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,* were domesticated well as floricultural plants. Of these, spontaneous mutants with various flower colors of *I. nil* and *I. purpurea* 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 (Figure 1). Almost all structural genes that encode enzymes to produce anthocyanidin 3-*O*-sophorosides for their flower pigmentation have been characterized, and the majority of their spontaneous mutations have been shown to be caused by insertions of DNA transposons. The transcriptional regulators for anthocyanin biosynthesis,

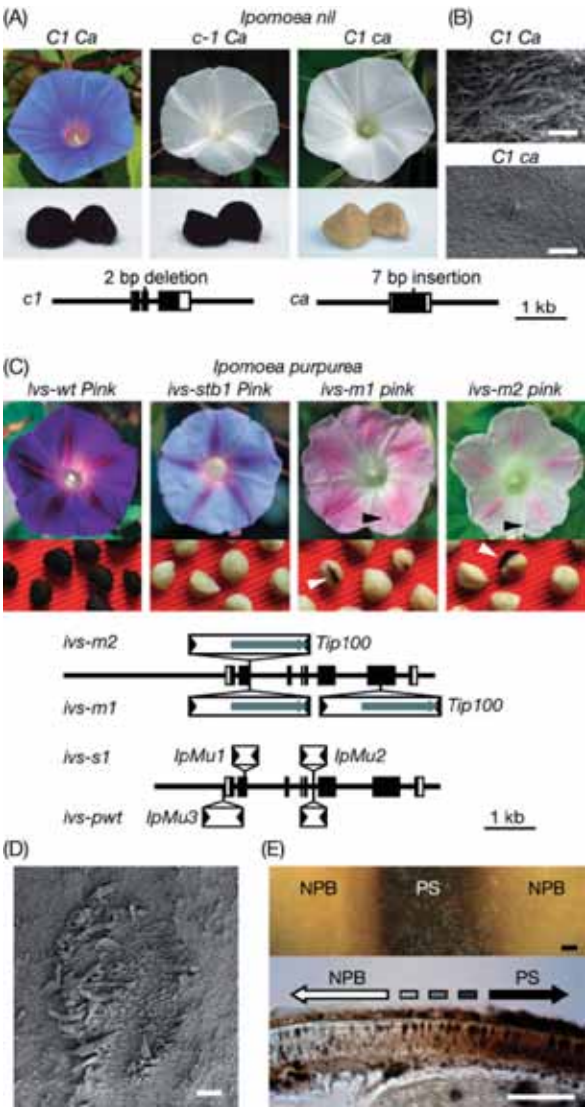

Figure1. Flower and seed phenotypes. (**A**) *I. nil*. (**B**) Scanning electron microscope (SEM) photographs of seeds. (**C**) *I. purpurea*. The black and white arrowheads indicate pigmented spots in flowers and seeds, respectively. (**D**) SEM photograph of small dark-brown reversion spot of a mature *ivs-m2* seed. (**E**) Magnified view (upper panel) and histological observation (lower panel) of the boundary region of a dark-brown reversion spot in the seed coats of the mutable PR43 *ivs-m2* line. The filled and open arrows with PS and NPB or grey broken bars indicate a pigmented spot and a non-pigmented background or a pigment-diffused area(s) on the seed coat, respectively. The scale bars indicate 50 μm.

which activate the structural genes, are known to include members of protein families containing R2R3-MYB domains, bHLH (basic helix-loop-helix) domains, and conserved WDR (WD40 repeats). Spacial and temporal expression of these structural genes encoding the enzymes for anthocyanin biosynthesis is determined by combinations of the R2R3-MYB, bHLH, and WDR factors and their interactions. In addition, their combinations and interactions also determine the set of genes for certain epidermal traits to be expressed.

In *I. nil*, the *c* mutants display white flowers with red stems and colored seeds, whereas the *ca* mutants exhibit white flowers with green stems and ivory seeds (Figure 1).

We showed that the recessive *c-1* and *ca* alleles are frameshift mutations caused by a 2-bp deletion and 7-bp insertions in the genes for the R2R3-MYB and WDR transcriptional regulators designated as InMYB1 and InWDR1, respectively. In the 2-bp deletion, dinucleotide AG was removed from a simple repeat sequence, AGAGAG. Regarding the 7-bp insertions, it is likely that they are footprints generated by independent excisions of a DNA transposon because the wild-type sequence, TAC, had changed into TAC(GGAG/TCCG)TAC. In addition to defects in flower, stem, and seed pigmentations, the *ca* mutants showed reduced trichome formation in seeds. Except for *CHS-E* in *ca* mutant, all structural genes tested coordinately reduced in both *c-1* and *ca* mutant flower limbs. However, slight but significant expression of *CHS-D*, *CHI*, and *F3H* for flavonol biosynthesis was detectable in *c-1* and *ca* mutants, whereas no such residual expression could be observed in other genes involved in the later anthocyanin biosynthesis pathway.

Spontaneous *ivory seed* (*ivs*) mutants of *I. purpurea* displaying pale pigmented flowers and ivory seeds are caused by insertions of DNA transposons in the *hAT* and the *Mutator* families into the *bHLH2* gene encoding a bHLH transcriptional regulator (Figure 1). A partial reduction in the expression of all structural genes for anthocyanin biosynthesis was observed in the young flower buds of these *ivs* mutants, whereas no reduction of *GST* was observed. The *bHLH2* expression appears to precede the expression of the structural genes for the proanthocyanidin biosynthesis in the seed coats. Interestingly, *CHS-E* rather than *CHS-D* is predominantly expressed in the seed coats, indicating that *CHS-E* is the first committed step of the proanthocyanidin biosynthesis pathway. The *DFR-B* and *ANS* transcripts were completely abolished in the *ivs* seed coats, whereas the early biosynthetic genes for flavonol biosynthesis remained active. The production and accumulation of both proanthocyanidin and phytomelanin pigments in their ivory seed coats were drastically reduced. Moreover, the unbranched trichomes in their ivory seeds were smaller in size and fewer in number than those in the wild-type dark-brown seeds, and the surface of the epidermis without trichomes in the dark-brown seeds looked rougher, due to the protruding tangential walls, than that in the ivory seeds. Combined with the results obtained in *I. nil* and *I. purpurea*, both bHLH2 and WDR1 transcriptional regulators in *Ipomoea* must control the biosynthesis and/or accumulation of anthocyanin, proanthocyanidin, and phytomelanin pigments as well as the formation of seed trichomes.

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 worldís population and 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 and succeeded in modifying the *Waxy* gene without the concomitant occurrence of ectopic events. While *Waxy* is a unique gene in the rice genome, 4 copies of the *Adh* gene are present, and *Adh1*, *Adh2* and *Adh3* reside on chromosome 11 in the same orientation, and highly repetitive *Copia*- and *Gypsy*-like retroelements are present adjacent to *Adh1* and *Adh2* (Figure 2). The *Adh* genes play a key function in response to an anaerobic condition, and only a single *adh1* mutant has been isolated in rice. We are attempting to modify the *Adh1* and *Adh2* genes, the coding sequences of which are similar to each other. While we were able to obtain transgenic plants having either *Adh1* or *Adh2* modified without the concomitant occurrence of undesirable ectopic events, the targeting frequency of *Adh1* appeared to be about one magnitude lower than those of *Waxy* and *Adh2,* indicating that *Adh1* appears to be a cold spot(s) for homologous recombination. To examine whether the repetitive *Gypsy*-like sequence precludes efficient homologous recombination-promoted gene targeting due to the occurrence of ectopic recombination, we used a vector for *Adh1* modification with short homologies lacking the *Gypsy*-like sequence and found no significant effects on targeting efficiency. The obtained *adh1* disrupted mutants showed very similar phenotype to the previously isolated *adh1* mutant. We also characterized structure and expression of the newly identified *Adh3* gene.

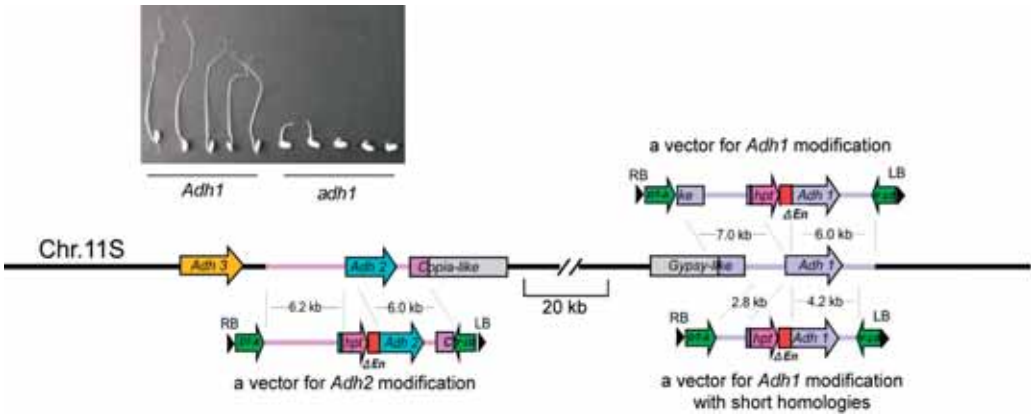

Figure 2. Strategy for gene targeting of the *Adh1* and *Adh2* genes in rice. The gene symbols *hpt* and *DT-A* on the T-DNA regions of the vectors used indicate the positive and negative selection markers, respectively. The sheedlings of the wild type and *adh1* mutants under the submerged condition are shown above the map.

III. Characterization of mutable *virescent* allele in rice

We have identified an active rice transposon *nDart1* as a causative transposon of a mutable *virescent* allele *pyl-v* conferring pale yellow leaves with dark green sectors in its seedlings (Figure 3A). 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*. While the cultivar Nipponbare does not carry the active *aDart* element, 5-azaC treatment induces transposition of the *nDart1* elements, suggesting that a dormant or an epigenetically silenced autonomous element(s) is present in the genomes. While tissue culture is necessary in all of the currently available rice reverse genetic approaches including transposon tagging systems employing exogenous or endogenous transposons, no somaclonal variation should occur in our *nDart/aDart* system because no tissue culture is involved in its activation. We examined the *nDart*-related elements in the Nipponbare genome with the latest 4.0 pseudomolecules (Figure 3). The *nDart*-related elements can be classified into three subgroups of about 0.6 kb nonautonomous elements (*nDart1-3*, *nDart1-101*, and *nDart1-201*) and four subgroups of elements longer than 2 kb (*iDart1/dDart1*, *iDart2*, *iDart3*, and *iDart4*) on the basis of their lengths and sequence characteristics. The copy numbers of the small *nDart1* elements of about 0.6 kb and longer *iDart/dDart* elements are 18 and 63, respectively, and both elements are distributed throughout chromosomes. We are currently developing an *nDart*-mediated transposon tagging system in rice and obtaining interesting mutants with new mutable alleles.

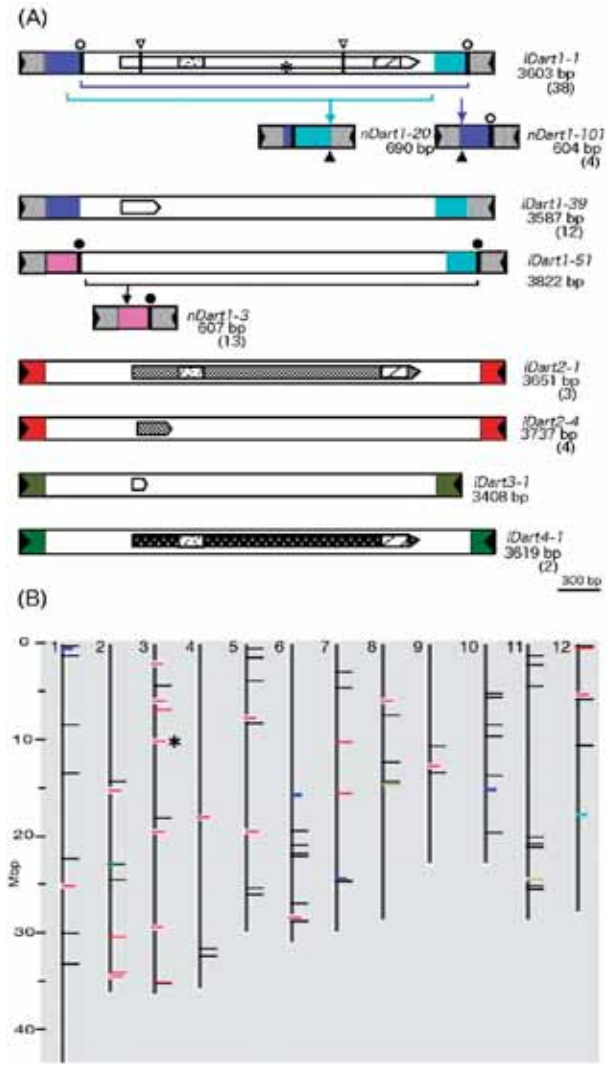

Figure 3. (**A**) Structures of *nDart*-related elements. Only typical structures in each representative element with its length are shown, and the numerals in parentheses indicate the copy numbers of a group of elements with closely related sequences. The horizontal filled arrowheads and the shadowed boxes at both ends indicate the terminal inverted repeats (TIRs) and subterminal regions. (**B**) Localization of *nDart*-related elements in Nipponbare. The short bars with pink-, dark-, and light-blue represent *nDart1* elements in the subgroups *nDart1-3*, *nDart1-101*, and *nDart1-201*, respectively, whereas the long bars with black, red, lime, and green indicate *iDart1/dDart1*, *iDart2*, *iDart3*, and *iDart4*, respectively. The asterisk indicates *nDart1-0* at the *pyl-v* allele. The open circles indicate the position of centromere.

Publication List:

Original papers

Tsugane, K., Maekawa, M., Takagi, K., Takahara, H., Qian Q., Eun, C.H., and Iida, S. (2006). An active DNA transposon *nDart* causing leaf variegation and mutable dwarfism and its related elements in rice. Plant J. *45*, 46-57.

Morita, Y., Saitoh, M., Hoshino, A., Nitasaka, E., and Iida, S. (2006). Isolation of cDNAs for R2R3-MYB, bHLH, and WDR transcriptional regulators and identification of *c* and *ca* mutations conferring white flowers in the Japanese morning glory. Plant Cell Physiol. *47*, 457-470.

Furukawa, T., Maekawa, M., Oki, T., Suda, I., Iida, S., Shimada, H., Takamure, I., and Kadowaki, K. (2006). The *Rc* and *Rd* genes are involved in proanthocyanidin synthesis in the rice pericarp. Plant J. *49*, 91-102.

Review articles

Chopra, S., Hoshino, A., Boddu, J., and Iida, S. (2006). Flavonoid pigments as tools in molecular genetics. In The Science of Flavonoid, E. Glotewold ed., (Springer), pp. 147-173.