Correction

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Correction for “Multiple organellar RNA editing factor (MORF) family proteins are required for RNA editing in mitochondria and plastids of plants,” by Mizuki Takenaka, Anja Zehrmann, Danil Verbitskiy, Matthias Kugelmann, Barbara Härtel, and Axel Brennicke, which appeared in issue 13, March 27, 2012, of Proc Natl Acad Sci USA (109: 5104–5109; first published March 12, 2012; 10.1073/pnas.1202452109).

The authors note that Fig. 2 appeared incorrectly. The corrected Fig. 2 and its legend appear below.

Fig. 2. The MORF family of proteins contains nine genes and a potential pseudogene in A. thaliana. (A) The cladogram of similarities between the MORF proteins shows that the plastid editing factors MORF2 and MORF9 are rather distant from each other and more similar to the mitochondrial proteins MORF3 and MORF1, respectively. Predictions (marked mt or cp) and experimental data obtained by GFP-fusion protein localization (only MORF2) or proteomics MS data (marked with an asterisk) for the respective organellar locations are indicated. The MORF8 protein encoded by At3g15000 has been found in mitochondria in three independent assays. Proteins investigated here for their function are boxed. The conserved ~100-amino acids domain is shaded; the other sequences show much less conservation (SI Appendix, Fig. S2). The potential pseudogene (At1g53260) contains only the C-terminal part of this conserved region. (B) Exon structures of the MORF3, MORF4 and MORF6 genes are similar to the MORF1 locus and contribute similar fragments but differ in their C-terminal extensions. MORF3 is a mitochondrial editing factor involved at more than 40 sites. Locations of the T-DNA insertions in mutants morf3-1, morf4-1, and morf6-1 are shown. LB denotes the location of the left border of the T-DNA. (C) Numbers of editing sites affected by T-DNA insertions in the respective MORF genes. In mutants morf4-1 and morf6-1, only one noncoding site each shows somewhat reduced editing.
Multiple organellar RNA editing factor (MORF) family proteins are required for RNA editing in mitochondria and plastids of plants

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RNA editing in plastids and mitochondria of flowering plants changes hundreds of selected cytidines to uridines, mostly in coding regions of mRNAs. Specific sequences around the editing sites are presumably recognized by up to 200 pentatricopeptide repeat (PPR) proteins. The here identified family of multiple organellar RNA editing factor (MORF) proteins provides additional components of the RNA editing machinery in both plant organelles. Two MORF proteins are required for editing in plastids; at least two are essential for editing in mitochondria. The loss of a MORF protein abolishes or lowers editing at multiple sites, many of which are addressed individually by PPR proteins. In plastids, both MORF proteins are required for complete editing at almost all sites, suggesting a heterodimeric complex. In yeast two-hybrid and pull-down assays, MORF proteins can connect to form hetero- and homodimers. Furthermore, MORF proteins interact selectively with PPR proteins, establishing a more complex edistosome in plant organelles than previously thought.

In all flowering plants, RNA editing alters more than 400 cytidines to uridines in the mRNAs of mitochondria and converts 30–40 cytidines in plastids (1, 2). In Lycopodiaceae, more than a thousand nucleotide identities in mitochondria and several hundred in plastids are changed (3). This process was recognized about 20 y ago (4–6), but only in recent years have the first determinants involved in the recognition of specific editing sites been identified (7). In target RNAs, the crucial sequence parameters that determine a nucleotide to be edited were identified by transgenic, in vivo, or in organello assays to be similarly structured in the two organelles (8–10). These cis targets, located mostly 5–20 nucleotides 5′ of the target cytidine, are postulated to be recognized by specific trans-acting proteins of the 450 members strong pentatricopeptide repeat (PPR) protein family (11–13).

Roughly 30 individual PPR proteins have been assigned to one or several targets by connecting a dysfunctional gene with the loss of RNA editing at specific sites (14, 15). These proteins, which are essential for processing of single or very few RNA editing sites, belong to a subgroup within the PPR family characterized by their patterns of repeats and C-terminal extensions. Some are extended by only an extension (E) domain; others contain an additional conserved region terminating with the name-giving amino acids DYW. This subgroup can supply up to 200 proteins for editing at specific sites, providing an explanation of how the numerous RNA editing sites in flowering plant mitochondria and plastids can be specifically addressed (12, 13). For the enzymatic reaction of converting a cytidine to a uridine, a deaminating activity is required. Because a separate enzyme has not been identified so far, it was proposed that possibly one of the additional C-terminal domains directly contributes the enzymatic activity, in cis when present and in trans through heterodimer formation (16, 17). We now find that an entirely unexpected class of proteins constitutes an additional, essential component of the plant organellar edistosome and is required for processing of almost all editing sites in plastids and of at least many sites in mitochondria.

Results

Mutation of MORF1 Affects Numerous Editing Sites in Plant Mitochondria. Our forward genetic screen of an ethylmethanesulfonate (EMS)-mutated population of Arabidopsis thaliana ecotype Columbia (Col) plants with a multiplexed single-nucleotide extension protocol yielded a number of mutant plants that have lost detectable editing at specific sites (18). The mutations were mapped and the nuclear encoded genes identified several of the site-specific trans factors of the PPR family (19). One of the mutants, however, shows reduced RNA editing at more than 40 mitochondrial sites, very different from PPR proteins, with only one overlapping site (Fig. 1 A–D and SI Appendix, Table S1). The effect of the mutation is specific to RNA editing defects; other RNA-processing steps and RNA stability are not affected (SI Appendix, Fig. S1). Genomic mapping in a cross of wild-type ecotype Landsberg erecta (Ler) plants and the mutant ecotype Col plant narrowed the locus to a region where no PPR protein is encoded. Sequence analysis revealed an EMS-typical mutation in an unassigned reading frame, At4g20020 (Fig. 1B). To confirm this identification, protoplasts from the mutant plant were transfected with the wild-type gene. In these assays, editing at the target sites was increased but not fully restored. In mutant plants stably transformed with the intact Col gene under control of the 3S promoter, RNA editing was fully recovered at all affected sites (Fig. 1C). The complementation of the editing defects at the target sites confirms that indeed the right locus has been identified. This gene was named MORF1 because it encodes a multiple organellar RNA editing factor. Homozygous mutant plants with a T-DNA insertion in the MORF1 gene are not viable (morf1-1; Fig. 1D and E). This finding suggests that the EMS mutant (morf1-1) is a “soft” mutation, which only partially disables the function of the encoded MORF1 protein. Therefore, presumably further, essential editing sites are also targeted by the MORF1 protein, and/or those RNA editing sites that are still partially processed in the EMS mutant morf1-1 are vitally required. The residual level of editing in this EMS mutant is sufficient for the viability of the plant.

MORF3, Another Member of the MORF Family, Is Required for Different RNA Editing Sites in Mitochondria. The MORF1 gene belongs to a small family of nine genes and one potential pseudogene (Fig. 2A and SI Appendix, Fig. S2). Of the encoded proteins, four are predicted by Predotar to be targeted to plastids (MORF2, At1g53260, MORF8, and MORF9). In three different protocole analyses, however, fragments of MORF8 were identified in mitochondrial extracts, thus correcting the theoretical prediction. In one of these investigations, the MORF8 protein as well as MORF3 were found

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The protein MORF2 has been expressed in the same ecotype and analyzed by mass spectrometry. The MORF1 protein is required for RNA editing at multiple sites. (A) A clonal analysis of the T-DNA insertion site in the MORF1 gene. The location of the first exon of the MORF1 gene is indicated. LB denotes the location of the left border of the T-DNA. The darker shading in the MORF protein marks the conserved MORF box. (B) Stable transformation of morf1-1 mutant plants with wild-type Col seed shows editing reduced to different degrees. (C) The T-DNA insertion line morf1-1 is homozygous lethal; homozygous seed growth is aborted (arrows) in pods on a selfed heterozygous plant. (D) The T-DNA insertion line morf1-1 is homozygous lethal; homozygous seed growth is aborted (arrows) in pods on a selfed heterozygous plant. (E) Wild-type Col plants show the full seed set.

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Fig. 1. The MORF1 protein is required for RNA editing at multiple sites. (A) Sample sequences of the more than 40 editing sites affected in morf1-1 EMS mutant plants. The first five sites show editing reduced to different degrees. At the last site, editing increases in the mutant in comparison with wild-type plants of Arabidopsis thaliana ecotype Columbia. (B) Structure of the MORF1 gene and the MORF1 protein. The location of the morf1-1 single-nucleotide alteration changing a proline to a serine codon and the T-DNA insertion site in morf1-1 are indicated. LB denotes the location of the left border of the T-DNA. The darker shading in the MORF protein marks the conserved MORF domain. (C) Stable transformation of morf1-1 mutant plants with the wild-type Col gene under control of a 35S promoter complements the editing defects. (D) The T-DNA insertion line morf1-1 is homozygous lethal; homozygous seed growth is aborted (arrows) in pods on a selfed heterozygous plant. (E) Wild-type Col plants show the full seed set.

Among mitochondrial proteins with affinity to cobalt ions (20), Genomic locus At1g53260 encodes a protein in which the first half of the otherwise conserved central domain of 100 amino acids (the MORF box) is missing and which is therefore likely not to be functionally competent (SI Appendix, Fig. S2). These findings leave MORF2 and MORF9 as functional plastid proteins and assign the seven other MORFs to mitochondria (MORF1 and MORF3–8).

We next investigated whether a second protein predicted for and found in a mitochondrial location, MORF3, is, like MORF1, involved in RNA editing. A T-DNA insertion in the first exon presumably disables the MORF3 gene in a mutant plant line (Fig. 2B). Unlike mutant morf1-2, this T-DNA line, morf3-1, is viable as a homozygous plant. morf3-1 plants grow a bit more slowly than wild-type plants but otherwise display no detectably altered morphological phenotype in the greenhouse. The analysis of about 400 mitochondrial editing sites showed that multiple sites are affected by the loss of MORF3. These are almost all different from the sites affected in morf1-1 (SI Appendix, Table S1).

Analogue investigation of RNA editing in homozygous mutant lines of MORF4 and MORF6 showed diminished editing levels at only one site each (Fig. 2B and C). These sites are silent; that is, they do not alter the encoded amino acids and are also in wild-type plants variably edited in different tissues of the plant. This finding does not exclude the participation of MORF4 and MORF6 in further editing events. Because MORF4 is similar to MORF1, and MORF6 is very similar to the MORF5 protein (Fig. 2A), the related proteins can potentially substitute for each other at some of their targets. Such functional substitutions seem to occur also in a number of mitochondrial PPR proteins at sites where editing in a knockout mutant of a given PPR gene is partially maintained, presumably by another specificity factor (21).

Mutants of Either MORF2 or MORF9 AreAffected at Almost All RNA Editing Sites in Chloroplasts. The protein MORF2 has been experimentally verified to be targeted to the plastid by in vitro import assays (22, 23), and dedicated proteomics analyses detected peptides of MORF2 and MORF9 in plastid proteins (24). Mutation of the MORF2 gene has been reported to influence mRNA and rRNA accumulation in chloroplasts of Arabidopsis with a phenotype similar to an apparent ortholog in Antirrhinum.
majus (22, 23). These genes have been tentatively assigned DAG (differentiation and greening) in Antirrhinum and DAG-like (DAL) in Arabidopsis (22, 23, 25).

To investigate whether the severe plastid developmental phenotypes of the dag and dal and of the morf2 mutants are caused by deficiencies in plastid RNA editing and to see whether a mutation of MORF9 also affects growth through RNA editing, we selected the respective T-DNA insertion lines (Fig. 3A) and analyzed the homozygous plants morf2-1 and morf9-1 (Fig. 3B and C–E). The morf2-1 mutant has severe problems in chloroplast development similar to the allelic dag and dal mutants. The morf2-1 mutant plants stay white, showing no sign of chlorophyll synthesis (Fig. 3B). The morf9-1 mutant also exhibits defects in greening in light, but with features distinct from the morf2-1 phenotype. When grown on sugar-supplying agar medium, the cotyledons of morf9-1 are uniformly green, whereas the first true leaves are white. In these, scattered green flecks arise occasionally with advancing age. Subsequent leaves develop a variegated pattern with about 30% green islands, eventually sufficient to sustain autotrophic growth in soil (Fig. 3 D–F).

For the analysis of RNA editing, white leaves were harvested from morf2-1 and young white first true leaves were collected from morf9-1. From these samples, total cellular RNA was purified and the cDNA was investigated for all 34 plastid editing sites documented in Arabidopsis. Surprisingly, in both mutants, nearly all plastid editing sites are affected (Fig. 3G and SI Appendix, Table S2). At several sites editing is completely lost, most editing events are reduced by 10–70%, and some sites are less affected in each mutant. Some of the sites for which MORF2 is essential are different from those that canonically require MORF9, but several sites cannot be edited at all without either MORF. Both MORF2 and MORF9 are thus required for full editing at almost all plastid editing sites. At some sites the reduction in editing is rather small, at the level of experimental variation, that is, less than 10% reduction (SI Appendix, Table S2). These findings suggest that MORF2 and MORF9 act together at most editing sites in plastids and that at many sites one can compensate for (or substitute) the respective other factor, except at those sites that remain unedited when one MORF factor is missing. The most parsimonious and straightforward explanation is a direct interaction between the two proteins in a heterodimeric or—at some sites—a homodimeric configuration.

MORF Proteins Can Interact with Each Other. The Arabidopsis interactome database predicts MORF9 to interact with MORF6 (26), although MORF9 is plastid-located and MORF6 is a mitochondrial protein. To investigate the possibility of direct interactions between the MORF proteins, we cloned several of these into yeast two-hybrid bait and prey vectors and tested various combinations (Fig. 4A). Indeed, most MORF proteins are able to interact with others and also with themselves to form hetero- or homodimers. The physical formation of homodimers was also investigated by pull-down assays with MORF1 as bait (Fig. 5A). The bait MORF1 protein was able to retain prey MORF1 molecules, confirming the ability to form homodimers. Among the heterodimers observed in yeast cells, the interaction between the two plastid proteins MORF2 and MORF9 is particularly noteworthy. This observation supports their in vivo potential to act in a heterodimeric connection. Pull-down assays with MORF1 as bait confirmed the general ability of MORF proteins to form heterodimers; the MORF1 protein was able to retain prey MORF2 molecules, although much less effectively than prey MORF1 molecules in the homodimeric assays (Fig. 5A). This promiscuous interaction between the mitochondrial MORF1 and the plastid MORF2 is also observed in the yeast two-hybrid assay (Fig. 4A). Furthermore, in a screen of an Arabidopsis cDNA expression library in yeast with mitochondrial MORF1 as bait, several clones of the plastid-located MORF2 were identified. The various combinations of interactions that are observed between plastid MORFs and mitochondrial MORFs suggest a flexible interactive binding that allows different combinations of MORFs in a given organelle. The slightly discriminating interactions of the plastid MORF2 and MORF9 proteins may still result in specific homo- and heterodimers of these two proteins because they seem to be the only MORFs present in this organelle.

In the mitochondrial compartment, the more than 40 sites affected by mutation of MORF1 and the likewise at least 40 sites addressed by MORF3 show almost no overlap; 92 of 95 sites are uniquely targeted (SI Appendix, Table S1). This observation suggests that one or more of the other as yet unassigned potential MORF proteins will be required for the remaining fully edited sites and may supply the residual activity for the partially affected sites. Alternatively, MORF1 and MORF3 substitute for each other at the partially affected sites as well as at the unaffected sites; for example, MORF1 potentially supplies the residual editing activities still available in the MORF3 mutant. These substitutions could be governed by the allowed MORF–

![Fig. 3. MORF2 and MORF9 are required for RNA editing in plastid mRNAs.](Image 0x1 to 19x816)

![Fig. 4. MORF Proteins Can Interact with Each Other.](Image 43x241 to 288x499)
MORF and MEF proteins can physically interact in yeast two-hybrid assays. (A) MORF proteins interact with each other. Reciprocal assays with the MORFs in bait (pGBK7) or prey (pGADT7) vectors in a yeast two-hybrid analysis reveal that these proteins can interact with themselves in homodimers and with each other in heterodimers. The least specific appears to be the mitochondrial MORF1 protein, which can contact all other MORFs in either direction. Another mitochondrial protein, MORF3, forms strong heterodimers only with the likewise mitochondrial MORF1. The plastid proteins MORF2 and MORF9 interact with each other and with the mitochondrial MORF1, but only weakly with the mitochondrial MORF3. "Empty" is the control for autoactivation. ++ indicates a strong interaction; + represents fewer, slower-growing colonies formed; - indicates no colonies. (B) MORF and MEF proteins interact in yeast two-hybrid assays. Respective MEFs are indicated for each plate, and their protein structures are shown. The MORFs tested for binding are numbered in their respective quadrants. The mitochondrial editing protein MORF1, for example, interacts with the mitochondrial editing factors MEF1 (weakly), MEF9, and MEF21, but not with MEF11. These results show that principally MORF and MEF proteins interact rather unspecifically, as, for example, the binding of the plastid proteins MORF2 and MORF9 with the mitochondrial MORF1, MEF9, and MEF21 PPR proteins shows. However, some combinations are preferred, and others do not occur. No-growth quadrants show that there is no autoactivation. (C) The interactions between MEF and MORF proteins documented in B are interpreted as strong interactions (+++), weak (+), or no (-) binding. The results show that the two DYW-containing proteins, MEF1 and MEF11, interact weakly or not at all with the MORF proteins, whereas the MEF9 and MEF21 proteins, which terminate after the E domain and do not contain a DYW extension, connect more readily and promiscuously with MORF proteins in the yeast two-hybrid assays. The specific target site of MEF21 (cox2-257) also requires MORF1, and MEF21 indeed does interact strongly with the MORF1 protein. pGBK7 is the bait and pGADT7 is the prey vector.

MORF heterodimer/homodimer combinations of which some may be less efficiently substituted by others and lead to loss of editing when one MORF is mutated. In yeast cells, MORF1 shows little discrimination in connecting to other MORFs, whereas MORF3 only interacts strongly with MORF1 but with none of the other MORF proteins (Fig. 4A). MORF3 does not seem to be able to form homodimers.

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MORF Proteins Can Interact with Site-Specific RNA Editing PPR Proteins. In plastids as well as in mitochondria, RNA editing is affected by the loss of individual MORF proteins at sites that also require individual PPR proteins for processing. In plastids, for example, editing at site ndhD-2 is lost when either MORF2 or MORF9 is disturbed, but also when the PPR protein chloro-respiratory reduction 4 (CCR4) is mutated (7). In mitochondria, editing site ccmB-566 requires mitochondrial editing factor 19 (MEF19) and MORF1; site cox3-257 needs MORF2 as well as MORF1 for processing (14). These coinciding requirements suggest that site-specific PPR proteins (e.g., CRRs and MEFs) are required in conjunction with one or more MORF proteins.

To directly investigate this potential connection between the specific editing factors and the MORF proteins, we tested whether MEF–PPR proteins can interact with MORF proteins in yeast two-hybrid assays (Fig. 4B) and in pull-down assays (Fig. 5B). Growth of yeast cells on respective selective media shows that most of the MEF proteins indeed interact with MORF proteins. Consistent with their “promiscuous” roles at most editing sites in their organelle, the plastid factors MORF2 and MORF9 interact with several mitochondrial MEFs. Similarly, the MORF1 protein, which is required for more than 40 RNA editing sites in mitochondria, interacts with MEF1, MEF9, and MORF2. This binding is, however, specific and selective, as the DYW–PPR protein MEF19 is not contacted by any of the MORFs investigated. Furthermore, MORF interaction with the second DYW-domain-containing MEF protein, MORF1, seems to be rather weak, as suggested by slower establishment and growth of the yeast cells.

The potential of MEF and MORF proteins to interact is supported by the results of a screen of a cDNA library derived from RNA isolated from young Arabidopsis seedlings with MORF9 as bait. Several clones of the MORF8 protein were identified in the total plant cDNA library, which strengthens the findings from the yeast two-hybrid assays that MORF and MEF proteins can interact. Both MEF9 and MORF8 are mitochondrially located proteins and may interact in this organelle.

To investigate potential MORF–MEF interactions by another experimental approach, we tested whether MEF21 and MEF19 are able to bind the mitochondrially located MORF1 and the plastid-targeted MORF2 proteins in pull-down assays (Fig. 5B). MEF21 can retain MORF1 but not MORF2, as similarly observed in the yeast two-hybrid assays, where MEF21 interacts strongly with MORF1 but only weakly with MORF2 (Fig. 4B). In mitochondria, MEF21 and MORF1 are both required for editing at site cox3-257 (14). We also probed the potential for physical interaction between the MEF19 PPR protein and the MORF1 protein, which both target the RNA editing site at ccmB-566 (Fig. 5B). In the pull-down assay, the MEF19 bait protein bound to matrix beads retains the prey MORF1 molecules but not the plastid-targeted MORF2 protein (Fig. 5B). The physical interaction between MORF1 and MEF19 can thus connect the site-specific MEF19 PPR protein and the MORF1 protein for RNA editing at their common target site at ccmB-566. In summary, these lines of evidence support the potential of MEF and MORF proteins to interact more or less specifically with each other.

Discussion

MORF Proteins Are Unique Components of the Plant Organelar “Editosomes.” The involvement of the family of MORF proteins suggests that the previous simple model of RNA editing in higher plant organelles has to be expanded to a more complex editosome model that contains more protein factors than envisaged: A PPR protein recognizes a specific sequence context in the RNA, binds there, and provides the attachment site for one or another of the MORF proteins. Or, vice versa, a MORF protein contacts an RNA molecule (provided MORF proteins bind RNA) and then attracts an MEF protein that then can play out its sequence specificity for binding to its cognate RNA motif. At the initiation step of MEF/MORF contacts to the RNA, additional, less specific ribonucleoproteins (RNPs) may be involved, such as CP31A and CP31B, which have been shown to be required for efficient RNA editing at several sites in plastids (27). These RNPs are partially redundant (27), a feature that seems to be common to MEF and MORF proteins. The interactions between PPR proteins and an RNA sequence appear to be specific yet fluid, because in some instances one PPR protein can be substituted by another: Those editing sites in mitochondria at which residual editing is still seen when a given PPR factor is disabled are not completely dependent on this single PPR protein; the remaining editing must be supported by another (PPR) protein (21).

MORF proteins must also partially overlap in their interactions with E or DYW–PPR proteins, as the numerous partial reductions in RNA editing at specific sites in plastids in the morf2 and morf9 mutants show. On the other hand, the complete loss of editing at several sites in the plastid when either MORF2 or MORF9 is disturbed indicates that at these sites both proteins are required and cannot substitute for each other. That both plastid MORF proteins are canonically required suggests that both should be contained within the editosome, potentially connecting each other directly in a heterodimeric arrangement. The observation that the MORF proteins interact selectively with each other corroborates this conclusion (Figs. 4/4 and S4).

MORF Proteins Are Present in Plants with Numerous Editing Sites. In evolutionary terms, this type of RNA editing and editosome seems to be a requirement specific to the land plant lineage. PPR proteins and MORF proteins expanded in plants in parallel with an increase of RNA editing sites. MORF-like proteins appear to be absent from other organisms; their presence is correlated with the evolutionary burst of editing site numbers. Genes for MORF proteins are only detected in flowering plants (SI Appendix, Fig. S3), and not in the moss Physcomitrella patens (17, 28). In this plant, only PPR proteins with C-terminal extended DYW domains are involved in the few editing events. This correlation raises the possibility that MORF proteins may be involved in compensating the loss of the DYW domain in some editing PPRs.

In the editosome, a MORF protein would connect to the RNA-binding PPR protein, either an E or a DYW moiety, and to the cytidine-deaminating or -transaminating activity. The latter may be another PPR protein with a DYW domain that acts as a deaminase, or may be another protein that performs the re-action. Inclusion of a second, different yet specific, PPR protein could have consequences for the decoding of the RNA sequence. Each PPR protein would have to contact just very few nucleotide identities in the cis element of the RNA, and only the combination of both PPR proteins would need to have full affinity. In the moss P. patens, single PPR proteins may recognize a given RNA nucleotide pattern, because a knockout of one PPR factor always results in full or no editing but never leads to partial loss of editing. In addition, no MORFs are present in the moss.

The involvement of the MORF proteins with many editing events in both organelles of flowering plants shows that the RNA editing processes in plant mitochondria and in plastids are similar and probably coevolved (SI Appendix, Fig. S3). In plastids, the process seems to be more stable, with all PPR proteins so far identified being required for fixed sites with no overlapping specificities of the PPR proteins manifested by residual editing at some sites upon the loss of a given PPR factor. Although this is superficially similar to the situation in the organelles of P. patens, in flowering plants MORF proteins are required and, for example, in the instance of site ndhD-2, two MORFs are needed for any editing to occur (Fig. 3). In mitochondria, a more fluid and flexible editing complex may adapt more rapidly to novel sites and new specificities by small modifications of the activities involved. The connection of the MORF proteins may provide an additional
safety level to avoid deleterious unwanted editing events caused by the often rather loose PPR specificity that is required to address sites with little sequence similarity (14, 15, 29).

Although the actual editing process in plant organelles with “just” C-to-U (but also U-to-C) nucleotide transitions seems biochemically much less complex than the U insertion/deletion editing in trypanosome mitochondria (30–32), likewise more and specialized protein factors such as the MORF proteins are required to make up a functional RNA editing complex.

Materials and Methods

Plant culture, mutant screening, handling of nucleic acids, RNA editing analysis, transfection, and transformation were as described (18, 29). Protein target predictions were analyzed with the Predotar program (urgi.versailles.inra.fr/predotar). Details of yeast two-hybrid and pull-down analyses, full methods, and associated references are described in SI Appendix, SI Materials and Methods.

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