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Title	Arabidopsis thalianaにおけるRNA編集関連ファミリータンパク質の組織特異的選択的スプライシングの研究
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Abstract

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.