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RNA editing machinery in plant organelles

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RNA editing is a type of post-transcriptional modification that includes nucleotide insertion/deletion or conversion. Different categories of RNA editing have been widely observed in distinct RNAs from divergent organisms. In flowering plants, RNA editing usually alters cytidine to uridine in plastids and mitochondria, playing important roles in various plant developmental processes, including organelle biogenesis, adaptation to environmental changes, and signal transduction. Numerous studies have demonstrated that a number of factors are involved in plant RNA editing, such as pentatricopeptide repeat (PPR) proteins, multiple organelle RNA editing factors (MORF, also known as RIP), organelle RNA recognition motif (ORRM) containing proteins, protoporphyrinogen IX oxidase 1 (PPO1) and organelle zinc finger 1 (OZ1). These factors play diverse roles in plant RNA editing due to their distinct characteristics. In this review, we discuss the functional roles of the individual editing factors and their associations in plant RNA editing.

RNA editing, editosome, plant organelles

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INTRODUCTION

RNA editing is a post-transcriptional process, where the nucleotides in a transcript differ from their encoding DNA sequences by nucleotide insertion/deletion or conversion (Takenaka et al., 2013). RNA editing phenomena have been observed in various types of RNAs from distinct organisms (Göringer, 2012; Nishikura, 2010; Takenaka et al., 2013). Nucleotide insertion/deletion, which is commonly exemplified by uridine insertion/deletion, is observed only within the mitochondrial transcripts of the kinetoplastid protozoa. The reactions involved are carried out by unique machinery, termed the editosome, which is a high-molecular-mass complex containing multiple protein factors (Göringer, 2012). Nucleotide conversion mainly occurs in two forms: A-to-I and C-to-U. These processes are catalyzed by several types of deaminase. A-to-I RNA editing is exclusively performed by adenosine deaminase acting on RNA (ADAR) enzymes, which always harbor one to three N-terminal double-stranded RNA binding domain(s) that associate with dsRNA and a C-terminal catalytic domain that deaminates the target adenine (Nishikura, 2010). C-to-U editing is best characterized in humans, and is catalyzed by the deaminase APOBEC1, which combines with APOBEC1 complementation factor (ACF) to form the editosome (Mehta and Driscoll, 2002). APOBEC1 cannot function alone, as it requires ACF, which itself contains three N-terminal RNA recognition motifs (RRMs). ACF recognizes sequences downstream of the edited cytidine and presents the target site to APOBEC1 for deamination (Maris et al., 2005).

In plants, C-to-U is the main type of RNA editing, which was first described in plant mitochondria in 1989 (Covello

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and Gray, 1989; Gualberto et al., 1989; Hiesel et al., 1989). Two years later, a similar phenomenon was observed in plant chloroplast (Hoch et al., 1991). Numerous C-to-U modifications have since been reported in these two organelles (Cai et al., 2009; Chateigner-Boutin et al., 2008; Hammani et al., 2011; Kim et al., 2009; Kotera et al., 2005; Sung et al., 2010; Zhou et al., 2009). To date, the modification of over 600 Cs in mitochondria and 45 Cs in plastids has been identified (Bentolila et al., 2013). C-to-U editing can introduce an initiation codon (ACG-to-AUG), or stop codon (CAA-to-UAA), or alter the encoded amino acid (Takenaka et al., 2013). This modification is thought to act as a corrective mechanism for DNA mutations at the RNA level. Abnormal RNA editing can result in a series of plant developmental defects, such as impaired chloroplast and mitochondrial biogenesis (Sosso et al., 2012; Zhou et al., 2009), retarded seedling growth (Lin et al., 2015), reduced embryo and endosperm development (Liu et al., 2013; Sosso et al., 2012), and hypersensitivity to various abiotic stresses (Zhu et al., 2014). These findings suggest that RNA editing is essential for normal plant growth and development. C-to-U deamination is thought to be orchestrated by the RNA editosome, but the nature of the deaminase involved remains unknown (Figure 1A).

A number of RNA editing factors have been identified thus far. In 2005, the first RNA editing factor, CRR4, a member of the PLS-type pentatricopeptide repeat (PPR) family protein, was discovered (Kotera et al., 2005). Subsequently, a number of other PLS-type PPR proteins were found to be involved in plant RNA editing. In 2012, a second RNA editing factor, multiple organellar RNA editing factor (MORF, also known

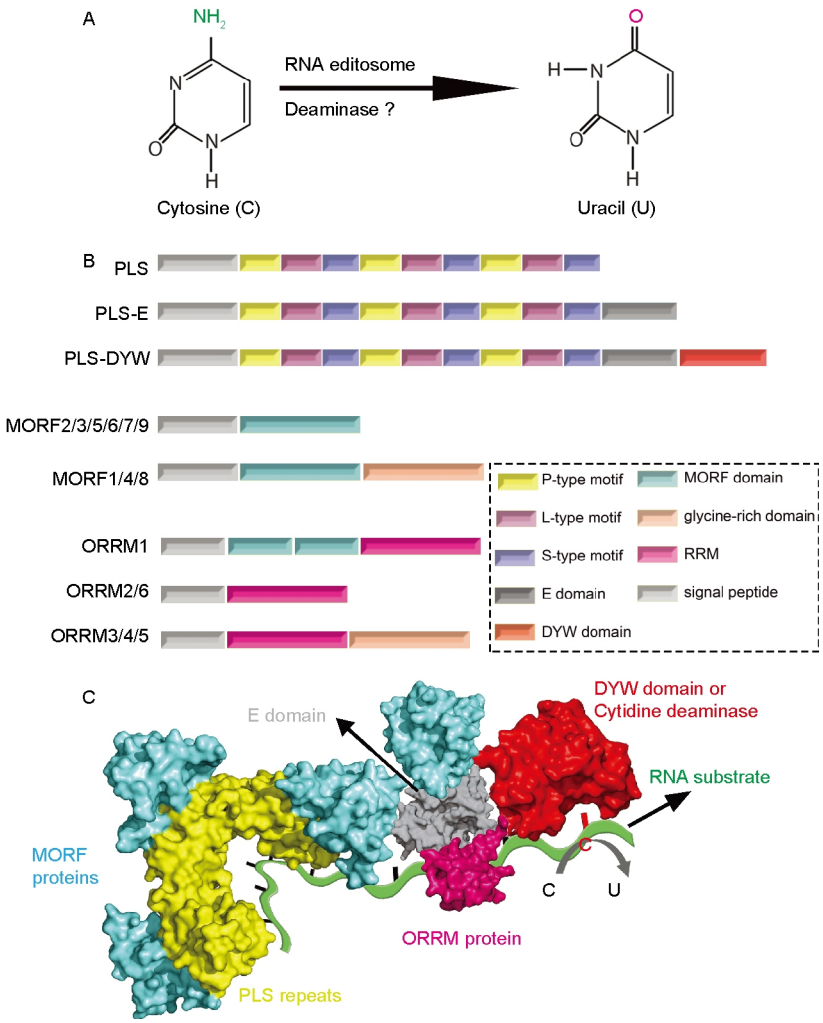

Figure 1 Components of RNA editing machinery in plants. A, A simplified schematic diagram of C-to-U RNA editing in plant organelles. The reaction is catalyzed by a multiple-component complex, termed RNA editosome, but the nature of the deaminase is not yet determined. B, Major RNA editing factors in plant organelles. Different kinds of editing factors are classified. The motif composition and arrangement of individual members are depicted by distinct colored boxes. All these RNA editing factors are encoded by the nuclear genome and transported into plastids or/and mitochondria. C, A putative RNA editosome performing C-to-U RNA editing on a specific RNA substrate. A DYW-containing PLS-type PPR protein recognizes the 5′ *cis* element upstream of the edited cytosine. The RNA-binding affinity of PPR repeats is further enhanced upon MORF binding. The E domain can also interact with MORF. ORRM members possess RNA-binding ability. We speculate that both the E domain and ORRM members may associate with the RNA base around the targeted C to contribute to the correct positioning of the edited C. The C-to-U deamination might be catalyzed by the DYW domain or an unidentified cytidine deaminase.

as RNA editing factor interacting protein (RIP)), was reported by two independent groups (Bentolila et al., 2012; Takenaka et al., 2012). In the past few years, several studies have demonstrated other types of editing factors, including organelle RNA recognition motif-containing (ORRM) proteins (Hackett et al., 2017; Shi et al., 2017; Shi et al., 2016; Shi et al., 2015; Sun et al., 2013), protoporphyrinogen IX oxidase 1 (PPO1) (Zhang et al., 2014), and organelle zinc finger 1 (OZ1) (Sun et al., 2015). All these factors in addition to potential unidentified editing factors constitute the editosome. Here, we review the functions ascribed to individual editing factors thus far and their functional assembly in plant RNA editing.

PPR PROTEINS: SITE-SPECIFIC FACTORS FOR TARGET RNA RECOGNITION

PPR proteins are protein family that is exclusively expanded in plants, with over 450 members in *Arabidopsis* and rice (Lurin et al., 2004). Virtually all the investigated PPR proteins are located in either plastids or mitochondria. It is generally acknowledged that PPR proteins function as RNA-binding proteins that specially bind to the *cis* element of the target RNA. PPR proteins have been shown to play various roles in organelle gene expression, including transcription, RNA splicing, RNA editing, RNA cleavage, RNA stabilization and translation (Barkan and Small, 2014). *PPR* mutants display various developmental defects (Jiang et al., 2015; Pyo et al., 2013; Sosso et al., 2012; Zhou et al., 2009).

PPR proteins contain a series of tandem array repeats. Based on the variation of such repeats, PPR proteins can be further divided into P and PLS subfamilies (Lurin et al., 2004). The P subfamily contains only the canonical P-type motif of 35 amino acids (aa), whereas the PLS subfamily also contain longer (L-type, 35–36 aa) or shorter (S-type, 31 aa) variant PPR motifs. The PPR proteins involved in RNA editing belong to the PLS subfamily, which is specific to terrestrial plants. On the basis of the C-terminal composition, the PLS-type PPR proteins can be further separated into smaller subclasses, including the E (extended) and DYW subtypes (Figure 1B) (Lurin et al., 2004).

A number of structural studies have illustrated the right-handed superhelical assembly of the P-type PPR proteins (Coquille et al., 2014; Gully et al., 2015; Ke et al., 2013; Shen et al., 2016; Yin et al., 2013). A recent study by our group revealed a similar overall architecture for the PLS-type PPR protein (Yan et al., 2017). Each P-, L- or S-type motif contains a pair of antiparallel α-helices. Nevertheless, structural alignment revealed distinguishing properties of L- and S-type motifs compared with P-type motifs. In the L-type motif, the second α-helix is shifted outward from the first; whereas in S-type motif, the second α-helix is reduced by one helical turn. Previously reported crystal structures of PPR-RNA complexes suggest that the 5th and 35th residues in each repeat play crucial roles in RNA base coordination through hydrogen bonding (Shen et al., 2016; Yin et al., 2013). Compared with the amino acid distance of 5th and 35th residues in the P-type motif, the distance is strikingly greater for the L-type motif (between the 5th and 35th residue), but comparable to that for the S-type motif (between the 5th and 31st residue) (Yan et al., 2017). These facts suggest that the S-type motif can efficiently recognize RNA bases, while the L-type motif might fail to form hydrogen bonds with bases unless it undergoes profound conformational changes. Thus, for PPR tracts of a similar length, it is reasonable for PLS-type PPR protein to display weak RNA-binding ability compared with their P-type PPR counterparts. Surprisingly, we have discovered that the L-type motif can undergo significant conformational changes in the presence of the MORF protein (another editing factor, discussed in the following section), which greatly facilitates RNA base coordination. This evidence not only provides a novel explanation for the molecular function of the L-type motif in PLS-type PPR proteins but also suggests that the RNA-binding affinity of PLS-type PPR proteins can be regulated by other factors.

In *Arabidopsis*, there are no more than 200 PLS-type PPR proteins involved in more than 600 C-to-U editing events in mitochondria and plastids, suggesting that one PLS-type PPR protein may target several RNA transcripts. For example, CLB19 contributes to the editing of rpoA and clpP transcripts (Chateigner-Boutin et al., 2008); OTP87 is essential for the editing of the nad7 and atp1 transcripts (Hammani et al., 2011); and CRR22 is involved in the editing events that take place in ndhB7, ndhD5 and rpoB3 (Okuda and Shikanai, 2012). The target elements of these PPR proteins show only partial similarity with their *cis* elements upstream of the edited Cs. However, the molecular mechanism underlying how a single PLS-type PPR protein target divergent RNA transcripts remains to be clarified. It is probable that many factors, such as the number of PPR repeats, motif types and organization modes, determines the RNA-binding ability of a PLS-type PPR protein. Further determination of the structures of PLS/PPR-RNA or MORF-PLS/PPR-RNA complexes will provide direct evidence of the base coordination and contribution of each motif.

In *Arabidopsis*, nearly half of the PLS-type PPR proteins contain DYW domains at the C-terminus (Lurin et al., 2004). The presence of a highly conserved signature HxE(x)nCxxC in most DYW domains resembling the zinc-binding active site motif of known cytosine deaminase, prompted speculation that the DYW domain contributes to an as-yet-unidentified cytidine deaminase activity (Boussardon et al., 2014). However, functional studies have suggested that the DYW domain is dispensable for a number of PPR proteins, such as CRR22 and CRR28 (Okuda et al., 2009), whereas it is

required for others, such as QED1 and RARE1 (Wagoner et al., 2015). Moreover, DYW1 consisting of only a DYW domain, without any PPR repeats, functions in RNA editing in conjunction with CRR4, which lacks the DYW domain (Boussardon et al., 2012). MEF8 and MEF8S harbor fewer than five PPR repeats, which is not sufficient for efficient targeted RNA-binding. However, MEF8 and MEF8S do contain a DYW domain, which is required for RNA editing (Verbitskiy et al., 2012). These findings suggest that the DYW domains have evolved divergently. However, whether the DYW domain has deaminase activity should be experimentally determined *in vitro*. PPR proteins carrying a DYW motif always exhibit preceding E motifs, which follow the PLS triplets (Lurin et al., 2004). Mutation of the E domain can abolish editing events, suggesting the essential role of this domain in plant RNA editing (Härtel et al., 2013). Previous studies have illustrated that PPR repeats recognize an RNA sequences four nucleotides upstream of the edited cytosine (Okuda et al., 2014). Considering its relevant position following the PLS triplets but upstream of the possible catalytic DYW domain, it is probable that the E domain is involved in contact with the RNA bases adjacent to and upstream of the edited cytosine. Further structural studies of PLS-E/PLS-DYW-RNA complexes will not only shed light on the function of the E domain but also reveal whether the DYW domain is a bona-fide deaminase that shares similar architectural folds with well-known cytosine deaminase.

MORF PROTEINS—ADAPTORS MEDIATING THE ASSEMBLY OF RNA EDITING COMPLEX

In 2012, two independent groups identified a second family of RNA editing factor, the MORF (also named RIP) proteins (Bentolila et al., 2012; Takenaka et al., 2012). MORF is a small protein family with 10 members in *Arabidopsis* (Table 1). MORF2, MORF9 and MORF10 are located in plastids, while MORF1 and MORF3–7 are located in mitochondria, and MORF8 is dual-targeted. Unlike the defects in PLS-type PPR proteins, which impair only one or a few editing sites, *morf* mutants usually show reduced editing efficiency at multiple sites (Takenaka et al., 2012). Disruption of *morf1*, *morf3* and *morf8* affects 19%, 26% and 72% of mitochondrial editing events, respectively, whereas mutants of either *morf2* or *morf9* exhibit reduced editing at nearly all sites in plastids (Bentolila et al., 2012; Takenaka et al., 2012). The distinct RNA editing deficiencies in these *morf* mutants suggest divergent roles of the individual MORF members.

All MORF members comprise a central conserved MORF domain that displays a globular architecture, consisting of a core of six anti-parallel β-sheets, flanked by three α-helices on one side and several loops on the other side (Haag et al., 2017; Yan et al., 2017). Both *in vivo* and *in vitro* studies have

Table 1 Distribution of non-PPR RNA editing factors in *Arabidopsis*

	Locus	aa	Organelle	Reference
MORF members				
MORF1	AT4G20020	419	Mitochondrion	(Takenaka et al., 2012; Bentolila et al., 2012)
MORF2	AT2G33430	219	Plastid	(Takenaka et al., 2012; Bentolila et al., 2012)
MORF3	AT3G06790	244	Mitochondrion	(Takenaka et al., 2012; Bentolila et al., 2012)
MORF4	AT5G44780	723	Mitochondrion	(Takenaka et al., 2012; Bentolila et al., 2012)
MORF5	AT1G32580	229	Mitochondrion	(Takenaka et al., 2012; Bentolila et al., 2012)
MORF6	AT2G35240	232	Mitochondrion	(Takenaka et al., 2012; Bentolila et al., 2012)
MORF7	AT1G72530	192	Mitochondrion	(Takenaka et al., 2012; Bentolila et al., 2012)
MORF8	AT3G15000	395	Plastid and mitochondrion	(Takenaka et al., 2012; Bentolila et al., 2012)
MORF9	AT1G11430	232	Plastid	(Takenaka et al., 2012; Bentolila et al., 2012)
MORF10	AT1G53260	271	Plastid	(Takenaka et al., 2012; Bentolila et al., 2012)
ORRM members				
ORRM1	AT3G20930	374	Plastid	(Sun et al., 2013)
ORRM2	AT5G54580	156	Mitochondrion	(Shi et al., 2015)
ORRM3	AT5G61030	309	Mitochondrion	(Shi et al., 2015)
ORRM4	AT1G74230	289	Mitochondrion	(Shi et al., 2016)
ORRM5	AT4G13850	158	Mitochondrion	(Shi et al., 2017)
ORRM6	AT1G73530	181	Plastid	(Hackett et al., 2017)
Other factors				
PPO1	AT4G01690	537	Plastid	(Zhang et al., 2014)
OZ1	AT5G17790	758	Plastid	(Sun et al., 2015)

shown that MORF members can form homodimers or heterodimers (Takenaka et al., 2012; Zehrmann et al., 2015). The C-terminal region of the central conserved MORF domain is required for the MORF-MORF connection (Zehrmann et al., 2015). The MORF domain in solution occurs as a monomer, but the crystal structure of MORF1 and MORF9 demonstrated a dimeric architecture (Haag et al., 2017). Hydrophobic interactions play major roles in homodimer formation (Haag et al., 2017). In addition to the MORF domain, a C-terminal glycine-rich region is also observed in some MORF members, such as MORF1/4/8 (Figure 1B). Previous research indicated that the glycine-rich domains exhibit RNA-binding activity or mediate protein-protein interactions (Ciuzan et al., 2015), but their exact roles within the MORF members remain largely unknown.

A series of studies have shown that MORF proteins could associate with PLS-type PPR proteins (Glass et al., 2015; Takenaka et al., 2012), but the detailed interaction is poorly understood. Recently, our group reconstitute the direct interactions using purified proteins via size exclusion chromatography analysis *in vitro* (Yan et al., 2017), and we further determined the crystal structure of MORF9 in complex with a PLS-type PPR protein. That study revealed that MORF9 interacts with the PLS triplets close to its concave surface (Yan et al., 2017). This interaction mainly involves D164 of MORF9 and R29 of the L-type motif from PLS triplet. In addition, extensive hydrophobic contacts are observed from residues W160 and L162 of MORF9, with relevant residues of the L- and S-type motif (Yan et al., 2017).

Structural alignment revealed that MORF9 could induce conformational changes of PLS-type PPR proteins and increases their RNA-binding activity (Yan et al., 2017). It is considered likely that the irregular organization of PLS motif mediates the lower RNA-binding activity of PLS-type PPR protein than that of their P-type counterparts (Barkan and Small, 2014). However, the high RNA-binding affinity of PLS-type PPR proteins is obviously favorable for the editing process. As many editing sites are located in coding regions, adequate dissociation of PLS-type PPR proteins from the edited transcript would facilitate subsequent translation. We speculate that the MORF proteins may be used as a molecular switch in the process of plant RNA editing. In the presence of MORF, the PLS-type PPR protein exhibits enhanced RNA-binding activity, which facilitates the editing reaction. After the reaction is complete, MORF dissociates from the editing complex, thus diminishing the binding affinity between the PLS-type PPR protein and the target RNA, so that the subsequent translation processes are not affected. A recent study demonstrated that the E domain, in addition to the PLS triplet, can interact with MORF (Bayer-Császár et al., 2017). However, the functional relevance of this association and whether the E domain undergoes conformational changes in the presence of MORF, similar to the PLS triplets, remain unclear. In conclusion, MORF proteins might play a delicate fine-tuning role in plant organelle RNA editing.

ORRM PROTEINS—A NEW TYPE OF RNA-BINDING PROTEIN IN PLANT RNA EDITING

Recently, a new protein family, referred to as organelle RNA recognition motif (ORRM) family, was reported to be required for plant organelle RNA editing (Sun et al., 2013). To date, six ORRM members have been described (Table 1), among which ORRM1 and ORRM6 are located in plastids (Hackett et al., 2017; Sun et al., 2013), while ORRM2/3/4/5 are targeted to mitochondria (Shi et al., 2017; Shi et al., 2016; Shi et al., 2015). *ORRM* mutants exhibit decreased RNA editing efficiencies but to variable degrees. Two plastid editing sites are impaired in *orrm6* mutants (Hackett et al., 2017), whereas over 30 mitochondrial sites display decreased RNA editing efficiency in transiently *orrm2*- and *orrm3*-silenced plants (Shi et al., 2015). ORRM1 and ORRM4 are major editing factors in plastids and mitochondria, respectively, and mutants of *orrm1* and *orrm4* show editing defects at dozens of editing sites (Shi et al., 2016; Sun et al., 2013). Moreover, disruption of *ORRM4*, *ORRM5* and *ORRM6* severely affects normal plant growth (Hackett et al., 2017; Shi et al., 2017; Shi et al., 2016), indicating the essential role of RNA editing in normal plant development.

ORRM members harbor an RNA recognition motif (RRM) that is responsible for their RNA-binding ability (Figure 1B) (Hackett et al., 2017; Sun et al., 2013). A well-known RRM-containing protein implicated in RNA editing is mammalian APOBEC1 complementation factor (ACF), which combines with APOBEC1 to form the minimal editosome (Mehta and Driscoll, 2002). ACF attaches the mooring sequence of the target transcript and presents the edited site to the deaminase APOBEC1 for deamination (Maris et al., 2005). However, the exact function of ORRM family members in plant RNA editing is poorly understood.

In addition to the RRM, some ORRM members contain additional domains (Figure 1B), indicating unique roles for individual ORRM members. ORRM1 harbors two truncated MORF domain at its N-terminus, which facilitate its interaction with some PLS-type PPR proteins (Sun et al., 2013). ORRM3, ORRM4 and ORRM5 have an additional C-terminal glycine-rich (GR) domain. Similar to MORF family proteins, ORRM members can form homodimers or heterodimers (Shi et al., 2016; Shi et al., 2015). The C-terminal GR domain of ORRM4 is responsible for mediating the interaction with ORRM3 and with itself (Shi et al., 2016). In addition, ORRM proteins can associate with other editing factors, and both ORRM3 and ORRM4 can interact with RIP1

(MORF8) (Shi et al., 2016; Shi et al., 2015). These findings reveal the multi-component assembly of the plant RNA editing machinery.

ADDITIONAL RNA EDITING FACTORS

In addition to the well-characterized core editing factors, other proteins, such as PPO1 and OZ1, have also been reported to be involved in RNA editing (Table 1). PPO1, which is an enzyme that catalyzes the transformation of protoporphyrinogen IX into protoporphyrin IX in the tetrapyrrole biosynthetic pathway, has been found to play important roles in plastid RNA editing (Zhang et al., 2014). Disruption of *PPO1* causes RNA editing defects in 18 of 34 plastid RNA targets, most of which encode subunits of the NADH dehydrogenase-like complex (NDH), further resulting in impairment of NDH complex accumulation and chlorophyll synthesis. In contrast to the PPR proteins, which interact with the central conserved MORF domain of MORF members, the results of Y2H experiments suggest that PPO1 interacts with the N-terminal fragments of MORF2 or MORF9 without the involvement of known domains (Zhang et al., 2014). The interaction with MORFs is required for the RNA editing function of PPO1. However, the actual role of PPO1 in plastid RNA editing requires further investigation.

OZ1 is a member of the RanBP2-type zinc finger protein family and has been characterized as an essential editing factor based on co-immunoprecipitation with ORRM1, followed by mass spectrometry (Sun et al., 2015). The *OZ1* mutant exhibits a yellow phenotype and show impairment of RNA editing at 14 sites and a decreased editing efficiency at another 16 sites in plastids (Sun et al., 2015), indicating its essential role in normal plant development and plastid RNA editing. Similar to MORF and ORRM members, OZ1 can form homodimers. In addition, Y2H analysis indicate that OZ1 can interact with other editing factors, such as ORRM1 and the PLS-type PPR proteins (Sun et al., 2015). These findings suggest involvement of OZ1 in plastid RNA editing complex assembly. However, the actual function of OZ1 in plant RNA editing requires further clarification.

DIVERSITY, COMPLEXITY AND DYNAMICS OF THE PLANT RNA EDITOSOME

The identification of various kinds of proteins as RNA editing factors expands our knowledge of the composition of the editing complex. It is probable that other potential editing factors, or even a deaminase, await identification in the future. All these editing factors are likely to constitute a functional editosome. A striking feature of the plant RNA editosome is its diversity and complexity in composition without consistency between individual RNA targets. Many lines of evidence support this view. First, different RNA substrates are generally recognized by distinct PLS-type PPR proteins. Second, the great number of PLS-type PPR proteins selectively interact with the small family of MORF proteins. It has been illustrated that MORF proteins can directly interact with the PLS triplets or the E domain of the PLS-type PPR proteins (Bayer-Császár et al., 2017; Yan et al., 2017). We can imagine that a single PLS-type PPR protein may simultaneously bind different MORFs via its distinct PLS triplets or the E domains. Third, the MORF members selectively interact with their homologs, and other editing factors such as ORRM proteins. Thus, all these RNA editing factors selectively cooperate with one another to form a complex network that catalyzes C-to-U deamination for individual target RNAs (Figure 1C).

RNA editing is a dynamic and precisely regulated process. Evidence has shown that non-PPR editing factors interact with one another and with PLS-type PPR proteins, to positively regulate both target RNA binding and the editing reaction. However, the functional editosome is expected to be disassembled once editing is complete, and the interaction network among these editing factors therefore also plays a negatively regulatory role in plant RNA editing. Further determination of the functional roles of each editing factor in editosome assembly and disassembly will elucidate this finely tuned process.

PERSPECTIVES

RNA editing has received particular attention because it challenges the central dogma of molecular biology by changing genetic information at the transcriptional level. Nearly thirty years have passed since its discovery, but the nature of the enzyme that catalyzes the C-to-U conversion in plant organelles is still an open question. Due to its conserved signature HxE(x)nCxxC similar to the zinc-binding active site in well-known cytosine deaminase, the DYW domain is always suggested to perform the deaminase activity. However, no direct evidence supports this hypothesis thus far. Moreover, amino acid alignment reveals little overall sequence conservation between the DYW domain and the cytidine deaminase. To address this issue, on one hand, crystal structure determination of the DYW domain will provide evidence whether it shares similar structural fold as the well-known cytosine deaminase; on the other hand, *in vitro* biochemical and high-throughput sequencing system should be set up to examine its C-to-U deamination activity. In addition, we cannot rule out the possibility that potential cytidine deaminase remains to be identified. Thus, great efforts should be made to characterize the other unknown editing factors in plant organelles.

To date, a number of editing factors have been identified as essential components of the plant RNA editing machinery, and these factors are considered to interact with one another to

form a large protein complex, termed RNA editosome. However, the actual function of individual factors and their associations in the process of RNA editing remain largely unknown. In recent years, tandem affinity purification (TAP) in combination with electron microscopy analyses has provided a picture of the editosome of trypanosomatid mitochondria (Golas et al., 2009; Li et al., 2009). With the recent rapid development of cryo-EM technology, we anticipate a fantastic surge in the structure determination of plant RNA editosome. And the complex structure will provide concrete evidence of the associations among the various editing factors and pave the way for further elucidation of the functions of individual members as well as their regulatory relationships. In the past few years, CRISPR-Cas9 has been successfully used for genome editing via RNA-guided target recognition and cleavage (Komor et al., 2017). Recently, a P-type PPR protein, SOT1, containing a C-terminal small MutS-related (SMR) domain, was reported to exhibit endonuclease activity. Additionally, code engineered SOT1 can efficiently recognize and cleave the predicted RNA substrates (Zhou et al., 2017), indicating its potential application for plant organelle RNA manipulation. It is conceivable that further determination of the composition and functional core of the plant RNA editosome will reveal broad prospects for its application. Organelle site-directed RNA editing not only facilitates the functional investigation of the genes involved in organelle biogenesis and plant development but also provides a valuable opportunity to generate cytoplasmic male sterile (CMS) lines to improve hybrid seed production.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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