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論文内容の要旨

Transposable elements constitute the major part of the genome in many eukaryotic organisms. Transposons and their remnants cause gene disruptions and genomic rearrangements. In addition, transposons often affect expression of neighboring genes. Furthermore, recent studies showed a part of transposon-derived sequences and proteins participate in host cellular processes.

In plants, most of these elements are not transcribed under normal conditions. A common feature of these sequences is heavy methylation. DNA methylation generally suppresses gene expression in both mammals and plants. In mammals, many cytosines in CG context are methylated. These methylated cytosines are involved in several epigenetic phenomena, e.g. X-chromosome inactivation, imprinting and silencing of transposable elements. In plants, cytosines in all contexts (CG, CHG and CHH; where H is A, C or T) have a potential to be methylated. In *Arabidopsis thaliana*, identified DNA methyltransferases appear to have preference for sequence contexts. *METHYLTRANSFERASE 1(MET1)* gene, which is a mouse *DNA methyltransferase 1* (Dnmt1) homolog, is responsible for cytosine methylation in the CG context by maintenance mechanism. *CHROMOMETHYLASE 3 (CMT3)* is thought to regulate it in the CHG context. *DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2)* is thought to be a *de novo* methyltransferase and to act on cytosines in all contexts. Defects in DNA methyltransferases result in transcriptional activation of transposons. However, the silencing mechanism of the transposon by host and the maintenance mechanism of transposon activity remain puzzling. For most transposons, the epigenetic alterations by defects of epigenetic modifiers often become heritable. These alterations are not recovered even after backcross to wild-type plants. In addition, the backcross brings epigenetically wild-type alleles into the F1 generation. Such epigenetic heterogeneities between epimutated allele and unaffected allele make it difficult to assess these epigenetic alterations.

Many phenomena as results of transposon mobility have been described in cultivar of maize, morning glory and snapdragon. It has long been known that maize transposons sometimes switch their phases between active and inactive without changes in nucleotide sequence. For instance, it has been described that the *Spm* transposon in maize transits among three phases: (i) cryptic (stable inactive), (ii) programmable (unsettled) and (iii) active phases. The correlation has been reported between these phases and their DNA methylation level. However, the molecular basis for the connection between the host regulator and transposon are poorly understood.

The *CACTA* transposon, which is a DNA-type transposon, transposes through "cut (excision) & paste (insertion)" mechanism. In *Arabidopsis*, the mobility of *CACTA* transposon is observed

in a mutant named *Decreased in DNA Methylation1* (*ddm1*). *DDM1* gene encodes a chromatin-remodeling factor but not DNA methylase. Although it is still unclear whether the reduction of DNA methylation is a direct trigger for the activation of *CACTA*, the *ddm1* mutant shows the genome-wide hypomethylation. Backcross analysis revealed that activated *CACTA* transposons maintain its activity even in wild-type background, suggesting epigenetic alterations induced by *ddm1* is also heritable. Behaviors of *CACTA* family in *Arabidopsis* are relatively easy to trace because of the low copy number and the availability of the comprehensive *Arabidopsis* genomic information. In addition, *CACTA* transposons are uniformly heavily methylated and silent in wild-type *Arabidopsis* plants, allowing us to investigate its activation process.

In the present study, I focused on the excision activity of *CACTA* transposon and DNA methylation status at early step of the activation by the *ddm1* mutation. The progressive reduction of DNA methylation level at *CACTA* loci and the progressive increment in the excision activity were observed in proceeding generations of the *ddm1* mutant. Additionally, a monitoring system using a reporter gene provided an evidence that transposases derived from endogenous locus were supplied in all tissues examined and that *trans*-acting factors contributed to the difference of the *CACTA* activity between generations. Furthermore, segregation of a non-autonomous copy *CACTA2 (CAC2)* from an autonomous copy *CACTA1 (CAC1)* leads to inefficient hypomethylation at the *CAC2* locus in the *ddm1* mutant background, especially at the 3′ terminal end. The excision activity of *CAC1* and *CAC2* that were exposed by the *ddm1* mutation increased even in the DDM1-functional background. This result suggests activated *CAC1* by the *ddm1* mutation can boost its own and its family activities. In further studies, I examined endogenous factors involved in regulation of the *CACTA* activity in the *ddm1* mutant background by examination of double mutants of *ddm1* and other epigenetic regulators. The results suggest that MET1 and CMT3, which are DNA methylases, and KYP and RTS1, which are histone modifiers, are also involved in suppression of the *CACTA* activity in the *ddm1* background. Although the small RNA pathway is a common regulator of transposons in animals and plants, the small RNA components did not affect in the *CACTA* activity. The progressive reduction of DNA methylation that were observed in the *ddm1* background could be due to the eventual positive-feedback of the *CACTA* activity as a result of the competition between active *CAC1* and other epigenetic factors. After exposure to the *ddm1* mutation, *CAC1* and *CAC2* are activated. Then, *CAC1* overcomes the remaining effects of other factors involved in the suppression of the *CACTA* activity. This study showed that active *CAC1* might hamper the DNA methylation as a host defense mechanism.

論文の審査結果の要旨

真核生物ゲノムに存在する転移性遺伝因子は転移に伴って挿入突然変異を起こすので、通常その活性は宿主細胞のエピジェネティックな機構により厳密に抑制されている。中村さんはシロイヌナズナの転移性因子 CACTA に着目し、CACTA が転移する機構と宿主が転移を抑制する機構について研究し、新たな知見を得た。

シロイヌナズナの CACTA は DNA 低メチル化変異体 ddm1 において転移活性を獲得する。その転移はゲノムからの CACTA 配列の切り出しと、新たな場所への挿入によって起きる。中村さんはまず PCR 法とレポーター遺伝子を用いた高感度の切り出し検出系を開発し、ddm1 変異体において CACTA の切り出しが様々な発生段階で様々な組織で起きること、またその切り出し活性が世代を追うごとに上昇することを見つけた。この切り出し活性の上昇は CACTA コピーの低メチル化状態と相関するが、戻し交配により野生型 DDM1 遺伝子を再導入してもメチル化は回復せず、切り出し活性の上昇も持続する。様々な戻し交配実験の結果、とくに活性化された自律性 CACTA コピーである CAC1 の存在が切り出し活性の維持に重要であった。また、興味深いことに非自律性コピーである CAC2 の 3' 部分の低メチル化状態が CAC1 の存在に依存していた。これは CAC1 から生産されるトランスの因子が CACTA 配列に作用し、切り出しに影響を与えることを示唆している。すなわち、一旦活性化された自律性 CACTA は、トランスの因子（おそらく転移酵素）を介する正のフィードバックにより、宿主の抑制機構に対抗していることが推測された。最後に、クロマチン再構成因子の変異である ddm1 に加えて、DNA メチル化酵素やヒストンメチル化酵素の遺伝子の変異が CACTA の切り出し活性を上昇させる一方、機能性小分子 RNA の経路はこの転移因子の切り出し抑制には関与しないことを示した。

審査員全員でこの論文を審査し、宿主細胞が転移因子を抑制するエピジェネティックな機構と、転移因子が自身の配列を切り出す機序に関する知見を大きく前進させたことを高く評価し、本大学院の学位の水準を十分に満たす論文であると判断した。