学位論文内容の要旨

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学位論文題名

Novel inactivation mechanisms of transposon uncovered by the *Old* and *New Stabiliser* loci in Tam3 system of *Antirrhinum*

(キンギョソウの Tam3 システムにおける *Old Stabiliser* と *New Stabiliser* 遺伝子座による新規のトランスポゾン抑制機構)

Antirrhinum majus is known to exhibit occasional instabilities that are manifested as variegations and morphological chimeras. In the 1980s and 1990s, several transposon-induced mutants in *Antirrhinum* affecting flower color, morphology, chlorophyll and other changes were identified. Of these mutants, Tam3 (the third transposon of *A. majus*) has been associated with the most of the gene discoveries involved in the mutants. Tam3 belongs to the hAT superfamily (Ac, hobo and Tam3) and is activated at low temperatures (around 15 ℃) while remaining stable at high temperatures (around 25 ℃). It exhibits low-temperature-dependent transposition (LTDT). Several flower variegation lines have been caused by Tam3 insertion into pigmentation loci responsible for anthocyanin synthesis. This variegation arises at 15 ℃ due to LTDT of Tam3; however, an inactive Tam3 at 25℃ displays no variegation in white or pale-colored petals. Here I studied Tam3 inactivation independent of LTDT due to genetic factors that immobilize Tam3 even at 15℃. It is known that certain *Antirrhinum* lines possess the Tam3 suppression factors named *Old Stabiliser* (*OSt*) and *New Stabiliser* (*NSt*).

In this study, I explored the mysterious field of Tam3 suppression by *OSt* and *NSt*, at low temperature. Firstly, I identified the two suppressor elements and analyzed their gene structures, chromosomal positions and linkage with Tam3 inactivation trait. Secondly, the mechanism(s) by which *OSt* and *NSt* suppress Tam3 transposition were investigated. Finally, I found an epigenetic phenomenon, paramutation, occurring at *NSt* locus in *Antirrhinum*.

Identification of *OSt* and *NSt* elements and possible mechanisms by which both *Sts* inactivate Tam3 transposition

Transposon tagging identified two *St* loci involving derivatives of Tam3 with unique structures: *OSt* has a pseudo-Tam3 copy whose 5' terminal region has been arranged compared to the cognate Tam3 element, and *NSt* consists of two intact copies of Tam3 in head-to-head orientation. Neither locus interferes with the production of the intact Tam3 TPase or the nuclear import of TPase. Both *OSt* and *NSt* produce specific sRNAs from their 5' terminal regions containing multiple TPase binding motifs. I performed sRNA induction experiments by TRV-mediated virus induced gene silencing targeting the 5' terminal region in the Tam3 sequence, resulted in the suppressed Tam3 transposition of the *ost*/*nst* plants Additionally, I employed S1-RNase H-S1 digestion system to detect whether *NSt*-derived sRNAs can target to Tam3 DNA in *NSt* plants grown at 15 ℃, but the sRNA-DNA-interacting triplexes cannot be observed. Instead, the sRNAs generated by *NSt* harbored several TPase binding motifs and can interact with synthesized Tam3 TPase *in vitro*. These results suggested that the specific sRNAs could repress Tam3 transposition by targeting the Tam3 TPase binding motifs or interacting with the TPase itself in *OSt* line. And *NSt* might suppress Tam3 transposition by generate the sRNAs to interact with the TPase itself instead of targeting Tam3 DNA.

Finding of paramutation phenomenon at *NSt* locus in *Antirrhinum*

In the progenies of *NSt* crossed with *nst-22* (HAM3 × HAM22), I observed paramutation where Tam3 transposition was suppressed even if *NSt* allele was absent. The *NSt*, *nst-22*, and *nst-5* alleles originated from HAM3, HAM22 and HAM5 lines, respectively. When crossing between HAM3 and HAM22, *NSt* stabilized Tam3 with *nst-22*, resulting in *NSt* becoming a paramutagenic allele and *nst-22* becoming a paramutable allele. The crossing between *NSt* and *nst-5* produced an active Tam3 linked to a homozygous *nst-5* allele, designated the neutral allele. After the *NSt* allele paramutates the *nst-22* allele, the Tam3-stabilized state of paramutated *nst-22* allele can be inherited to progeny and be maintained at a high level in successive generations. Moreover, the structure of *nst-22* is distinct from *NSt* and *nst-5* because no Tam3 reside in the *nst-22* allele. I expected that the *NSt*-specific sRNAs could function in paramutated *nst-22*/*nst-22* plants, and thus sRNA-seq was performed to examine these sRNAs. However, I cannot detect these sRNAs in the paramutated *nst-22*/*nst-22* plants. The reason for the difficulty in the sRNA detection might be that the amount of *NSt*-specific sRNAs decreases during inheritance. The mechanism involved in the paramutation may provide new insight to how transposons are inactivated in the plant genomes.