氏　　名	島谷　善平
学位（専攻分野）	博士（学術）
学位記番号	総研大 1293 号
学位授与の日付	平成２１年９月３０日
学位授与の要件	生命科学研究科　基礎生物学専攻 学位規則第６条第１項該当
学位論文題目	Characterization of autonomous *Dart1* transposons belonging to the *hAT* superfamily in rice
論文審査委員	主　査　教授　長谷部　光泰 教授　川口　正代司 教授　鳥山　欽哉（東北大学） 教授　西村　幹夫

論文内容の要旨

As a major provision for more than half of the world's population, rice (*Oryza sativa* L.) is one of the most important plant species and has great economic importance. Also, rice has been focused on as an excellent model plant for cereal genomics studies due to its following features; 1) the smallest genome size (389 Mb) among the cereal grasses, 2) syntenic relation with other agronomically important *Poaceae* species such as maize, barley and wheat, 3) available varied resources including a large number of genetic markers, genomic libraries, many mutant lines and retrotransposon tagged lines, 4) there exists well developed technologies for rice genome manipulation such as *Agrobacterium tumefaciens*-mediated gene transformation and gene targeting with homologous recombination. Consequently, fine quality map-based genome sequencing of rice was completed in 2005.

One of the most challenging goals for the plant-research community going forward is to identify the function and regulation of rice genes. Thus, both forward and reverse genetic approaches have been developed to elucidate these functions. However, because the tissue culture process is a necessary step in most of the currently available procedures used in rice genome research, somaclonal variations, which refer to genetic and epigenetic mutations induced by tissue culture, can hamper these approaches. Therefore, a lot of attention has been given to recently identified endogenous DNA transposons that are active under natural growth conditions, a character that is quite useful to development of more efficient rice transposon tagging as a functional genomics tool free from somaclonal variation.

One such DNA transposon, *nDart1-0* (*non-autonomous DNA-based active rice transposon one-zero*) in the *hAT* superfamily, had been identified as a causative element of spontaneous leaf variegation shown in the mutant line pyl-v (pale-yellow-leaf variegated). This mutable phenotype is caused by the disruption and restoration of the nuclear-coded essential chloroplast protease gene, *OsClpP5*, due to the insertion and subsequent excision of *nDart1-0*. As a typical

non-autonomous transposon in the *hAT* superfamily, *nDart1-0* can transpose only when the *trans*-acting transposase is supplied from an autonomous element, *aDart* (*active autonomous Dart*). On the other hand, an indicator line, pyl-stb (pyl-stable) shows uniform pale-yellow leaves with no *nDart1-0* excision due to a lack of an *aDart*. The result of test crosses between pyl-v and pyl-stb lines indicated that the pyl-v line carries an *aDart* element in its genome.

In the published genomic sequence of the cultivar Nipponbare, there are 38 candidate autonomous *Dart* elements that have putative transposase genes with no apparent nonsense or frameshift mutations. However, from the result of test crosses with the pyl-stb line, it was shown that Nipponbare carries no *aDart* elements in its genome. Meanwhile, the excision of some endogenous *nDart1* elements in Nipponbare and pyl-stb was induced by treatment with a DNA methylation inhibitor, 5-azacytidine. Hence, these lines were predicted to carry epigenetically silenced autonomous elements, *iDart*s (*inactive autonomous Dart*s).

The first aims of this study were identifying the *aDart* element in the pyl-v line and demonstrating its molecular criteria as an autonomous element. To this end, he performed map-based cloning and revealed that the *aDart* element in the pyl-v line coincides with one of the 38 candidate autonomous elements, *iDart1-27*, residing on chromosome 6 in Nipponbare. Also, he has found that all of the examined transcripts of the *Dart* transposase gene were derived from *Dart1-27* in the pyl-v line. These results strongly suggested that *Dart1-27* in pyl-v acts on *nDart1-0* as an active *aDart* element. Then, he demonstrated that *iDart1*-27 cloned from the Nipponbare genome can be converted to an active *aDart* element in *Arabidopsis thaliana* plants when its methylation status was eliminated during the cloning process; *Dart1-27* excised *nDart1-0* as well as itself from the introduced vectors and integrated into various sites of the *A. thaliana* genome. These results clearly indicated that *Dart1-27* is a functional autonomous element, and it is active as an *aDart* element in the pyl-v line whereas epigenetically silenced as *iDart1-27* in Nipponbare. Furthermore, he showed other *Dart* elements, *Dart1-1*, *Dart1-20*,

Dart1-28 and *Dart1-52* are also functional autonomous elements, but they are epigenetically silenced as *iDart*s in Nipponbare.

Next, in order to study if there are any regulatory mechanisms that control the activity of the *Dart/nDart* system in the pyl-stb line, he introduced *Dart1-27* derivatives into the pyl-stb line and evaluated their activity. As a prerequisite for this transgenic approach, he carefully confirmed that during each step of the *A. tumefaciens*-mediated transformation process the endogenous *iDart* elements in the pyl-stb genome are almost never activated (0.1%). Based on this confirmation, he introduced *Dart1-27* derivatives into pyl-stb and demonstrated that they can mobilize *nDart1-0* elements from the *OsClpP5* gene as well as from an introduced *GUSPlus* gene at a high frequency in transgenic pyl-stb plants. This result reconfirmed that *Dart1-27* is a functional autonomous element able to act on *nDart* elements when its methylation status is eliminated, as shown in *A. thaliana* plants. From the results of phenotypic analysis of transgenic pyl-stb plants, it was suggested that there is a development-dependent regulation of *Dart* activity in regenerated pyl-stb plants; most of the transgenic pyl-stb plants introduced with *Dart1-27* derivatives were the pyl-stb phenotype at their 4-6 leaves stage, but almost all of them became the pyl-v phenotype at their 7-10 leaves stage.

In this manuscript, he has unambiguously demonstrated that the active autonomous element in the pyl-v line is *Dart1-27* on chromosome 6 and that the rice genome contains multiple potential autonomous *Dart* elements silenced epigenetically. From analysis of transgenic pyl-stb plants, he has also indicated a development-dependent regulation that could be a key to further elucidating *Dart/nDart* regulation mechanisms in the rice genome. He believes these results will facilitate an effective gene tagging system using the *Dart/nDart* elements in rice.

博士論文の審査結果の要旨

イネは食料として有用であり、穀物の中で最も早くゲノム解読が完了した。この状況の元、突然変異体をスクリーニングし、有用形質を制御する遺伝子を同定し、将来の優良品種作製に結びつけていくことが求められている。従来、イネの突然変異体作製には細胞培養を必要とし、培養中の体細胞突然変異が問題であった。この点を解決するためには、内在性のDNAトランスポゾンを用いた突然変異体作出が効果的である。

*nDart1*はhAT型非自立性DNAトランスポゾンであり、斑入り葉を形成するイネpyl-v突然変異体系統の原因遺伝子として同定されていた。pyl-v系統において*nDart1*は葉緑体形成に必須な*OsClpP5*遺伝子座位に挿入しており、葉緑体形成阻害を引き起こし、葉緑体不全細胞を形成する。pyl-vは活性型トランスポゼース*aDart*をゲノム中に持ち、*aDart*の働きで、細胞によってpyl-vが*OsClpP5*遺伝子座から転移し、*OsClpP5*遺伝子が活性化され野生型葉緑体を持つ細胞を形成する。その結果、斑入り葉が形成されることとなる。

本論文では、マップベースクローニングによってpyl-vの*aDart*遺伝子を同定し、葉で発現していることを確認した。さらに、*aDart*遺伝子はゲノム解読の終了している*Nipponbare*系統の*iDart1-27*遺伝子とオルソロガスであることがわかった。*iDart1-27*遺伝子を活性型トランスポゼースを持たないpyl-v系統に導入したところ、*nDart1*の転移が確認できた。この際に、発生段階に応じて、転移頻度が異なることが示唆され、今後、発生過程とトランスポゾン転移との関連についての研究が期待できる。これらのことから、今後、イネにおける突然変異体の作出において本トランスポゾンを利用することによって、責任遺伝子同定が効率的に進むことが期待できる。

さらに、本DNAトランスポゾンタギング系を真正双子葉植物のモデルであるシロイヌナズナにおける応用を試みた。*nDart1*と*aDart*をともにシロイヌナズナに導入し、*nDart1*がシロイヌナズナゲノム内の異なった場所に転移することを確認した。このことから、本系はシロイヌナズナにおいても有用であることが期待できる。

以上より本研究は、*aDart*遺伝子を同定し、その作用機作を解明することによって、発生過程とトランスポゾン転移との関係を示唆した点、イネおよびシロイヌナズナにおける効率的な責任遺伝子同定系を確立した点において、植物科学に対する重要な貢献であると高く評価できる。従って、審査委員会は本論文が学位論文として十分な内容を持つものと判定した。