DIVISION OF GENE EXPRESSION AND REGULATION I

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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. Although there are many elements affecting organization and reorganization of the genomes, we are currently focused on mobile genetic elements in general and plant transposable controlling elements in particular.

I. Identification and characterization of mutable alleles in the Japanese morning glory

The Japanese morning glory is a traditional horticultural plant in Japan, and extensive physiological and genetical studies on the plant have been conducted. A number of mutants related to the color and shape of its flowers and leaves have been isolated since the 17th century, and more than 200 genetic loci including about 20 mutable loci have been documented. We have identified that the mutable *flecked* allele bearing white flowers with colored flecks is the *DFR-B* gene having the *En/Spm*-related transposable element *Tpn1* inserted into its second intron. The non-autonomous *Tpn1* element carries a part of the genomic sequence for an HMG-box. Thus *Tpn1* can be regarded as a specialized transducing transposon.

It is known that the frequency and the timing of the flecking phenotype tend to be heritable by their progeny, although conversion of these phenotypes is sometimes observed. From the variegated flower lines, white variants bearing white flowers free from variegation throughout their plant lives occasionally appeared. Since some of the selfed progeny of the white variants produced a few flecked flowers together with white flowers (others bore only white flowers), the plants were called as variants rather than mutants. We have examined the structure of the *DFR-B* region in the white variants and found that *Tpn1* excision rarely occurs in the variants. DNA methylation in the subterminal repetitive regions of *Tpn1* in the *DFR-B* gene of the white variant appeared to be similar to that of a heavily variegated line, suggesting that the DNA methylation within *Tpn1* is probably not the primary cause of deficiency in excision and that the *Tpn1*-related autonomous element in the white variant is likely to become inactive. The results also suggest that the frequency and the timing of the flecking phenotype of the variegated flowers in the mutable *flecked* line are mainly dependent on the activity of the *Tpn1*-related autonomous element.

The plants with the recessive mutable *speckled* allele, in the presence of the dominant *Speckled-activator*, produce colorless flowers with fine and round colored spots distributed over the corolla, while plants carrying the *speckled* allele without active *Speckled-activator* bear pale yellow flowers. We have found that the *speckled* allele is the *CHI* gene containing a *Tpn1*-related non-autonomous element of 6.5 kb, termed *Tpn2*. Interestingly, *Tpn2* is also a specialized transducing transposon containing a part of the genomic sequence encoding β-galactosidase. The results indicate that the dominant *Speckled-activator* is the *Tpn1*-related autonomous element acting on not only *Tpn2* on the mutable *speckled* allele but also *Tpn1* on the mutable *flecked* allele.

II. Identification of mutable alleles in the common morning glory

The mutable *flaked* line of the common morning glory also bears white flowers with colored flakes and sectors. We showed that the mutable *flaked* allele is caused by insertion of a 3.9 kb transposable element, *Tip100*, into the *CHS-D* gene intron and that *Tip100* belongs to the *Ac/Ds* family. It has been postulated that the timing and the frequency of the variegation are determined by the active state of another genetic element *modulator*. As an initial step to elucidate the genetic system to determine the timing and the frequency of the flower variegation, we examined whether *Tip100* is able to transpose in transgenic tobacco plants and found that *Tip100* can be excised from the introduced vector carrying *Tip100*. The results strongly indicate that *Tip100* is an autonomous element.

III. Characterization of the genes for anthocyanin pigmentation in morning glories.

The *CHS* genes encoding chalcone synthase for flavonoid biosynthesis comprise a multigene family in the common and Japanese morning glories. Among these *Ipomoea CHS* genes, the *CHS-D* gene is the most abundantly expressed in the pigmented young flower buds and is primarily responsible for flower pigmentation. The majority of the remaining *CHS* transcripts in the flower buds are produced from the *CHS-E* gene. Moreover, the *CHS-D* and *CHS-E* genes are expressed predominantly in flower limbs and tubes, respectively. The recombinant CHS-D and CHS-E proteins obtained by expressing the *CHS-D* and *CHS-E* genes in *Escher-*

ichia coli with various expression systems showed CHS activity to produce naringenin chalcone. These results are consistent with the notion that the *CHS-D* and *CHS-E* genes encode the chalcone synthases for anthocyanin biosynthesis in flowers (see also II).

We have characterized the genomic DNA segments of the *CHS-D* and *CHS-E* genes in the Japanese and common morning glories. Both genes have two exons with identical intron positions and carry several copies of two mobile element-like sequences with short terminal inverted repeats, *MELS3* and *MELS6* of around 200 - 300 bp. Small tandem repeats were also found in these *CHS* gene regions. Gene duplication and subsequent divergence are regarded to play important roles in evolution of multiple genes. Comparison of the genomic sequences suggests that gene duplication and major divergence in these *CHS* genes occurred prior to the speciation of the Japanese and common morning glories. Subsequent DNA rearrangements are likely to have taken place after the speciation, and the *MELS* elements appear to play a role in generating such DNA rearrangements.

IV. An efficient transformation system and homologous recombination in rice.

In higher plants, efficient and reliable gene targeting procedures for “reverse genetics” remains to be established. As an initial step to develop such procedures, we are trying to develop an efficient rice transformation system and searching for factors to enhance homologous recombination in rice. We employed an Agrobacterium-mediated transformation system using vigorously dividing calli derived from mature seed scutellum. Under the optimum condition, approximately 1200 independent transformed calli were obtainable from 150 matured seeds.

Publication List:

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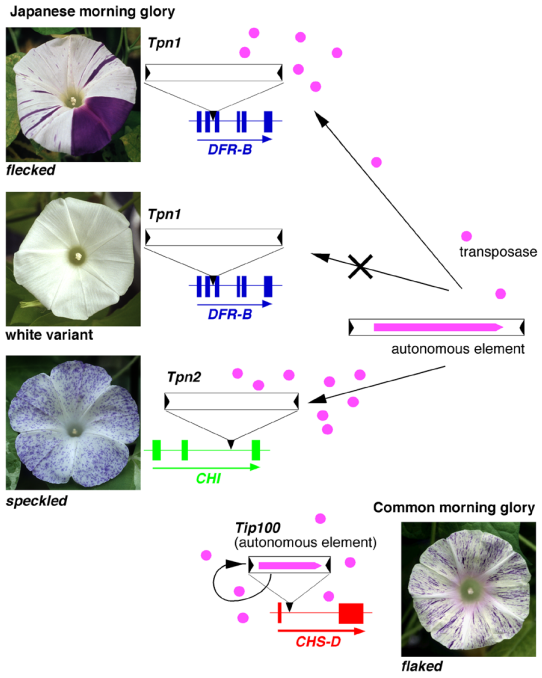

Fig.1. Mutable alleles and transposable elements in the Japanese and common morning glories. In the Japanese morning glory, activities of a putative autonomous element act on the non-autonomous *Tpn1* and *Tpn2* elements and determine the frequency and the timing of flower variegation. In the common morning glory, *Tip100* is an autonomous element.