LABORATORY OF GENE EXPRESSION AND REGULATION

Head: Takashi Horiuchi

The laboratory consists of four regular divisions and conducts research into regulatory mechanisms of gene expression in microorganisms, plants and animals.

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 genome, we are currently focused on mobile genetic elements in general and plant transposable controlling elements in particular.

I. Spontaneous mutants in the Japanese morning glory.

The Japanese morning glory (*Ipomoea nil* or *Pharbitis nil*), displaying blue flowers, is believed to be originated from southeast Asia and has an extensive history of genetic and physiological studies. The plant had been introduced into Japan from China in about 8th century as a medicinal herb, seeds of which were utilized as a laxative, and has become a traditional horticultural plant in Japan since around 17th century. A number of its spontaneous mutants related to the colors and shapes of the flowers and leaves have been isolated, and about 10% of these mutants carry mutable alleles conferring variegated phenotypes. Several lines of evidence indicate that an *En/Spm*-related transposable element *Tpn1* and its relatives, which we termed *Tpn1*-family elements, are major sources of these spontaneous mutations. Indeed, we have succeeded to identify two of these mutable alleles for flower pigmentation, *flecked* and *speckled*, which are caused by integration of *Tpn1*-related elements, *Tpn1* and *Tpn2*, respectively. Both *Tpn1* and *Tpn2* are non-autonomous elements and their transposition is mediated by a *Tpn1*-related autonomous element. Among selfed progeny of a mutable *flecked* line, a white variant displaying white flowers occasionally appeared. Some of the selfed progeny of the white variant bore only white flowers whereas others produced a few flecked flowers together with white flowers. In these white variant derivatives, the excision of *Tpn1* occurred rarely. We are speculating that appearance of the white variant is probably due to epigenetic inactivation of the autonomous element. In accordance with this notion, we also found that the apparent stable *r-1* allele conferring white flowers is caused by insertion of a non-autonomous *Tpn1*-family element, *Tpn3*, into the *CHS-D* gene encoding a chalcone synthase for anthocyanin biosynthesis.

II. A new procedure for isolation of a gene tagged by a transposable element belonging to the *Tpn1* family in the Japanese morning glory.

Transposable elements are regarded as a powerful mutagen and as an effective tool to isolate genes tagged by transposon insertions. The Japanese morning glory contains around 500-1000 copies of an *En/Spm*-related element *Tpn1* and its relatives, which act as major spontaneous mutagens. We have previously developed an amplified restriction fragment length polymorphism (AFLP)-based mRNA fingerprinting (AMF) procedure which is based on the systematic comparison of differently expressed transcripts in the same tissue in different lines, and succeeded in applying AMF for the identification of a new mutable allele caused by integration of a transposable element into an anthocyanin biosynthesis gene. Since transposon mutagenesis has become a powerful tool for the isolation of genes of interest, we have attempted to develop a new protocol for identifying tagged genes by insertion of *Tpn1*-related elements in the Japanese morning glory. Our transposon tagging method, named simplified transposon display (STD), was based on our AMF procedure and is simple and requires neither biotinylated oligonucleotides nor streptavidin-capturing which are essential in other transposon display methods published recently.

III. Identification and characterization of the *Purple* gene encoding a vacuolar Na^+/H^+ exchanger, InNHX1, for blue flower coloration in the Japanese morning glory.

We have applied STD for identification of a mutable allele, *purple-mutable* (*pr-m*), which confers purple flowers with blue sectors (Fig. 1). The flower variegation is regarded to be due to recurrent somatic mutation from the recessive purple to the blue revertant allele, *Purple-revertant* (*Pr-r*) and we assumed that the *pr-m* allele is caused by insertion of a *Tpn1*-family element. To characterize the *Purple* (*Pr*) gene, we chose pairs of siblings carrying either the *pr-m* or *Pr-r* allele homozygously. No alterations were detected in the anthocyanin pigment compositions between the *pr-m* and *Pr-r* lines. The *pr-m* mutant showed partial increase in the vacuolar pH during flower opening and its reddish-purple buds change into purple open flowers. The vacuolar pH in the purple open flowers of the mutant was significantly lower than that in the blue open flowers, indicating that the *pr* mutant fails to increase the vacuolar pH.

We have succeeded in identifying the *pr-m* mutation that is caused by integration of an *En/Spm*-related transposable element, *Tpn4*, into the *Pr* gene encoding a

vacuolar Na^+/H^+ exchanger, InNHX1 (Fig. 1). The *Pr* gene comprises 15 exons, and *Tpn4* is integrated into the first untranslated exon. The genome of *I. nil* carries one copy of the *Pr* (or *InNHX1*) gene and its pseudogene. The *Pr* gene is most abundantly expressed at around 12 h before flower opening in the petals, which must correlate with the increase in the vacuolar pH for the blue flower coloration. The isolated *Pr* gene is able to show functional complementation to a deletion mutation in the *NHX1* gene encoding a vacuolar Na^+/H^+ exchanger in yeast (*Saccharomyses cerevisiae*), indicating that the Pr gene product bears the NHX1 activity (Fig. 1). The NHX1 proteins are shown to be important for salt tolerance and intracellular protein trafficking in yeast and plants. We have thus added a new biological role for blue flower coloration in the Japanese morning glory by the vacuolar alkalization. The vacuolar pH has been regarded to play an important role in the blue flower coloration, and InNHX1 is the first major component characterized for increasing vacuolar pH responsible for blue flower coloration.

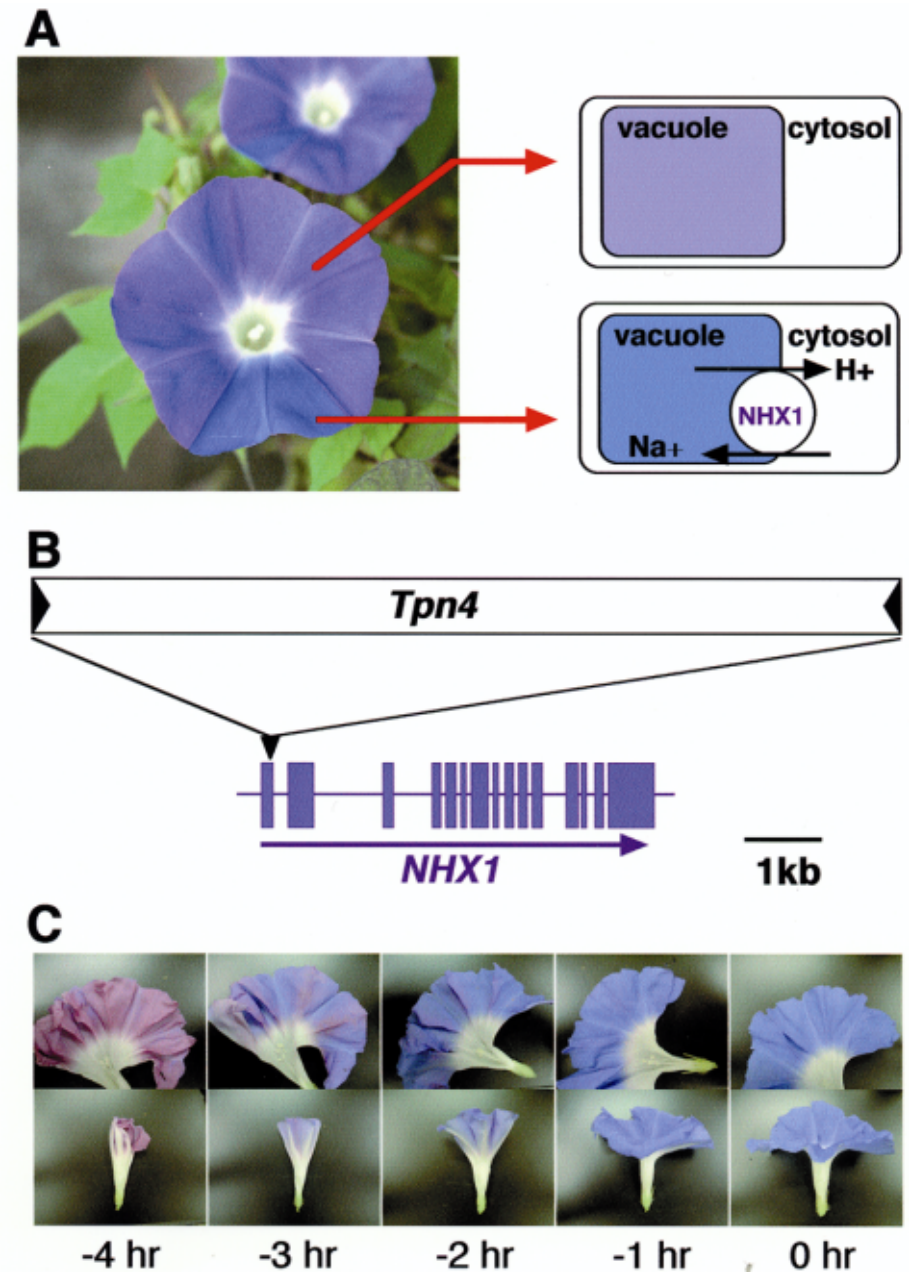

Figure 1. Blue flower coloration and the vacuolar pH in the Japanese morning glory.
A, Flower variegation and the *Pr* gene product, InNHX1, for blue flower coloration. B, The genomic structure of the mutable *pr-m* allele. C, Blue flower coloration during flower opening in the *Pr-r* plant. Petals in the upper line are the artificially opened petals of the buds in the lower line.

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