DIVISION OF MOLECULAR GENETICS

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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

Considerable attention has recently been paid to the morning glory genus *Ipomoea* because of the experimental versatility of its floral biology including the genetics of floral variation, flavonoid biosynthesis, and transposon-induced mutations. 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, spontaneous mutants of *I. nil* and *I. purpurea* with various flower colors have been isolated and cultivated since the 17th century in Japan and Europe, respectively. The wild-type *I. nil* and *I. purpurea* display blue and dark-purple flowers, respectively, both of which contain polyacylated and polyglycosylated cyanidin-based anthocyanins, and both plants exhibit red stems and dark-brown seeds. Almost all structural and regulatory

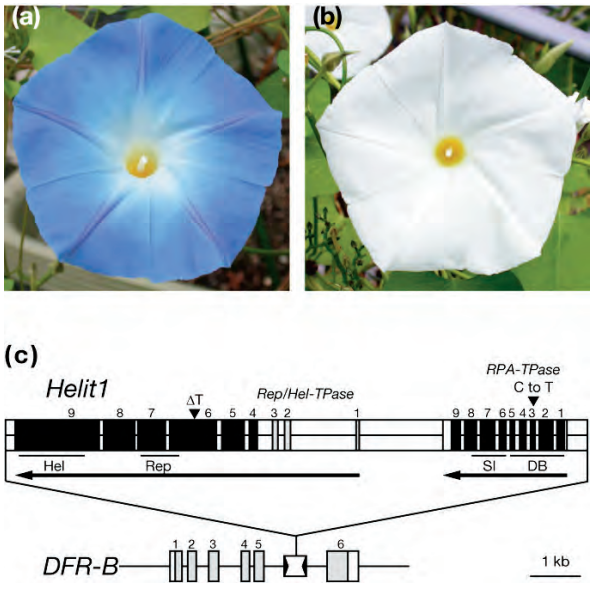

Figure 1. Pigmentation phenotype of the *pearly-s* mutant and structure of the *pearly-s* mutation. (a) Wild-type cultivar Heavenly Blue. (b) A *pearly-s* mutant displays stable white flowers. (c) Structure of the *pearly-s* mutation having the *Hel-It1* transposon integrated into the *DFR-B* gene. The boxes with numerals and the shadowed parts indicate exons and the coding region of the *DFR-B* gene, respectively. The rectangle with the filled arrowheads shows the *MELS2* element. The boxes in *Hel-It1* indicate the predicted exons for the putative wild-type *RPA-TPase* and *Rep/Hel-TPase* genes, respectively. The horizontal lines under the boxes indicate functional domains of these proteins, and the symbols DB, SI, Rep, and Hel represent DNA-binding, subunit interaction, Rep, and Hel domains, respectively. The predicted single-base substitution and deletion are shown by vertical arrowheads with C to T and ΔT, respectively.

genes, which encode enzymes to produce anthocyanidin 3-*O*-sophorosides for their flower pigmentation, and transcriptional regulators, which activate the structural genes for anthocyanin biosynthesis, as well as their spontaneous mutations, have been identified and characterized. The majority of their spontaneous mutations have been shown to be caused by insertions of various DNA transposons belonging to the *hAT*, CACTA, and *MuLE* superfamilies.

Helitrons are newcomers of eukaryotic DNA transposons and were originally identified by computational analysis in the genomes of Arabidopsis, rice, and nematode. They are distinguished from other transposons in their structural features, and their proposed transposition mechanisms are involved in rolling circle replication. Computer-predicted autonomous *Helitrons* with conserved terminal sequences, 5'-TC and CTRR-3', are presumed to encode a putative transposase, *Rep/Hel-TPase*, which contains a characteristic nuclease/ligase domain for the replication initiation protein (Rep) and a DNA helicase domain (Hel). Plant *Helitrons* are thought to carry an additional transposase gene, *RPA-TPase*, which is related to the largest subunit of the replication protein A (RPA70). Although *Helitrons* are found in diverse genomes, neither an autonomous element nor a transposition event has been reported. We found that a spontaneous *pearly-s* mutant of *Ipomoea tricolor*, cultivar Pearly Gates,

exhibiting white flowers and isolated in approximately 1940, has an 11.5-kbp novel *Helitron*, named *Hel-It1*, integrated into the *DFR-B* gene for anthocyanin pigmentation (Figure 1). *Hel-It1* shows the predicted plant *Helitron* structure for an autonomous element with the conserved termini and carries the two putative transposase genes, *Rep/Hel-TPase* and *RPA-TPase*, which contain a nonsense and a frameshift mutation, respectively. *Hel-It1*-related elements are scattered in the *Ipomoea* genome, and only a fraction of the *pearly-s* plants was found to carry *Hel-It1* at another insertion site. The *pearly-s* mutant appears to bear an autonomous element and express the wild-type *RPA-TPase* transcripts, indicating that an *Ipomoea Helitron* also acts as a spontaneous mutagen.

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

Rice (*Oryza sativa* L.), 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. We have developed efficient and reproducible gene targeting by homologous recombination with a large-scale *Agrobacterium*-mediated transformation and a strong positive-negative selection using the *hpt* and *DT-A* genes and have succeeded in modifying the *Waxy* and *Adh2* genes to generate true gene targeting (TGT) repeatedly without the concomitant occurrence of ectopic events, such as one-sided invasion (OSI) and ectopic gene targeting (EGT) (Figure 2). While *Waxy* is a unique gene in the rice genome, the 3 *Adh* genes, *Adh1*, *Adh2*, and *Adh3*, reside on chromosome 11 in the same orientation. The targeting frequencies of *Waxy* and *Adh2* were about 1% and 2% per surviving callus, respectively. To gain information about surviving calli that were not targeted homologous recombinants, a series of PCR analyses was performed to determine the randomly integrated transgene segments present in these calli. All the surviving calli examined contained the selective *hpt* sequence, and none of them carried the intact *DT-A* sequence, indicating that none of the surviving calli was an escapee from the positive-negative selection. The analyses prompt us to speculate the following integration processes of transgenes associated with gene targeting in *Agorbacterium*-mediated transformation (Figure 2).

In *Agrobacterium*-mediated transformation, which has been used by gene targeting in higher plants, T-DNA appears to integrate randomly throughout the plant genome as a single molecule or multiple sequences ligated with each other in various orientations. The majority of the randomly integrated single-copy T-DNA molecules mediated by nonhomologous end-joining are known to contain the entire T-DNA segment with a well-conserved right border, and a left-border sequence that is either conserved or slightly truncated, which we termed as border-associated random integration (BARI) (Figure 2). There appears to be another type of random integration with relatively large deletions at both ends of the T-DNA segment without the border proximal regions, which we termed border-independent random integration (BIRI) (Figure 2). BIRI appears to occur much less frequently than BARI. Since a significant portion of single-stranded T-DNA imported into the plant nucleus can become double-stranded in *Agrobacterium*-mediated transformation, it has been speculated that such BIRI processes must share common recombination mechanisms with random-integration processes by direct DNA delivery methods using double-stranded DNA. Although the targeting frequencies in higher plants have been thought to be low as calculated by TGT events per BARI-mediated transformant, such frequencies may be too low to calculate the proper targeting frequency because *Agrobacterium*-mediated transformation via BARI of T-DNA is a highly efficient process.

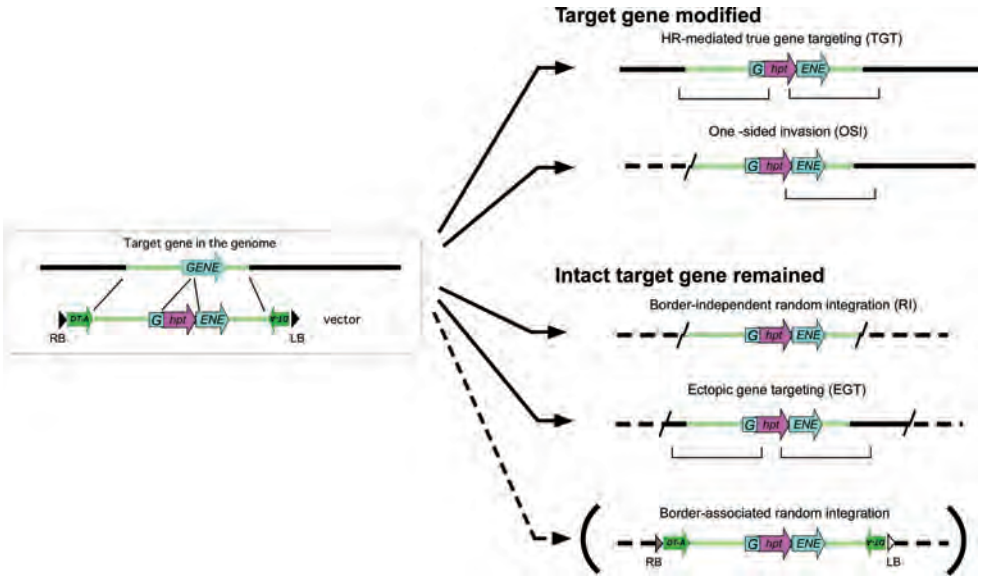

Figure 2. Integration events of a transgene associated with homology-dependent gene targeting with positive-negative selection. The large black arrowheads with RB and LB indicate the right and left borders, respectively. The most efficient BARI events are shown in large parentheses because BARI-mediated calli are killed by the action of TD-A. Zigzag lines represent breakpoints generated by nonhomologous events.

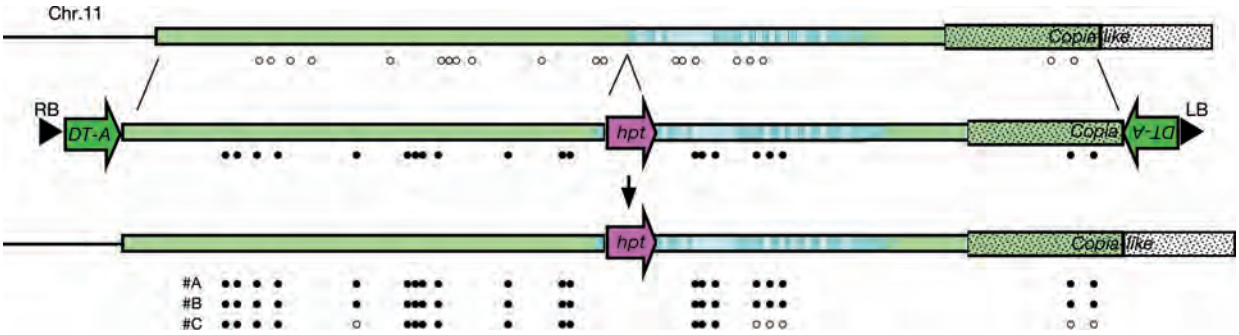

Figure 3. Transfer of base changes from a vector to the rice genome. The rice *Adh2* locus on chromosome 11 bears a *Copia*-like retroelement, and the vector used contains the 6.2-kb *Adh2* promoter sequence including a 0.1-kb 5'-untranslated region, *hpt*, 4.0-kb *Adh2* region, and 2.0-kb 3' part of a *Copia*-like retroelement; the *DT-A* genes were placed at both ends of T-DNA in order to efficiently eliminate BARI. 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. Homologous crossover sites can be deduced by determining these alternative sequences in the targeted recombinant allele.

To facilitate the molecular analysis of the recombination processes, we began to characterize crossover sites in the targeting of *Adh2* with a vector carrying multiple base changes (Figure 3). Preliminary results indicate that the efficient transfer of base changes (point mutations) occurs from the vector to the rice genome.

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; its transposition can be induced by crossing with a line containing an active autonomous element *aDart* and stabilized by segregating *aDart*. Mapping data indicated that *aDart* resides within 170-kb region on chromosome 6. The most likely candidate element, *Dart1-27*, was cloned and reintroduced into a stable *virescent* mutant *pyl-stb* (*pyl-stable*) exhibiting pale yellow leaves due to the a deficiency of the *aDart* activity. Clear leaf variegation could be observed in a significant portion of the *pyl-stb* derivatives, in which the demethylated *Dart1-27* element by growing in *Escherichia coli* had been introduced. We can thus conclude that *Dart1-27* is the active autonomous *aDart* element in the mutable *virescent pyl-v* plants. In consistent with this notion, the 5'-terminus of *Dart1-27* in the *pyl-v* lines is less DNA methylated than that in the pyl-stb plants, even though the sequences of the *Dart1-27* elements in *pyl-v* and *pyl-stb* are found to be identical to each other.

Publication List

〔Original papers〕

- Choi, J.D., Hoshino, A., Park, K.I., Park, I.S., and Iida, S. (2007). Spontaneous mutations caused by an active *Helitron* transposon, *Hel-It1*, in morning glory, *Ipomoea tricolor*. Plant J. *49*, 924-934.
- Furukawa, T., Maekawa, M., Oki, T., Suda, I., Iida, S., Shimada, H., Takamure, I., and Kadowaki, K. (2007). The *Rc* and *Rd* genes are involved in proanthocyanidin synthesis in the rice pericarp. Plant J. *49*, 91-102.
- Park, K.I., Ishikawa, N., Morita, Y., Choi, J.D., Hoshino, A., and Iida, S. (2007). A *bHLH* regulatory gene in the common morning glory, *Ipomoea purpurea*, controls anthocyanin biosynthesis in flowers, proanthocyanidin and phytomelanin pigmentation in seeds, and seed trichome formation. Plant J. *49*, 641-659.
- Takagi, K., Ishikawa, N., Maekawa, M., Tsugane, K., and Iida, S. (2007). Transposon display for active DNA transposons in rice. Genes Genet. Syst. *82*, 109-122.
- Terada, R., Johzuka-Hisatomi, Y., Saitoh, M., Asao, H., and Iida, S. (2007). Gene targeting by homologous recombination as a biotechnological tool for rice functional genomics. Plant Physiol. *144*, 846-856.

〔Review article〕

- Iida, S., Johzuka-Hisatomi, Y., and Terada, R. (2007). Gene targeting by homologous recombination for rice functional genomics. In Rice Functional Genomics-Challenges, Progress and Prospects, N.M. Upadhyaya ed., (Springer), pp. 273-289.