigenetic regulatory and evolutionary aspects of autonomous elements in the *nDart/aDart* system.

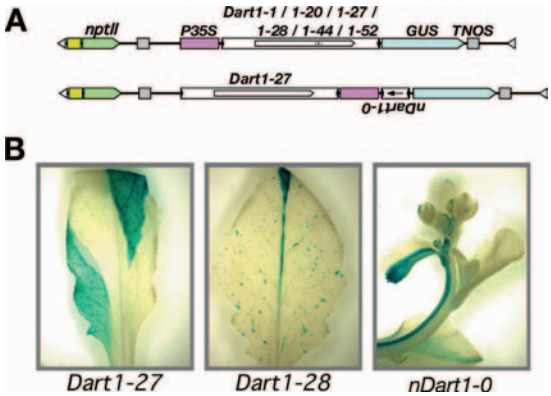

Figure 4. Transposision of *nDart1-0* and *Dart1* elements in Arabidopsis. (**A**) Structures of the T-DNA regions of the vectors used. (**B**) Excision activities of the introduced *nDart1-0* and *Dart1* elements examined in transgenic Arabidopsis plants.

Publication List

〔Original papers〕

- Johzuka-Hisatomi, Y., Terada, R., and Iida, S. (2008). Efficient transfer of base changes from a vector to the rice genome by homologous recombination: involvement of heteroduplex formation and mismatch correction. Nucleic Acids Res. *36*, 4727-4735.
- Kitazawa, D., Miyazawa, Y., Fujii, N., Hoshino, A., Iida, S., Nitasaka, E., and Takahashi, H. (2008). The gravity-regulated growth of axillary buds is mediated by a mechanism different from decapitation-induced release. Plant Cell Physiol. *49*, 891-900.
- Yamauchi, T., Moritoh, S., Johzuka-Hisatomi, Y., Ono, A., Terada, R., Nakamura, I., and Iida, S. (2008). Alternative splicing of the rice *OsMET1* genes encoding maintenance DNA methyltransferase. J. Plant Physiol. *165*, 1774-1782.
- Nishimura, H., Ahmed, N., Tsugane, K., Iida, S., and Maekawa, M. (2008). Distribution and mapping of an active autonomous *aDart* element responsible for mobilizing nonautonomous *nDart1* transposons in cultivated rice varieties. Theor. Appl. Genet. *116*, 395-405.

〔Review article〕

- Johzuka-Hisatomi, Y., Maekawa, M., Takagi, K., Eun, C.H., Yamauchi, T., Shimatani, Z., Ahmed, N., Urawa, H., Tsugane, K., Terada R., and S. Iida (2008). Homologous recombination-dependent gene targeting and an active DNA transposon *nDart*-promoted gene tagging for rice functional genomics. Biotechnology in Agriculture and Forestry *62*, 81-94