The unexpected diversity of plant organelle RNA editosomes

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Keywords

chloroplasts, mitochondria, protein complex, pentatricopeptide, RNA recognition motif, zinc finger motif

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19 **Flowering plants convert many hundreds of organelle Cs to Us during post-transcriptional**
20 **RNA editing. Pentatricopeptide Repeat (PPR) proteins dictate specificity by recognizing**
21 **RNA sequences near C targets. However, the complete mechanism of the editing machinery**
22 **is not yet understood. Recently, non-PPR editing factors (RIP/MORFs, ORRM, OZ and**
23 **PPO1) have been identified as components of the plant RNA editosome, which is a small**
24 **RNA/protein complex. Surprisingly, plant editosomes are highly diverse with regard not**
25 **only to the PPR proteins they contain, but also in the non-PPR components that are**
26 **present. Here we review the most recent progress in the field and discuss the implications**
27 **of the diversity of plant editosomes for evolution of RNA editing and for possible future**
28 **applications.**

30 **Plant RNA editing- a correction mechanism with high specificity**

31 Once believed to be merely an apprentice of DNA, RNA is now known to undergo **RNA editing**,
32 a process that differentiates the RNA sequence from its DNA master [1-3]. In vascular plants,
33 RNA editing converts many hundred cytidines (C) to uridines (U) at specific positions in plastid
34 and mitochondrial transcripts [4-7]. Editing often restores conserved codons or creates start or
35 stop codons, serving as a correction mechanism for otherwise defective organellar transcripts [5].
36 Despite some examples of unimportant editing events that are either silent or in non-coding
37 regions, many RNA editing events have been shown to be essential for proper function of
38 proteins and optimal growth of plants [8-14].

39 Cytidine can be converted to uridine by a simple deamination reaction. In animals and
40 humans, two deaminase-mediated RNA editing mechanisms, the C-to-U editing by **APOBEC**
41 and A-to-I editing by **ADAR**, have been identified [15-24]. While APOBEC- and ADAR-
42 mediated editing has been shown to create multiple proteins from differentially edited
43 transcripts, no evidence supports the same function in plants [11, 25]. Although unedited or
44 partially edited transcripts can load onto plant mitochondrial ribosomes, there are no examples of
45 useful protein diversity produced by translation of incompletely edited transcripts. In two cases
46 that have been investigated carefully, the plant proteins that accumulate are specified primarily
47 by edited transcripts, likely due to instability of aberrant proteins resulting from translation of
48 improperly edited transcripts [26-28]. Instead of creating protein polymorphism, plant RNA

editing is believed to be a correction mechanism to cope with detrimental T-to-C mutations inherited from the **organelle** genome.

Editing events are being gained and lost over evolutionary time. A C in one species is sometimes not edited in another because a T is already genomically encoded [5, 9, 14]. While RNA editing would not be needed if all detrimental T-to-C mutations were changed back to Ts, once many have accumulated, converting all of them becomes less likely, resulting in retention of the RNA editing apparatus [29, 30]. Nevertheless, overall, plant RNA editing events are gradually becoming lost; in general, lower plants and gymnosperms exhibit a larger number of target sites than angiosperms [6, 31, 32]

Another distinct property of the plant RNA editing activity is the high specificity governed by RNA-binding Pentatricopeptide Repeat (**PPR**) proteins [31, 33]. Human ADAR editing can be rather promiscuous across many thousands of adenosine targets residing in double-stranded RNAs [34]. While the human APOBEC system contains an RNA sequence recognition factor, only a few targets can be specified [11, 17, 19]. In contrast, the plant editing apparatus (the **editosome**) can specifically target hundreds of individual sites. Unlike the APOBEC system, plant RNA editosomes are able to incorporate different RNA-binding proteins that recognize a variety of RNA sequences near the C targets of editing. Vascular plants such as arabidopsis (*Arabidopsis thaliana*) encode hundreds of PPR proteins, which provide an unparalleled capacity for specific editing [31, 35].

Modular RNA/protein complexes that carry targeting specificity and enzymatic activity hold great potential for bioengineering. One of the most successful examples is the CRISPR/Cas9 system originally found in bacteria [36-38]. Initial efforts have been made to use mammalian ADAR combined with guiding RNA to correct mutations in cell lines [39]. Given its novel properties, the plant RNA editing system potentially offers an alternative mechanism for engineering purposes. The discovery of a recognition code for specifying the RNA sequences recognized by PPR motifs have been followed by some recent examples of reprogramming PPR proteins to target desired RNA sequences [33, 40-42]. All of the *trans*-factors needed for RNA editing in chloroplasts are nuclear-encoded [43, 44]. However, unlike the CRISPR/Cas9 system, to date the composition of the plant editosome is not fully understood; further application will be obstructed without more information about plant editosomes.

Starting from the discovery of **RIP/MORF** editing factors, great progress has been made in identifying the other protein components of the editosome, now including the ORRM and **OZ** protein families. Unexpectedly, editosomes across different targets differ not only in their PPR recognition factors but also in other components. This review will focus on the recent progress in the plant editing field, with a focus on the non-PPR editing factors that point to an unexpected diversity of plant editosomes.

PPR proteins

Compared to merely 7 members in humans, the PPR-motif containing family has substantially increased in the plant kingdom, with over 400 members in a typical flowering plant [30, 35, 45, 46]. Each member carries multiple ~35 amino acid repeats (PPR motif) in tandem, which recognize specific **cis-elements** for editing in a combinatorial pattern (Figure 1A) [40, 47, 48]. The PPR proteins involved in editing belong to a subclass that has slightly longer or shorter PPR motifs and additional domains other than PPR motifs [14, 30, 46]. Two functional domains- the Extension (E) domain and the **DYW domain** have been identified in this subclass of PPR proteins (Figure 1A). In Arabidopsis, approximately 193 PPR members contain an E domain, while 87 contain both E and DYW domains [35]. The E domain has been shown to be essential for editing, especially the PG region [49-51].

Because the sequence of the DYW domain exhibits some similarity to cytidine deaminase domains found in other systems, it was proposed to be the catalytic factor for plant editing [52]. As well as their presence in plants, DYW domains have been discovered in protists [53]. However, while *in vitro* editing systems using chloroplast or mitochondrial extracts and synthetic RNA have been established [54-58], no deaminase activity has been detected through expression of recombinant DYW-type PPR editing factors [55-57, 59]. Divalent cations are evidently required for editing, since EDTA and EGTA severely inhibit the editing activity [55]. Notably, plant editing *in vitro* is sensitive to zinc chelators, indicating zinc ion is an essential co-factor, similar to other deaminase systems [55, 57].

An essential role of the DYW domain was challenged by the finding that DYW domains in certain PPR proteins are dispensable for editing [51, 60]. In one case, an Arabidopsis gene encoding a protein with a DYW domain, but no PPR motifs, was found to be necessary for editing of a C target recognized by a E-type PPR protein (Figure 1A), which lacks a DYW

domain [61]. This raises the possibility that the other C targets whose *cis*-elements are recognized by E-type PPR proteins are also utilizing DYW-type PPR proteins to provide the deaminase activity. However, examples of C targets that require more than one PPR protein are rare [62-64], even though a number of PPR-protein-encoding genes have been subjected to mutagenesis. A possible explanation is redundancy of the DYW-type PPR proteins that are able to provide the enzyme activity to an editosome carrying a site-specific E-type PPR protein recognition factor. In that case, even if one DYW-type PPR protein is not available due to mutation, others would be able to carry out the deamination task. Alternatively, there may be proteins, which are as yet unidentified, that supply catalytic activity to editosomes.

While deletion analysis has shown that the DYW domain is dispensable in some DYW-type PPR proteins, editing activity and zinc binding is lost when the DYW domain is missing or removed from certain PPR proteins [49]. Identification of PPR-DYW proteins in which the DYW domain was essential allowed three different groups to examine the importance of conserved residues in the DYW domains of those PPR proteins. Site-specific mutagenesis within the conserved motifs of DYW domains greatly reduced editing extent [49, 61, 65] and binding of zinc, a recognized feature of known deaminases [61, 66], which is required for editing *in vitro* [55, 58].

Until recently, all identified editing factors belonged to the PPR family [5, 14, 67]. Given that PPR proteins could possibly carry both RNA binding activity and the enzymatic activity, a model could be envisioned in which multimers of PPR proteins alone could constitute the editosome [52]. However, this hypothesis was completely overturned by the discovery of essential non-PPR protein components in the editosome—first the RIP/MORF family, then the ORRM family and later a member of the OZ family (Table 1) [4, 68-73].

RNA editing factor-interacting proteins lacking PPR motifs

When a chloroplast extract was subjected to size exclusion chromatography, the 70 kDa PPR protein RARE1 was found to be present in a small protein complex that separated between 200 and 443 kDa protein markers [68]. Immunoprecipitation of an epitope-tagged RARE1 resulted in the identification of one non-PPR protein that strongly interacted with RARE1—RNA editing factor Interacting Protein 1 (RIP1) [68]. In a *rip1* hypomorph Arabidopsis mutant, editing extent of over 400 editing Cs in mitochondria and 11 editing sites in chloroplasts was reduced, making

it the most influential editing factor in plants yet identified [4]. Other members of the RIP1 family were identified as Multiple Organellar RNA editing Factors (MORFs) through genetic screening for mitochondrial editing defects in a collection of Arabidopsis EMS mutants [73]. In contrast to genes encoding PPR proteins, mutagenesis of a single gene encoding a RIP/MORF protein affects dozens or even hundreds of editing sites. In order to analyze such large number of editing targets, high-throughput methods based on next generation sequencing and mass spectrometry are particularly valuable methods [4, 80]. Of the 10 members of the RIP/MORF family in Arabidopsis, 5 are major editing factors for mitochondria or/and plastids, while the remaining family members have minor or no effect on editing [4]. In plastids, RIP2/MORF2 and RIP9/MORF9 are major players, required for almost every editing site [4, 73]. In mitochondria, RIP1/MORF8 affects over 70% of all the editing events and RIP3/MORF3, RIP8/MORF1 affect 26% and 19% respectively (Table 1) [4, 68, 73].

While a motif of unknown function is conserved across RIP/MORF members, RIP/MORF proteins do not have any annotated domain, and some contain an extended C-terminus (Figure 1B)[4, 73]. RIP/MORF proteins interact with the PPR factors that address the same editing sites, via the connection to the PPR tract or other regions [68, 81]. Different RIP/MORF editing factors may affect different sets of editing events, while some editing events can be affected by two or more different RIP/MORFs [4]. This may be due to a requirement of a homomer of RIP/MORF for some sites but a heteromer for others. Such types of interaction were indeed observed among RIP/MORF members through protein-protein interaction studies (Table 2) [73, 81]. The function of this family is still unknown, though speculation exists that RIP/MORF proteins bridge the PPR factor and the deaminase activity in the editosome [81].

Like the PPR family, the RIP/MORF family is widespread among angiosperms. However, genes encoding RIP/MORF proteins are missing in some ferns and moss species, which also carry out organelle RNA editing [68, 73, 82]. Why angiosperm RNA editing requires these proteins, but other plants do not, is a mystery; less information is available about non-PPR editing factors in non-vascular plants.

Organelle RNA Recognition Motif proteins

Organelle RNA Recognition Motif protein1 (ORRM)1, the first ORRM editing factor, was found *in silico* as a protein related to the RIP family; it has two truncated RIP domains in

addition to an RRM domain [69]. ORRM1 controls >60% of plastid editing sites in Arabidopsis (Table1). Surprisingly, the ORRM1 RRM domain alone can restore editing to an *orrm1* mutant, according to a transient complementation assay [69]. A genetic screening of photosynthetic mutations in a maize insertional mutant collection identified the maize ortholog of *ORRM1*, which was confirmed as a plastid editing factor as well, implying a conserved editing function across species [69]. Although the RRM is a highly common motif in eukaryotes, the motif in ORRM1 belongs to a distinct clade of approximately 20 members in Arabidopsis, most of which are organelle-targeted. Except for ORRM1, none of the other members carries a RIP domain. Instead, many contain glycine-rich regions [69] (Figure 1C).

Four ORRM proteins have been reported to date to be RNA editing factors (Table 1) [69, 71, 72, 74]. ORRM1 is a major factor in plastids, while ORRM2, ORRM3 and ORRM4 are mitochondrial editing factors [74]. Similar to RIP/MORF factors, ORRM1 affects many editing events in a site-specific manner, though ORRM4 also broadly affects editing events in the same transcripts [69, 71, 72]. ORRMs can interact with RIP/MORF factors and form homo- or heterodimers [70-72]. ORRM1 associates with PPR editing factors that control the common editing events, while the interaction between ORRM2-4 and PPR factors might not be direct, but rather mediated via RIP/MORF or other components of the editosome (Table 2) [69, 71, 72]. The function of the RRM domain in editing is still an open question. ORRM1 can bind near some editing *cis*-elements [69]. Why a plant editosome would require a second RNA binding activity besides PPR factor is not clear. One speculation is that RRM binds in the vicinity of editing sites, preparing the RNA for entry of additional factors or enhancing the specificity of the PPR-protein mediated *cis*-element recognition [78]. Alternatively, instead of binding RNA, perhaps some RRM bind other protein components of the editosome.

Organelle Zinc finger proteins (OZ)

Analogous to the discovery of RIP1, OZ1 was found in an ORRM1 co-purified protein complex [70]. Loss of *OZ1* in Arabidopsis leads to editing defects at 30 plastid sites, which eventually results in a virescent phenotype (Table 1). Four members comprise the OZ family, all of which are organelle targeted. The only annotated domain of the family is the RanBP2 type zinc finger, which is repeated in various numbers in the OZ members (Figure 1D); however, there is another conserved domain of unknown function in the four OZ proteins.

OZ1 selectively interacts with the PPR specificity factors and also strongly associates with ORRM1, but does not appear to directly bind RIP/MORFs. In addition, OZ1 can dimerize (Table 2) [70]. The function of the zinc finger and other uncharacterized regions of the OZ proteins in the editosome awaits further investigation. Plant C-to-U editing has been shown to be zinc ion dependent, possibly due to the requirement of Zn^{2+} in the active center of cytidine deaminase [58, 83]. It is not yet known whether zinc fingers in OZ actually bind Zn^{2+} and whether such binding affects editing. The widespread OZ family can be detected in many plant lineages, including *Selaginella*, suggesting an evolutionally conserved function in the plant editosome [70].

Additional factors

Three additional proteins — CP31, **PPO1** and OCP3--have been found to affect RNA editing efficiency in plastids. Loss of *CP31* leads to reduced editing levels at multiple plastid sites and immunodepletion of the CP31 protein also inhibits *in vitro* editing of some substrates (Table 1) [78]. Given that CP31 is an RRM containing protein, whether CP31 and ORRM operate in a similar fashion is an intriguing question. However, CP31 does not belong to the ORRM clade, nor does it affect editing in a site-specific pattern that is seen in *orrm1* [69, 78]. Moreover, transcript abundance is greatly reduced in *cp31* mutant, which suggests CP31 may primarily be an RNA stability factor [78, 79].

Protoporphyrinogen Oxidase 1 (PPO1), a key enzyme for tetrapyrrole metabolism, was shown to have a surprising role in plastid RNA editing [76]. Reduced editing was observed for 18 plastid sites when *PPO1* is mutated, and such defects can be rescued by a truncated PPO1 protein that lacks oxidase enzymatic activity (Table 1). PPO1 directly interacts with the plastid RIP/MORF proteins but not with the PPR factors (Table 2). However, except for one site, none of the affected sites completely loses editing when PPO1 is absent [76]. Most of the affected sites are in the *ndh* gene transcripts

Overexpression of Cationic Peroxidase 3 (OCP3) affects editing of multiple sites in the plastid *ndhB* transcript (Table 1) [84]. Though *ocp3* mutant exhibit only mildly reduced editing extent, it seemed sufficient to impair NDH activity, which consequently enhanced the plant's resistance to fungal infection [84].

The diversity of plant RNA editosomes

RNA editing must be confined to particular Cs to prevent detrimental changes in transcripts. The specificity of recognition of 5' *cis*-elements is clearly explained by the polypeptide code for recognition of particular nucleotides by the repeat motifs [40, 47, 85-87]. Editing is often maintained when a few nucleotides are altered in the *cis*-elements, thus allowing a few different C targets carrying similar but not identical *cis*-elements to be recognized by a single PPR protein. This flexibility in recognition site makes sense evolutionarily; as new T to C mutations arise, the plant must evolve new PPR proteins for recognition, perhaps based on a protein that provides weak binding and partial editing. The flexibility also explains how <200 editing-type PPR proteins can specify >600 C targets in Arabidopsis.

Up to about 200 different editosomes could exist in Arabidopsis organelles, if all the other components are identical. An early hypothesis was that the plant editing system consisted of one or more PPR proteins that recruited a single editing activity to the proper location, analogous to the RNA-binding protein that binds 3' to *cis*-elements and determines where APOBEC1 performs C-to-U editing in mammalian systems [11]. Now that many non-PPR editing factors have been discovered, there are evidently far more than 200 types of editosomes in plant organelles.

To date, three families of proteins that lack PPR motifs have been identified as essential components of the plant editosome. Most of these factors affect many more editing targets than any individual PPR protein. Thus, most non-PPR factors are evidently present in a larger number of editosomes than a single PPR protein, reflected by their greater expression levels compared to PPR proteins. For example, 77% of the mitochondrial C targets require RIP1 in their editosomes, while 26% and 19% require RIP3 and RIP8, respectively [4, 73]. Why particular editing factors associate with specific PPR proteins is not understood, though yeast two-hybrid, bimolecular fluorescence complementation, and pull-down assays (Table 2) indicate that certain interactions occur and others do not. One way of maintaining selective interaction is segregation of editing factors into different organelles by targeting of import from the cytoplasm. Most factors enter only the plastid or the mitochondrial compartments, though exceptions exist, such as RIP1, which is found in both organelles [68].

Although loss of expression of *orrm1*, *oz1*, and *rip/morf* gene expression can sometimes cause complete absence of editing of certain sites, some family members are functionally

redundant. Removing one factor often results in a partially reduced editing efficiency of particular C targets, suggesting that other family members can fulfill editing functions in the absence of another member. Direct evidence for this hypothesis comes from a study on two mitochondrial factors ORRM2 and ORRM3, which share many cognate sites. While residual editing levels were observed in an *orrm3* mutant, silencing of *ORRM2* in the *orrm3* mutant further reduced the editing efficiency [72]. ORRM2 and ORRM3 form heterodimers in yeast two-hybrid assays, and ORRM3 can form a homodimer [72]. Possibly, an editosome carrying ORRM3 homodimer can substitute for an ORRM2-ORRM3 heterodimer when ORRM2 is missing, though the ensuing editosome is not as efficient or not sufficiently abundant.

A similar situation occurs in editing of plastid *ndhG* C50, in which the editosome is predicted to contain at least RIP9, ORRM1 and OZ1 and likely often RIP2 as well. No editing of *ndhG* C50 occurs when *RIP9* is absent, while 40% editing remains in the *rip2* null mutant [73]. Possibly the *ndhG* C50 editosomes are more efficient when they contain a heteromer of RIP2 and RIP9, but one without RIP2 still can operate (Figure 2). Alternatively, in the absence of RIP2, there may be inadequate amounts of RIP9 for efficient editing of *ndhG* C50. Some evidence that RIP/MORF heteromers may sometimes associate with PPR proteins comes from an experiment with mitochondrial factors. Expression of MORF8/RIP1 along with the PPR protein MEF13 increased interaction of MEF13 and MORF3/RIP3 in a yeast 3-hybrid system [88].

Concluding Remarks and Future Perspectives

Unlike its counterparts in mammals, the plant editosome varies at most editing sites, not only in the PPR site-specific recognition factor, but also in the non-PPR factors (Figure 3, Key Figure). For some sites whose editosome components from each of the four families have been identified, the combined size of all factors, postulating each as a monomer, is at the low end of the observed size of editosome in size exclusion chromatography (~200kDa). As some of the proteins may be present as multimers, possibly all of the proteins present in such editosomes have been identified, which thus awaits experimental confirmation through reconstitution of the editing complex *in vitro*. Alternatively, there might still be more unknown editing factors for some, or all sites (see also “Outstanding Questions”). A number of ORRM and OZ family members still remain uncharacterized. There are also “orphan sites” whose editing extents are not substantially affected in any *rip*, *orrm*, or *oz* mutants or in tissue silenced for a known editing

factor. These orphan sites could be ones that can be operated upon by many different editing factors, so that redundancy prevents expression of a mutant phenotype—or they could be sites for which some actual editing factors have not yet been identified.

Why are plant editosomes so diverse in composition? Consider the plight of a plant organelle whose DNA has undergone a T-to-C mutation. If the amino acid affected seriously impairs the function of a protein, there would be strong selection for organelles that can overcome the deficiency through RNA editing. Perhaps an existing PPR protein may exhibit weak binding to *cis*-element near a new T-to-C mutation. Other organelle proteins that exhibit chance binding to the PPR protein or the *cis*-element may be recruited to enhance the initial inefficient editing. Further selection could strengthen these interactions, thus enlisting a variety of organelle proteins in the mission to rescue the organelle from a detrimental mutation. The unexpected diversity of plant RNA editosomes may reflect haphazard placement of temporary bandages onto defective RNA encoded by damaged DNA.

While evolution created a complex and diverse set of editosomes in order to deal with organelle DNA mutations, modern genetic engineers are not confined by selective constraints. The PPR recognition code, along with a PPR protein structure [89], has provided insights into designing proteins able to bind particular sequences. With further understanding of the minimal requirements for the factors that must be requisitioned to supply PPR proteins with catalytic activity, we should be able to design simpler editing complexes to direct editing events to targets of our choice. With intelligent design, perhaps the unexpected complexity of plant RNA editosomes will be shown to be unnecessary.

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Figure Legends

Figure 1. Schematic representation of essential editing factors. (A) *Arabidopsis* has approximately 193 editing-type PPR proteins. PPR proteins contain tandem PPR motifs and additional C terminal domains—the E domain and DYW domain. E-type editing factors do not have the DYW domain. DYW1 has a truncated E domain and a DYW domain. (B) Five RIP proteins play a major role in editing. RIP proteins have a conserved motif at N termini while RIP1 has an extended C terminus with unknown function. (C) To date, four ORRMs have been shown to be editing factors. ORRM1 has two degenerate RIP motifs and one RRM motif while other ORRMs do not have RIP motifs. ORRM3 and ORRM4 have glycine rich regions. (D) There are four members in the OZ family and OZ1 is the only protein that has been studied in RNA editing. OZ1 has a region at its N terminus that is conserved with other family members as well as two RanBP2 type zinc fingers.

Figure 2. Possible editosomes acting on *ndhG* C50. The PPR protein OTP82 recognizes the *cis*-element (pink line) 5' to the target C (red letter). Additional factors from the RIP, ORRM and

OZ families are also required. Optimal editing efficiency (~80%) is observed when both RIP2 and RIP9 are present. When there is no expression of *RIP2*, 40% editing of *ndhG* C50 remains, thus RIP9 monomers or multimers may be able to partially substitute for RIP2. The actual composition and stoichiometry of the subunits in an editosome is unknown, nor is it known whether additional proteins are required.

Figure 3. Diversity of plant editosomes. The protein components of the editosome are nuclear-encoded, cytosol-translated and translocated into each organelle. The diversity among plant editosomes is depicted in, but not restricted to, the following examples. Shape variation indicates different families, while color and pattern variation indicates different members within a family. A question mark suggests possible involvement of a second PPR protein. 1) Members of non-PPR families (RIP, ORRM, and OZ) are partially redundant. The major factor (solid circles) is indispensable although another family member (dashed circles) may replace it with a reduced activity. Mutation in a dispensable factor (dashed) leads to a minor editing defect, while loss of a major factor (solid) causes major loss of editing. 2) Different members of non-PPR families are fully redundant. Any component can be dispensable (dashed circles) since another family member can be incorporated into the editosome. Loss of one factor will not cause severe editing defects. 3) No redundancy. Editosome requires multiple copies of non-PPR factors. Loss of any factor will severely affect editing efficiency. 4) The editosome contains unknown factors which play equivalent roles to known non-PPR factors. The presence of OZ proteins in mitochondrial editosomes is currently hypothetical. Printed with permission from a professional artist © Li Yao.

Glossary

ADAR: adenosine deaminase acting on RNA, an enzyme that binds to double-stranded RNA and converts adenosine (A) to inosine (I).

APOBEC-1: apolipoprotein B mRNA editing enzyme, catalytic. The enzyme that carries out *apoB* editing as a homodimer in mammalian systems, in complex with other proteins.

cis-element: The RNA sequence adjacent to the editing sites that can be recognized by the editing machinery. It has been shown that the 15-20 nucleotides 5' to the editing sites are the most critical *cis*-element and are specific targets of PPR proteins.

DYW domain: A highly conserved C-terminal domain in some PPR proteins. The domain generally terminates with triplet amino acids Asp-Tyr-Trp (D-Y-W). This domain contains a characteristic zinc binding motif (CXXC, HXE) found in cytidine deaminases that convert Cs to Us.

Organelle: a specialized subunit within a cell that has a specific function. In plants, two types of organelles—chloroplast and mitochondrion have their own genomes. Plant RNA editing occurs in these two organelles.

OZ: Organelle Zinc finger proteins, a small protein family that are involved in plant RNA editing. All members are targeted to organelles and share the conserved RanBP2 type zinc finger motifs.

Pentatricopeptide Repeat (PPR): a 35 amino-acid degenerate repeat present in a number of RNA-binding proteins.

PPO1: Protoporphyrinogen Oxidase 1, an enzyme required for tetrapyrrole metabolism

RNA editing: A form of RNA processing that makes discrete changes to specific nucleotide sequences within an RNA molecule. Editing events can include insertion, deletion, or base substitution of nucleotides. In higher plants Cytidine-to-Uridine editing occurs in chloroplast and mitochondrial RNAs.

RNA editing factor Interacting Protein (RIP)/Multiple Organellar RNA editing Factor (MORF): a small protein family based on sequence similarity. Several family members are essential RNA editing factors. Different nomenclatures reflect their initial reporting in two independent studies.

RNA editosome: The molecular machinery that conducts RNA editing. In plants, it is a small protein complex that operates on specific editing sites.

597 **Table 1. List of identified non-PPR editing factors**

Protein family	Protein	Alias	Mature protein size kDa ^a	Subcellular localization	% affected sites of all chloroplast sites	% affected sites of all mitochondrial sites	Refs.
RIP/MORF	RIP1	MORF8	37	Dual	22%	77%	[4, 68]
	RIP2	MORF2; DAL	19	Chloroplast	100%	NA	[4, 73]
	RIP3	MORF3	23	Mitochondrion	NA	26%	[4, 73]
	RIP8	MORF1	42	Mitochondrion	NA	19%	[4, 73]
	RIP9	MORF9; DAG	20	Chloroplast	97%	NA	[4, 73]
ORRM	ORRM1		36	Chloroplast	62%	NA	[69]
	ORRM2		15	Mitochondrion	NA	6% ^b	[72]
	ORRM3	GR-RBP3; At-mRBP2b	30	Mitochondrion	NA	19%	[72, 74]
	ORRM4	GR-RBP5; At-mRBP2a	29	Mitochondrion	NA	44%	[71]
OZ	OZ1	VAR3	82	Chloroplast	81%	NA	[70, 75]
	PPO1	PPOX1	56	Chloroplast	50%	NA	[76]
	OCP3		32	Chloroplast	12%	NA	[77]
	CP31		26	Chloroplast	38%	NA	[78, 79]

^aMature protein is the protein without chloroplast/mitochondrial transit peptide. Sizes are predicted based on protein annotation in PPDB (ppdb.tc.cornell.edu)

^bResults from silencing experiments instead of null mutants

NA: not applicable

Table 2. Protein-protein interaction matrix between known editing factors in each organelle

	PPR factors	RIP2	RIP9	RIP1	ORRM1	OZ1	PPO1
Chloroplast							
PPR factors	Weak interaction; dimer in crystal structure	-	-	-	-	-	-
RIP2	Interaction	Homodimer	-	-	-	-	-
RIP9	Interaction	Heterodimer	Homodimer	-	-	-	-
RIP1	Interaction	Heterodimer	Heterodimer	Homodimer	-	-	-
ORRM1	Interaction	Interaction	Not tested	Interaction	No	-	-
OZ1	Interaction	No	No	Weak	Interaction	Homodimer	
PPO1	No	Interaction	Interaction	Interaction	Not tested	Not tested	Not tested
Mitochondrion							
PPR factors	Not tested	-	-	-	-	-	-
RIP1	Interaction	Homodimer	-	-	-	-	-
RIP3	Interaction	Heterodimer	Homodimer	-	-	-	-
RIP8	Interaction	Heterodimer	Heterodimer	Homodimer	-	-	-
ORRM2	No evidence ^a	No	No	Not tested	No	-	-
ORRM3	No evidence ^a	Interaction	No	Not tested	Heterodimer	Homodimer	-
ORRM4	No evidence ^a	Interaction	Not tested	Not tested	No	Heterodimer	Homodimer

^aIndividual factors are listed except for the PPR family. “No evidence” indicates that a few PPR factors have been tested and gave negative interaction results but the data are not sufficient to be conclusive.

Interaction results are summarized here based on references [49] [65] [68-73] [76] [87-88].

Figure 1

Key:

Figure 1

Key:

Figure 2

Figure 3