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学位論文題目　Studies on anti-silencing mechanisms of non-TIR transposons in Arabidopsis

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(別紙様式 2)
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論文内容の要旨

Summary of thesis contents

Transposable elements (TEs) constitute significant portions of genomes in vertebrates and plants. TEs are classified into two types according to their transposition manner; copy-and-paste (Class I) or cut-and-paste (Class II). Since TE movement is mutagenic, most of them are silenced by epigenetic mechanisms, such as histone modifications and DNA methylation. In the TEs of plants, both CG and non-CG contexts of cytosine can be methylated. The CG methylation is maintained by a MET1 DNA methyltransferase, a homolog of mammalian DNA methyltransferase DNMT1. Methylation at non-CG sites is catalyzed by plant specific Chromomethylases (CMT2/3) through as self-reinforcing loop with Histone H3 lysine 9 methylation (H3K9me). H3K9me is a histone modification typically associated with transcriptionally silent and condensed chromatin, called heterochromatin. DNA methylation for both CG and non-CG sites at heterochromatin is maintained by a chromatin remodeler Decrease in DNA Methylation 1 (DDM1). Mutation of DDM1 leads drastic transcriptional activation and proliferation of TEs.

Decades of studies have shown that TEs have adopted various strategies to survive in genome. Some TEs have evolved site-specific integration systems to minimize host damage. *Ty5* LTR retrotransposons in *Saccaromyces cerevisiae* specifically integrate into gene-poor heterochromatin regions by directly target to one of heterochromatin components, Sir4. Co-option, or domestication, of TEs to their host is other well-described strategies for survival. Substantial amount of functional regulatory elements in genome are derived from TEs. Some TE-derived proteins also play pivotal roles. For example, V(D)J recombination during development of immune systems is mediated by transposase-derived proteins, RAG1 and RAG2.

Intriguingly, some TEs also have developed mechanisms to counteract the defense systems in the host. For example, McClintock's *Suppressor-mutator* (*Spm*) element in maize encodes a protein TnpA, which induces loss of DNA methylation at a promoter region of *Spm* and reactivates it *in trans*. In the case of *Mutator* (*Mu*), another well-characterized TE in maize, an autonomously mobile copy named *MuDR* also reactivates silent copies of *Mu*. *MuDR* contains two genes, *mudrA* and *mudrB*, the former encoding a transposase responsible for the loss of DNA methylation and transposition of *Mu* TEs. TEs similar to the maize *Mu* are widespread in eukaryotes and they are referred to as *Mu*-like elements (MULEs). ORFs related to *mudrA* are generally found in autonomous copies of these MULEs.

While all MULEs in maize contain conserved ~220bp TIRs, some MULEs in *Arabidopsis* genomes lack any recognizable TIRs and are classified as non-TIR-MULEs. Phylogenic analyses indicate that non-TIR-MULEs in *Arabidopsis* genomes form large families and they are derived from TIR-MULEs and proliferated

recently. They generally possess several ORFs in addition to an ORF encoding *Mutator*-like transposase domain. In *Arabidopsis thaliana*, a group of non-TIR-MULEs called *VANDAL21* transposes in background of DNA methylation deficient mutants. An autonomous copy of *VANDAL21*/*AT2TE42810*, referring to as *Hiun* (*Hi*), encodes three ORFs; *VANA*, which is a

putative transposase, *VANB*, and *VANC*. Importantly, a transgene of *VANC* induced hypomethylation, transcriptional activation, and excision of endogenous *Hi*, indicating that *VANC* is a novel anti-silencing factor. Furthermore, full-length of *Hi* transgene induces the loss of DNA methylation specifically in *Hi* and other *VANDAL21* members. These observations indicate that *Hi* harbors sequence-specific anti-silencing system, which is in contrast to the viral counter-defense systems that globally interrupt host surveillance. Because TEs cannot be horizontally transferred, reduction of host fitness by disruption of the host surveillance system would be deleterious for survival of TEs. However, it is unknown how the sequence-specificity is established. It is particularly interesting that even with high target specificity of the anti-silencing system, non-CG methylation is reduced in the entire region of *VANDAL21* TEs, which are more than 8kb in length. Another important question is the evolutionary dynamics of the sequence-specific anti-silencing system.

In this thesis, I show effect of VANC in vivo and localization and biochemical characteristics of VANC. The results reveal molecular and evolutionary basis for the sequence-specific anti-silencing systems. Anti-silencing is also known in many viruses, but these anti-silencing in viruses is generally not sequence-specific and the general anti-silencing severely damages host fitness. Compared to viruses, horizontal transfer is rare in TEs, and the proliferation without host damage would be important for survival of TEs. My findings provide novel insights into evolutionary aspects of TEs for their survival in the host genome by adopting the sequence-specific anti-silencing.

(別紙様式 3)
(Separate Form 3)

博士論文の審査結果の要旨

Summary of the results of the doctoral thesis screening

トランスポゾン（TE）は動植物ゲノムの重要な構成要素のひとつである。しかし、その転移は遺伝子破壊を誘発するなど宿主にとって有害であり、通常TEの転移活性は宿主のエピジェネティックな機構により抑制されている。一方、宿主による抑制機構に対抗するTE側の機構が存在する。保坂さんは、シロイヌナズナの*VANDAL21*ファミリーに属するTEが持つ抗転移抑制機構について研究を行った。

*VANDAL21*ファミリーに属するTEのひとつ*Hiun*は、３つのタンパク質VANA、VANB、VANCをコードし、VANCが*Hiun*自身の抗転移抑制に重要であることが報告されている。学位論文申請者の保坂碧君は、形質転換植物を用いてVANC産物の効果を調べるとともに、クロマチン免疫沈降法を用いてVANCタンパク質の分布を調べた。その結果、このタンパク質とその局在機構が、速い進化を説明できることを見出した。

エピジェネティックな抑制に対抗する機構は、ウイルスで良く研究されているが、ウイルスによる抗抑制は一般に、配列特異性が低く、宿主の適応度を大きく低下させる。ウイルスと比べて、トランスポゾンは水平伝播が稀であり、宿主の適応度にダメージを与えないことがトランスポゾンの生き残りに重要である。保坂君の結果は、配列特異的で、かつ速い進化をする抗抑制系の分子機構を明らかにしたものであり、エピジェネティクス分野においても、進化生物学的にも、極めて新規性の高い重要な成果である。今後、本論文の研究を発展させることで、新規のエピジェネティック制御経路や、TEとのせめぎ合いによる宿主のゲノム進化などに関する新たな知見が得られることも期待できる。

上記を総合して、本論文は博士論文として十分な水準に達していると審査員全員が判断した。