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学位論文題目 Molecular characterization of genes essential for early
development of germ cells in rice

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論文内容の要旨

In flowering plants, after the transition from vegetative growth to reproductive development, primordial germ cell initials differentiate from somatic cells in flowers. Several genes that control the early stages of germ-cell development have been identified, however, little is known about the molecular mechanism determining the germ cell fate during sexual reproduction in plants. The *MEL1* (*MEIOSIS ARRESTED AT LEPTOTENE1*) is the only plant ARGONAUTE (AGO) gene specifically expressing at the primordial germ cells. AGO proteins are known to be a key player in gene-silencing pathways guided by small RNAs. PIWI-domain containing subfamily has important roles in maintaining germ stem cells in animal. In plant, however, there is no AGO gene which belonging to PIWI subfamily. Therefore, a specific AGO gene system in plant germ cell development would be suggested.

In this study, I focused on the characterization gene expressed at early stages of germ cells development specifically in relation to the MEL1 function. MEL1 is well-characterized ARGONAUTE family that has essential functions in germ cell development. To date the rice *MEL1* is the only gene available for investigating a genetic system conducting plant germ cell initiation and maintenance. A large portion of the genes identified specific in very early stages in germ cell development had unknown function. However, comparison of gene expression profiles between wild-type and *mell-1* mutant down- or up-regulated in *mell-1* mutant revealed many possible causal genes for *mell-1* dysfunction, i.e. most of which are not characterized previously. Of these genes, the *OsSPL14*, one of *SQUAMOSA* (*SQUA*) promoter-binding-like (*SPL*) family genes, encoding a putative transcription factor, revealed to up-regulated in the *mell-1* mutant in both Affymetrix (Fold change=4.37) and Agilent (=3.01) microarray. Sequence and experimental analyses indicated that eleven *OsSPL* genes including *OsSPL14* were putative targets of *OsmiR156*, suggesting a possibility that the *OsSPL14* should be directly targeted by the rice MEL1 AGO with *miR156* microRNA as a guide molecule. Moreover, the volume of *miR156* RNA detected in the co-IP fraction with the anti-MEL1 antibody 3.2-fold more than in that with the pre-immune serum. The detection level of *miR156* depended on that of the MEL1 protein, as the *miR156* co-IP with the MEL1 immune complex protein, but few or not with precipitates recovered using *mell-1* mutant or preimmune serum. These data strongly suggest that the MEL1 forms a complex with the *miR156* microRNA *in vivo*. These results strongly suggested that the *miR156* microRNA was one of good candidates of guide RNA molecules of the MEL1 AGO.

Analysis performed in each section and results obtained from the analysis are summarized as follows.

In the Section 1, the whole transcriptome profiles of plant reproductive process, including early stages that are difficult to be dissected in *Arabidopsis*, was obtained by using the Affymetrix rice genome array analysis and provided as a dataset of rice reproductive expression atlas. In addition,

using the atlas data, the gene expression patterns of several genes that are highly expressed in early development stage were investigated. A large part of genes that are expressed in early reproductive development remains uncharacterized because most of pre-meiotic stage specific genes were not categorized to any functions by GO analysis. However, the expression patterns of other meiosis-related genes were well corresponded to previous reports. The specific genes that were found in this study may have the crucial function at early germ cells development stages.

In the section 2, to confirm the spatial and temporal expression of the *MEL1* gene, mRNA *in situ* hybridization was performed on the anther sections with the *MSPI* gene as a cellular marker of young anther tissues. Expression of the *MEL1* was earlier than that of the *MSPI*, which clearly indicates that the *MEL1* mRNA expression start at the archesporial initials. The *MEL1* expression is not required for the germ-cell initiation, but for the maintenance of germ cells. In the Section 2, *in situ* hybridization of the *OsNOZZLE* (*OsNZZ*), a putative rice ortholog of *Arabidopsis* *SPOLOCYTELESS/NOZZLE* was also performed. Different from the *MEL1* and the *MSPI*, the *OsNZZ* mRNA was expressed both in developing anther wall layers and sporogenous cells similar to that of *Arabidopsis* *SPL/NZZ*, but it was not detected in archesporial cells. Although their function is not the same as *Arabidopsis* *SPL/NZZ*, *OsNZZ* may have function in early germ cell development.

In the Section 3, the microarray at 1-cm young panicle, several genes responsive for environmental stresses and/or hormone-responsive genes were up-regulated. It suggests that absence of the *MEL1* may cause stressful condition, thus many stress-response genes and ethylene signaling-related gene are induced. The *MEL1* may inhibits stress responses in germ cells to accomplish precise germ cell division and meiosis. In addition, some transposable element-like transcripts were also up-regulated in *mell-1* mutant. It was demonstrated that *Drosophila piwi* mutations impact retrotransposon mobility. *MEL1* may also suppress the activity of transposable elements during rice germ-cell development. On the other hand, many genes related to cell structure and cell cycle were down-regulated in the *mell-1* mutant. These results may indicated that in *mell-1* mutant, the failure of pre-meiotic mitosis of sporogenous cells in the *mell-1* mutant anther (Nonomura et al. 2007) was caused by down-regulation of these genes. At pre-meiotic S/G2 stage of young anthers, few genes were affected by the *mell-1* mutation. Most of up- and down-regulation of genes in the later stages of pre-meiotic germ-cell development might be secondary effects of *mell-1* mutation.

Finally, the Section 4 suggested a possibility that the rice *MEL1* AGO directly regulated the *OsSPL14* gene with the plant specific microRNA, *miR156* as described above.

Results obtained in this thesis will provide new and useful information to understand the gene functions in initiation of germ cells development and maintenance of germ cell identity in rice.

論文の審査結果の要旨

植物では葉をつける栄養成長から、外的要因の変化に応じて生殖成長へと移行し、同一生殖器官内のすべての生殖細胞が受精に向けて同調的な発生を行う。この過程における分子的なメカニズムは、*Arabidopsis* で幾つか遺伝子が見つかったものの、まだ不明のままである。上田さんはゲノム情報が豊富なイネを用いて、初期生殖過程で発現する遺伝子群の網羅的な解析を進め、Argonote ファミリーの MEL1 が始原生殖細胞の維持と発達に重要な役割をもつことを示すとともに、その制御の一端を解析した。

第1章では、イネの生殖過程で、archesporial cell の形成から減数分裂の終了までの初期過程を、葯の長さを指標に5段階に分けて、花組織における詳細なマイクロアレイ解析を行った。その結果、段階特異的な発現を示す遺伝子や減数分裂に至る過程で発現が変化する遺伝子群を同定した。これはイネの初期生殖過程で発現する遺伝子を詳細に記載したデータセットとして貴重である。

第2章では、1章で見つけられた MEL1 と MSP1 に加えて、OsNZZ (*Arabidopsis* SPL/NZZ のホモログ、変異体では archesporial cell の分裂異常となる) の葯における発現パターンを *in situ* ハイブリダイゼーションにより解析し、MEL1 の発現が archesporial cell に限局することを認めた。これは植物の生殖細胞で Argonote が発現していることを組織学的に明確に示した最初の知見である。

第3章では、上田さんの所属する植物遺伝研究室で単離されていた *mell-1* 突然変異体を用いてマイクロアレイ解析を進めた。その結果、変異体ではストレス関連遺伝子とホルモン応答に関わる遺伝子の発現が上昇し、細胞周期や細胞壁、花粉形成に関わる遺伝子の発現が下がっていることがわかった。これらは *mell-1* 変異体が不稔になることとよく一致している。さらに、*mell-1* 変異体で発現が上昇している遺伝子として *OsSPL14* をみつけた。この遺伝子は相補的な配列の miR156 により制御を受ける可能性が報告されていたが、どの Argonote により制御されるかは不明であった。

そこで、第4章では MEL1 が *OsSPL14* を制御する可能性を検討し、*mell-1* 変異体において *OsSPL14* と miR156 の発現量が上昇することをリアルタイムPCR法により確認した。さらに、抗 MEL1 抗体を用いた免疫沈降実験を行い、まだ例数は少ないものの miR156 が MEL1 に結合することも認めた。これらの結果は MEL1 が miR156 をガイド分子として *OsSPL14* を制御する可能性を示すものであり、今後の詳細な解析を行ううえでの先導的な成果と判断される。

最近の研究から、動物では生殖細胞の発達に小さな RNA 分子 piRNA が必要であることが明らかになっている。上田さんの研究は植物の生殖細胞でも同様の制御機構がある可能性を示し、詳細なマイクロアレイ解析結果を含めて、この分野の発展に貢献する成果である。以上の理由から、上田弥生さんの博士論文は博士号授与の要件を満たすと判断した。