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論文の内容の要旨

Arabidopsis is most useful model plants in molecular biology. RNA editing is a post-transcriptional modification of genes that commonly occur in plant plastids and mitochondria. Alternative splicing is a post and co-transcriptional regulation of gene expression. Pentatricopeptide repeat (PPR) family proteins were recently found to be involved in RNA editing in plants. The aim of this study was to investigate the tissue-specific expression and alternative splicing of *PPR* family genes and their effects on PPR motif and functionality. Of the 27 *PPR* genes in *Arabidopsis thaliana*, I selected six *PPR* genes of the P subfamily that are likely alternatively spliced, which were confirmed by sequencing. Four of these genes show intron retention, and the two remaining genes have 3' alternative-splicing sites. Alternative-splicing events occurred in the coding regions of three genes and in the 3' UTRs of the three remaining genes. I also identified five previously unannotated alternatively spliced isoforms of these *PPR* genes, which were confirmed by PCR and sequencing. Among these, three contain 3' alternative-splicing sites, one contains a 5' alternative-splicing site, and the remaining gene contains a 3'-5' alternative-splicing site. The new isoforms of two genes affect protein, and three other alternative-splicing sites are located in 3' UTRs. These findings suggest that tissue-specific expression of different alternatively spliced transcripts occurs in Arabidopsis, even at different developmental stages.

Recently it has been revealed that, not only PPR family proteins but also other additional family proteins MORF/RIP, ORRM and OZ are involved in RNA editing. The aim of this study is to find out the tissue-specific expression and alternative splicing of *ZnF* family genes and their effect on protein and functionality. Out of 25 *ZnF* genes, I randomly selected seven which are probably alternatively spliced and most of the genes are located in protein coding region which is determined using Arabidopsis database. Among these, alternative splicing in 7 genes of *ZnF* family was confirmed by sequencing. Out of which five genes with intron retention, one gene with 3' alternative splice site and another one genes

exon skipping were detected. Alternative splicing events were located in six genes in the coding region and one gene in 3' UTR region. Here I also reported three unannotated and new alternatively spliced isoforms from these *ZnF* genes that were confirmed by PCR and sequencing. Among these, one is with 3' alternative splice site and two with intron retention. New unannotated isoforms affecting protein in one gene and another one alternate splice located in 3' UTR region. This study suggests that tissue-specific expression of different alternatively spliced transcript happen even in different developmental stages.

RNA editing illustrated as any site-specific alteration in RNA sequences containing insertion or deletion and base substitution and has been broadly investigated in animals. In plant, RNA editing is a post-transcriptional modification of genes that commonly occur in plastids and mitochondria. In case of flowering plants, it is reported that not only PPR but also non-PPR proteins like MORF/RIP, ORRM and OZ partake in diverse RNA editing complex. Previously predicted 12 types RNA editing patterns may exist in the nuclear transcript, chloroplast and mitochondria in Arabidopsis. In the course of study of alternative splicing, tissue-specific RNA editing events were found in RNA editing related family genes. I collected samples of different tissues of different developmental stages from Arabidopsis. Such as seedling (whole plant) 4, 8, 12 days; 16, 21, 27 and 32 days old leaf, stem and root. Extraction of total RNA, cDNA synthesis and PCR were performed. After PCR, the targeted band was cut from PAGE then sequencing was performed. I found 9 types of RNA editing events these are C-to-U, U-to-C, A-to-I(G), A-to-C, A-to-U, G-to-A, G-to-C, U-to-A and U-to-G in targeted genes. Most of the editing events in seedling and leaf and less in stem tissues. Extensive editing U-to-C (60%) was detected in seedling 12 days, A-to-I(G) (54%) in leaf 21 days. This is the first experimental report that RNA editing could be regulated in tissue and development specific manner. During plant development, RNA editing machinery may play important role in proteins diversity and functionality thus ultimately affecting plant physiology.
Keywords: RNA editing, Alternative splicing, PPR, Zinc-finger motif, RNA editing events.

論文審査の結果の要旨

高等生物にはRNA editingと呼ばれる転写後RNAの遺伝コード変換システムが存在する。哺乳動物ではA⇒I（G）およびC⇒Uのみが観られるが、植物ではU⇒C、G⇒Cなど様々な塩基置換が知られており、組織や成長段階特異的に様々な調節を受けている。本論文では植物におけるRNA editingの機序を明らかにする端緒として、シロイヌナズナにおけるRNA editingに関与するPPRタンパク質mRNAおよびZnFタンパク質mRNAの発現がどの様に変化するかを詳細に研究している。Qulsum氏はシロイヌナズナの組織および時系列の異なる19の試料におけるこれら遺伝子群の選択的splicingを解析し、新たにPPRタンパク質で5種、ZnFタンパク質で3種の新しいisoformを発見した。これらはsplicingの違いによってそれぞれ異なる機能を持つと考えられた。例えばPPR5 mRNAの選択的splicingでは、播種後16日の根でintron7が保持されたisoformの発現が顕著であった。選択的splicingによってRNAの塩基認識部位であるPPR部位が1つ欠失したPPR5bとなり、異なる標的RNAを認識することが示

唆された。また、ZnF3 mRNA でも intron2 が保持された ZnFb が様々な組織で発現していた。Intron 保持によって 28 アミノ酸が付加されるが、その配列は Zinc Finger タンパク質で高度に保存されていることから機能的に重要であることが示唆された。

また、これら isoform の塩基配列解析の結果、RNA editing 関連タンパク質遺伝子が RNA editing を受けることも明らかにした。これら遺伝子の選択的 splicing 部位のみという限定的な解析にも関わらず、C⇒U、U⇒C、A⇒I（G）、A⇒C、A⇒U、G⇒A、G⇒C、U⇒A、U⇒G の 9 種類の RNA editing が観察された。これら RNA editing のほとんどは幼弱な苗と葉で観られ、茎組織では観られなかった。U⇒C の RNA editing は播種後 12 日の組織に顕著（60%）であっただけでなく、他の組織、成長段階では観察されなかったことから、当該成長段階で特異的に U⇒C 変換を触媒する機構が存在することが明らかとなり、U⇒C 変換機構の解明とその利用の可能性を拓いた。一方、A⇒I（G）は 21 日の葉で顕著（54%）であった。これらの結果は、RNA editing が組織および成長段階によって調節され得るという最初の実験的報告である。植物の生育において、RNA editing 機構はタンパク質の多様性と機能の調節において重要な役割を果たすことが示唆された。

以上、本論文は、シロイヌナズナにおける RNA editing 関連遺伝子群の発現について解析したものであり、学術的に貢献するところが大きい。よって博士（マテリアルサイエンス）の学位論文として十分価値あるものと認めた。