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A truncated MEF11 protein shows site-specific effects on mitochondrial RNA editing

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RNA editing in flowering plant mitochondria post-transcriptionally alters several hundred nucleotides from C to U, mostly in mRNAs. We recently identified the nuclear encoded gene *MEF11* which is involved in RNA editing of three sites in mRNAs coding for subunits of respiratory chain complexes in *Arabidopsis thaliana*. In the *mef11-2* mutant a T-DNA insert alters the C-terminal part of the DYW domain. This insertion blocks RNA editing at sites *cox3*-422 and site *nad4*-124 completely, but only partially at *ccb203*-344. Transcription analysis shows that RNA editing is not defective due to disturbance of transcripts from the *mef11-2* locus. These observations suggest that the enzymatic activity or its recruitment is not compromised by the alteration of the C-terminus of MEF11.

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RNA editing in mitochondria of flowering plants was recognized 20 years ago, but the first nuclear encoded specificity factors have been identified only very recently. These mitochondrial editing factors (MEFs) are required for specific RNA editing events and thus presumed to mediate editing-site recognition in the affected RNA molecule in plant mitochondria.[1,2]

Two presently characterized mitochondrial editing factors MEF1 and MEF11 in *Arabidopsis thaliana*[1,2] and the OGR1 factor in rice[3] are pentatricopeptide repeat proteins (PPR proteins) of the DYW-subgroup, while the MEF9 protein terminates with the E-domain.[4] Several of the specific nuclear factors identified for RNA editing events in plastids are also members of this class of DYW-PPR proteins, others are PPR proteins but do not contain the DYW extension.[5-14] About 140 of the 450 PPR proteins in *Arabidopsis thaliana* are extended at their C-termini by DYW domains.[15-17] This DYW domain contains signature amino acids characteristic of Zn-containing cytidine deaminases and has thus been proposed to be enzymatically involved in the C to U RNA editing.[18,19] On the other hand, the DYW-motif in a PPR protein of as yet unknown function has RNase activity,[20] and DYW-domains can be deleted in PPR proteins involved in plastid editing with no effect on the processing of their editing targets.[21]

To gather further information on the function of the DYW-domains, we have started to analyze the requirement of this region in RNA editing factor MEF11. The nuclear encoded MEF11 protein is required for RNA editing at three distinct sites in three different mitochondrial mRNAs, sites *cox3*-422, *nad4*-124 and *ccb203*-344. Inactivation of this RNA editing protein surprisingly enhances isoprenoid biosynthesis, suggesting retrograde signalling from mitochondria to the cytoplasm.[2,22,23]

A Point Mutation in Mutant Line *mef11-1* and a T-DNA Insertion Line *mef11-2* Differentially Affect Specific RNA Editing

The single amino acid exchange in the second PPR repeat in the *mef11-1* mutant eliminates RNA editing competence at the three identified target sites *cox3*-422, *nad4*-124 and *ccb203*-344. Surprisingly, the T-DNA insertion in the DYW-domain in line *mef11-2* completely blocks RNA editing at just the two sites *cox3*-422 and

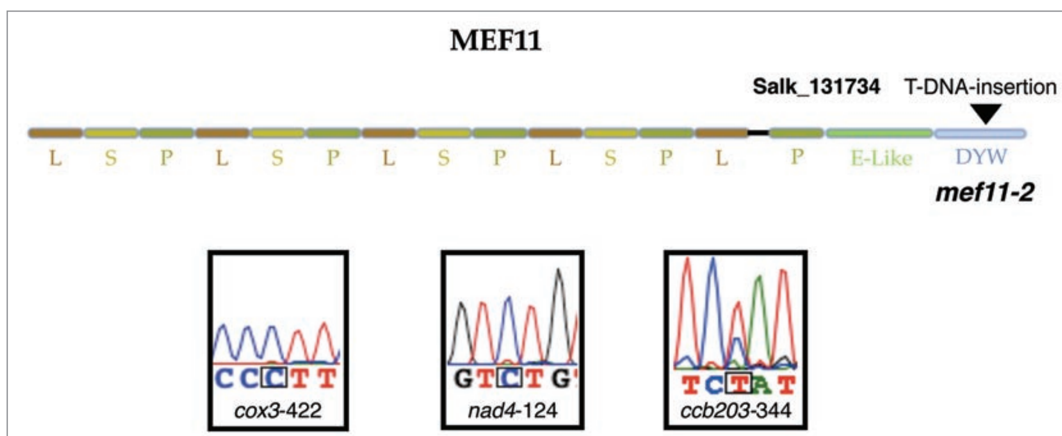

Figure 1. Mutant mef11-2 differentially affects editing at the MEF11 target sites. The top part shows the modular structure of the MEF11 protein with the insertion site of the T-DNA in the C-terminal region of the reading frame in mutant *mef11-2*. Specific cDNA fragments were generated by RT-PCR amplification following established protocols.[24,25] The cDNA sequences around the three target sites are compared for C to T differences resulting from RNA editing. The *mef11-2* mutant plants have lost the ability of C to U editing at sites *nad4*-124 and *cox3*-422. In the third target site *ccb203*-344 editing is still occurring albeit only at about 60%. The edited U nucleotide is seen as a red trace, the unedited C is seen as a blue trace.

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OGR1     495 ELGYEPETSNVLHDIGEEEKQYALCYHSEKLAIAFGLIATPPGETLRVIKNLRICGDCHVVAKLISKAYGRVIVIRDRARFHRFEDGQCSCRDYW
MEF1     494 KAGYIADTSYVLREVDGDEKNQTIRYHSERLAIAFGLITFPADRPIRVMKNLRVCGDCHNAIKFMSVCTRRVIIVRDNNRFHRFEDGKCSCNDYW
MEF11    590 AAGYKPDLKLSLYDLEEEEKAAEVSHHSEKLALAFGLLSLPLSVPIRITKNLRICGDCHSFFKFVSGSVKREIIVRDNNRFHRFKDGICSCKDYW
MEF11-2  590 AAGYKPDLKLSLYDLEEEEKAAEVSHHSEKLALAFGLLSLPLSVPIRITKNLRICGDCHSFFKFVSGSVQDILWCKQIDA
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Figure 2. The T-DNA insertion alters the MEF11-2 protein in mutant mef11-2. Aligned are the C-terminal amino acid sequences of the three DYW-PPR proteins presently identified as specific RNA editing factors for plant mitochondria with the MEF11-2 protein encoded by the mutant *mef11-2*. OGR1 is from rice,[23] MEF1 and MEF11 are from Arabidopsis.[1,2] The conserved signatures of cytidine deaminases are underlined, the amino acids altered by the insertion of the T-DNA in the C-terminal region in MEF11-2 are boxed.

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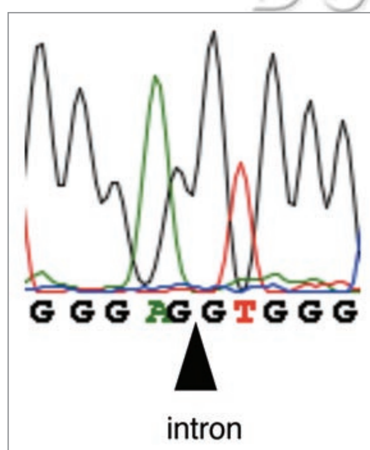

Figure 3. Analysis of splicing of the mutant mef11-2 mRNA coding for the MEF11-2 protein. The cDNA sequence analysis across the exon junctions after RT-PCR shows that the gene for MEF11-2 is transcribed and also correctly spliced in mutant *mef11-2*. The location of the excised single intron is indicated.

nad4-124, whereas editing at site *ccb203*-344 is only diminished to about 60% of the total *ccb203* transcript population (**Fig. 1**).

This differential effect of the two mutants on editing may shed some light on the role of the MEF11 protein in RNA editing at its target sites. Sequence analysis of the genomic location of the T-DNA insertion in the At4g14850 gene in line *mef11-2* shows that this insertion has removed 26 amino acids from the C-terminus of the DYW region and has added 11 different amino acids instead (**Fig. 2**). Among the missing 26 amino acids are the name-giving DYW and other blocks of well conserved amino acids, while all of the amino acids which have been proposed to resemble the cytidine deaminase signature are still present in the mutated protein.

To test the possibility that expression of the MEF11-2 protein is compromised by the alteration of the C-terminus of the DYW region and the further downstream T-DNA derived sequences, we analysed the presence and maturation of transcripts from the *mef11-2* mutant gene. Processing of the transcripts does not seem to be affected, since analysis of the *mef11-2* cDNA by RT-PCR reveals that the mRNA is correctly spliced (**Fig. 3**) and that it is transcribed into the T-DNA insert (Not shown). This suggests that the effect of the T-DNA insertion more likely acts on the MEF11-2 protein level, which will need to be analysed in more detail. To determine the in vivo expression level of the MEF11-2 protein, specific antibodies will have to be raised and in vitro expressed MEF11-2 protein will have to be functionally tested with respect to its RNA binding and editing properties.

These results suggest a role of the C-terminal amino acids and the DYW-motif in modulating the contact to specific motifs in the target RNAs. Since in the investigated *mef11-2* mutant the cytidine deaminase signature is still intact, a deaminating enzymatic activity of this region can at present not be ruled out, at least for the MEF11 protein.

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References

1. Zehrmann A, van der Merwe JA, Verbitskiy D, Brennicke A, Takenaka M. A DYW-domain containing PPR-protein is required for RNA editing at multiple sites in mitochondria of *Arabidopsis thaliana*. Plant Cell 2009; 21:558-67.
2. 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 2009; 61: 446-455.
3. Kim S-R, Yang J-I, Moon S, Ryu C-H, An K, Yim J, An G. Rice OGR1 encodes a pentatricopeptide repeat-DYW protein and is essential for RNA editing in plant mitochondria. Plant J 2009; 59:738-49.
4. Takenaka M. MEF9, an E subclass PPR protein, is required for an RNA editing event in the nad7 transcript in mitochondria of *Arabidopsis thaliana*. Plant Physiol 2009; 152(2):939-47.
5. Sasaki T, Yukawa Y, Wakasugi T, Yamada K, Sugiura M. A simple in vitro RNA editing assay for chloroplast transcripts using fluorescent dideoxynucleotides: distinct types of sequence elements required for editing of ndh transcripts. Plant J 2006; 47:802-10.
6. Shikanai T. RNA editing in plant organelles: machinery, physiological function and evolution. Cell Mol Life Sci 2009; 63:689-708.
7. Kotera E, Tasaka M, Shikanai T. A pentatricopeptide repeat protein is essential for RNA editing in chloroplasts. Nature 2005; 433:326-30.
8. Okuda K, Myouga R, Motohashi K, Shinozaki K, Shikanai T. Conserved domain structure of pentatricopeptide repeat proteins involved in chloroplast RNA editing. Proc Natl Acad Sci USA 2007; 104:1878-83.
9. Okuda K, Habata Y, Kobayashi Y, Shikanai T. Amino acid sequenmce variations in Nicotiana CRR4 orthologs determine the species-specific efficiency of RNA editing in plastids. Nucleic Acids Res 2008; 36:6155-64.
10. Chateigner-Boutin A-L, Ramos-Vega M, Guevara-Garcia A, Andrés C, Gutierrez-Nava MdlL, Cantero A, et al. CLB19, a pentatricopeptide repeat protein required for editing of rpoA and clpP chloroplast transcripts. Plant J 2008; 56:590-602.
11. 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.
12. Yu Q-B, Jiang Y, Chong K, Yang Z-N. AtECB2, a pentatricopeptide repeat protein, is required for chloroplast transcript accD editing and early chloroplast biogenesis in *Arabidopsis thaliana*. Plant J 2009; 59:1011-23.
13. Zhou W, Cheng Y, Yap A, Chateigner-Boutin A-L, Delannoy E, Hammami K, et al. The Arabidopsis gene YS1 encoding a DYW protein is required for editing of rpoB transcripts and the rapid development of chloroplasts during early growth. Plant J 2008; 58:82-96.
14. Hammani K, Okuda K, Tanz SK, Chateigner-Boutin A-L, Shikanai T, Small I. A study of new Arabidopsis chloroplast RNA editing mutants reveals general features of editing factors and their target sites. Plant Cell 2009; tpc.109.071472v1.
15. Small ID, Peeters N. The PPR motif—A TPR-related motif prevalent in plant organellar proteins. Trends Biochem Sci 2000; 25:46-7.
16. Lurin C, Andrés C, Aubourg S, Bellaoui M, Bitton F, Bruyère C, et al. Genome-wide analysis of Arabidopsis pentatricopeptide repeat proteins reveals their essential role in organelle biogenesis. Plant Cell 2004; 16:2089-103.
17. Schmitz-Linneweber C, Small ID. Pentatricopeptide repeat proteins: a socket set for organelle gene expression. Trends Plant Sci 2008; 13:663-70.
18. Rüdinger M, Polsakiewicz M, Knoop V. Organellar RNA editing and plant-specific extensions of pentatricopeptide repeat proteins in Jungermaniid but not in Marchantiid liverworts. Mol Biol Evol 2008; 25:1405-14.
19. Salone V, Rüdinger M, Polsakiewicz M, Hoffmann B, Groth-Malonek M, Szurek B, et al. A hypothesis on the identification of the editing enzyme in plant organelles. FEBS Letts 2007; 581:4132-8.
20. Nakamura T, Sugita M. A conserved DYW domain of the pentatricopeptide repeat protein possesses a novel endoribonuclease activity. FEBS Letts 2008; 582:4163-8.
21. Okuda K, Chateigner-Boutin A-L, Nakamura T, Delannoy E, Sugita M, Myouga F, et al. Pentatricopeptide repeat proteins with the DYW motif have distinct molecular functions in RNA editing and RNA cleavage in Arabidopsis chloroplasts. Plant Cell 2009; 21:146-56.
22. Kobayashi K, Susuki M, Tang J, Nagata N, Ohyama K, Seki H, et al. LOVASTATIN INSENSITIVE 1, a novel pentatricopeptide repeat protein, is a potential regulatory factor of isoprenoid biosynthesis in Arabidopsis. Plant Cell Physiol 2007; 48:322-31.
23. Tang J, Kobayashi K, Suzuki M, Matsumoto S, Muranaka T. The mitochondrial PPR protein LOVASTATIN INSENSITIVE 1 plays regulatory roles in cytosolic and plastidial isoprenoid biosynthesis through RNA editing. Plant J 2009;61: 446-455.
24. Takenaka M, Brennicke A. RNA editing in plant mitochondria: Assays and biochemical approaches. Meth Enzymol 2007; 424:439-58.
25. Takenaka M, Brennicke A. Multiplex single base extension typing to identify nuclear genes required for RNA editing in plant organelles. Nucleic Acids Res 2009; 37:13.

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