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Transposable elements (TEs) are found in genomes of essentially every organisms examined. Mobile TEs are potentially mutagenic and deleterious for stability of the host genome, but most of TEs in eukaryotes are silenced by epigenetic mechanisms of host, such as RNA interference, histone modifications, and DNA methylation. The importance of DNA methylation in TE repression has recently been demonstrated in both mammals and plants by molecular genetic approaches. Less investigated research area is mechanisms of TEs to counter-act the host defense. Here I report activity of a plant TE to counter-act DNA methylation and silencing by the host.

VANDAL21 is a group of DNA TEs found in the genome of *Arabidopsis thaliana*. By structural similarity of encoded genes, *VANDAL21* has been classified as a member of *Mutator*-like elements (*MULE*s). Previous results of Southern analysis suggest that some of *VANDAL21* copies transpose in the background of reduced genomic DNA methylation in *A. thaliana* mutant *ddm1* (decrease in DNA methylation) (Tsukahara et al 2009). Using genomic DNA of the self-pollinated *ddm1* mutants, first I identified a mobile copy of *VANDAL21* family by PCR-based methods and whole-genome re-sequencing. I renamed the mobile *VANDAL21* copy *Hiun* (*Hi*; Japanese for "flying cloud").

Unlike most of other mobile *MULE*s, the mobile copy does not have terminal inverted repeat (TIR). This is unusual because TIR has generally been thought to be the substrate for transposase. Despite the unorthodox structure, *Hi* excised and transposed as other typical *MULE*s with TIR. As other *MULE*s, *Hi* integrated with generating target site duplication around 9-bp long. In addition, it integrated preferentially near transcription start site. A unique behavior of *Hi* is that the integration has bias in the orientation, which may be related to the asymmetry in the terminal sequences of *Hi*. Excision of *Hi* often occurs with leaving the structure of the target locus before the integration, suggesting that the terminal positions in both sides are precisely determined even without TIR.

Hi has three open reading flames (ORFs), which I named *hiA*, *hiB* and *hiC*. The *hiA* encodes a protein with high sequence similarities to transposases found in other *MULE*s. Two other ORFs, *hiB* and *hiC*, do not have sequence similarity to any characterized proteins. These ORFs are silent in wild type background, where *Hi* is immobile. In order to see if transcriptional de-repression of these ORFs is sufficient for *Hi* mobilization, I introduced *Hi* transgene into wild type background. In the *Hi* transgene, all three ORFs are transcribed and the transgene induced excision of endogenous *Hi*. In addition, the *Hi* transgene induced loss of DNA methylation in terminal regions of endogenous *Hi*.

In order to know role of each ORF in the trans-acting effects of *Hi* transgene, I generated

three types of transgenes with deletions for each of the three ORFs. The DNA de-methylation effect was abolished in transgene with deletion of *hiC* (Δ*hiC*), suggesting that *hiC* essential for the demethylation. On the other hand, Δ*hiB* (transgene with deletion of *hiB*) had the de-methylation activity indistinguishable from full length *Hi* transgene. Δ*hiA* (transgene with deletion of *hiA*) had de-methylation activity for one of the two terminals of endogenous *Hi*, which is upstream of *hiC*, but de-methylation activity was much reduced in the terminal region upstream of *hiA*.

I also examined effect of Δ*hiA* for the mobilization of endogenous *Hi*. In most of the transgenic lines, I could detect excision of endogenous *Hi*. The results were surprising, because *hiA* encodes a putative transposase, which presumably catalyses the transposition. However, subsequent analyses revealed that *hiA* transcript accumulates in Δ*hiA* lines, suggesting that endogenous *hiA* was de-repressed in the presence of Δ*hiA* transgene. Δ*hiA* transgene contains two ORFs, *hiB* and *hiC*. In order to test if *hiC* is sufficient for the transcriptional re-repression of endogenous *hiA*, I introduced a transgene with deletion of both *hiA* and *hiB* (Δ*hiA;B*). Δ*hiA;B* induced transcription of endogenous *hiA* and *hiB*. Δ*hiA;B* also induced excision of endogenous *Hi*, and DNA de-methylation of one terminal of endogenous *Hi*. These trans-acting effects are indistinguishable from those by Δ*hiA*, suggesting that *hiC*, rather than *hiB*, is responsible for these trans-acting effects of the *Hi* transgene.

In summary, I identified a mobile copy of *MULE*s without TIR and named that *Hiun* (*Hi*). When *Hi* is transformed into wild type plant, silent endogenous *Hi* copy was excised, suggesting that *Hi* is supplying factor(s) necessary for the transposition. *hiC*, one of the *Hi*-encoded gene, induced transcriptional activation, excision and DNA de-methylation of the repressed *Hi* copy. These trans-acting effects of *hiC* would contribute for counter-acting DNA methylation and silencing by the host.

博士論文の審査結果の要旨

生命が誕生して約40億年、生物のゲノムは絶え間なく変化し、その変化が生物進化の原動力になってきた。ゲノムの変化は様々な要因によってもたらされるが、トランスポゾンなどの反復配列はゲノム変化に大きな貢献をしてきたと考えられている。トランスポゾンは、ゲノム中で移動したり、コピー数を増加させたりする。このようなトランスポゾンの転移は、多くの場合、ホスト生物にとって有害であるため、ホスト生物によって厳密にコントロールされていることが、近年、分子レベルで明らかになりつつある。

付煜さんの所属する角谷研究室では全ゲノムにわたってDNAのメチル化のレベルが低下したシロイヌナズナ (*Arabidopsis thaliana*) の変異体*ddm1*の解析をおこなってきた。この結果、DNAメチル化の低下のような エピジェネティック変化よって、シロイヌナズナのさまざまなトランスポゾンが再活性化し、転移することを突き止めている。付さんは、転移が確認されたトランスポゾンの中で、DNAトランスポゾンである*VANDAL21*について詳細な解析をおこなった。*VANDAL21*はDNAトランスポゾンのなかでも*Mutator*-like elements (*MULE*s) のファミリーに属する。

付さんはまず、*ddm1*変異下において最も転移活性のある*VANDAL21*の１つのコピーを同定し、*Hiun* (*Hi*)と名付けた。この*Hi*について、付さんが得た主な知見は以下の通りである。1. *Hi*は他の*MULE*sとは異なり、転移に必要と考えられていたterminal inverted repeat (TIR) 配列を持たなかった。2. *Hi*は３つのopen reading flames (ORFs), *hiA*, *hiB*, *hiC*を持っていた。*hiA*はその相同性より転移酵素と思われたが、*hiB*, *hiC*は有意な相同性を持つ既知のタンパク質は見出せなかった。3. *Hi*を野生株に導入したトランスジェニック植物では、内在性*Hi*付近のDNAメチル化が極度に低下し、内在性*Hi*の切り出しが確認された。4. 導入された*Hi*の３つのORFのうち、*hiA*は転移酵素と思われたが、内在性*hiA*の発現や、*Hi*の切り出しには必須ではなかった。5. 導入された*Hi*の*hiC*が内在性*Hi*付近のDNAメチル化を低下させ、*Hi*の転写を活性化し、*Hi*切り出しに関与すると思われた。

本研究は、今まであまり解析がおこなわれてこなかったDNA トランスポゾンのゲノムワイドな転移制御に焦点を当てた。そしてトランスポゾン転移におけるエピジェネティックな制御メカニズムの一端を明らかにした。トランスポゾン転移は、多くの場合、ホスト生物にとって有害となるため、ホスト生物はDNA メチル化によって、トランスポゾンを不活化しようとする。本研究は、これに対するトランスポゾン側の「対抗手段」の発見とも言え、非常に興味深いものである。また、その「対抗手段」を担う*hiC*は機能未知のタンパク質であるため、新しいメカニズムが明らかになるだろう。よって付煜さんの学位提出論文は、博士号授与の要件を満たすと審査員全員一致で判断した。