RESEARCH PAPER

RNA Biology 10:9, 1520–1525; September 2013; © 2013 Landes Bioscience

Quantitative trait locus mapping identifies REME2, a PPR-DYW protein required for editing of specific C targets in Arabidopsis mitochondria

Stéphane Bentolila*, Arianne M Babina†, Arnaud Germain, and Maureen R Hanson

Department of Molecular Biology and Genetics; Cornell University; Ithaca, NY USA

†Current affiliation: Biology Department; Boston College; Chestnut Hill, MA USA

Keywords: plant mitochondria, QTL mapping, virus-induced gene silencing, RPS3, RPS4

Targeted RNA editing by C-to-U alteration occurs at hundreds of sites in the mitochondrial transcriptome of flowering plants. By using natural variation and positional cloning on a population of Arabidopsis recombinant inbred lines between the ecotypes Col and L*er*, we found that two of these occurrences involve the Arabidopsis PPR-DYW protein REME2 (*Required for Efficiency of Mitochondrial Editing2*). The analysis of a knockdown mutant along with silenced tissues confirms the specificity of REME2 for both sites located in mitochondrial ribosomal protein genes (*rps3*-1534 and *rps4*-175). The conservation level of both *cis* elements is relatively high, as is the amino acid conservation among flowering plants for both genes in that location, underlining the importance of these editing events and REME2.

Introduction

Nuclear-encoded proteins control the efficiency of RNA editing in plant organelles. In Arabidopsis mitochondria, particular members of the RIP family of RNA editing factors[1] (also named MORF proteins[2]) affect editing of many individual Cs. In addition, members of the PLS subclass of the large family of pentatricopeptide repeat (PPR) proteins are site-specific factors, each of which is responsible for determining the C to be edited at a small number of targets. A recent review described 12 different mitochondrial PPR proteins required for editing of 21 different C targets.[3] The number of mitochondrial C targets controlled by these PPR proteins is likely underestimated, given that the entire RNA editome was not analyzed in some of the reports. With over 500 mitochondrial C targets in Arabidopsis,[4,5] most PPR proteins are likely to control editing at multiple locations.

In order to identify nuclear-encoded RNA editing factors, we pioneered the use of natural variation between Arabidopsis ecotypes for mapping of quantitative trait loci (QTLs) affecting editing.[5,6] These loci provide an unbiased means to search for editing factors, as genes encoding members of protein families not previously known to be editing factors can potentially be identified. Furthermore, genes can be identified even though knockout mutations would cause lethality due to the essential functions of many plant mitochondrial proteins. QTL mapping with 30 recombinant inbred lines, progeny of a cross between the two parental accessions Col and L*er*, detected 12 major QTLs for 11 of the 13 editing traits analyzed.[5] Positional cloning of one of the QTLs already identified *REME1* (*Required for Efficiency of Mitochondrial Editing1*) unambiguously as the gene controlling editing of *nad2*-558 and *mttb*-552.[7] *REME1* encodes a PPR-DYW protein that is predicted to be targeted to mitochondria by both Predotar[8] and TargetP[9] and, thus, is an example of a member of this subclass of PPR protein implicated in mitochondrial editing.[10-12] Here we report on the use of QTL mapping for identification of REME2, another PPR-DYW protein involved in the editing of two other mitochondrial sites, *rps3*-1534 and *rps4*-175.

Results and Discussion

Identification of *REME2* as putative editing QTL controlling the editing of *rp3*-1534 and *rps4*-175. Two non-silent sites, *rps3*-1534 and *rps4*-175, show a difference in editing extent between Col and L*er*, two widely used Arabidopsis accessions (**Fig. 1A**); in addition, the level of editing extent for both sites was very highly correlated among the parental accessions and the recombinant inbred lines (RILs[5]), suggesting that both traits could be controlled by the same factor. This prediction was further supported by the co-localization of both QTL controlling these two editing traits in the same interval on chromosome 4 between markers 7900K and 9500K (**Fig. 1B**). Assays of editing extent for *rps3*-1534 and *rps4*-175 in three additional RILs exhibiting recombination in the QTL interval further delineated the area comprising the QTL between 8810K and 9160K (**Fig. 1C**). This genomic area contains 105 genes (http://www.ncbi.nlm.nih.gov/projects/mapview/maps.

*Correspondence to: Stéphane Bentolila; Email: sb46@cornell.edu
Submitted: 03/29/2013; Revised: 06/03/2013; Accepted: 06/06/2013
http://dx.doi.org/10.4161/rna.25297

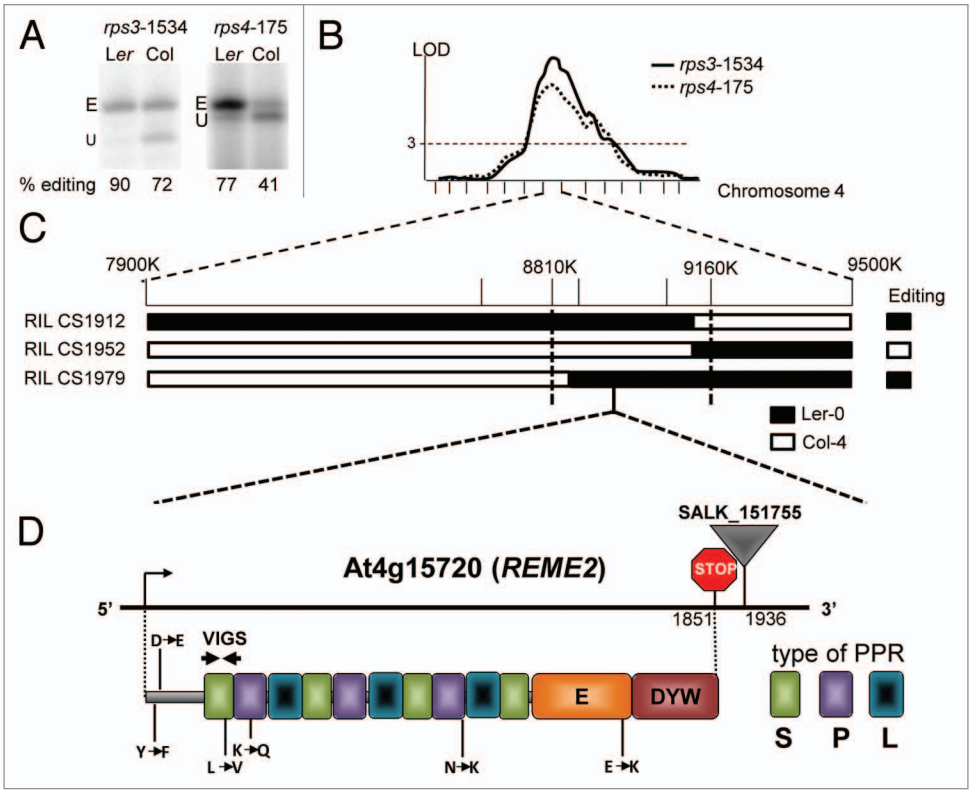

Figure 1. Mapping strategy used to identify the PPR-DYW protein encoded by At4g15720 as a possible mitochondrial editing factor. (**A**) Two Arabidopsis accessions, L*er* and Col, exhibit a differential editing extent at two mitochondrial sites, *rps3*-1534 and *rps4*-175. Poisoned Primer Extension (PPE) gels show a more intense unedited (U) band for Col than for L*er* at the two sites assayed. The percentage of editing [edited (E)/edited (E) + unedited (U)] is given below each lane. (**B**) QTL mapping identifies an editing QTL that controls the editing of *rps3*-1534 and *rps4*-175 on chromosome 4 in the interval between markers 7900K and 9500K. The curves for the likelihood odds ratio (LOD) scores, a statistic used to detect a QTL, are very similar for the two editing traits analyzed; in particular the maximum LOD scores, above the significance threshold of 3, are located in the same interval, 7900K-9500K, for both *rps3*-1524 and *rps4*-175. (**C**) Three recombinant inbred lines (RILs) that have recombined genomes in the QTL interval allow the further localization of the editing factor between 8810K and 9160K. (**D**) Among the 105 genes lying in the 8810K-9160K interval, only one, At4g15720, encodes a PPR protein which belongs to the DYW subclass. The localization of the T-DNA used in this study is shown on the gene model in the 3'UTR, 85 nucleotides downstream of the stop codon. The modular structure of the predicted protein encoded by At4g15720 with the type of PPR (P, S and L) symbolized by squares, is represented below the gene model. The 190-nucleotide region of At4g15720 RNA targeted by VIGS is delimited by facing arrows. The six amino acids substitutions occurring between REME2 encoded by Col and L*er* are indicated (Col → L*er*).

cgi?TAXID=3702&CHR=4&MAPS=cntg-r,clone,tair_marker,genes[8810000.00%3A9160000.00]&CMD=TXT#1), among which only one, At4g15720, encodes a PPR-containing gene belonging to the DYW subclass (**Fig. 1D**). Because of the precedence for PPR-DYW proteins to be editing *trans*-factors, we decided to first test At4g15720 as a potential candidate editing factor, instead of carrying out more precise mapping of the QTL location.

Knockdown of At4g15720 both by T-DNA insertion and silencing confirms its role as an editing factor. We looked for an insertional mutation in At4g15720 in collections of T-DNA mutants. Among several T-DNA insertional mutants present in the SALK T-DNA express browser (signal.salk.edu/cgi-bin/tdnaexpress), only one, SALK_151755, was reported to be located in the coding sequence and to be homozygous. Both these statements were erroneous: SALK_151755 is a segregating population comprising the three possible genotypic classes: homozygous wild-type, heterozygous and homozygous mutants. Sequencing of the insertion amplified from a homozygous mutant revealed that it is located 85 nucleotides from the stop codon, in the 3'UTR as depicted in **Figure 1D**. Expression of At4g15720 is downregulated in the homozygous mutant compared with the wild-type as demonstrated by semi-quantitative RT-PCR (data not shown). Like most genes encoding PPR-PLS proteins, At4g15720 is expressed at low levels in most tissues.[13]

Assays of editing extent in the SALK_151755 population showed a very significant reduction of editing extent for *rps3*-1534 in homozygous mutants relative to the wild-type, on average 63% vs. 86%, respectively (**Fig. 2A**). Because a second independent T-DNA mutant was not available in the coding sequence of At4g15720, we tested the effect of silencing At4g15720 on the editing extent of *rps3*-1534. Virus-induced gene silencing (VIGS) of At4g15720 resulted in a significant decrease of editing extent for *rps3*-1534 in silenced plants compared with the uninoculated plants, 49% vs. 77%, respectively (**Fig. 2C**). Analysis of both the T-DNA mutant and the silenced plants demonstrate that At4g15720 encodes the editing *trans*-factor promoting the editing extent of *rps3*-1534; we therefore named this gene *REME2*. Although Predotar and

TargetP did not predict an organelle localization of REME2, we observed that 100 amino acids of the N-terminus of REME2 could target GFP to mitochondria in onion epidermal cells (**Fig. 3**).

The editing extent of *rps4*-175 in the T-DNA mutant and in silenced plants was significantly decreased in both types of plants when compared with either the wild-type or the uninoculated controls (**Fig. 2B and D**). REME2 therefore controls the editing of both mitochondrial sites *rps3*-1534 and *rps4*-175.

The effect of REME2 on the editing extent of *rps3*-1534 and *rps4*-175 is specific as other mitochondrial sites do not show reduction of their editing extent in the *reme2* mutant when compared with the wild-type (**Fig. 2E and F**).

The putative *cis* elements of *rp3*-1534 and *rps4*-175 show high similarity. The current model of C recognition for editing in plant organelles proposes that a *cis* element that is mostly upstream of the targeted C is recognized through binding of a PPR *trans*-factor. Binding of a PPR protein factor to the *cis* element has been demonstrated in vitro for several sites[14-16] and a code for recognition between the RNA target sequence and the PPRs has been elucidated.[17,18] We checked for sequence similarity in the putative *cis* element around the two sites (–20 to +5) under the control of REME2 (**Fig. 4A**). A majority of the nucleotides, 64% (16/25), are conserved between the *cis* elements surrounding the two sites, thus supporting the model of recognition of these similar sequences by REME2. Compared with other putative *cis* elements recognized by the same PPR *trans*-factor, the level of nucleotide conservation observed between *rps3*-1534 and *rps4*-175 is relatively high; for example, the nucleotide conservation ranges from 44–64% in the putative *cis* elements recognized by SLO1, REME1 and MEF7 (**Fig. 4A**).[7,19,20]

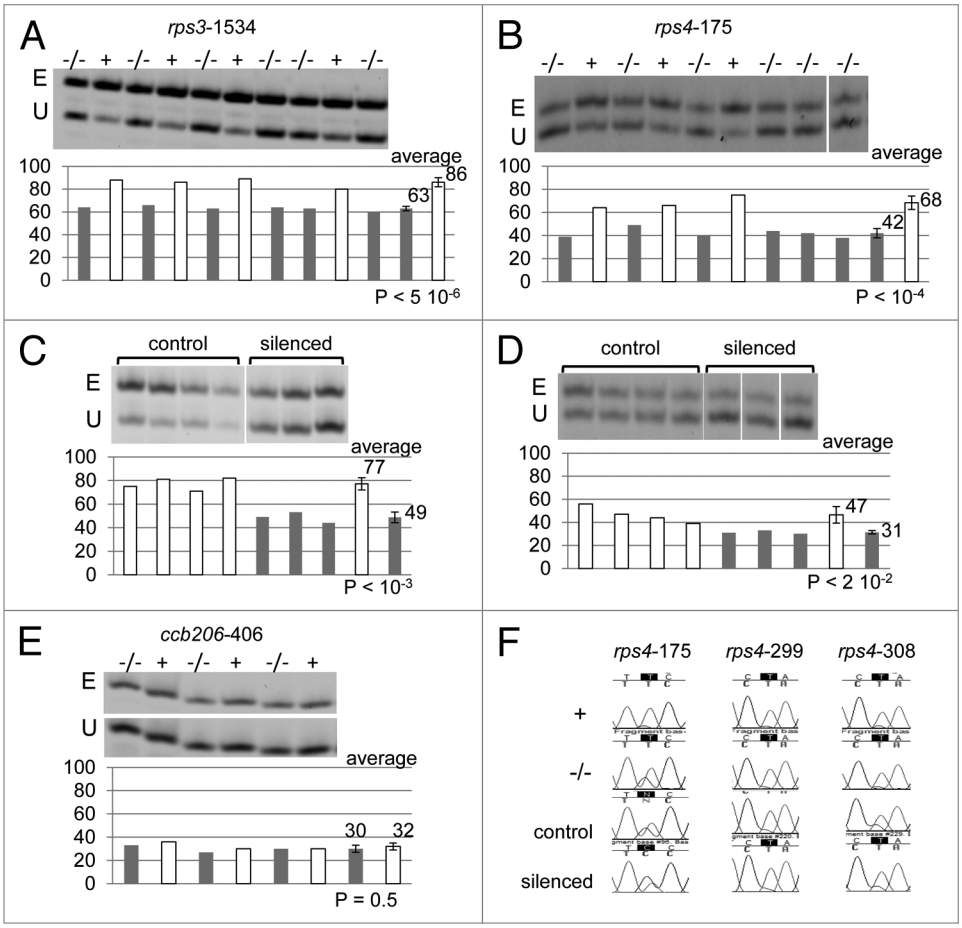

Figure 2. Analysis of *rps3*-1534 and *rps4*-175 editing extent in a mutant and in tissue silenced for *REME2* demonstrate a specific control of these two sites by REME2. A T-DNA insertional mutant in the *REME2* 3'UTR exhibits a significant reduction of editing extent for *rps3*-1534 (**A**) and *rps4*-175 (**B**). Upper panels, PPE products separated on an acrylamide gel; E, edited; U, unedited; –/–, T-DNA mutant; +, wild-type. Below, the PPE gels are shown the quantification of editing extent based on the intensity of the E and U bands. Each bar corresponds to the editing extent based on the products of the lane directly above: gray bar, mutant; white bar, wild-type. On the right of the graphs are shown the averages with error bars (s.d.). Silencing of *REME2* by VIGS results in a significant decrease of editing extent of *rps3*-1534 (**C**) and *rps4*-175 (**D**) in silenced plants. Upper, PPE gel; E, edited; U, unedited; control, uninoculated plants; silenced, *REME2*-silenced plants. As for (**A and B**), the graphs below the PPE gel show the quantification of editing extent corresponding to each lane: gray bar, silenced; white bar, control. Analysis of sites not controlled by REME2 does not reveal any reduction of their editing extent in *reme2* or in the *REME2*-silenced plant. (**E**) PPE gel shows a similar editing extent in *reme2* and the wild-type for *ccb206*-406. (**F**) RT-PCR bulk sequencing demonstrates the specificity of REME2 on *rps4*-175 editing extent as other sites on the same transcript do not exhibit any difference between the wild-type (+) and *reme2* mutant (–/–) or between the control and the silenced plant.

Adopting the same model as Barkan et al.[17] to predict the nucleotides on the RNA target binding to REME2, we found that the nucleotides in the putative *cis* element of *rps3*-1534 fit the model perfectly (**Fig. 4B**). By contrast, two of the guanines found in the putative *cis* element of *rps4*-175 are not the expected nucleotides based on the recognition code. The discrepancy between the observed nucleotides in *rps4*-175 and the nucleotides expected to bind REME2 might explain the reduced editing extent of *rps4*-175 in both Col and L*er* when compared with *rps3*-1534 (**Fig. 1A**).

SNPs in the Col and L*er* alleles of *REME2* might explain the differential editing extent of *rps3*-1534 and *rps4*-175 in the two accessions. The L*er* REME2 allele was retrieved by using the genome browser (gbrowse.cbio.mskcc.org/gb/gbrowse/thaliana-19magic) representing the database from sequencing the genomes and transcriptomes of 18 natural *A. thaliana* accessions.[21] Among the 12 SNPs found in the coding sequence of *REME2* between

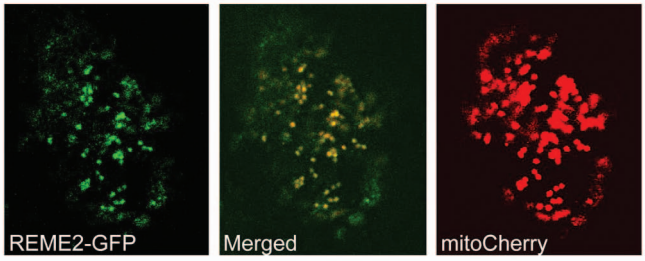

Figure 3. REME2 is targeted to mitochondria. The left panel shows the subcellular localization in onion epidermal cells of REME2 fused to GFP while the right panel is the mitoCherry fluorescent marker localized to the mitochondria. The middle panel is a merged figure showing co-localization of both fluorescent markers.

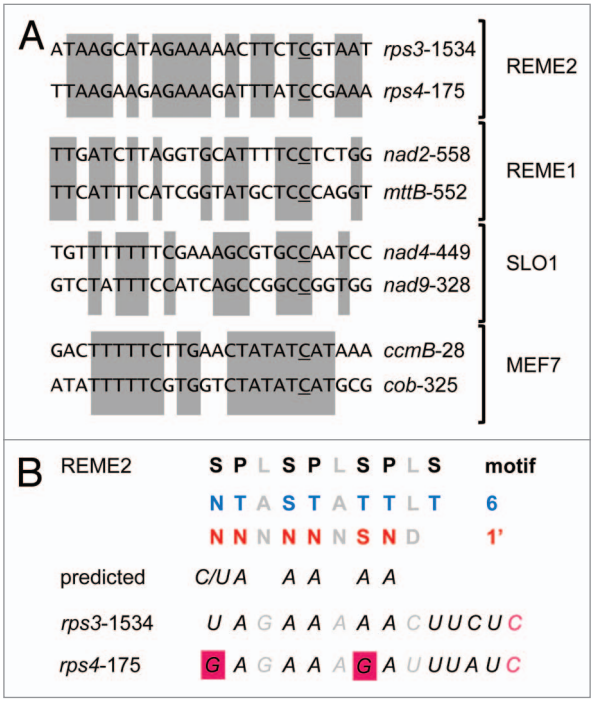

Figure 4. Analysis of the putative *cis* elements of the RNA targets recognized by REME2. (**A**) Nucleotide alignment of the putative *cis* element (–20 +5) of the two editing sites under the control of REME2, and those of sites under the control of three other PPR *trans*-factors. The target C for editing is underlined. Conserved nucleotides are highlighted in gray. The names of editing sites (gene-position) are given on the right of each sequence, along with the corresponding PPR *trans*-factors. (**B**) Predicted RNA nucleotides binding to REME2 according to the recognition code.[17] The first row shows the modular structure of REME2 in relation to the nature of the PPR motifs, S, P, or L. Row 6 indicates the identity of the residues found at position 6 of each PPR motif, while row 1' shows the residue found at position 1 of the next PPR motif. The expected nucleotides binding to REME2 are predicted based on the identities of the residues at positions 6 and 1' in PPR motifs S and P only. The targeted C for editing is shown in magenta and nucleotides in the *rp4*-175 putative *cis* element that do not fit the recognition model are highlighted by a magenta box.

Table 1. SNPs found in REME2 encoded by Col and Ler alleles

Nucleotide position	Col→*Ler* nucleotide	Col→*Ler* amino acid	PPR motif	Residue position
65	A→T	Y→F		
72	C→T	silent		
75	C→G	D→E		
225	C→T	silent		
255	A→G	silent		
256	C→G	L→V	S	24
328	A→C	K→Q	P	17
471	G→T	silent		
582	T→C	silent		
1002	T→G	N→K		
1335	C→A	silent	P	33
1510	G→A	E→K	E	

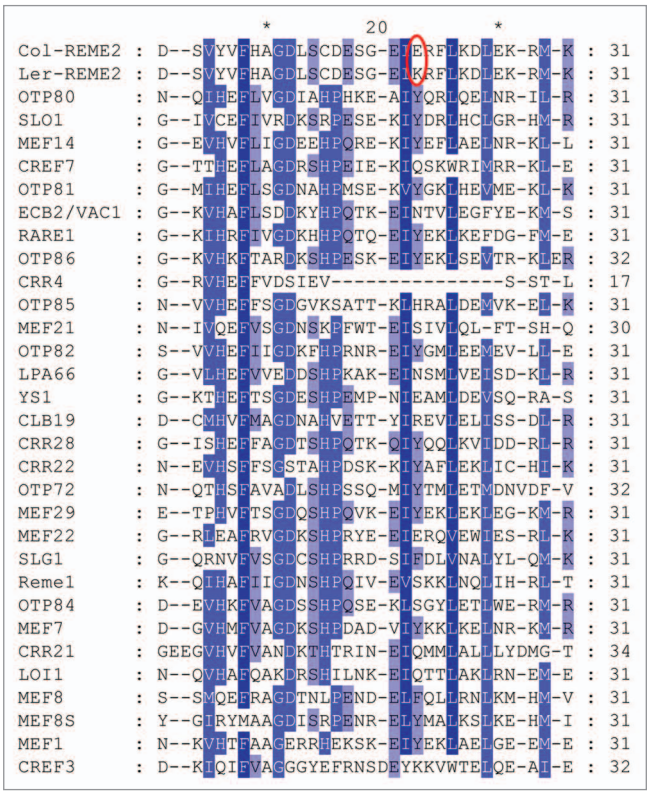

Figure 5. Alignment of the E+ motifs found in organelle editing *trans* factors. The amino-acid substitution between the Col and *Ler* REME2 proteins is circled. All known PPR protein editing factors containing an E+ domain are aligned.

Col and *Ler*, six result in non-synonymous codons, thus causing a change in the encoded amino acid (**Table 1**). Three of the SNPs are in PPR motifs that are predicted to be important for recognition of nucleotides in the *cis*-elements (**Fig. 4B**). However, none of the amino-acid substitutions between Col and *Ler* occur in a residue position that has been shown to be important for RNA recognition, namely positions 1 or 6 in S or P motifs (**Table 1**). It is

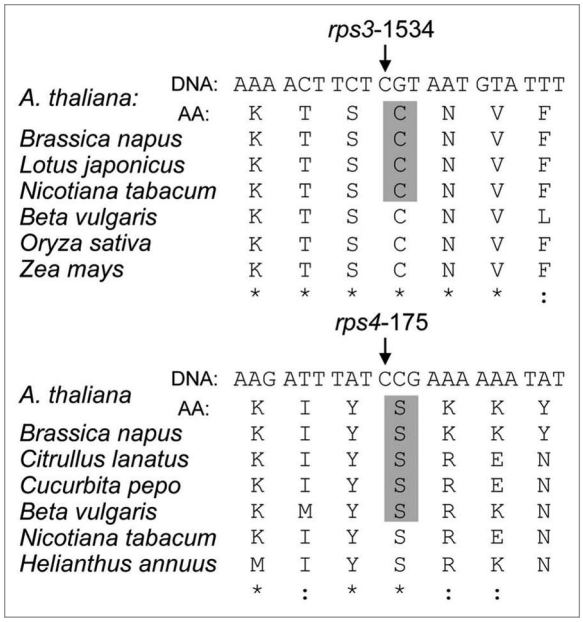

Figure 6. Amino acid conservation between flowering species for the region around *rps3*-1534 and *rps4*-175. The amino acid C or S for *rps3*-1534 and *rps4*-175, respectively, resulting from editing of C targets by REME2, are highlighted in gray. The corresponding amino acid from species where editing is not necessary because the T is genomically encoded are not highlighted. An arrow indicates the position of the edited C in both transcripts for the Arabidopsis DNA sequence displayed above the amino acid alignment.

still possible that some amino acids differing between Col and L*er* are important for the folding of the REME2 protein and result in the L*er* version being more efficient in recognizing the RNA or the assembly of the editosome. One difference between accessions is in an amino acid of the E motif, which is essential for RNA editing, and has been proposed to recruit the editing enzyme.[22] However, Glu is present in Col vs. Lys in L*er*; not only do these amino acids have similar properties, but the amino acid difference also occurs at a position in the E motif that is not strongly conserved (**Fig. 5**). This difference is therefore unlikely to account for the more efficient editing *trans* factor activity of the L*er* protein.

Alternatively, the amount of the editing *trans* factor might influence the level of editing extent, especially given the generally low level of PPR expression. We have previously shown that overexpression of the high editing Col *REME1* allele in a L*er* background can result in a level of editing extent of its targets that is higher than in Col.[7] Numerous SNPs and three indels in the *REME2* promoter region (1 kb upstream the start codon) might result in the L*er* *REME2* being more expressed than the Col allele. Indeed, the amount of RNA-seq reads matching *REME2* is higher for L*er* than for Col (gbrowse.cbio.mskcc.org/gb/gbrowse/thaliana-19magic).

A functional REME2 is likely to be essential to plant development. We were unable to find a null mutant for *REME2* in any of the mutant collections we searched. The mutant we characterized carries the T-DNA insertion in the 3'UTR, resulting in the knockdown of *REME2* expression. This finding suggests that REME2 is essential to plant development and that homozygous mutants in the coding sequence of *REME2* are not viable. Disruption of either RPS3 or RPS4 could potentially affect ribosome function and mitochondrial translation. We examined other flowering plants for which editing data are available in order to find out whether the two sites under REME2 control are also edited. In all such species, if a C is genomically encoded, the transcript undergoes editing, thus restoring the amino acid that is encoded by species carrying a T in their DNA sequences (**Fig. 6**). Editing at *rps3*-1354 and *rps4*-175 converts a codon for arginine to a cysteine codon and a codon for proline to a serine codon respectively, both non-conservative changes. Taken together, these facts indicate that editing under the control of REME2 is likely to be important for the proper function of both RPS3 and RPS4.

Materials and Methods

Plant material and preparation of nucleic acids. Seeds of Arabidopsis (*A. thaliana*) accession Col-4, Ler-0, and the RILs (CS1899) were obtained from the Arabidopsis Biological Resource Center (ABRC) (www.biosci.ohio-state.edu/:plantbio/Facilities/abrc/abrchome.htm). Plants were grown under conditions and nucleic acids were prepared as previously described.[5]

CAPs markers. The development of the CAPs markers, 8810K and 9160K to genotype the RILs was done by amplifying the DNA with primers resulting in amplicons of different length depending on the parental allele, Col or L*er*.

8810K-F1 = 5'-CGTAGATTCC AGGACAATAG AGAT-3'
8810K-R1 = 5'-TACCAGTTGG TATATGCCAC TATC-3'
9160K-F1 = 5'-CCACATCTCT AAGCTCCTCA TACG-3'
9160K-R1 = 5'-TAAGCGATAT GACTGGACCT GAAT-3'

Intracellular localization of REME2. The first 100 codons of REME2 were cloned and transferred into pEarleygate 103 before being transiently introduced into onion (*Allium cepa*) epidermal cells as previously described.[7]

Measurement of editing extent and VIGS. Measurement of editing extent by PPE and the primers used for *rps3*-1534 and *rps4*-175 were done as previously described.[5] The protocol and materials used for VIGS are detailed by Robbins et al.[23] The silencing fragment for REME2 was designed by the CATMA database[24] and amplified by PCR using the following gene sequence tag primers:

REME2-F1: 5'-CGACTTCTTT CATCTCAAAA CC-3'
REME2-R1:5'-AAACAGTTTG CGTGCAGT-3'

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Huijun Yang and Amirali Sattarzadeh for assistance with transient expression and imaging of REME2-GFP.

Financial support

This work was supported by the National Science Foundation, from the Molecular and Cellular Biosciences, Gene and Genome Systems program, grants MCB-1020636 (to SB) and MCB-0929423 (to MRH).

References

1. Bentolila S, Heller WP, Sun T, Babina AM, Friso G, van Wijk KJ, et al. RIP1, a member of an Arabidopsis protein family, interacts with the protein RARE1 and broadly affects RNA editing. Proc Natl Acad Sci USA 2012; 109:E1453-61; PMID:22566615; http://dx.doi.org/10.1073/pnas.1121465109
2. Takenaka M, Zehrmann A, Verbitskiy D, Kugelmann M, Härtel B, Brennicke A. Multiple organellar RNA editing factor (MORF) family proteins are required for RNA editing in mitochondria and plastids of plants. Proc Natl Acad Sci USA 2012; 109:5104-9; PMID:22411807; http://dx.doi.org/10.1073/pnas.1202452109
3. Gutmann B, Gobert A, Giege P, Marechal-Drouard L, ed. *Mitochondrial Genome Evolution and the Emergence of PPR Proteins.* Advances in Botanical Research: Academic press; 2012:253-313.
4. Giegé P, Brennicke A. RNA editing in Arabidopsis mitochondria effects 441 C to U changes in ORFs. Proc Natl Acad Sci USA 1999; 96:15324-9; PMID:10611383; http://dx.doi.org/10.1073/pnas.96.26.15324
5. Bentolila S, Elliott LE, Hanson MR. Genetic architecture of mitochondrial editing in *Arabidopsis thaliana.* Genetics 2008; 178:1693-708; PMID:17565941; http://dx.doi.org/10.1534/genetics.107.073585
6. Bentolila S, Chateigner-Boutin AL, Hanson MR. Ecotype allelic variation in C-to-U editing extent of a mitochondrial transcript identifies RNA-editing quantitative trait loci in Arabidopsis. Plant Physiol 2005; 139:2006-16; PMID:16286447; http://dx.doi.org/10.1104/pp.105.069013
7. Bentolila S, Knight WE, Hanson MR. Natural variation in Arabidopsis leads to the identification of REME1, a pentatricopeptide repeat-DYW protein controlling the editing of mitochondrial transcripts. Plant Physiol 2010; 154:1966-82; PMID:20974892; http://dx.doi.org/10.1104/pp.110.165969
8. Small I, Peeters N, Legeai F, Lurin C. Predotar: A tool for rapidly screening proteomes for N-terminal targeting sequences. Proteomics 2004; 4:1581-90; PMID:15174128; http://dx.doi.org/10.1002/pmic.200300776
9. Emanuelsson O, Nielsen H, Brunak S, von Heijne G. Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. J Mol Biol 2000; 300:1005-16; PMID:10891285; http://dx.doi.org/10.1006/jmbi.2000.3903
10. Kim SR, Yang JI, Moon S, Ryu CH, An K, Kim KM, et al. Rice OGR1 encodes a pentatricopeptide repeat-DYW protein and is essential for RNA editing in mitochondria. Plant J 2009; 59:738-49; PMID:19453459; http://dx.doi.org/10.1111/j.1365-313X.2009.03909.x
11. Zehrmann A, Verbitskiy D, van der Merwe JA, Brennicke A, Takenaka M. A DYW domain-containing pentatricopeptide repeat protein is required for RNA editing at multiple sites in mitochondria of *Arabidopsis thaliana.* Plant Cell 2009; 21:558-67; PMID:19252080; http://dx.doi.org/10.1105/tpc.108.064535
12. Verbitskiy D, Zehrmann A, van der Merwe JA, Brennicke A, Takenaka M. The PPR protein encoded by the LOVASTATIN INSENSITIVE 1 gene is involved in RNA editing at three sites in mitochondria of *Arabidopsis thaliana.* Plant J 2010; 61:446-55; PMID:19919573; http://dx.doi.org/10.1111/j.1365-313X.2009.04076.x
13. Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, et al. Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. Adv Bioinformatics 2008; 2008:420747; PMID:19956698; http://dx.doi.org/10.1155/2008/420747
14. Okuda K, Nakamura T, Sugita M, Shimizu T, Shikanai T. A pentatricopeptide repeat protein is a site recognition factor in chloroplast RNA editing. J Biol Chem 2006; 281:37661-7; PMID:17015439; http://dx.doi.org/10.1074/jbc.M608184200
15. Hammani K, des Francs-Small CC, Takenaka M, Tanz SK, Okuda K, Shikanai T, et al. The pentatricopeptide repeat protein OTP87 is essential for RNA editing of *nad7* and *atp1* transcripts in Arabidopsis mitochondria. J Biol Chem 2011; 286:21361-71; PMID:21504904; http://dx.doi.org/10.1074/jbc.M111.230516
16. Tasaki E, Hattori M, Sugita M. The moss pentatricopeptide repeat protein with a DYW domain is responsible for RNA editing of mitochondrial *ccmFc* transcript. Plant J 2010; 62:560-70; PMID:20163555; http://dx.doi.org/10.1111/j.1365-313X.2010.04175.x
17. Barkan A, Rojas M, Fujii S, Yap A, Chong YS, Bond CS, et al. A combinatorial amino acid code for RNA recognition by pentatricopeptide repeat proteins. PLoS Genet 2012; 8:e1002910; PMID:22916040; http://dx.doi.org/10.1371/journal.pgen.1002910
18. Yagi Y, Hayashi S, Kobayashi K, Hirayama T, Nakamura T. Elucidation of the RNA recognition code for pentatricopeptide repeat proteins involved in organelle RNA editing in plants. PLoS One 2013; 8:e57286; PMID:23472078; http://dx.doi.org/10.1371/journal.pone.0057286
19. Sung TY, Tseng CC, Hsieh MH. The SLO1 PPR protein is required for RNA editing at multiple sites with similar upstream sequences in Arabidopsis mitochondria. Plant J 2010; 63:499-511; PMID:20497377; http://dx.doi.org/10.1111/j.1365-313X.2010.04258.x
20. Zehrmann A, van der Merwe J, Verbitskiy D, Härtel B, Brennicke A, Takenaka M. The DYW-class PPR protein MEF7 is required for RNA editing at four sites in mitochondria of *Arabidopsis thaliana.* RNA Biol 2012; 9:155-61; PMID:22258224; http://dx.doi.org/10.4161/rna.18644
21. Gan X, Stegle O, Behr J, Steffen JG, Drewe P, Hildebrand KL, et al. Multiple reference genomes and transcriptomes for *Arabidopsis thaliana.* Nature 2011; 477:419-23; PMID:21874022; http://dx.doi.org/10.1038/nature10414
22. Shikanai T. Why do plants edit RNA in plant organelles? In: Bullerwell C, ed. Organelle genetics: evolution of organelle genomes and gene expression. Berlin; Heidelberg: Springer, 2012:381-397.
23. Robbins JC, Heller WP, Hanson MR. A comparative genomics approach identifies a PPR-DYW protein that is essential for C-to-U editing of the Arabidopsis chloroplast *accD* transcript. RNA 2009; 15:1142-53; PMID:19395655; http://dx.doi.org/10.1261/rna.1533909
24. Crowe ML, Serizet C, Thareau V, Aubourg S, Rouzé P, Hilson P, et al. CATMA: a complete Arabidopsis GST database. Nucleic Acids Res 2003; 31:156-8; PMID:12519971; http://dx.doi.org/10.1093/nar/gkg071