How to establish a mutually beneficial relationship between a transposon and its host: lessons from Tam3 in *Antirrhinum*

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The transposon Tam3 of *Antirrhinum* (snapdragon) has acquired properties that distinguish it from other transposons. Mobile DNA, commonly referred to as a transposable element or transposon, is considered to be synonymous with a selfish factor. That is, a transposable element increases in copy number and moves copies of itself independently of the survival of the host organism. Therefore, the host collectively regulates the transposition activities of most transposable elements in its genome by epigenetic means. However, our analyses of the structure and behavior of Tam3, as shown by the following five results, provide evidence that it does not behave in a selfish manner in relation to the host. 1) Active transposable elements normally increase the abundance of their non-autonomous elements, whereas Tam3 is known to have no non-autonomous elements, and a limited number of around 10 copies of autonomous elements present in the genome have been isolated as active copies. 2) Tam3 does not transpose at 25 °C, which is the optimal growth temperature for *Antirrhinum*. Transposition of Tam3 occurs only at low temperatures of about 15 °C, which is stressful for *Antirrhinum*. 3) Few strains of *Antirrhinum* have been found to contain genes that specifically suppress Tam3 transposition. 4) Most of the Tam3 insertions found in *Antirrhinum* genes do not affect the host genome, and the expression of these host genes is not completely suppressed. 5) Transcription and translation of the Tam3 transposase gene are not epigenetically regulated by the host. These five experimental results constitute evidence that Tam3 retains features that are dissimilar to those of many other transposons and that it does not behave in a selfish manner that is detrimental to the survival of the host. In this review, we consider what kinds of behavior are required if transposons are to establish a mutually beneficial relationship with their hosts, with reference to Tam3.

Key words: Tam3, LTDT, transposition regulation, snapdragon (*Antirrhinum majus*)

INTRODUCTION

Prominent biologists in 19th-century Europe were fascinated by spotted strains of snapdragon (*Antirrhinum majus*; hereafter, *Antirrhinum*) that exhibited anomalies in their flower shapes and colors. The first mention of the spotted *Antirrhinum* can be traced to 1838 in “Paxton’s Magazine of Botany”. Later, Darwin and de Vries also described the red-spotted-flower variegation. The fact that the spotted-flower lineage of *Antirrhinum* often produces mutant offspring may have been one reason for their interest in the species. During the height of transposon research in the 1980s and 1990s, a number of transposons involved in flower color, morphology, chlorophyll and other facilitative changes were isolated in *Antirrhinum* and identified under the name Tam (transposon of *Antirrhinum majus*) with a number corresponding to the order of their discovery. Tam1 was published...
in 1984, and Tam9 was reported in 1992. Many Tams were isolated in association with the high mutability of these traits in Antirrhinum. Except for Tam3, all other Tams are classified as CACTA elements. Of these CACTA elements, Tam1 is the only autonomous element, so the other Tams are likely non-autonomous elements derived from Tam1. Tam3 was the third element and was discovered in a mutant niv-98 line, which showed unstable flower color with variegation attributable to the Nivea (Niv) locus encoding chalcone synthase (Sommer et al., 1985). Tam3 is a DNA transposon and belongs to the hAT (hobo, Ac, Tam3) superfamily (Calvi et al., 1991). Tam3 copies identified from various mutated strains of Antirrhinum exhibit the same structure, and non-autonomous factors such as the CACTA elements have not been found so far. Tam3 has also been shown to insert into various genes, demonstrating that it is an active transposon. Uniquely, Tam3 only transposes in a low-temperature-dependent manner. Our group has also characterized other unique properties of Tam3, all of which indicate that Tam3 is not detrimental to the host. As evidence of this, we have shown that the host does not epigenetically regulate Tam3. Taking these findings together, we believe that a mutually beneficial relationship exists between Tam3 and the host. This review describes the mechanisms by which an intimate relationship is established between Tam3 and the host.

**STRUCTURAL CONSERVATION OF TAM3 COPIES IN THE GENOME**

Tam3 copies are exceptionally structurally conserved. The estimated organization of the Tam3 family consists of 40–60 copies in the diploid genome, and 40 independent Tam3 clones, of which 10 copies were identified as active elements, were isolated from an Antirrhinum plant (Martin et al., 1989; Kishima et al., 1999). However, copies with a deleted internal sequence (non-autonomous copies) have not been found in Tam3, in contrast to the heterogeneous organization of other transposon families (Kishima et al., 1999). Yamashita et al. (1999) suggested that the extreme homogeneity of the Tam3 copies is determined by important structures at the Tam3 termini. Tam3 integrated into the Antirrhinum genome at least five million years ago, which is a sufficiently long period to allow the production of aberrant copies by gap repair, but non-autonomous Tam3 copies have not occurred during the long evolutionary history of the element (Yamashita et al., 1999). The 5’ and 3’ end regions of the Tam3 sequence contain 11 and five hairpin structures, respectively, which form highly stable structures because of the extremely low free energies (SantaLucia et al., 1998; Yamashita et al., 1999; Table 1). These secondary structures in the Tam3 termini are responsible for the absence of non-autonomous copies in the Tam3 copies. They inhibit the gap repair of synthesis-dependent strand annealing after Tam3 excision, resulting in extremely short Tam3 segments, which are no longer than 150 bp from the Tam3 ends (Yamashita et al., 1999; Fig. 1). As ordinary non-autonomous elements capable of transposition have not been generated, this mechanism ensures the conservation of the Tam3 family.

**TEMPERATURE-DEPENDENT TAM3 TRANSPOSITION**

The mechanism of transposition for each transposon is one of the most interesting and attractive topics in this research field. Researchers have paid attention to the Tam3 transposition mechanism due to the unique nature of the transposition. Unlike most other cut-and-paste-type transposons, Tam3 transposition is tightly controlled by environmental temperature and exhibits a low-temperature-dependent transposition (LTDT) phenomenon: Tam3 is active at low temperatures (around 15 °C) and is stable at high temperatures (around 25 °C; Harrison and Fincham, 1964; Carpenter et al., 1987). In the Antirrhinum genome, several intact Tam3 copies are closely associated with pigmentation loci that are required for anthocyanin synthesis in the flower. Tam3 insertions strongly inhibit the expression of these pigmentation genes, resulting in a light-colored or even white background of the flower petal (Fig. 2). When the Antirrhinum growth temperature is shifted from 25 °C to 15 °C, Tam3 excision events occur and the expression of these pigmentation genes reverts to wild-type level, which eventually causes magenta flakes and stripes to appear on petals having a light-colored background (Carpenter et al., 1987; Fig. 2). Tam3 copies are distributed at different positions including the above-mentioned pigmentation loci within the Antirrhinum genome, but transposition ability or activity is not uniform among all

| Transposon | Free energy in 300-bp end regions |
|------------|----------------------------------|
| Tam3       | −210.1                           |
| En/Spm     | −116.8                           |
| hobo       | −100.8                           |
| Ac         | −89.7                            |
| Mu         | −89.0                            |
| P          | −50.3                            |
| Tcl        | −46.1                            |

Note: This table is adapted from Table 2 in Yamashita et al. (1999).

Free energy (kcal/mol) was calculated according to SantaLucia (1998). Values shown are the sum of the free energies in both 300-bp end regions.
Mutual relationship of transposon Tam3 with host

Fig. 1. Model for processes after TE excision. (A) Synthesis-dependent strand annealing (SDSA) processes after the excision of ordinary DNA transposons. Insertion is due to strand invasion into a nonhomologous template (striped line), while deletion results from annealing of incomplete repair products at short repeats (short vertical bar). (B) Inhibition of SDSA processes after Tam3 excision. SDSA stops within hairpin regions located in the termini of Tam3. These short stretches search for complementary motifs (short vertical bar), to which they anneal. Each thin line and thick line represents a single strand of genomic DNA and transposon DNA, respectively.

Fig. 2. Model for the LTDT mechanism and corresponding phenotypes of flower petals in *Antirrhinum majus*. At 25 °C, host factor(s) inhibit the nuclear import of Tam3 TPase and the flower petals display a light-colored background, while at 15 °C, Tam3 TPase can be transported into the nucleus and the flower petals display a variegated phenotype.

intact Tam3 copies: the most active copy exhibits a transposition frequency more than 100-fold higher than that of the least active copy (Kishima et al., 1999; Kitamura et al., 2001). Nevertheless, all the Tam3 copies behave in the manner of LTDT. Moreover, the position effect on Tam3 transposition is correlated with the methylation state of the end regions of each Tam3 copy: copies with DNA hypomethylation have high excision activi-
ties, whereas copies with DNA hypermethylation show low excision activities, and the degree of methylation is dependent on the chromosomal position of each copy (Kitamura et al., 2001).

What kind of factor(s) can determine Tam3 LTDT? Hashida et al. (2003) examined the methylation level of Tam3 sequences in an Antirrhinum DNA genome from the same plant exposed to different temperature conditions and found that the methylation state of Tam3 also reversibly varies with the temperature shift, mainly in the end regions. The methylation level of Tam3 is positively correlated with the growth temperature of the Antirrhinum plant: Tam3 exhibits the hypermethylation state at 25 °C, but the hypomethylation state at 15 °C (Yamashita et al., 1999; Hashida et al., 2003). Although DNA methylation has a great impact on the suppression of the Tam3 transposon, Hashida et al. (2005) revealed that DNA methylation is not the direct cause for the inactivation of Tam3 at high temperature, because Tam3 transposition frequency did not increase in Antirrhinum calli treated with methylation inhibitors. The transposition process of DNA transposons mainly contains four parts: transcription of the transposase (TPase) gene, transposase (TPase) synthesis, nuclear import of TPase protein and binding between TPase and its target sites on the transposon sequence. When any part is inhibited, transposition of DNA transposons is not possible. Hashida et al. (2003) and Uchiyama et al. (2008) examined Tam3 transcription by Northern analysis and RT-PCR, and Tam3 TPase activity by transient assay and immunoblotting, and their results suggested that LTDT is not associated with either transcriptional or translational regulation of the Tam3 TPase gene. After being synthesized in the cytoplasm, TPase should be imported into the nucleus under the guidance of three nuclear localization signals (NLSs) and catalyze transposition by interacting with the Tam3 sequence. However, the nuclear import frequency of Tam3 TPase exhibits a substantial difference at high and low temperatures: import is strictly restricted in Antirrhinum cells at 25 °C, whereas nuclear localization of the Tam3 TPase can reach approximately 20% in Antirrhinum cells at 15 °C (Fujino et al., 2011; Fig. 2). Thus, Fujino et al. (2011) suggested that the LTDT mechanism is involved in the nuclear import process of Tam3 TPase, which necessarily contains a nuclear localization inhibitory domain (NLID) (Fig. 2). It was assumed that the NLID could completely abolish the function of the NLSs of Tam3 by interacting with Antirrhinum-specific factor(s) at high temperature. Furthermore, the Tam3 TPase can bind to a probe containing three GCHCG motifs, multiple copies of which occur on both ends of the Tam3 sequence (Hashida et al., 2006). In Antirrhinum plants grown at 25 °C, the Tam3 TPase was not transported into the nucleus, while TPase bound to the GCHCG motifs was detected in the nucleus in plants grown at 15 °C (Hashida et al., 2006). Zhou et al. (2017) identified the NLID of the Tam3 TPase, which is a BED-zinc finger domain (Znf-BED) located near the N terminus of the polypeptide. They also demonstrated that at high temperature, Znf-BED strongly prevented the nuclear import of the Tam3 TPase, and localized it to the plasma membrane (PM), whereas low temperature allowed the TPase to localize in the nucleus (Fig. 2). Therefore, we suppose that Antirrhinum-specific factor(s) target Znf-BED at the high temperature and detain Tam3 TPase at the PM (Zhou et al., 2017). Its consequence finally results in LTDT where Tam3 transposition is silenced at high temperature and occurs at low temperature.

**TWO HOST GENES INDEPENDENTLY SUPPRESS TAM3 TRANPOSITION**

In addition to LTDT regulation of Tam3 activity, two controlling elements discovered in the Antirrhinum genome exhibit a strong suppression effect on Tam3 transposition at low temperature (Fig. 3). One suppressor locus is Stabiliser (St), described by Harrison and Fincham (1968) in the Antirrhinum line carrying the

![Fig. 3. Phenotypes of flower petals in st/nst, St and NSt Antirrhinum lines and the corresponding activity of Tam3 at high and low temperatures.](image-url)
nivea\textsuperscript{rec}::Tam3 (niv\textsuperscript{rec}) allele, which represses the genetic instability of variegation in flower petals. This phenomenon of decreasing spotting frequency is controlled by St alone, and St is not linked to the Niv locus (Harrison and Fincham, 1968). The second element is the New Stabiliser (NS\textit{St}) locus derived from progeny of the niv\textsuperscript{rec} line that showed a high frequency of sites or sectors at 15 °C (Fig. 3). Tam3 transposition is almost completely repressed by NS\textit{St}, and thus the NS\textit{St} line does not show variegated petals at low temperature (Hashida et al., 2006). Besides, the suppression effect of NS\textit{St} on Tam3 transposition is dominant, while the genetic effect of St on Tam3 is semi-dominant (Uchiyama et al., 2008). Moreover, several crossing experiments performed by Hashida et al. (2006) indicated that NS\textit{St} is not linked to the Niv or St loci. Both suppressor elements are genetically independent, implying that they reside at different chromosomal positions in the \textit{Antirrhinum} genome. Thus, mechanisms by which St and NS\textit{St} suppress Tam3 excision should be distinct. Uchiyama et al. (2008) suggested that St and NS\textit{St} elements inhibit Tam3 transposition at the post-translational stage.

**LEAKY PHENOTYPE IS OFTEN OBSERVED IN HOST GENES WITH A TAM3 INSERTION**

Insertion of the Tam3 transposon into various genes of \textit{Antirrhinum} can confer leaky phenotypes, called permissive alleles (Table 2). Interestingly, most of the genes with Tam3 insertions in noncoding regions were not completely suppressed, but appeared to show permissive transcription (Uchiyama et al., 2009; Table 2). Among the alleles with Tam3 insertions, Tam3-permissive alleles (11 alleles) outnumber non-permissive alleles (six alleles; Uchiyama et al., 2009). Among the 11 Tam3-permissive alleles, the number of alleles with Tam3 inserted in the same orientation as the host gene (6 alleles) exceeds that of alleles with Tam3 inserted in the reverse orientation (2 alleles) (Table 2). The magenta phenotype of \textit{Antirrhinum} flowers is determined by anthocyanin, whose synthesis is cooperatively controlled by two genetic loci: Niv, encoding chalcone synthase (CHS), and Pal, encoding dihydroflavonol-4-reductase (DFR) (Stickland and Harrison, 1974). Insertions of an intact Tam3 in the promoter region of Niv and Pal give rise to two new alleles, niv\textsuperscript{rec} and pal\textsuperscript{rec} (Fig. 4). When these two alleles are stable at high temperature, the petals are pale red and ivory, respectively (Martin et al., 1985; Sommer and Saedler, 1986; Fig. 4). The niv\textsuperscript{rec} allele displays a leaky phenotype, indicating a permissive allele, and pal\textsuperscript{rec} indicates a non-permissive allele where a no-anthocyanin phenotype appears on the petals. These different phenotypes are due to transcriptional activities at the niv\textsuperscript{rec} and pal\textsuperscript{rec} alleles, which have Tam3 at a similar position in the promoter, and are attributable to the opposite Tam3 inser-

| Condition | Locus | Allele or line | Tam3 Position | Direction |
|-----------|-------|----------------|---------------|-----------|
| Permissive | Nivea | niv\textsuperscript{rec} | Promoter | Forward |
| Olive | ali-605 | Promoter | Forward |
| Plena | ple-627 | Intron | Reverse |
| Fimbriata | fm-619 | Promoter | Forward |
| Centroradialis | cen-594 | Intron | Unknown |
| Dag | dag::Tam3 | 5' UTR | Forward |
| Hirz | Hirz-d153 | Intron | Unknown |
| Ina | Ina-d1 | 5' UTR | Unknown |
| Cincinnati | cin-757 | Promoter | Forward |
| Cincinnati | cin-758 | 5' UTR | Forward |
| Cincinnati | cin-759 | Promoter | Reverse |
| Pullida | pal\textsuperscript{rec} | Promoter | Reverse |
| Floricaula | flo-613 | Coding region | Unknown |
| Plena | ple-625 | Intron | Forward |
| Fimbriata | fm-620 | Promoter | Unknown |
| Lipless1 | lip1 | Coding region | Reverse |
| Lipless2 | lip2 | Coding region | Forward |

Note: This table is adapted from Table 1 in Uchiyama et al. (2009). Forward and reverse directions indicate that the orientation of Tam3 is, respectively, the same as and opposite to that of the host gene.
NON-EPIGENETIC REGULATION OF TAM3 TRANSPOSITION IN THE ANTIRRHINUM GENOME

Research results accumulated to date indicate that host organisms initiate transposon silencing mainly through two epigenetic regulatory pathways: transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS). In the TGS strategy, host organisms employ RNA-directed DNA methylation (RdDM) to increase DNA methylation and histone modifications of the sequences of transposons and thus inhibit transcription of the TPase gene (Fig. 5). In the PTGS strategy, although TPase mRNA can be produced, TPase transcripts are cleaved and degraded under the guidance of small interfering RNAs (siRNAs) and then transposition activity is stopped due to the lack of TPase protein (Lisch, 2009; Fig. 5). One well-known TGS case is the silencing of a short interspersed nuclear element (SINE) transposon residing in the promoter of the FWA gene in Arabidopsis (Kinoshita et al., 2007). DNA methylation of a short, tandemly duplicated fragment of this SINE transposon limits its transposition and then the presence of the SINE element indirectly inhibits transcription of the FWA gene (Kinoshita et al., 2007). The best example of PTGS is the suppression of MuDR by Mu killer (Muk) in maize (Slotkin et al., 2005). MuDR (Slotkin et al., 2005). The read-through transcription of Muk activated by the promoter of the neighboring acm1 gene produces a long hairpin RNA molecule which is processed into small RNAs, and the transcript of the MuDR transposase gene is then digested under the guidance of these small RNAs, which finally triggers the silencing of MuDR elements (Slotkin et al., 2005). However, the Antirrhinum host does not take these two strategies to inhibit Tam3 transposition activity. Hashida et al. (2006) provided evidence that DNA hypomethylation did not affect Tam3 transposition associated with LTDT. The suppressive actions of the two suppressor genes, St and NSt, were not associated with transcription or translation of the Tam3 TPase gene. The silencing of Tam3 is due to the failure of nuclear import of the Tam3 transposase product at high temperature, as described above (Zhou et al., 2017; Zhou and Kishima, 2017; Fig. 5). Hence, Antirrhinum manages Tam3 transposition in a non-epigenetic regulatory manner, a unique aspect that is different from the epigenetic regulation of most transposons in other species.

PROSPECT: RECONSIDERATION OF THE RELATIONSHIP BETWEEN HOST SPECIES AND TRANSPOSONS

The transposition activity of transposons, in general, plays both negative and positive roles in the host species. On the negative side, transposons are considered...
as parasites of host genomes because their excision and insertion activities generally cause genome instability of hosts and even threaten the hosts’ survival; on the positive side, transposons’ activity can provide numerous mutagens to hosts and help hosts to adapt to their living environment quickly when host species suffer from strong biotic or abiotic stress (Lanciano and Mirouze, 2018; Schrader and Schmitz, 2019; Klein and Anderson, 2022). Although transposons may make some contributions to the evolution of the host genome, it seems that active transposon elements are gradually silenced by their hosts in several complete silencing manners, and thus the relationship between transposons and hosts is usually considered as parasitic (Lisch, 2013; Nishihara, 2020). However, active Tam3 elements are not completely silenced in the Antirrhinum genome. Two features of Tam3, namely its structural conservation and incomplete suppression of host genes, can partially account for why Tam3 is still active in the Antirrhinum genome. The Tam3 family has not given rise to non-autonomous copies, implying a limited perturbation of the Antirrhinum genome. Even though Tam3 is closely associated with some functional genes and has a repressive effect on the expression of these genes, this impact is not strong and these host genes are still permitted to transcribe and play their functional roles. In addition, the Antirrhinum host flexibly switches Tam3 transposition activity on and off based on environmental stress, or instead limits its activity to a certain extent to maintain the balance between hosts and transposons. Extreme temperature change is a serious abiotic stress for the growth or survival of Antirrhinum plants. A growth temperature of 25 °C is suitable for Antirrhinum, while 15 °C or lower is stressful. LTDT of Tam3 seems to satisfy Antirrhinum, which adapts to different temperature conditions. Antirrhinum plants may have gradually adapted to the stressful low-temperature environment and then required the silencing of Tam3 to maintain genome stability even at low temperatures, like the occurrence of St and NSt. Taken together, these observations indicate that the relationship between Antirrhinum and Tam3 should be considered as mutualism. In conclusion, the beneficial relationship between them should lead us to reconsider the meaning of the relationship between a transposon and its host, and the behavior of Tam3 should be explored to reveal new aspects of the regulatory mechanisms of transposons.

**CONCLUSION**

The experimental results described above about the structure and behavior of Tam3 imply that the Tam3 DNA transposon is distinct from other transposons. Tam3 possesses several unique properties – structural conservation of Tam3 organization, low-temperature-dependent transposition and the incomplete suppression of host genes with Tam3 insertion – that make it stand out from most transposons. Moreover, compared with other transposons, Tam3 has maintained an intact structure during its long lifetime in the Antirrhinum genome. An obvious difference in Tam3 from the other transposons is that the transposition of Tam3 is regulated in a non-epigenetic manner. These unique structural and behavioral features of Tam3 may be a prerequisite for establishing a mutualistic relationship between transposons and their hosts.

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