DIVISION OF MOLECULAR GENETICS

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The main interest of the group is in 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, and many mutants displaying various flower pigmentation patterns were isolated. The wild-type *I. nil* displays blue flowers (Figure 1A) that contain the peonidin (3' methoxyl cyanidin) derivative named Heavenly Blue Anthocyanin or HBA.

I. nil was introduced into Japan from China in the 8th century as a medicinal herb, the seeds of which were used as a laxative, and the plant became a traditional floricultural plant in Japan around the 17th century. The plant has an extensive history of genetic studies, and a number of its spontaneous mutants related to the color and shape of the flowers have been isolated. Genetic studies on the color of *I. nil* have shown that blue flower coloration was mainly controlled by two genetic loci, *Magenta* and *Purple*. Recessive *magenta* and *purple* mutants bloom magenta and purple flowers, respectively, and double mutants carrying both *magenta* and *purple* alleles display red flowers (Figure 1C). The *Magenta* gene encodes flavonoid 3'-hydorxylase, which hydroxylates the 3' position of the B-ring of anthocyanidin precursors. The *Purple* gene encodes a vacuolar Na^+/H^+ antiporter called InNHX1 that increases the vacuolar pH during flower opening, causing a shift towards the bluer coloration. While most of the plant *NHX* genes characterized are generally expressed in leaves, stems and roots and induced by NaCl treatment, the *InNHX1* gene is predominantly expressed in the flower limbs at around 12 hour before flower-opening and is very scarcely expressed in leaves, stems and roots, and no induction occurs in response to NaCl treatment. We also found that the InNHX1 proteins could catalyze both Na^+ and K^+ transport into vacuoles.

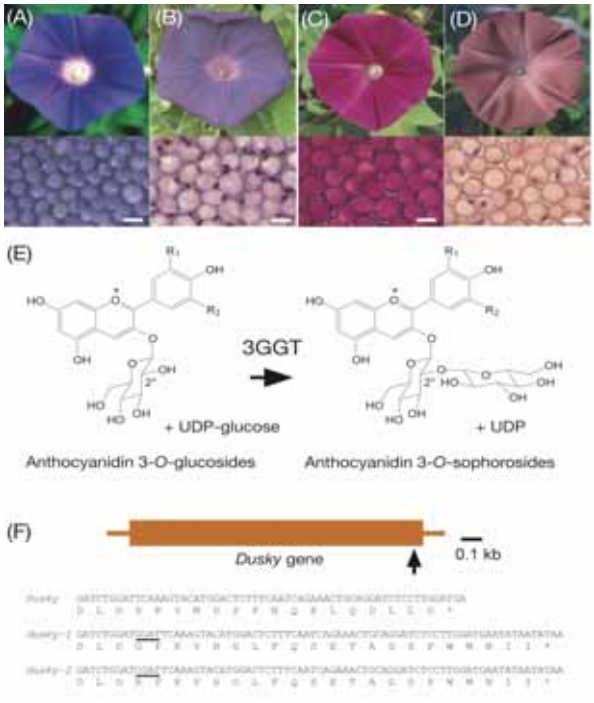

Figure1. Flower phenotypes and the *Dusky* gene for the 3GGT enzyme in *I. nil*. **A-D.** Flower phenotypes (above) and microscopic photographs of adaxial epidermal cells of flower petals (below). (**A**) The wild-type line with the *Magenta*, *Purple*, and *Dusky* alleles. (**B**) The line with the *Magenta*, *Purple*, and *dusky-1* allele. (**C**) The line with the *magenta*, *purple*, and *Dusky* alleles. (**D**) The line with the *magenta*, *purple*, and *dusky-1* allele. Scale bars in microscopic photographs indicate 30 μm. **E.** Reaction mediated by the *Dusky* gene products, 3GGT. **F.** The 3GGT gene and the dusky mutations. The large vertical arrow indicates the site of 4-bp insertions (above), and the 4-bp insertions underlined in the *dusky* mutants (below) result in frameshift mutations.

Among the various colors of *I. nil* flowers, the favorite hue for Japanese floriculturists has been reddish-brown or purplish-grey petals (Figure 1B and D) since the early 19th century, and the flower coloration is mainly caused by recessive *dusky* mutations. We noticed that the petals in all *dusky* mutants often contained intensely pigmented globules, which appeared to affect flower hue. We found that the *Dusky* gene encodes UDP-glucose:anthocyanidin 3-*O*-glucoside-2''-*O*-glucosyltransferase (3GGT), which catalyzes the the conversion of anthocyanidin 3-*O*-glucosides into anthocyanidin 3-*O*-sophorosides (Figure 1E) and that all of the *dusky* mutants tested carry the 4-bp insertion mutations GGAT or CGAT at an identical position near the 3' end of the gene, which resulted in frameshift mutations (Figure 1F). The expected 3GGT enzymatic activities were found in the crude extracts of *Escherichia coli*, in which the *3GGT* cDNA was expressed, and the introduced *3GGT* cDNA could efficiently produce 3GGT that could convert cyanidin 3-*O*-glucoside into cyanidin 3-*O*-sophoroside in transgenic petunia plants.

The transcriptional regulators for anthocyanin biosynthesis include members of proteins containing an R2R3-MYB domain, a bHLH (basic helix-loop-helix) domain, and conserved WD40 repeats. We also found that the recessive *c-1* and *ca* alleles conferring white flowers are frameshift mutations caused by a 2-bp deletion and 7-bp insertions in the genes for the R2R3-MYB and WD40 repeats transcriptional regulators, respectively.

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 a model plant for other cereal species. We have developed a large-scale *Agrobacterium*-mediated transformation procedure with a strong positive-negative selection and succeeded in efficient and reproducible targeting of the *Waxy* gene by homologous recombination without concomitant occurrence of ectopic events, which must be an important first step for developing a precise modification system of the genomic sequences in rice. While the *Waxy* gene is a unique gene in the rice genome, 3 copies of the *Adh* gene are present, and both *Adh1* and *Adh2* genes reside on chromosome 11 in the same orientation with an interval of 30 kb and flank highly repetitive *Copia*- and *Gypsy*-like retroelements (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. By improving our transformation procedure further, we are attempting to modify the *Adh1* and *Adh2* genes, the coding sequences of which are similar to each other. We obtained 9 independent transformed calli having the *Adh2* gene modified and subsequently isolated 8 fertile transgenic plants without concomitant occurrence of undesirable ectopic events. Although we were able to obtain transformed calli with modified *Adh1*, the frequency of homologous recombination at *Adh1* appeared to be about one magnitude lower than that at *Adh2*, indicating that *Adh2* contains a more active hot spot(s) for efficient homologous recombination than *Adh1*.

III. Characterization of mutable *virescent* allele in rice

Leaves of seedlings in the *virescent* mutant of rice are initially pale yellow green due to partial deficient in chlorophyll and gradually become green with the growth of the mutant. We have been characterizing a spontaneous mutable *virescent* allele, *pale yellow leaf-variegated (pyl-v)*, conferring pale yellow leaves with dark green sectors in its seedlings (Figure 3A). The *pyl-v* mutant was isolated among progeny of a hybrid between *indica* and *japonica* rice plants. The leaf variegation is regarded as a recurrent somatic mutation from the recessive pale yellow allele to the dark green revertant allele. From the *pyl-v* line, we also obtained a stable *pyl-stb* (*pyl-stable*) line that exhibits pale-yellow leaves without variegation (Figure 3B), which appeared to carry no active autonomous element acting on the nonautonomous DNA element inserted into the *Pyl* gene. The availability of the genomic sequences of both *japonica* and *indica* subspecies facilitates map-based cloning of the *pyl-v* allele. We identified an active nonautonomous DNA transposon of about 0.6 kb, named *nDart1* (*nonautonomous DNA-based active rice transposon one*), in the untranslated exon 1 of the *Pyl* gene on chromosome 3 (Figure 3D), and excision of the new DNA transposon from the *pyl* gene appears to be responsible for conferring the leaf variegation. We also

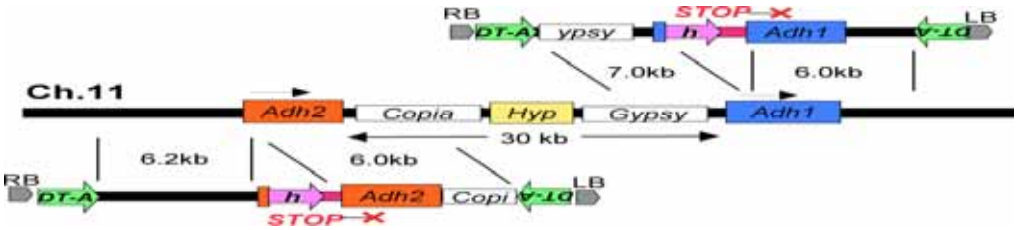

Figure 2. Strategy for gene targeting of the *Adh1* and *Adh2* genes in rice. The symbols *h* and *DT-A* on the T-DNA regions of the vectors used indicate the positive and negative selection markers, respectively. The hypothetical gene flanked by the retroelements on chromosome 11 is indicated by *Hyp*.

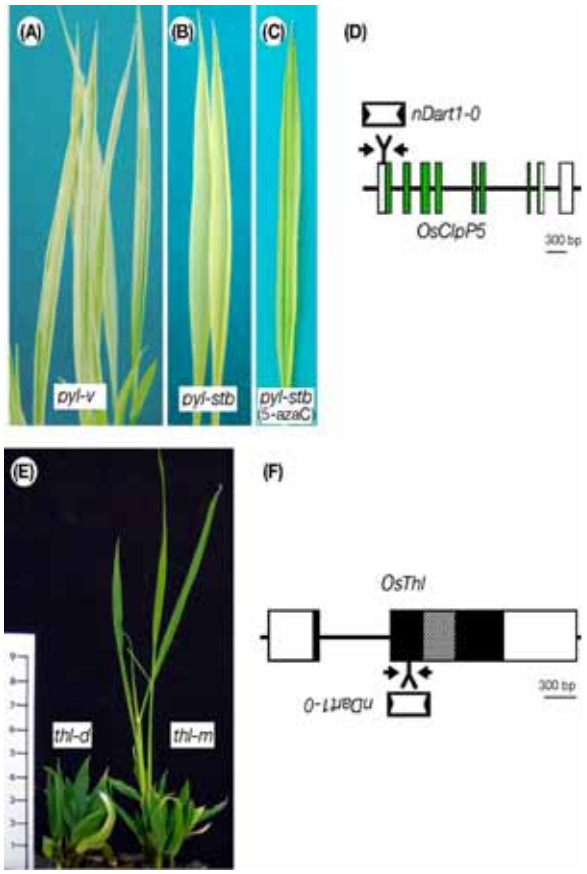

Figure 3. **A-C.** Leaf phenotypes of the mutable *pyl-v* allele, the stable *pyl-stb* allele, and the stable *pyl-stb* allele treated with 5-azaC. Dark-green sectors in a pale-yellow leaf can be seen in *pyl-v* and *pyl-stb* treated with 5-azaC. **D.** Structure of the *pyl-v* allele carrying the *nDart1* insertion. The white and green boxes represent the *Pyl* exons and coding region, respectively. The upper box and small horizontal arrows indicate the *nDart1* element and the positions of the primers to detect the *nDart1* insertion. **E.** A stable *thl-d* mutant and a mutable *thl-m* plant producing a normal revertant tiller. **F.** Structure of the *thl-m* allele carrying the *nDart1* insertion. The open, black and shadowed boxes represent the *Thl* exons, cording region, and a putative esterase/lipase domain.

demonstrated that the transposition of *nDart* could be induced by crossing with a line containing an active autonomous element, *aDart*, and stabilized by segregating out of *aDart* under natural growth conditions. Not only *pyl-stb* but also the *japonica* cultivar Nipponbare carries no *aDart*, although they contain epigenetically silenced *Dart* elements that can be activated by the treatment of 5-azaC (Figure 3C). We also identified a novel mutable dwarf allele, *thl-m* (*thambelina-mutable*), which conferred a tiny and gibberellin-insensitive dwarf phenotype (around 4 cm in height, shorter than a thumb) with occasional appearance on a normal fertile tiller (Figure 3E) and was caused by an *nDart1* insertion (Figure 3F). No somaclonal variation should occur in mutant lines induced by our newly characterizing endogenous element, because no tissue culture is involved in its activation. In this respect, it is important to emphasize here that tissue culture is necessary in all of the currently available rice reverse genetic approaches including transposon tagging systems employing exogenous or endogenous transposons. We are currently attempting to develop a novel transposon tagging system in rice.

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Original papers

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