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REVIEW

RNA editing of plastid-encoded genes

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Abstract

RNA editing is post-transcriptional modification to RNA molecules. In plants, RNA editing primarily occurs to two energy-producing organelles: plastids and mitochondria. Organelle RNA editing is often viewed as a mechanism of correction to compensate for defects or mutations in haploid organelle genomes. A common type of organelle RNA editing is deamination from cytidine to uridine. Cytidine-to-uridine plastid RNA editing is carried out by the RNA editing complex which consists of at least four types of proteins: pentatricopeptide repeat proteins, RNA editing interacting proteins/ multiple organellar RNA editing factors, organelle RNA recognition motif proteins, and organelle zinc-finger proteins. The four types of RNA editing factors work together to carry out RNA editing site recognition, zinc cofactor binding, and cytidine-to-uridine deamination. In addition, three other types of proteins have been found to be important for plastid RNA editing. These additional proteins may play a regulatory or stabilizing role in the RNA editing complex.

Additional key words: *Arabidopsis thaliana*; cytidine deaminase; *trans* factor.

Introduction

RNA editing is post-transcriptional modification to the nucleotide sequences of RNAs, an important yet under-appreciated biological process. Examples of RNA editing include uracil insertion and deletion, deamination from cytidine to uridine, deamination from adenosine to inosine, and reverse RNA editing from uridine to cytidine (Covello and Gray 1993, Brennicke *et al.* 1999, Takenaka *et al.* 2013, Hackett and Lu 2017). RNA editing has been reported in different types of RNAs, such as mRNA, tRNA, rRNA, and even microRNA (Covello and Gray 1993, Gray and Covello 1993, Blow *et al.* 2006, Yang *et al.* 2006, Kawahara *et al.* 2007, Cui *et al.* 2015). RNA editing occurs in viruses and a wide variety of organisms, including primitive eukaryotes, fungi, land plants, and vertebrates (Takenaka *et al.* 2013, Hackett and Lu 2017). RNA editing may take place in the nucleus, the cytoplasm, as well as energy-producing organelles mitochondria and plastids. In plants, RNA editing is limited to mitochondria and plastids. Organelle RNA editing occurs in almost all land plants, including liverworts, mosses, hornworts, lycopods, ferns, and flowering plants (Takenaka *et al.* 2013). However, RNA editing has not been reported in algae. It was postulated that plant organelle RNA editing evolved during the transition from water to land, independently from the evolution of RNA editing in primitive eukaryotes, fungi, and vertebrates (Takenaka *et al.* 2013). Over evolutionary time, different RNA editing events arise and disappear (Takenaka *et al.* 2013, Sun *et al.* 2016). Generally, flowering plants have fewer organelle RNA editing sites than lower land plants and gymnosperms (Takenaka *et al.* 2013, Sun *et al.* 2016). In the plastids and mitochondria of flowering plants, two types of RNA editing have been reported: cytidine-to-uridine (C-to-U) editing in mRNAs and tRNAs and

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Abbreviations: APOBEC – apolipoprotein B mRNA editing enzyme catalytic polypeptide-like; CDA – cytidine deaminase; CLB – chloroplast biogenesis; CP31A – 31 kD chloroplast protein A; CP31B – 31 kD chloroplast protein B; CRR – chlororespiratory reduction; C-to-U – cytidine-to-uridine; DYW – aspartate, tyrosine, and tryptophan domain; E – extension domain; ECB – early chloroplast biogenesis; L – long motif; LPA - low PSII accumulation; MORF – multiple organellar RNA editing factor; OCP3 – overexpressor of cationic peroxidase; ORRM – organelle RNA recognition motif; OTP – organelle transcript processing; OZ – organelle zinc-finger; P – pentatricopeptide; PPO/PPOX – protoporphyrinogen oxidase; PPR – pentatricopeptide repeat; QED – quintuple editing factor; RanBP – Ran-binding-protein; RARE – required for *accD* RNA editing; RIP – RNA editing interacting protein; RRM – RNA recognition motif; S – short motif; SMR – PPR-small MutS-related; THA – thylakoid assembly; T-to-C – thymine-to-cytosine; VAC – vanilla cream.
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adenosine-to-inosine editing in tRNAs (Fey *et al.* 2002, Delannoy *et al.* 2009, Karcher and Bock 2009, Zhou *et al.* 2014, Hackett and Lu 2017). This review focuses on plastid C-to-U mRNA editing in land plants, *Arabidopsis thaliana* in particular.

Organelle RNA editing is a mechanism of correction to compensate for defects/mutations in haploid organelle genomes

Why did RNA editing evolve in the plastid and mitochondrion of land plants? First, due to their haploid and uniparental nature, recombination rarely occurs to plastid and mitochondrial genomes (Lynch and Blanchard 1998, Neiman and Taylor 2009). Second, plastid and mitochondrial DNAs are exposed to many highly reactive intermediates of the electron transport chain (Foyer and Harbinson 1994, Foyer 1997, Blokhina and Fagerstedt 2010). Therefore, plastid and mitochondrial genomes are more susceptible to mutations than the nuclear genome (Lynch and Blanchard 1998, Neiman and Taylor 2009). For instance, a detrimental thymine-to-cytosine (T-to-C) mutation may occur to the coding region of an essential gene in the plastid genome. This mutation cannot be repaired by homologous recombination. RNA editing, however, is able to change the T-to-C mutation back to T. Because RNA editing often restores the function of the corresponding RNA and protein, it is considered as a mechanism of correction to compensate for defects/mutations in the genome (Takenaka *et al.* 2013, Sun *et al.* 2016).

Different RNA editing sites demonstrate different editing extents

The editing extents at different RNA editing sites vary substantially. For example, in mature rosette leaves of four-week-old Columbia wild-type *A. thaliana* plants, some plastid C-to-U RNA editing sites exhibit 3–8% editing, some exhibit near-complete editing, while the majority of plastid RNA editing sites exhibit intermediate-level editing extents (Fig. 1). Among the six plastid RNA editing sites that exhibit <8% editing, two result in synonymous mutations, one is located in the intergenic region, one is located in the intron, and two are located in the 3'-untranslated region (*see* Table 1 in Hackett and Lu 2017). The low editing extents at these sites could be the result of (*1*) weak binding between the RNA editing complex and the RNA target, or (*2*) inefficient editing of the RNA editing complex (Sun *et al.* 2016). Because these RNA editing sites are located in the nonessential region of a gene, there is no strong selection for organelles with higher editing extent at these sites.

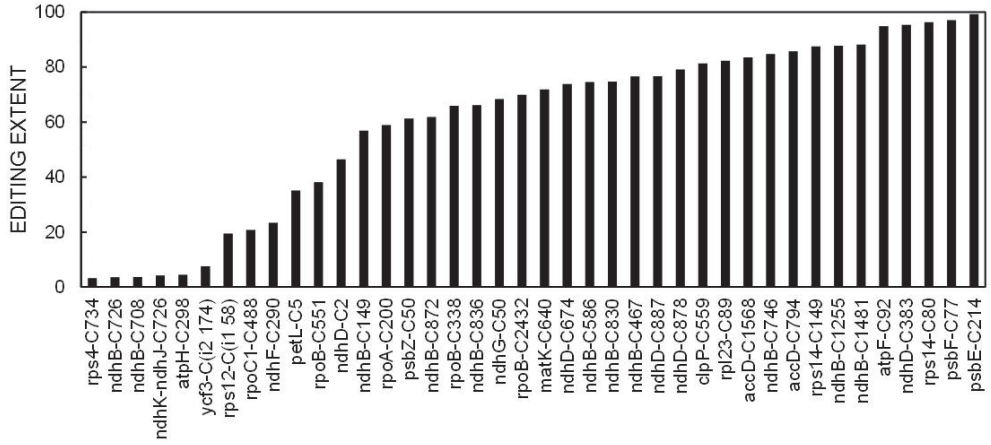

Fig. 1. Editing extents at 40 plastid RNA editing sites in mature rosette leaves of 4-week-old Columbia wild-type *Arabidopsis thaliana*.

The pattern of plastid RNA editing sites varies among different plant species

Different plant species may have different sets of plastid and mitochondrial RNA editing sites. For example, >40 plastid and >600 mitochondrial C-to-U RNA editing sites have been reported in *A. thaliana* (Chateigner-Boutin and Small 2007, Bentolila *et al.* 2013, Ruwe *et al.* 2013). Among the 41 plastid C-to-U RNA editing sites in Columbia wild-type *A. thaliana*, only nine are subject to editing in rice (*Oryza sativa*) (Table 1). About the other 32 sites, two are located in a gene that is absent in rice, 21 have nucleotide T encoded genomically in rice, three are not edited to uridine in rice, and six have not yet been analyzed in rice. Among the three *A. thaliana* sites, which are not edited in rice, *ndhB*-C872 and *psbZ*-C50 exhibit ~60% editing in Columbia wild-type *A. thaliana* and

rps12-C(i1 58) is located in the intron and only exhibits ~20% editing in Columbia wild-type *A. thaliana* (*see* Table 1 in Hackett and Lu 2017). Similar trends are observed in rice. Among the 22 plastid C-to-U RNA editing sites in rice, only eight are subject to editing in *A. thaliana* (Table 1). About the other 14 sites, 13 have nucleotide T encoded genomically in *A. thaliana* and one (*ndhB*-C611) is not edited to uridine in *A. thaliana*. Interestingly, *ndhB*-C611 is only partially edited in rice. Because plastid RNA editing is much more abundant in early-branching angiosperms, the pattern of plastid RNA editing sites among modern-day flowering plants is viewed as the result of divergent losses of RNA editing sites over evolutionary time (Stern *et al.* 2010, Hein *et al.* 2016).

Table 1. Plastid RNA editing sites in *Arabidopsis* and rice. Ab – the gene is absent in rice; NA – editing is not analyzed; NE – the C target exists but it is mostly not edited in the wild type; PE – partially edited (<50%) in the wild type. [a]The *Arabidopsis* plastid genome reference sequence (NCBI accession number NC_000932.1) was used to indicate the genome positions of the editing sites. [b]The rice plastid genome reference sequence (NCBI accession number NC_031333.1) was used to indicate the genome positions of the editing sites. [c]Synonymous codons.

Gene	*Arabidopsis* genome position[a]	Edited nucleotide	Δ amino acid	Rice genome position[b]	Edited nucleotide	Δ amino acid
accD	57868	C794	S265→L265	Ab	Ab	Ab
accD	58642	C1568	3'-UTR	Ab	Ab	Ab
atpA	10314	T1148	L383	35317	C1148	S383→L383
atpF	12707	C92	P31→L31	32791	T92	L31
atpH	13210	C298[PE]	3'-UTR	NA	NA	NA
clpP	69942	C559	H187→Y187	67681	T559	Y187
matK	2931	C706	H236→Y236	2558	T646	Y216
ndhA	121534	T476	L159	112206	C473	S158→L158
ndhA	119866	T1064	F355	110621	C1070	S357→F357
ndhB	97016	C149	S50→L50	87448	T149	L50
ndhB	96698	C467	P156→L156	87130	C467	P156→L156
ndhB	96579	C586	H196→Y196	87011	C586	H196→Y196
ndhB	96554	C611[NE]	S204	86986	C611[PE]	S204→L204
ndhB	96461	T704	F235	86913	C704	S235→F235
ndhB	96457	C708[PE]	I236→I236[c]	NA	NA	NA
ndhB	96439	C726[PE]	F242→F242[c]	NA	NA	NA
ndhB	96428	T737	L246	86880	C737	P246→L246
ndhB	96419	C746	S249→F249	86871	T746	F249
ndhB	95650	C830	S277→L277	86055	C830	S277→L277
ndhB	95644	C836	S279→L279	86049	C836	S279→L279
ndhB	95608	C872	S291→L291	86013	C872[NE]	S291
ndhB	95225	C1255	H419→Y419	85630	T1255	Y419
ndhB	94999	C1481	P494→L494	85404	C1481	P494→L494
ndhD	117166	C2[PE]	T1→M1	107864	T2	M1
ndhD	116785	C383	S128→L128	107483	T383	L128
ndhD	116494	C674	S225→L225	107192	T674	L225
ndhD	116290	C878	S293→L293	106988	C878	S293→L293
ndhD	116281	C887	P296→L296	106979	T887	L296
ndhF	112577	T62	L21	103536	C62	S21→L21
ndhF	112349	C290[PE]	S97→L97	103308	T290	L97
ndhG	118917	T(-10)	5'-UTR	109732	C(-10)	5'-UTR
ndhG	118858	C50	S17→F17	109673	T50	F17
ndhG	118561	T347	L116	109573	C347	P116→L116
ndhK-ndhJ	49209	C726[PE]	Intergene	NA	NA	NA
petL	65716	C5[PE]	P2→L2	63489	T5	L2
psbE	64109	C214	P72→S72	62075	T214	S72
psbF	63985	C77	S26→F26	61950	T77	F26
psbZ	35800	C50	S17→L17	11925	C50[NE]	S17
rpl2	154478	T2	M1	132430	C2	T1→M1

Table 1 continues on the next page.

Table 1 continued

Gene	*Arabidopsis* Genome position[a]	Edited nucleotide	Δ amino acid	Rice Genome position[b]	Edited nucleotide	Δ amino acid
rpl23	86055	C89	S30→L30	132217	T89	F30
rpoA	78691	C200	S67→F67	75100	T200	F67
rpoB	25992	C338	S113→L113	19505	T332	F111
rpoB	25857	T473	L158	19640	C467	S156→L156
rpoB	25779	C551[PE]	S184→L184	19718	C545	S182→L182
rpoB	25764	T566	L189	19732	C560	S187→L187
rpoB	23898	C2432	S811→L811	21614	T2441	L814
rpoC1	21806	C488[PE]	S163→L163	22926	T488	L163
rps4	45095	C734[PE]	3'-UTR	NA	NA	NA
rps8	80291	T182	L61	76881	C182	S61→L61
rps12-intron	69553	C(i1 58)[PE]	intron	88507	C(i1 54)[NE]	intron
rps14	37161	C80	S27→L27	36501	C80	S27→L27
rps14	37092	C149	P50→L50	36432	T149	L50
ycf3	43350	C(i2 174)[PE]	intron	NA	NA	NA
ycf3	43693	T185	M62	42863	C185	T62→M62

Cytidine deaminase-related enzymes

C-to-U RNA deamination is analogous to C-to-U single nucleotide conversion, which is catalyzed by classic cytidine deaminase (CDA) (Takenaka *et al.* 2013). Phylogenetic analysis revealed that there are four families of CDA-related enzymes: classic CDA, nucleoside (adenosine, guanine, and cytosine) deaminase, deoxycytidylate deaminase, and APOBEC (apolipoprotein B mRNA editing enzyme catalytic polypeptide-like)-type cytidine deaminase (Xu and Messing 2006).

The classic CDA was initially a candidate for C-to-U RNA deamination in land plant plastids and mitochondria. Amino acid sequence alignment showed that CDAs in *A. thaliana*, *Escherichia coli*, and *Haemophilus influenzae* are twice as long as CDAs in other organisms (Faivre-Nitschke *et al.* 1999). According to crystal structures, the longer type of CDAs acts as homodimers and the short type of CDAs acts as homotetramers (Betts *et al.* 1994, Faivre-Nitschke *et al.* 1999, Chung *et al.* 2005). Long or short, each CDA peptide contains a substrate-binding site and a catalytic site with a zinc-finger motif [HXE(X)$_n$PCXXC] (Faivre-Nitschke *et al.* 1999, Boussardon *et al.* 2014). The *A. thaliana* nuclear genome contains nine cytidine deaminase-like genes (Chen *et al.* 2016). Only one of them (CDA1) is functional and the other eight are pseudogenes (Faivre-Nitschke *et al.* 1999, Vincenzetti *et al.* 1999, Chen *et al.* 2016). CDA1 is targeted to the cytosol, and it is not involved in RNA editing (Faivre-Nitschke *et al.* 1999, Vincenzetti *et al.* 1999). Activity assays of the recombinant CDA1 protein and phenotypic characterization of loss-of-function *cda1* mutants revealed that CDA1 is required in pyrimidine homeostasis (Faivre-Nitschke *et al.* 1999, Vincenzetti *et al.* 1999).

Nucleoside deaminase and deoxycytidylate deaminase are not able to act on polynucleotide chains either (Navaratnam *et al.* 1995, Xu and Messing 2006). APOBEC-type cytidine deaminase within the CDA superfamily then became a candidate for C-to-U RNA deamination in the plastids and mitochondria of land plants. This protein family probably evolved from classic CDA, after acquiring the ability to act on polynucleotide chains (Navaratnam *et al.* 1995, Xu and Messing 2006). Mammalian APOBEC is a well-established example of C-to-U RNA editing enzymes (Navaratnam *et al.* 1995). APOBEC-type cytidine deaminase has been reported in other vertebrates, but not in plants (Xu and Messing 2006). Plastid RNA editing is carried out by the RNA editing complex that are between 200 and 400 kD in size (Bentolila *et al.* 2012, Shi *et al.* 2016). As described below, the RNA editing complex may consist of at least four types of proteins: pentatricopeptide repeat (PPR) proteins, RNA editing interacting proteins/multiple organellar RNA editing factors (RIPs/MORFs), organelle RNA recognition motif (ORRM) proteins, and organelle zinc-finger (OZ) proteins. In addition to the four common types of organelle RNA editing factors, three other types of proteins have been found to be important to plastid RNA editing.

PPR and PPR-related proteins

PPR proteins have recently became a prime candidate for C-to-U RNA editing in land plant plastids and mitochondria. PPR proteins exist ubiquitously in eukaryotes, but are absent in most prokaryotes (Barkan and Small 2014). Although most eukaryotes only have 5–10 PPR proteins, land plants contain 400–1000 PPR proteins. Basically all PPR proteins are predicted (or have been shown) to be mitochondrion/plastid-targeted. PPR proteins can be classified into four subfamilies: P-type, PPR-SMR-type, PPR-E-DYW-type, and PPR-E-type (Fig. 2*A*) (Barkan and Small 2014, Sun *et al.* 2016). P-type PPR proteins have a PPR domain with tandem P (pentatricopeptide) motifs. PPR-SMR-type PPRs are composed of a PPR domain with tandem P motifs and a SMR (PPR-small MutS-related) domain. PPR-E-DYW-type PPRs contain a PPR domain with triplets of P, L (long), and S (short) motifs, an E (extension) domain, and a DYW domain. PPR-E-type PPRs have a PPR domain with triplets of P, L, and S motifs and an E domain. In addition to these four PPR subfamilies, a PPR-related protein, DYW1, was also found to be involved in C-to-U RNA editing in land plant plastids and mitochondria (Boussardon *et al.* 2012, Boussardon *et al.* 2014). The DYW1 protein does not contain a PPR domain ; however, it does contain the C-terminal region of the E domain and a complete DYW domain (Fig. 2*A*).

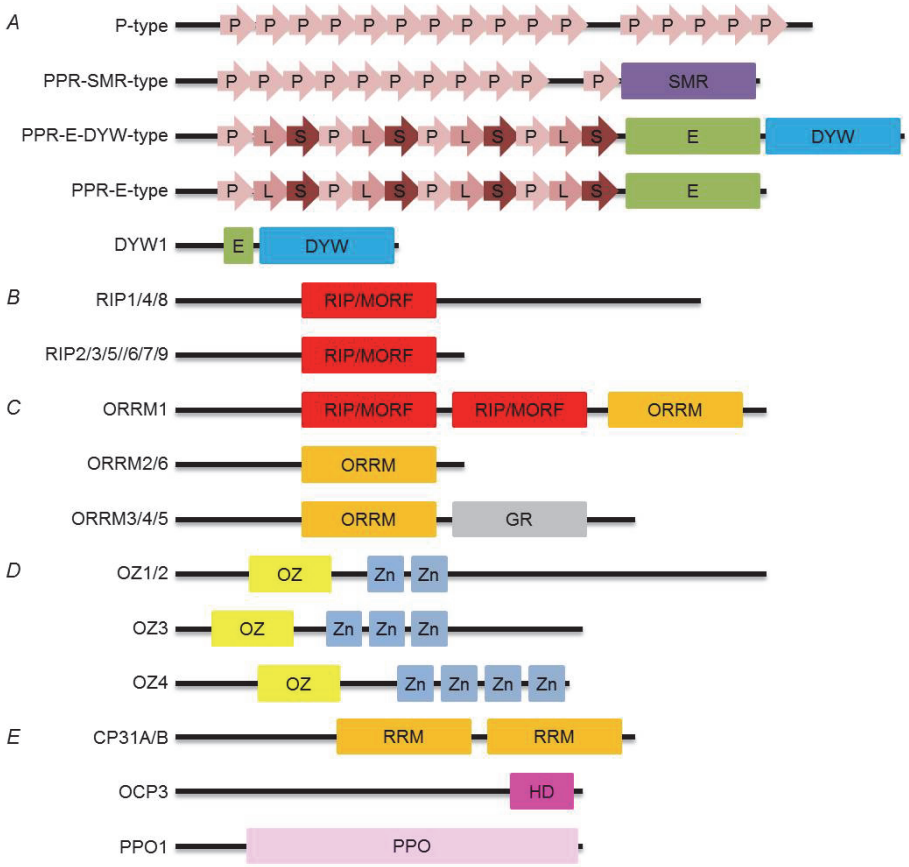

Fig. 2. Domain composition of five classes of organelle RNA editing factors and related proteins. (*A*) Domain composition of the P-type, PPR-SMR-type, PPR-E-DYW-type, and PPR-E-type PPR proteins and the DYW1 protein. P-type PPRs contain tandem P (pentatricopeptide) motifs. PPR-SMR-type PPRs contain tandem P motifs and a SMR (PPR-small MutS-related) domain. PPR-E-DYW-type PPRs contain triplets of P, L (long), and S (short) motifs, an E (extension) domain, and a DYW domain. PPR-E-type PPRs contain triplets of P, L, and S motifs and an E domain. DYW1 contains the C-terminal region of the E domain and a complete DYW domain. (*B*) Domain composition of RIP/MORF proteins. All RIP/MORFs contain a RIP/MORF domain. RIP1/MORF8, RIP4/MORF4, and RIP8/MORF1 also contain an extended C-terminus with unknown function. (*C*) Domain composition of ORRM proteins. All ORRMs contain an ORRM domain. ORRM1 also contains two RIP/MORF domains at the N-terminus. ORRM3 and ORRM4 also contain a glycine-rich domain at the C-terminus. (*D*) Domain composition of OZ proteins. All OZs contain an N-terminal OZ conserved domain and multiple Ran-Binding-Protein2-type zinc (Zn) fingers. OZ1 and OZ2 contain two RanBP2-type zinc fingers. OZ3 and OZ4 contain three and four RanBP2-type zinc fingers, respectively. (*E*) Domain composition of additional RNA editing factors. CP31A and CP31B each contain two RRM domains. OCP31 contains a homeodomain (HD). PPO1/PPOX1 contains a protoporphyrinogen oxidase (PPO) domain.

Table 2. Identified RNA editing factors in the *Arabidopsis* plastid. [a]The editing extent at this site is increased in the loss-of-function mutant(s).

Gene	Edited nucleotide	PPR and PPR-related	RIP/MORF	ORRM	OZ	Other factors
accD	C794	RARE1, ECB2	RIP1, RIP2, RIP9	ORRM6	OZ1	
accD	C1568	ECB2, OTP81	RIP2[a], RIP9[a]	ORRM1	OZ1	
atpF	C92	AEF1	RIP2, RIP9			
clpP	C559	CLB19	RIP2, RIP9	ORRM1	OZ1	
matK	C706	OTP81	RIP2, RIP9	ORRM1	OZ1	CP31A
ndhB	C149		RIP2, RIP9		OZ1	
ndhB	C467	CRR28	RIP1, RIP2, RIP9	ORRM1	OZ1	PPO1, CP31A, OCP3
ndhB	C586		RIP1, RIP2, RIP9	ORRM1	OZ1	OZ1, PPO1, OCP3
ndhB	C726			ORRM1	OZ1	OZ1
ndhB	C746	CRR22	RIP1, RIP2, RIP9	ORRM1	OZ1	OCP3
ndhB	C830	ELI1	RIP1, RIP2, RIP9	ORRM1	OZ1	PPO1
ndhB	C836	OTP82	RIP1, RIP2, RIP9	ORRM1	OZ1	PPO1, CP31A, CP31B, OCP3
ndhB	C872	OTP81	RIP2, RIP9	ORRM1	OZ1	PPO1
ndhB	C1255	CREF7	RIP2, RIP9	ORRM1	OZ1	PPO1, CP31A
ndhB	C1481	OTP84	RIP1, RIP2, RIP9		OZ1	PPO1, CP31A, CP31B
ndhD	C2	CRR4, DYW1	RIP1, RIP2, RIP9	ORRM1	OZ1	PPO1
ndhD	C383	CRR21	RIP1, RIP2, RIP9			PPO1
ndhD	C674	OTP85	RIP2, RIP9	ORRM1	OZ1	PPO1, CP31A
ndhD	C878	CRR28	RIP2, RIP9	ORRM1	OZ1	PPO1, CP31A, CP31B
ndhD	C887	CRR22	RIP2, RIP9	ORRM1	OZ1	PPO1, CP31A, CP31B
ndhF	C290	ECB2, OTP84	RIP1, RIP2, RIP9		OZ1	PPO1, CP31A
ndhG	C50	OTP82	RIP2, RIP9	ORRM1	OZ1	PPO1
ndhK-ndhJ	C726			ORRM6[a]		
petL	C5		RIP1, RIP2, RIP9		OZ1	PPO1, CP31A
psbE	C214	CREF3	RIP2			
psbF	C77	LPA66	RIP2, RIP9	ORRM6	OZ1	
psbZ	C50	OTP84	RIP2, RIP9		OZ1	CP31A, CP31B
rpl2	T2					
rpl23	C89	OTP80	RIP2, RIP9		OZ1	PPO1
rpoA	C200	CLB19	RIP2, RIP9	ORRM1	OZ1	
rpoB	C338	YS1	RIP2, RIP9	ORRM1	OZ1	CP31A
rpoB	C551	CRR22	RIP2, RIP9	ORRM1	OZ1	CP31A, CP31B
rpoB	C2432	OTP81	RIP1[a], RIP2, RIP9	ORRM1	OZ1	CP31A, CP31B
rpoC1	C488	DOT4	RIP1[a]		OZ1[a]	PPO1, CP31A, CP31B
rps12-intron	C(i1 58)	OTP81	RIP1[a], RIP2, RIP9	ORRM1	OZ1	
rps14	C80	OTP86	RIP2, RIP9			
rps14	C149		RIP2, RIP9	ORRM1		PPO1, CP31A

The PPR domain binds to RNAs in a sequence-specific manner (Barkan and Small 2014). The extension domain serves as a link between the PPR domain and the DYW domain and it is only found in RNA-editing PPR proteins, namely, PPR-E-DYW-type and PPR-E-type PPRs, as well as DYW1 (Barkan and Small 2014). P-type and PPR-SMR-type PPR proteins are not involved in organelle RNA editing in plants. The DYW domain is named after the three conserved amino acids aspartate, tyrosine, and tryptophan at the C-terminus (Barkan and Small 2014). The DYW domain contains a [HXE(X)$_n$CXXC] motif, similar to the signature zinc-finger motif [HXE(X)$_n$PCXXC] in classic CDAs (Faivre-Nitschke *et al.* 1999, Boussardon *et al.* 2014). The proline residue in classic CDAs is not conserved in the DYW domain of PPR proteins (Boussardon *et al.* 2014).

A total of 21 PPR and PPR-related proteins have been reported to be required in plastid RNA editing in *A. thaliana* (Tables 2, 3; Fig. 3*A*). Among them, three are PPR-E-type PPRs: CHLOROPLAST BIOGENESIS 19 (CLB19), CHLORORESPIRATORY REDUCTION 4 (CRR4), and CRR21; one is DYW1; the other seventeen are PPR-E-DYW-type PPR proteins. (Okuda *et al.* 2007, Chateigner-Boutin *et al.* 2008, Boussardon *et al.* 2012,

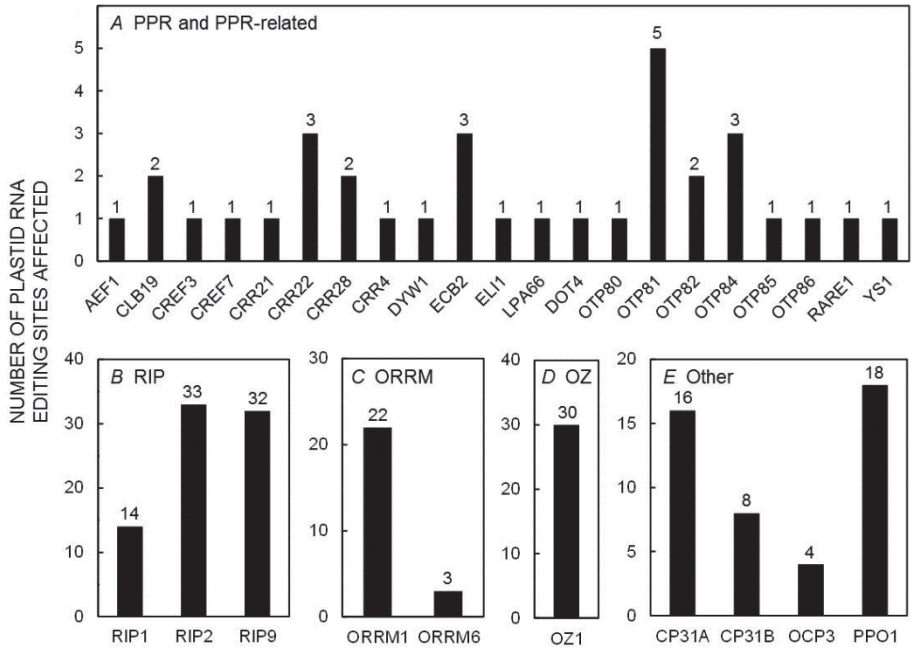

Fig. 3. Numbers of plastid RNA editing sites affected by individual plastid RNA editing factors. (*A–E*) numbers of plastid RNA editing sites affected by individual proteins in the PPR (*A*), RIP/MORF (*B*), ORRM (*C*), OZ (*D*), and other (*E*) families of plastid RNA editing factors.

Boussardon *et al*. 2014, Okuda *et al*. 2014). Fourteen of the 21 plastid RNA editing PPR and PPR-related proteins are required for a specific plastid RNA editing site (Table 2; Fig. 3*A*) (Kotera *et al.* 2005, Okuda *et al.* 2007, Cai *et al.* 2009, Hammani *et al.* 2009, Robbins *et al.* 2009, Zhou *et al.* 2009, Boussardon *et al.* 2012, Hayes *et al.* 2013, Yagi *et al.* 2013, Yap *et al.* 2015). The seven other PPR proteins, CLB19, CRR22, CRR28, EARLY CHLOROPLAST BIOGENESIS2/VANILLA CREAM1 (ECB2/VAC1), ORGANELLE TRANSCRIPT PROCESSING 81/QUINTUPLE EDITING FACTOR 1 (OTP81/QED1), OTP82, and OTP84, participate in editing at two, three, two, three, five, two, and three plastid RNA editing sites, respectively (Table 2; Fig. 3*A*) (Chateigner-Boutin *et al.* 2008, Hammani *et al.* 2009, Okuda *et al.* 2009, Yu *et al.* 2009, Tseng *et al.* 2010, Cao *et al.* 2011). The relatively small number of specific plastid RNA editing sites, which are edited by individual RNA-editing PPR proteins (Table 3), is consistent with sequence-specific binding between the PPR protein and the RNA target. Consequently, RNA-editing PPR proteins are characterized as site-specific RNA recognition factors (Barkan and Small 2014, Sun *et al.* 2016).

At most plastid RNA editing sites, only one PPR protein has been identified (Table 2). This is not surprising because most of the PPR proteins identified for these sites are PPR-E-DYW-type PPRs. A PPR-E-DYW-type PPR protein could accomplish both editing site recognition and cytidine deamination without the help from another PPR-E-DYW-type PPR protein. As mentioned previously, CLB19 and CRR21 are PPR-E-type PPR proteins. They do not contain the DYW domain required for cytidine deamination. Although CLB19 was identified as the PPR protein for editing at *clpP*-C559 and *rpoA*-C200, and CRR21 was identified as the PPR protein for editing at *ndhD*-C383, a DYW domain-containing PPR protein might be needed to assist these PPR-E-type PPR proteins.

Table 3. Anti-correlation between the number of identified proteins in each family of plastid-targeted RNA editing factors and the average number of plastid RNA editing sites affected. [a]Only plastid-targeted RNA editing factors are included in this column.

Protein family	Number of identified/ characterized proteins[a]	Average number of plastid RNA editing sites affected
PPR and PPR-related	21	1.6
RIP/MORF	3	26.3
ORRM	2	12.5
OZ	3	30.0
Others	4	17.6

Indeed, it was recently found that editing at four plastid RNA editing sites requires more than one PPR protein: editing at *accD*-C794 requires REQUIRED FOR ACCD RNA EDITING (RARE1) and ECB2/VAC1, editing at *accD*-C1568 requires ECB2/VAC1 and OTP81/QED1, editing at *ndhD*-C2 requires CRR4 and DYW1, and editing at *ndhG*-C50 requires ECB2/VAC1 and OTP84 (Table 2) (Kotera *et al.* 2005, Hammani *et al.* 2009, Robbins *et al.* 2009, Yu *et al.* 2009, Tseng *et al.* 2010, Cao *et al.* 2011, Boussardon *et al.* 2012). Among these RNA-editing PPR proteins, CRR4 and DYW1 are unique. CRR4 has a canonical PPR domain and a partial E domain but it lacks a DYW domain and 14 C-terminal amino acid residues of the E domain (Fig. 2*A*) (Boussardon *et al.* 2012). On the contrary, DYW1 has a canonical DYW domain and the C-terminal region of the E domain but it does not contain a PPR domain (Fig. 2*A*). DYW1 interacts with CRR4 in bimolecular fluorescence complementation assays (Table 4). Therefore, Boussardon *et al.* (2012) proposed that CRR4 and DYW1 act together to edit *ndhD*-C2: the PPR domain in CRR4 is responsible for binding to the RNA target in a sequence-specific manner, while the DYW domain in DYW1 might be responsible for cytidine deamination. To test whether the combined action of CRR4 and DYW1 is equivalent of a canonical PPR-E-DYW-type PPR protein, Boussardon *et al.* (2012) generated a chimeric *CRR4-DYW1* construct and used it to complement the *crr4 dyw1* double mutant. As expected, the complementation experiment results in >80% restoration of editing at *ndhD*-C2 (Boussardon *et al.* 2012). This study also revealed that the three essential domains of RNA-editing PPR proteins, *i.e.*, the PPR, E, and DYW domains, do not have to be encoded in one transcription unit.

Apart from the interaction between CRR4 and DYW1, two partial PPR proteins, no other plastid RNA-editing PPR proteins have been found to interact with each other (Table 4). These observations are consistent with PPR proteins being the *trans* factors that recognize specific RNA editing sites. A PPR-E-DYW-type PPR protein could accomplish editing site recognition and deamination without the help from another PPR-E-DYW-type PPR protein. It is intriguing that editing of *accD*-C794, *accD*-C1568, and *ndhF*-C290 requires two different PPR-E-DYW-type PPR proteins at each site (Table 2). One possibility is that the PPR domain of one PPR-E-DYW-type PPR proteins is responsible for editing site recognition and the DYW domain of the other PPR-E-DYW-type PPR protein might be responsible for cytidine deamination. This hypothesis may be tested by complementing double knockout mutants with progressively truncated PPR-E-DYW-type PPR proteins (Hayes *et al.* 2015).

Multiple studies showed that P-type PPR proteins act as homodimers (Ke *et al.* 2013, Yin *et al.* 2013). PPR10, a P-type PPR protein, which protects target RNAs from exonucleases in maize, forms an antiparallel homodimer (Yin *et al.* 2013). THYLAKOID ASSEMBLY 8 (THA8), a P-type PPR protein required for splicing the *ycf3* transcript, forms an asymmetric homodimer (Ke *et al.* 2013). It is conceivable that RNA-editing PPR proteins may form homodimers as well. Interestingly, OTP81/QED1, a PPR-E-DYW-type PPR protein required for editing at *rpoB*-C2432 and *rps12*- C(i1 58), interacts with itself in yeast-two-hybrid assays (Wagoner *et al.* 2015).

RNA editing interacting proteins/multiple organellar RNA editing factors

The second class of proteins required for plastid RNA editing contains a conserved RIP/MORF domain (Fig. 2*B*). RIP/MORF proteins are only found in the mitochondrion and plastid of flowering plants (Takenaka *et al.* 2012). The *A. thaliana* nuclear genome encodes nine functional RIP/MORF proteins: RIP1/MORF8 is dual-targeted to plastids and mitochondria, RIP2/MORF2 and RIP9/MORF9 are plastid-targeted, and the other six are mitochondrion-targeted (Takenaka *et al.* 2012). Among the nine RIP/MORF proteins, RIP1/MORF8, RIP4/MORF4, and RIP8/MORF1 have an extended C-terminus with unknown function (Fig. 2*B*). This extended C-terminus is absent in the other six RIP/MORF proteins, which are much shorter.

Loss-of-function mutations in the *RIP1/MORF8* gene affect editing at 14 plastid and 266 mitochondrial RNA editing sites (Table 2; Fig. 3*B*) (Bentolila *et al.* 2012). Loss-of-function mutations in the *RIP2/MORF2* and *RIP9/MORF9* genes affect editing at 33 and 32 plastid RNA editing sites, respectively (Table 2; Fig. 3*B*) (Takenaka *et al.* 2012).

Pairwise yeast-two hybrid, *in vitro* pull down, and bimolecular fluorescence complementation assays consistently demonstrated that RIP/MORF proteins may form homo- and heterodimers (Table 4) (Takenaka *et al.* 2012, Zehrmann *et al.* 2015). Furthermore, yeast-two-hybrid assays with progressively truncated versions of the MORF1 protein revealed that the C-terminus of the RIP/MORF domain is necessary for RIP/MORF-RIP/MORF protein interactions (Zehrmann *et al.* 2015). RIP/MORF proteins do not contain any RNA-binding domains. Therefore, RIP/MORF proteins probably are not capable of binding to RNAs directly. Based on the organelle RNA editing patterns of loss-of-function mutants and the protein-protein interaction data of RIP/MORF proteins, it is very likely that RIP/MORF proteins act as a scaffold, bridging different components of the RNA editing complex together.

Table 4. Protein-protein interactions among different RNA editing factors. Cells for within-group interactions are shaded in gray.

	AEF1	CLB19	CREF3	CREF7	CRR21	CRR22	CRR28	CRR4	DOT4	DYW1	ECB2	ELI1	LPA66	OTP80	OTP81	OTP82	OTP84	OTP85	OTP86	RARE1	YS1	RIP1	RIP2	RIP9	ORRM1	ORRM6	OZ1	CP31A	CP31B	OCP3	PPO1
AEF1																															
CLB19										-																					
CREF3																															
CREF7																															
CRR21																															
CRR22																															
CRR28																							+	+	+						-
CRR4										+													-	-							-
DOT4																															
DYW1		-						+		-													+	+							-
ECB2																															
ELI1																															
LPA66																							+	+		-					
OTP80																							-	-							-
OTP81															+							+	+	+	-						
OTP82																							+	+	+						-
OTP84																									-						
OTP85																															
OTP86																															
RARE1																						+			-	-					
YS1																															
RIP1															+					+		+	+	+	+	+	+				+
RIP2							+	-		+			+	-	+	+						+	+	+	+	+	-				+
RIP9							+	-		+			+	-	+	+						+	+	+		+	-				+
ORRM1							+								-	+	-			-		+	+		-	-	+				
ORRM6													-							-		+	+	+	-	+	+				
OZ1																						+	-	-	+	+	+				
CP31A																															
CP31B																															
OCP3																															
PPO1							-	-		-				-		-						+	+	+							

Organelle RNA recognition motif proteins

The third class of proteins required for plastid RNA editing contains an ORRM (Fig. 2*C*). ORRM proteins are a subset of organelle-located RNA recognition motif (RRM) proteins (Shi *et al.* 2017). RRM-containing proteins bind to RNAs and they exist in virus, bacteria, and most importantly, eukaryotes (Maris *et al.* 2005). The *A. thaliana* nuclear genome encodes six ORRM proteins: ORRM1 and ORRM6 are plastid-targeted; ORRM2, ORRM3, ORRM4, and ORRM5 are mitochondrion-targeted (Shi *et al.* 2017). Among the six ORRM proteins, ORRM1 has two RIP/MORF domains at the N-terminus, ORRM3, ORRM4, and ORRM5 have a glycine-rich domain at the C-terminus (Fig. 2*C*) (Shi *et al.* 2017).

Loss-of-function mutation in the *ORRM1* gene affects editing at 22 plastid RNA editing sites (Table 2, Fig. 3*C*) (Sun *et al.* 2013). By contrast, loss-of-function mutations in the *ORRM6* gene only affect editing at three plastid RNA editing sites: *accD*-C794, *psbF*-C77, and *ndhK*-*ndhJ*-C726 (an intergenic cytidine target between *ndhK* and *ndhJ*) (Table 2, Fig. 3*C*) (Hackett *et al.* 2017, Hackett and Lu 2017). It should be noted that the three plastid RNA editing sites affected in the *orrm6* mutants are not affected in the *orrm1* mutant (Table 2). Because the *psbF* gene encodes the β-subunit of cytochrome b_{559}, an essential protein in PSII, the *orrm6* mutants are much sicker and smaller than the *orrm1* mutant (Hackett *et al.* 2017).

Electrophoretic mobility shift assays demonstrated that the recombinant ORRM1 protein is capable of binding to ORRM1-dependent RNA editing sites (Sun *et al.* 2013). Likewise, the recombinant ORRM6 protein can bind to *accD* and *psbF* transcripts near the two editing sites that are affected in the *orrm6* mutants: *accD*-C794 and *psbF*-C77 (Hackett *et al.* 2017).

Bimolecular fluorescence complementation assays demonstrated that ORRM6 interacts with itself but it does not interact with ORRM1 (Table 4) (Hackett *et al.* 2017). These data suggest that ORRM6 may form homodimers or homooligomers. Unlike ORRM6, ORRM1 does not interact with itself (Shi *et al.* 2017). This is somewhat surprising because: (*1*) ORRM6, the other plastid-targeted ORRM protein, interacts with itself (Hackett *et al.* 2017); (*2*) ORRM1 contains two RIP/MORF domains and RIP/MORF domain-containing proteins tend to form homo- and heterodimers (Takenaka *et al.* 2012, Zehrmann *et al.* 2015). ORRM1 showed a weak interaction with RIP1/MORF8 in yeast-two-hybrid assays (Table 4) (Sun *et al.* 2015). No interactions were observed between ORRM1 and the two plastid-targeted RIP/MORF proteins (RIP2/MORF2 and RIP9/MORF9). It is possible that the presence of both ORRM and RIP/MORF domains in ORRM1 causes the unique interaction pattern of ORRM1.

Yeast-two-hybrid assays demonstrated that ORRM1 interacts with PPR-E-DYW-type PPR proteins CRR28 and OTP82 (Table 4) (Sun *et al.* 2013). CRR28 is the RNA-editing PPR protein at *ndhB*-C467 and *ndhD*-C878, while OTP82 is the RNA-editing PPR protein at *ndhB*-C836 and *ndhG*-C50 (Hammani *et al.* 2009, Okuda *et al.* 2009). Interestingly, these four plastid RNA editing sites are also affected in the *orrm1* mutant (Sun *et al.* 2013). ORRM6 does not interact with LOW PSII ACCUMULATION 66 (LPA66) or RARE1, the two PPR-E-DYW-type PPR proteins at *psbF*-C77 and *accD*-C794, respectively (Table 4) (Cai *et al.* 2009, Robbins *et al.* 2009, Hackett *et al.* 2017). These two plastid RNA editing sites are substantially affected in the *orrm6* mutants (Hackett *et al.* 2017, Hackett and Lu 2017). The absence of interaction between ORRM6 and the two RNA-editing PPR proteins could be explained by the lack of RIP/MORF domains in ORRM6. ORRM6 interacts with plastid-targeted RIP2/MORF2 and RIP9/MORF9 and plastid/mitochondrion-dual targeted RIP1/MORF8, in reciprocal bimolecular fluorescence complementation assays (Table 4) (Hackett *et al.* 2017). Because RIP/MORF proteins interact with PRR proteins directly (Bentolila *et al.* 2012), ORRM6 might be indirectly associated with LPA66 and RARE1, the two PPR-E-DYW-type PPR proteins at *psbF*-C77 and *accD*-C794, respectively.

Organelle zinc-finger proteins

OZ proteins are the fourth class of proteins required for plastid RNA editing (Fig. 2*D*). The first OZ protein, OZ1, was identified as a component of the plastid RNA editing complex *via* mass spectrometry analysis of immunoprecipitation products from *A. thaliana orrm1* mutants complemented with a FLAG-tagged ORRM1 protein (Sun *et al.* 2015). OZ proteins are found in many land plant lineages, including mosses and flowering plants. The *A. thaliana* nuclear genome encodes four OZ proteins. According to TargetP, OZ1, OZ2, and OZ4 are predicted to be plastid-targeted and OZ3 is predicted to be mitochondrion-targeted. The plastid-localization of OZ1 was confirmed with confocal microscopic analysis of *A. thaliana* protoplast expressing the Yellow Fluorescent Protein-OZ1 fusion protein. OZ proteins contain multiple Ran-Binding-Protein2 (RanBP2, $CXXCX_{10}CXXC$)-type zinc-finger domains: OZ1 and OZ2 have two, OZ3 has three, and OZ4 has four (Fig. 2*D*) (Sun *et al.* 2015). RanBP2-type zinc-fingers have been demonstrated to bind to RNAs in a sequence-specific manner (Nguyen *et al.* 2011). However, whether the zinc-finger domains in OZ proteins actually bind to zinc and whether they confer OZ proteins the ability to bind to RNAs in a sequence-specific manner have not been tested. In addition to RanBP2-type

zinc-finger domains, each OZ protein also contains an N-terminal conserved domain of unknown function (Sun *et al.* 2015).

Loss-of-function mutation in the *OZ1* gene affects editing at 30 plastid RNA editing sites (Table 2, Fig. 3*D*) (Sun *et al.* 2015). Yeast-two-hybrid assays revealed that OZ1 interacts with PPR-E-DYW-type PPR proteins CRR28 and OTP82 and the RIP1/MORF8 protein (Table 4) (Sun *et al.* 2015). However, OZ1 does not appear to interact with RIP2/MORF2 or RIP9/MORF9 directly (Sun *et al.* 2015). It is possible that OZ1 is associated with RIP2/MORF2 or RIP9/MORF9 *via* interaction with plastid-targeted ORRM proteins. As expected, yeast-two-hybrid and reciprocal bimolecular fluorescence complementation assays showed that OZ1 interacts with ORRM1 and ORRM6 (Table 4) (Sun *et al.* 2015, Hackett *et al.* 2017).

Additional factors

Three additional types of proteins have been found to be required in plastid RNA editing: 31 kD CHLOROPLAST PROTEIN A and B (CP31A and CP31B), OVEREXPRESSOR OF CATIONIC PEROXIDASE 3 (OCP3), and PROTOPORPHYRINOGEN OXIDASE 1 (PPO1/PPOX1) (Table 2, Fig. 2*E*) (Coego *et al.* 2005, Tillich *et al.* 2009, Kupsch *et al.* 2012, García-Andrade *et al.* 2013, Zhang *et al.* 2014).

CP31A and CP31B are organelle-located RRM proteins, each with two RRM motifs (Fig. 2*E*). Organelle-located RRM proteins are known to be involved in organelle RNA processing and stabilization (Tillich *et al.* 2009, Shi *et al.* 2017). Indeed, transcript levels of some plastid-encoded genes, such as *ndhF*, *ycf1*, and *rrn23*, are substantially reduced in the *cp31a* mutants (Tillich *et al.* 2009). By contrast, the *cp31b* mutant showed little deviation in the transcript levels of plastid-encoded genes from the wild type. These data demonstrated that CP31A is required for stabilizing transcripts of certain plastid-encoded genes. CP31, the homolog of CP31A and CP31B in tobacco, is required for editing at two plastid RNA editing sites *in vitro* (Hirose and Sugiura 2001). Therefore, Tillich *et al*. (2009) investigated whether CP31A and CP31B play a role in plastid RNA editing. In the *cp31a cp31b* double mutant, 16 plastid RNA editing sites showed different degrees of reduction in editing efficiency (Tillich *et al.* 2009). These sites are either less severely affected or not affected in *cp31a* and *cp31b* single mutants, indicating that CP31A and CP31B act in a combinatorial manner. Detailed comparison of editing efficiency among the wild type, single mutants, and the double mutant suggested that CP31A and CP31B are involved in editing at 16 and 8 plastid RNA editing sites, respectively (Table 2; Fig. 3*E*). Taken together, CP31A and CP31B are involved in plastid RNA editing in a site-specific fashion, possible *via* stabilizing specific transcript targets during the editing process (Tillich *et al.* 2009). However, it is yet to be investigated whether CP31A and CP31B interact with other RNA editing factors.

OCP3 is a homeodomain-containing protein originally thought to function as a nuclear transcription factor (Coego *et al.* 2005). However, confocal microscopic analysis of *Nicotiana benthamiana* protoplast expressing fluorescently-tagged OCP3 protein demonstrated that this protein is plastid-targeted (García-Andrade *et al.* 2013). Loss-of-function mutations in the *OCP3* gene result in reduced editing extent at four plastid RNA editing sites: *ndhB*-C467 *ndhB*-C586, *ndhB*-C746, and *ndhB*-C836 (Table 2) (García-Andrade *et al.* 2013). Consistent with reduced editing at these four *ndhB* RNA editing sites, the OCP3 protein was found to associate with the *ndhB* transcript *in vivo*. Because OCP3 does not have any domain or motif characteristic of other known RNA editing factors, Garcia-Andrade *et al.* (2013) hypothesized that the association of OCP3 with the *ndhB* transcript is indirect and presumably *via* interaction with other RNA editing factors. Garcia-Andrade *et al.* (2013) also proposed that OCP3 may play a regulatory role on the RNA editing complex.

PPO1/PPOX1 contains a protoporphyrinogen oxidase (PPO) domain (Fig. 2*E*); it is a key enzyme in tetrapyrrole biosynthesis (Zhang *et al.* 2014). Surprisingly, loss-of-function mutations in the *PPO1* gene result in reduced editing at 18 plastid RNA editing sites (Table 2; Fig. 3*E*) (Zhang *et al.* 2014). In addition, yeast-two-hybrid assays revealed that PPO1 interacts with plastid RNA editing factors RIP1/MORF8, RIP2/MORF2, and RIP9/MORF9 (Table 4) (Zhang *et al.* 2014). Zhang *et al.* (2014) also found that a 22-amino acid N-terminal region in PPO1/PPOX1 is essential for its interaction with RIP/MORF proteins and its role in plastid RNA editing. Therefore, Zhang *et al.* (2014) proposed that PPO1/PPOX1 is required for plastid RNA editing by modulating the stability of RIP/MORF proteins in the editing complex.

Summary

Organelle RNA editing could be viewed as a mechanism of correction to compensate for defects or mutations in the haploid plastid and mitochondrial genomes of land plants. C-to-U deamination is the most common type of RNA editing in land plants. In mammals, C-to-U RNA editing is carried out by APOBEC–type cytidine deaminases (Xu and Messing 2006). Unlike APOBECs, the composition of the C-to-U RNA editing complex in land plant organelles varies extensively (Sun *et al.* 2016). At least four types of proteins have been found to be essential for plastid RNA

editing in land plants: PPRs, RIP/MORFs, ORRMs, and OZs. Interactions exist between PPRs and RIP/MORFs, PPRs and ORRMs, RIP/MORFs and ORRMs, RIP/MORFs and OZ1, as well as ORRMs and OZ1. The interactions among different types of plastid RNA editing factors suggest these factors may form RNA editing complexes within the plastid.

Interactions also exist within the same types of proteins. For example, plastid-targeted RIP/MORF proteins interact with each other and themselves, suggesting that RIP/MORF proteins may form homo- or heterooligomers in the RNA editing complex. Among the two plastid-targeted ORRM proteins, ORRM1 does not interact with ORRM6 or itself. By contrast, ORRM6 interacts with itself but not with ORRM1. Interactions among different PPRs are relatively understudied. To date, only one interaction has been experimentally demonstrated: the interaction between a partial PPR protein CRR4 and a PPR-related protein DYW1. The absence of extensive interaction among different PPR proteins is consistent with PPRs being site-specific RNA recognition factors.

Among these four types of RNA editing factors, PPRs and ORRMs contain RNA-binding domains and are capable of binding to RNA targets in a sequence-specific manner. It is not yet known whether OZs bind to RNA targets. However, RanBP2-type zinc-finger domain, the signature motif in OZ proteins, has been shown to bind to RNAs (Nguyen *et al.* 2011). Taken together, PPRs, ORRMs, and OZs may work together in recognizing RNA targets. Because RIP/MORF proteins do not contain any potential RNA-binding domain, RIP/MORF proteins probably do not play a role in recognizing RNA targets. Instead, this type of proteins might be essential in bridging other RNA editing factors together in the RNA editing complex. Cytidine deamination requires zinc ions as cofactors. Among the four types of RNA editing factors, PPR-E-DYW-type PPR proteins and OZs contain motifs for zinc coordination. OZ proteins may assist PPR-E-DYW-type PPR proteins during C-to-U RNA editing.

Three additional types of proteins have been reported to be required in plastid RNA editing: CP31A and CP31B, OCP3, and PPO1/PPOX1. These additional plastid RNA editing factors may play a regulatory or stabilizing role on RNA editing complexes. CP31A and CP31B each contain two RRM domains, suggesting that CP31A and CP31B may bind to RNA targets directly. OCP3 and PPO1/PPOX1 do not contain any RNA-binding domain; these two proteins probably do not bind to RNA targets directly. PPO1/PPOX1 interacts with plastid-targeted RIP/MORF proteins; they may regulate the stability of RIP/MORF proteins in the RNA editing complex.

Future perspectives and outstanding questions

Although PPR and PPR-related proteins are currently the prime candidate for C-to-U RNA deamination in land plant plastids and mitochondria, the C-to-U deamination activity of PPR and PPR-related proteins has not yet been demonstrated *in vitro*. OZ proteins are one of the newest members of C-to-U plant organelle RNA editing factors. Like PPR and PPR-related proteins, the exact function of OZ proteins could be investigated further. For instance, it is not yet known whether the zinc-finger domain of OZ proteins binds to zinc and RNAs. Many of the previously characterized C-to-U plant organelle RNA editing factors contain additional domains. It would be interesting to know whether these domains are dispensable. The composition of the C-to-U RNA editing complex at different plant organelle RNA editing sites varies. However, there must be a minimal requirement of necessary components for the editing complex to be functional. One of the biggest challenges thus far is to reconstitute the C-to-U RNA editing activity *in vitro*.

Taken together, a number of outstanding questions await to be resolved: (*1*) Does the DYW domain of PPR and PPR-related proteins actually possess deaminase activity? (*2*) Does the zinc-finger domain in OZ proteins have the ability to bind to RNAs? (*3*) What are the functions of the unknown domains or motifs in the five known types of plant organelle RNA editing factors? (*4*) What is the minimal composition and stoichiometry of a C-to-U plant organelle RNA editing complex? (*5*) Are there other types of RNA editing factors? (*6*) Can we reconstitute a C-to-U plant organelle RNA editing complex *in vitro* and demonstrate it editing activity?

Accession numbers

CDA1, At2g19570; AEF1, At3g22150; CLB19, At1g05750; CREF3, At3g14330; CREF7, At5g66520; CRR4, At2g45350; CRR21, At1g11290; CRR22, At1g11290; CRR28, At1g59720; DOT4, At4g18750; DYW1, At1g47580; ECB2/VAC1, At1g15510; ELI1, At4g37380; LPA66, At5g48910; OTP80, At5g59200; OTP81/QED1, At2g29760; OTP82, At2g29760; OTP84, At3g57430; OTP85, At2g02980; OTP86, At3g63370; RARE1, At5g13270; THA8, At3g27750; YS1, At3g22690; RIP1/MORF8, At3g15000); RIP2/MORF2, At2g33430; RIP3/MORF3, At3g06790; RIP4/MORF4, At5g44780; RIP5/MORF5, At1g32580; RIP6/MORF6, At2g35240; RIP7/MORF7, At1g72530; RIP8/MORF1, At4g20020; RIP9/MORF9, At1g11430; ORRM1, At3g20930; ORRM2, At5g54580; ORRM3, At5g61030; ORRM4, At1g74230; ORRM5, At4g13850; ORRM6, At1g73530; OZ1, At5g17790; OZ2, At1g55040; OZ3, At1g70650; OZ4, At1g48570; CP31A, At4g24770; CP31B, At5g50250; OCP3, At5g11270; PPO1/PPOX1, At4g01690.

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