ARTICLE ADDENDUM

Organelle RNA recognition motif-containing (ORRM) proteins are plastid and mitochondrial editing factors in Arabidopsis

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ABSTRACT

Post-transcriptional C-to-U RNA editing occurs at specific sites in plastid and plant mitochondrial transcripts. Members of the Arabidopsis pentatricopeptide repeat (PPR) motif-containing protein family and RNA-editing factor interacting Protein (RIP, also known as MORF) family have been characterized as essential components of the RNA editing apparatus. Recent studies reveal that several organelle-targeted RNA recognition motif (RRM)-containing proteins are involved in either plastid or mitochondrial RNA editing. ORRM1 (Organelle RRM protein 1) is essential for plastid editing, whereas ORRM2, ORRM3 and ORRM4 are involved in mitochondrial RNA editing. The RRM domain of ORRM1, ORRM3 and ORRM4 is required for editing activity, whereas the auxiliary RIP and Glycine-Rich (GR) domains mediate the ORRM proteins’ interactions with other editing factors. The identification of the ORRM proteins as RNA editing factors further expands our knowledge of the composition of the editosome.

Characterization of four ORRM proteins as RNA editing factors

We have recently discovered an additional group of trans-factors required for editing of particular Cs in chloroplasts and mitochondria. Due to the presence of an RNA recognition motif (RRM), we have named these trans-factors as Organelle RRM (ORRM) proteins. The founder of this family, ORRM1 (Organelle RNA recognition motif-containing protein 1), is a plastid editing factor that was discovered through a database search with the protein sequence of the major plastid and mitochondrial editing factor RIP1.13,19 Twenty-one plastid sites exhibit a reduction of editing in the orrm1 homozygous mutant relative to the wild-type (Table 1).19 Subsequently, mitochondrial RNA editing factors ORRM2, ORRM3 and ORRM4 were identified based on their sequence similarity to ORRM1 in the RRM domain (Fig. 1).20,21 Transient silencing of ORRM2 and ORRM3 resulted in reduced editing efficiency at over 30 of the mitochondrial sites (Table 1).21 ORRM4 was characterized as a major mitochondrial editing factor because ORRM4 mutation causes editing defects at 270 mitochondrial C targets (Table 1).20 The alteration of ORRM1, ORRM2 and ORRM3 expression did not cause any morphological defects in the corresponding mutant or silenced tissue, whereas a slow growth

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and late flowering phenotype was observed in the orrm4 mutant (Table 1). The impact of ORRM4 on plant growth and flowering indicates that adequate mitochondrial editing is necessary for normal plant development.

**Interaction between ORRM proteins and other editing factors**

RNA editing is performed by editosomes found between the 200 and 400 kD markers on size exclusion columns. Protein-protein interaction assays have provided us with some information about the composition of RNA editosomes. ORRM1 interacts with the PPR proteins that are required for editing of the same C target as ORRM1 (Fig. 2A). For instance, both ORRM1 and OTP82 are required for the editing at ndhG C50 and ndhB C836, and as expected, ORRM1 interacts with OTP82. ORRM1 is dispensable for the editing at the sites controlled by RARE1, thus no interaction was observed (Fig. 2A). In addition to PPR proteins, ORRM1 interacts with RIP1, plastid editing factors RIP2 and OZ1 as shown in Fig. 2A. Unlike ORRM1, mitochondrial editing factors ORRM2, ORRM3 and ORRM4 do not interact with the tested PPR proteins in yeast two-hybrid assays. ORRM3 interacts with ORRM4, and both of them interact with RIP1 and with themselves (Fig. 2B). ORRM2 interacts with ORRM3, but does not interact with ORRM4, RIP1 or itself (Fig. 2B).

| Gene ID   | Location | Name of T-DNA mutant or VIGS line | Growth phenotype                                           | Number of editing sites affected |
|-----------|----------|----------------------------------|------------------------------------------------------------|----------------------------------|
| ORRM1     | At3g20930| P                                | SALK_072648                                                | No phenotypic defect             | 21 of 34                        |
| ORRM2     | At5g54580| M                                | ORRM2-VIGS                                                 | No phenotypic defect             | 35 of 618                       |
| ORRM3     | At5g61030| M                                | ORRM3-VIGS                                                 | No phenotypic defect             | 32 of 618                       |
| ORRM4     | At1g74230| M                                | ORRM4-VIGS                                                 | No phenotypic defect             | 110 of 576                      |
| CP31A     | At4g24770| P                                | SALK_109613, SAIL_258_N02                                   | No phenotypic defect             | 51 of 564                       |
| CP31B     | At5g50250| P                                | WiscDsLocX8389                                             | Slow growth and late flowering   | 270 of 618                      |
| CP29A     | At3g53460| P                                | SALK_003066                                                | Pale leaves under cold stress    | 13 of 34                        |
|           |          |                                  |                                                             | conditions                      |                                  |

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| ORRM3     | At5g61030| M                                | ORRM3-VIGS                                                 | No phenotypic defect             | 32 of 618                       |
| ORRM4     | At1g74230| M                                | ORRM4-VIGS                                                 | No phenotypic defect             | 110 of 576                      |
| CP31A     | At4g24770| P                                | SALK_109613, SAIL_258_N02                                   | No phenotypic defect             | 51 of 564                       |
| CP31B     | At5g50250| P                                | WiscDsLocX8389                                             | Slow growth and late flowering   | 270 of 618                      |
| CP29A     | At3g53460| P                                | SALK_003066                                                | Pale leaves under cold stress    | 13 of 34                        |
|           |          |                                  |                                                             | conditions                      |                                  |

**Functional analysis of the RRM domain and the GR domain in ORRM proteins**

The RRM domain is a conserved ~80 amino-acid domain that binds RNA molecules with a variety of specificities and affinities. Each of the four ORRM proteins contains one RRM domain either at its N terminus or C terminus (Fig. 3). Complementation tests revealed that the RRM domain of ORRM1, ORRM3 and ORRM4 is necessary for the editing ability of the
protein.\textsuperscript{19-21} thus the RRM domain plays a key role in both plastid and mitochondrial RNA editing. In addition to the RRM domain, ORRM1 carries a truncated RIP-RIP domain at its N terminus, while ORRM3 and ORRM4 contain a C-terminal Glycine-Rich (GR) domain (Fig. 3). The RIP domain or the GR domain is dispensable for the editing activity of the protein. However, the N-terminal RIP domain is required for ORRM1 to interact with PPR proteins (Fig. 2A), and the GR domain of ORRM4 is responsible for mediating its interaction with ORRM3 and with itself.\textsuperscript{19,20}

**Functional redundancy between the mitochondrial ORRM proteins**

In our study, the editing extent was reduced but not eliminated by the knockdown or knockout of ORRM2, ORRM3 and ORRM4 in mutants or silenced tissue,\textsuperscript{20,21} likely due to the redundant function of the ORRM proteins. We have shown that the editing efficiency of sites under the control of both ORRM2 and ORRM3 was further impaired by transiently silencing ORRM2 in the \textit{orr3} mutant background.\textsuperscript{21} Thus, the redundancy hypothesis is plausible for ORRM2 and ORRM3. Additionally, overexpression of ORRM3 or the RRM domain of ORRM3 improves the editing defects in the \textit{orr4} mutant,\textsuperscript{20} suggesting that ORRM3 is able to compensate (at least partially) for the function of ORRM4 in the editosome.

**Other plant RRM-containing proteins**

The RRM domain is one of the most abundant protein domains in eukaryotes, and is involved in multiple biological functions, such as pre-mRNA processing, splicing, mRNA stability, translation regulation, and RNA editing.\textsuperscript{25} Other RRM-containing proteins have been previously analyzed in terms of their function in RNA editing. CP31A, CP31B and CP29A belong to a small family of chloroplast ribonucleoproteins (cpRNPs) that are characterized by a twin RRM domain.\textsuperscript{26,27} The tobacco protein CP31 was reported to be a plastid editing factor in an \textit{in vitro} RNA editing system.\textsuperscript{28} However, its close relatives in \textit{Arabidopsis}, CP31A, CP31B and CP29A, only have a minor impact on editing (Table 1).\textsuperscript{29,30} Compared to drastic alterations of RNA accumulation that occurred, \textit{cp31a} and \textit{cp29a} mutants showed minor editing defects under cold stress conditions.\textsuperscript{29}

The effect of CP31B on editing is mostly dependent on \textit{CP31A} mutation.\textsuperscript{30} Although the RRM domains of the ORRM proteins show high sequence similarity with the one in these cpRNPs, they are in separate phylogenetic groups (Fig. 1), have a distinct domain arrangement (Fig. 3), and different expression pattern under stress conditions. For instance, cold stress does not affect \textit{ORRM4} expression but it induces the accumulation of \textit{CP29A} and \textit{CP31A}.\textsuperscript{29,31} These facts may point to different functions of ORRM proteins and the RRM-containing cpRNPs.

The identification of the ORRM proteins as RNA editing factors further expands our knowledge of the composition of the editosome. There are now three major protein families known to be required for plant organelle RNA editing: the PPR protein family, the RIP/MORF family and the ORRM family. Further experiments investigating the interaction between mRNA and the ORRM proteins, analyzing the redundancy between the ORRM proteins, and identifying other components of the RNA editosome should give us more insights into the RNA editing apparatus.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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