A novel E-Subgroup Pentatricopeptide Repeat Protein DEK55 is Responsible for RNA Editing at 15 Sites and Splicing of nad1 and nad4 in Maize

Ru Chang Ren
Shandong Agricultural University
Xu Wei Yan
Shandong Agricultural University
Ya Jie Zhao
Shandong Agricultural University
Yi Ming Wei
Shandong Agricultural University
Xiaoduo Lu
Qilu Normal University
Jie Zang
Shandong Agricultural University
Jia Wen Wu
Shandong Agricultural University
Guang Ming Zheng
Shandong Agricultural University
Xin Hua Ding
Shandong Agricultural University
Xian Sheng Zhang
Shandong Agricultural University
Xiang Yu Zhao (zhxy@sdau.edu.cn)
Shandong Agricultural University https://orcid.org/0000-0002-6809-6542

Research article

Keywords: defective kernel, maize, mitochondrion, pentatricopeptide repeat proteins, RNA processing, splicing

Posted Date: May 21st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-26875/v1

Version of Record: A version of this preprint was published on December 9th, 2020. See the published version at https://doi.org/10.1186/s12870-020-02765-x.

Abstract

Background Pentatricopeptide repeat (PPR) proteins is a large protein family, which participate in RNA processing in organelles and plant growth. Previous reports have generally considered E-subgroup PPR proteins as an editing factors for RNA editing. However, the underlying mechanisms and effects of E-subgroup PPR proteins remain to be investigated.

Results In this study, we recognized and identified a new maize kernel mutant with arrested embryo and endosperm development, *defective kernel 55* (*dek55*). Genetic and molecular evidences suggest that the defective kernels resulted from a mononucleotide alteration (C to T) at + 449 in the open reading frame (ORF) of Zm00001d014471 (hereafter referred to as *DEK55*). *DEK55* encodes an E-subgroup PPR protein within mitochondria. Molecular analyses suggest that DEK55 plays crucial roles in RNA editing at multiple sites of *ribosomal protein S13*, *ATP synthase subunit1*, *NADH dehydrogenase-6* (*nad6*), and *nad9* transcripts as well as in splicing of *nad1* and *nad4*. The mutation of DEK55 lead to the dysfunction of mitochondrial complex I.

Conclusions Our results demonstrate that the *DEK55* mutation is responsible for the *dek55* mutant phenotypes, as it affects mitochondrial function that is essential for maize kernel development. This study also provides novel insight into the molecular function of E-subgroup PPR proteins in plant organellar RNA metabolism.

Background

The pentatricopeptide repeat (PPR) protein family is a large protein family in most land plants, and over 450 members have been identified in *Arabidopsis thaliana*, *Oryza sativa*, and *Zea mays* [1–5]. These proteins include standard tandem degenerate repeat motifs, which form a helix-loop-helix structure with approximately 35 amino acids. PPR proteins are mainly classified into P and PLS type subfamilies according to the amino acid sequence of their PPR repeat motifs [2, 6, 7]. P subfamily PPR proteins only contain classical “P” motif repeats in tandem, while PLS subfamily PPR proteins consist of alternant repeats of three different longer PPR motifs. These are usually divided into PLS, E, E+, and DYW subgroups based on the presence of E, E+, or DYW domains on the carboxy-terminal end [2]. A new class of PPR-small MutS-related proteins has been discovered, which contains small MutS-related domains on the carboxy-terminal end [8, 9].

P-type PPRs are considered to participate in the splicing of group II introns, RNA stabilization, cleavage, translational activation, and transcript accumulation, whereas PLS-type PPRs play important roles in the conversion of cytidine (C) to uridine (U) at specific sites of organelle transcripts [4, 10, 11]. Most plant PPR proteins are targeted to mitochondria, chloroplasts, or both and regulate the functions and development of these organelles [10]. In mitochondria, the oxidative phosphorylation system is comprised of five complexes (I – V) [12]. Normal assembly of these complexes is essential to maintain mitochondrial function, which requires normal processing of mitochondrial pre-RNAs, including RNA

editing and intron splicing [13, 14]. Numerous PPRs are responsible for RNA post-transcriptional processes in mitochondria [15–21].

The E-subgroup PPR proteins (f.e., slow growth1 (SLO1), organelle transcript processing 87 (OTP87), mitochondrial editing factor 3 (MEF3), MEF9, MEF12, and mitochondrial PPR25 (MPR25)) play vital roles in mitochondrial RNA editing and plant development [22–27]. Besides, a few E-subgroup proteins in Arabidopsis or rice are also implicated in RNA splicing [28, 29]. In maize, five E-subgroup PPR proteins have been characterized, and all of them are involved in RNA editing. Small kernel 1 (SMK1) is critical for *nad7*-836 editing in mitochondria, which is conserved in maize and rice [14]. SMK4 is critical for RNA editing of *cytochrome c oxidase1* (*cox1*) at position + 1489 [30]. Empty pericarp 7 (EMP7) is responsible for RNA editing of $ccmF_N$ at position + 1553, which is critical for cytochrome c maturation [31]. DEK39 is necessary for RNA editing of *nad3* transcript in mitochondria [32]. Dek10 is responsible for RNA editing of three sites of *nad3* and *cox2* transcripts [33]. However, it is still unclear whether E-subgroup PPRs are involved in RNA editing and intron splicing in maize organelles.

Here, we identified the maize mutant *dek55* with an embryo-lethal phenotype with arrested endosperm development, which is caused by the mutation of the mitochondria-localized E-subgroup PPR protein DEK55. In the *dek55* mutant, the splicing efficiency of *nad1* intron 1 and intron 4 *trans*-splicing and *nad4* intron 1 *cis*-splicing were decreased. Moreover, the editing ratio of multiple editing sites in *ribosomal protein S13* (*rps13)*, *ATP synthase subunit1* (*atp1)*, *NADH dehydrogenase-6* (*nad6)*, and *nad9* transcripts was also significantly reduced. Our results suggest that the E-subgroup PPR protein DEK55 participates in both RNA editing and group II intron splicing in maize mitochondria.

Results

Genetic and phenotypic analysis of the dek55-1 mutant

A mutant with a defective kernel phenotype was isolated from an ethyl methane sulfonate-induced maize B73 background population, named *defective kernel 55 − 1* (*dek55-1*). The *dek55-1* kernels were segregated from self-pollinated progenies of *dek55-1/+* heterozygotes in a 1:3 ratio (Fig. 1a, Additional file 1: Table S1). This mutant was confirmed in other populations generated from *dek55-1/+* heterozygotes crossed with the inbred lines C733 or S162 (Additional file 1: Table S1). These results suggest that *dek55-1* as a recessive phenotype is caused by a monogenic mutation.

The *dek55-1* kernels could be distinguished from wild type (WT) kernels at 15 days after pollination (DAP) (Fig. 1a). The *dek55* mutant kernels exhibited a whitened pericarp and were smaller than WT kernels, which exhibited a yellow color (Fig. 1a). At the maturity stage, *dek55-1* kernels became smaller and more shriveled (Fig. 1b, c). To further dissect the mutant phenotype, both WT and *dek55-1* kernels were longitudinally sliced at different developmental stages. At 15 DAP, the pericarp of WT kernels, but not *dek55-1* mutant kernels, was filled with endosperm cells (Fig. 1d, e). Furthermore, *dek55-1* exhibited a smaller mature embryo and a decreased proportion of hard endosperm than that in WT (Fig. 1f, g). *dek55-*

1 kernels could not germinate in the experimental field (0/100), implying that the arrested embryo was lethal in mutants. In addition, the kernel weight of *dek55-1* was reduced by approximately 70% compared to that of WT kernels (Fig. 1h).

To further investigate the developmental structure of *dek55-1* kernels, we examined the tissue structure of WT and *dek55-1* kernels at 12 and 18 DAP (Fig. 1i – l). At 12 DAP, WT embryos contained visible coleoptiles, shoot apical meristems, scutella, and two leaf primordia (Fig. 1i). In contrast, *dek55-1* embryos only had a small scutellum that was arrested at the coleoptile stage (Fig. 1j). Moreover, WT kernels were filled with endosperm cells, whereas a large interspace between endosperm and seed coat in *dek55-1* was observed (Fig. 1i, j). At 18 DAP, WT embryos had developed into relatively complete structures containing four leaf primordia, shoot apical meristems, and a clearly seen root apical meristem (Fig. 1k), while *dek55-1* embryos only generated one leaf primordium (Fig. 1l). Less starch grains were accumulated in *dek55-1* than in WT endosperm cells at this stage (Fig. 1k, l). In addition, a cavity was observed in *dek55-1* endosperm (Fig. 1l). These results indicate that developmental defects in embryo and endosperm are present in *dek55-1* mutants.

Map-based cloning of DEK55

To identify the *DEK55* gene, we performed the classical map-based cloning strategy to detect F_2 mutant kernels, which were segregated from self-pollinated filial 1 (F_1) hybrid ear. Four genomic DNA pools (10 mutant kernels per pool) and both parents were used for correlation analysis with polymorphic simple sequence repeats. The six simple sequence repeats at chromosome 5 were highly correlated with defective kernel phenotypes, implying that the candidate gene may be at chromosome 5. Further analysis showed that the *DEK55* gene is located between umc1705 and umc2302 on chromosome 5 (Fig. 2a). Six polymorphic molecular markers in this region were used to analyze 1868 mutant kernels from the F_2 population. Finally, the *DEK55* gene was located on an approximately 1.29 Mb region between molecular label M3 and M4 (Fig. 2a). There are 25 putative protein-coding genes in this region (http://ensembl.gramene.org/Zea_mays/Info/Index). To identify the mutated genes, genomic DNA of 25 candidate genes were amplified and sequenced. This revealed that the E-subgroup PPR protein gene (Zm00001d014471) has a single nucleotide change (C to T) at + 449 in *dek55-1*, which might result in an amino acid replacement (Ser to Phe) in the protein sequence but not in expression level of *DEK55* change (Fig. 2a – d). To validate this result, we obtained a new mutant, *dek55-2*, from the maize ethyl methane sulfonate-induced mutant database [34]. The *dek55-2* mutant had a single nucleotide mutation (G to A) at + 729 bp (Fig. 2b), which leads to protein truncation (Fig. 2d). The mutant *dek55-2* also exhibited defective kernels with small and white pericarps (Fig. 2e). The allelic test between *dek55-1* and *dek55-2* heterozygotes revealed that normal and mutant kernels were segregated with the expected 3:1 ratio (normal/mutant; 450/143; $P = 0.62$) in the F_1 ear (Fig. 2e). As a control, all the kernels from the ear that were crossed between *dek55-2* heterozygote and WT were normal (Fig. 2e). These results indicate that the PPR gene Zm00001d014471 mutation is responsible for defective kernel phenotype, and the annotated gene was designated *DEK55*.

Dek55 Is A Mitochondrial E-subgroup Ppr Protein

Sequence alignment demonstrated that the *DEK55* gene has one exon containing an 1893 bp ORF, which encodes a 630 amino acid residue protein containing 13 PPR motifs and an E domain on the carboxy-terminal end (Fig. 2b – d and Additional file 1: Fig. S1). Mutated sites in *dek55-1* and *dek55-2* were located in the third and fifth PPR motifs, respectively (Fig. 2d). The mutation in *dek55-2* resulted in a truncated DEK55 protein without the last eight PPR motifs or E domain.

To examine the subcellular localization of DEK55, the *p35S*:DEK55-EGFP vector was constructed and transformed into maize protoplasts. The fluorescence signal of DEK55-EGFP overlapped with Mito Tracker, which is a mitochondria-specific dye (Fig. 3a), suggesting that the DEK55 protein is a mitochondrial PPR protein in maize (Fig. 3a). In addition, *DEK55* expression analysis in various maize tissues demonstrated that *DEK55* is highly expressed in root, anther, and ear, but lowly expressed in stem, leaf, silk, tassel, and kernel (Fig. 3b).

DEK55 is involved in the C-to-U editing of rps13, atp1, nad6, **and** nad9 **transcripts at multiple sites**

Usually, PPR proteins take part in modifying organelle transcripts [10]. It has been reported that E-subgroup PPRs participate in the C-to-U editing of mitochondrial pre-RNA [14, 32, 33]. To explore whether DEK55 is involved in this processing, the transcriptional levels of 35 maize mitochondrial genes encoding functional proteins were analyzed in WT and *dek55-1*. RNA editing of these transcripts in WT and *dek55* was detected by amplification sequencing. Direct sequencing of the PCR products and monoclonal sequencing revealed that the C-to-U editing ratio of 15 editing sites in the four transcripts *rps13*, *atp1*, *nad6*, and *nad9* were significantly reduced in *dek55*. The C-to-U editing at the *rps13*-56 site was about 78.2% in WT kernels, whereas the editing efficiency of *rps13-56* was dramatically decreased in *dek55-1* (4.5%) and *dek55-2* (0%) mutants (Fig. 4). The editing efficiency at the *atp1*-1490 and *nad6*-159 sites was dramatically decreased in *dek55* (Fig. 4). The editing efficiencies of the two editing sites in WT were 100% and 68.8%, respectively, whereas they were reduced to 43.3% and 16.8% in *dek55*, respectively. In WT, the C-to-U editing of *atp1*-1490 changed the Pro codon (CCU) to the Leu codon (CUU), and the editing of *nad6*-159 kept the same amino acids (Phe) at this position (Fig. 4). Interestingly, the C-to-U editing ratio at 12 *nad9* editing sites (*nad9*-14, *nad9*-92, *nad9*-113, *nad9*-167, *nad9*-190, *nad9*-233, *nad9*-298, *nad9*-311, *nad9*-328, *nad9*-356, *nad9*-368, and *nad9*-398) was dramatically decreased in *dek55* (Fig. 4). The above results indicate that DEK55 is necessary for editing at *rps13*-56, *atp1*-1490, *nad6*-159, and 12 *nad9* editing sites.

DEK55 is essential for the trans**-splicing of** nad1 **introns 1 and 4 and for the** cis**-splicing of** nad4 **intron 1**

The transcript levels of 35 maize mitochondrial genes were examined, and *nad1* and *nad4* were significantly downregulated in the *dek55* mutant (Fig. 5a). The genomic DNA of *nad1* contains four group II introns; intron 2 is a *cis*-splicing intron and the others are *trans*-splicing introns (Fig. 5c). The genomic DNA of *nad4* has three *cis*-splicing introns (Fig. 5d) [13, 35]. The full maturation of *nad1* and *nad4* transcripts requires complete intron splicing. We further detected the intron splicing efficiency of *nad1*,

nad4, and other genes in WT and *dek55-1* by qRT-PCR. Compared with that in WT, the splicing efficiency of the first and fourth introns of *nad1* and the first intron of *nad4* in *dek55-1* mutant were decreased (Fig. 5b). Furthermore, we amplified each intron and full transcripts of *nad1* and *nad4* by RT-PCR (Fig. 5c, d). The transcriptional abundance of *nad1* exon 1–2, exon 4–5, and full-length DNA fragments were significantly decreased (Fig. 5c). The unspliced DNA fragments (1F + 2R, 3F + 4R, 4F + 5R) were not amplified by RT-PCR in WT and *dek55*, as *nad1* introns 1, 3, and 4 are too long (Fig. 5c). The unspliced intron 2 fragments of *nad1* in *dek55* mutants were similar to those in WT (Fig. 5c). The abundance of *nad4* spliced exon 1–2 and full-length DNA transcript fragments were significantly decreased, and the abundance of *nad4* unspliced intron 1 transcript was significantly increased (Fig. 5d). This suggests that the significant decrease in the *nad4* and *nad1* transcript abundance in *dek55* mutants was caused by the abnormal splicing of *nad4* intron 1, *nad1* intron 1, and intron 4, respectively (Fig. 5a – d). Therefore, DEK55 is necessary for the *trans*-splicing of two *nad1* introns (1 and 4) and *cis*-splicing of the first *nad4* intron in maize.

dek55-1 mutant exhibits reduced complex I activity and increased alternative respiratory pathway activity

The four genes *nad1*, *nad4*, *nad6*, and *nad9* encode the subunits of complex I NAD1, NAD4, NAD6, and NAD9, respectively [35]. The *rps13* gene encodes ribosomal protein, and *atp1* encodes the ATP1 subunit of ATP synthase F1 [35]. Defects in post-transcriptional processing of these genes may impair the biosynthesis of mitochondrial complexes [17, 36–38]. We performed blue native polyacrylamide gel electrophoresis (BN-PAGE) and the in-gel NADH dehydrogenase activity assay to investigate the accumulation level and activity of mitochondrial complexes in WT and *dek55*-1 endosperm. BN-PAGE showed that the abundance of complex I and super-complex I + III_2 in *dek55-1* mutants significantly decreased (Fig. 6a). However, no significant differences were observed in the abundance of complex V between WT and *dek55-1* (Fig. 6a). Furthermore, *dek55-1* deficiency the activities of complex I and I + III_2 (Fig. 6b). These results indicate that defects in mitochondrial transcript splicing and editing might affect the abundance and activity of mitochondrial complex I.

The mitochondrial respiratory chain in plants contains the cytochrome *c* and alternative oxidase (AOX) pathways [39]. When the main cytochrome *c* pathway is blocked, AOX activity can be increased to compensate respiration pathways [40]. In *dek55-1*, the functions of complex I were abolished (Fig. 6a, b). Thus, we performed qRT-PCR to detect the expression levels of *Aox* genes in WT and *dek55-1*. The expression of the *Aox2* gene was increased approximately 512-fold in the *dek55-1* mutant (Fig. 6c). Taken together, our results indicate that the respiration pathway is severely blocked in *dek55-1* mitochondria.

Discussion

DEK55 is required for maize kernel development

Previous reports have shown that PPR proteins play important roles in maize kernel development and that the loss of function of some PPR proteins results in empty pericarp as well as small and defective kernel phenotypes of different genetic backgrounds [13, 14, 17–21, 30, 31, 38, 41, 42]. These *ppr* mutants exhibit developmentally arrested embryos and endosperm. The embryos usually reached the coleoptilar stage or the leaf stage 1 (L1), and endosperm exhibited significantly reduced starch and protein levels [14, 33, 42]. The *dek55* mutant kernels exhibited a shriveled pericarp and small size (Fig. 1a – c). The *dek55-1* mutant kernels also exhibited smaller embryos and a decreased proportion of hard endosperm compared with WT (Fig. 1d – l). In particular, *dek55-1* embryos were severely arrested and only had one leaf primordium. Thus, the mutant kernel could not germinate in the field. Allelic tests indicated a nonsense mutant *dek55-2*, an allelic mutant with *dek55-1* and *dek55-1/dek55-2* heterozygous kernels, which displayed a similar phenotype to *dek55-1*. This suggests that *dek55* dysfunction is responsible for defective kernel phenotype and that DEK55 is required for kernel development in maize.

The E-subgroup PPR proteins are characterized by an E domain on the carboxy-terminal end that might be responsible for interactions between proteins [43, 44]. In the *dek55-1* mutant, there is a single nucleotide change (C to T) at + 449 in *dek55*, which caused phenylalanine (Phe) to replace serine (Ser) on the third PPR motif of DEK55 at 150 amino acid sites (Fig. 2b, d; Additional file 1: Fig. S1). This mutation altered the affinity of amino acids to water, from hydrophilic to hydrophobic amino acids. Our evidence suggests that the amino acid change (Ser→Phe) is responsible for defective kernels in *dek55* mutants. Therefore, the amino acid change at this site in DEK55 might cause altered conformation and function loss. In *dek55-2* mutants, the mutation resulted in a loss of the last eight PPR motifs and E domain on the carboxy-terminal end of the DEK55 protein (Fig. 2b, d), which might prevent it from forming complexes with other proteins and from binding to targets.

DEK55 is necessary for C-to-U editing of multiple sites in the mitochondrial transcripts of maize

PPR proteins, including DYW2, EMP21, NUWA, and MEF8, are involved in C-to-U editing at multiple sites [45–48]. In this study, DEK55 participated in RNA editing at 15 sites, suggesting it might be a novel E-subgroup PPR protein. However, PPR proteins do not share uniform protein features. Among them, DYW2 and MEF8 harbor only five PPR repeats and belong to an atypical DYW subgroup [45, 46]. NUWA belongs to the P-class of PPR proteins [45, 47]. EMP21 contains 11 PPR-motifs in addition to E and DYW domains and belongs to PPR-DYW proteins [48]. DEK55 is considered as an E-subgroup PPR protein that contains the canonical E domain. Therefore, PPR proteins that target multiple sites for editing might not share similar structures.

Multiple organellar RNA editing factors (MORFs) participate in RNA editing at numerous editing sites and can directly interact with PPR proteins [48–50]. In Arabidopsis, MEF13 (an E-subgroup PPR protein) can interact between MORF3 and MORF8. The protein complex is responsible for RNA editing of the same sites between *morf3*, *morf8*, and *mef13* mutants [50]. EMP21 is necessary for the editing of ~ 17% of mitochondrial target Cs in maize [48]. More interestingly, 34 editing sites overlap in maize *emp21* mutants and Arabidopsis *morf8* mutants, and eight editing sites overlap in maize *emp5* mutants and *morf8*

mutants. The ortholog of MORF8, ZmMORF8 (GRMZM2G169384) in maize, was proved to directly interact with EMP21 and EMP5, suggesting that EMP21 and EMP5 participate in the editing of some sites by interacting with ZmMORF8. However, EMP5 and EMP21 did not directly interact in yeast, and it is possible that other P-type PPR proteins or editing factor(s) are required for the interaction between EMP5 and EMP21 [48]. In our study, DEK55 participated in C-to-U editing of four mitochondrial transcripts at the following 15 editing sites: *rps13*-56, *atp1*-1490, *nad6*-159, *nad9*-14, *nad9*-92, *nad9*-113, *nad9*-167, *nad9*-190, *nad9*-233, *nad9*-298, *nad9*-311, *nad9*-328, *nad9*-356, *nad9*-368, and *nad9*-398 (Fig. 4). Comparative analyses of these mitochondrial transcript C-to-U editing events in both Arabidopsis and maize indicated that the sites at *nad9*-14, *nad9*-113, *nad9*-223, *nad9*-311, *nad9*-356, and *nad9*-368 were not edited in Arabidopsis, as these sites are “Ts”, and *rps13* is absent in the Arabidopsis genome [35]. Editing was also substantially impaired in *morf8* mutants of Arabidopsis at the following seven sites: *atp1*-1484 (*atp1*-1490 in *dek55* mutant), *nad9*-92, *nad9*-167, *nad9*-190, *nad9*-298, *nad9*-328, and *nad9*-398 [51]. This implies that DEK55 might directly interact with ZmMORF8 to function at these RNA editing sites in maize. In addition to ZmMORF8, other MORFs might also interact with DEK55 to form an editing complex for other editing sites.

DEK55 is involved in group II intron splicing in maize mitochondria

The E-subgroup PPR proteins are usually considered to be editing factors for RNA editing in organelles [10], whereas few of these proteins are considered to play a role in splicing [28, 29, 52]. SLO4 is necessary for RNA editing of *nad4* and the efficient splicing of *nad2* intron 1 in Arabidopsis mitochondria [29]. AEF1/MPR25 not only participates in RNA editing of *atpF* and *nad5*, but also modulates *atpF* splicing in both Arabidopsis and rice [28]. Furthermore, the plastid PPR protein OTP70 only participates in the intron splicing of the *rpoC1* transcript [52]. Interestingly, in this study, DEK55 (an E-subgroup PPR protein) participated in both RNA editing of 15 sites and group II intron splicing in maize mitochondrial transcripts (Figs. 4, 5a – d). However, the RNA editing of *nad1* and *nad4* transcripts was not affected. It has been reported that intron splicing is usually mediated by RNA editing events, in which the key sites of introns are edited [53, 54].

Some proteins have been identified that participate in the splicing of *nad1* and *nad4* pre-RNAs. The nuclear maturases 1 [55], DEK2 [42], and EMP11 [41] participate in *trans*-splicing of *nad1* intron 1. EMP11, EMP8, and ZmSMK3 are required for *nad1* intron 4 *trans*-splicing [41]. The proteins include NMS1 [56], DEK35 [19], EMP8 [13], DEK43 [20], EMP602 [57], and ZmSMK3 [58] and are implicated in *cis*-splicing of *nad4* intron 1. In our study, we have demonstrated that DEK55 is involved in both *trans*- and *cis*- dual splicing. It appears that the splicing of one intron possibly requires the involvement of multiple factors to constitute a putative spliceosome. This is supported by the finding that PPR-small MutS-related-1 can interact with the Zm-mCSF1 formation protein complex. The protein complex participates in the intron splicing of multiple transcripts within mitochondria [59]. Therefore, DEK55 might interact with P-type PPR proteins or other splicing factors involved in group II intron splicing.

Conclusions

In this study, we have demonstrated that DEK55 is a mitochondria-localized E-subgroup PPR protein. Mutation of *DEK55* lead to embryo-lethal and arrested endosperm development phenotype in maize. DEK55 is required for editing at *rps13*-56, *atp1*-1490, *nad6*-159, and 12 *nad9* editing sites. Meanwhile, DEK55 is responsible for the *trans*-splicing of two *nad1* introns (intron 1 and intron 4) and *cis*-splicing of the *nad4* intron 1 in mitochondria. Our results suggest that the E-subgroup PPR protein DEK55 plays an important role in RNA editing and splicing of introns of maize mitochondrial transcripts. These results provide novel view for understanding the molecular function of E-subgroup PPR proteins in RNA processing in plant organelles.

Methods

Plant materials

Maize *dek55-1* was identified from ethyl methane sulfonate populations from a B73 background, which was provided by Prof. Xiaoduo Lu of Qilu Normal University. The allele mutant *dek55-2*,which original material name is EMS4-073342. The EMS4-073342 was purchased from the maize ethyl methane sulfonate-induced mutant database (http://www.elabcaas.cn/memd/) [34]which can be found by searching for gene ID (Zm00001d014471). To purify the genetic background of the *dek55-1* mutant, *dek55-1* was crossed into the B73 inbred line twice to harvest BC_2F_1. BC_2F_2 kernel was used for further experiments. The *dek55-1* heterozygote as the male parent was crossed with our laboratory inbred lines C733 and S162, then F_1 progenies were self-pollinated to generate the F_2 population that was used for gene mapping. Ru Chang Ren and Xu Wei Yan undertook the formal identification of the plant materials. All plant materials were planted in the experimental station of Shandong Agricultural University (Taian, Shandong province).

Histological Analysis

The WT and defective kernels were obtained from the self-pollinated heterozygous plant at 12 and 18 DAP, respectively. The middle part of the kernel along the longitudinal axis was selected and placed in formalin-acetic acid-alcohol solution for at least 12 h on ice. This was followed by treatment with 50%, 70%, 85%, 95%, and 100% ethanol as well as 100% xylene for 2–4 h. After dehydration, materials were treated in paraffin for 72 h at 60 °C and then embedded in paraffin. The paraffin-embedded samples were cut into 12 μm thin slices using a microtome (Leica RM2235, Germany). Section staining was performed based on the methods of Ren et al. (2020). Finally, the sections were photographed with a light microscope (OLYMPUS DP72).

Map-based Cloning

The *DEK55* locus was identified using 1868 F_2 defective kernels from the self-pollinated F_1 population (C733 × *dek55-1/+*). For preliminary mapping, 73 polymorphic simple sequence repeat markers were selected from the entire genome with which the parent F_1 individual plant and F_2 defective kernel DNA pools were analyzed. New molecular markers were selected according to the parent DNA sequences used for fine mapping. The website (http://ensembl.gramene.org/Zea_mays/Info/Index) was used to search for gene annotations between candidate regions in *Zea mays* (B73_RefGen_v4) [60]. Phanta EVO Super-Fidelity DNA polymerase (Vazyme Code: P503-d1) was used to clone all candidate gene genomic DNA sequences and sequencing. The primers were designed according to candidate gene reference sequences. The primer sequences for cloning of full length *DEK55* genomic DNA and map-based cloning are shown in Additional file 1: Table S2.

Rna Extraction, Rt-pcr, And Qrt-pcr

Total RNA of WT and *dek55* mutant kernels without pericarp and other tissues were extracted with the Ultrapure RNA Kit (CWBIO, China). The residual DNA in the total RNA was removed by DNase. Complementary DNA was obtained by reverse transcription. RT-PCR was performed to amplify mitochondrial transcripts, splicing efficiency of *nad1*, and *nad4* introns. The DNA fragments obtained by RT-PCR were directly sequenced and shifted into the *Escherichia coli* strain (TOP10) for monoclonal sequencing. The transcripts were amplified according to the primers previously reported [61]. Primers used to amplify introns of *nad1* and *nad4* are shown in Additional file 1: Table S2.

The qRT-PCR equipment and reaction system were used according to a previous report [20]. All qRT-PCR assays were performed with three samples and technical repeats. The primers were designed for group II intron splicing efficiency analysis in mitochondria according to previous reports [17, 18, 61]. The primers used to analyze *DEK55* expression levels are shown in Additional file 1: Table S2.

Subcellular Localization

The termination codon was removed from the whole coding sequence of *DEK55* and imported into the pM999-EGFP vector generating a DEK55-EGFP recombinant vector driven by the CaMV 35S promoter. The subcellular localization experiment was performed as previously described [62]. Protoplasts of maize mesophyll were obtained from etiolated leaves by enzymatic hydrolysis as described previously [21]. Recombinant vector (20 μL, 15–20 μg) was added into maize protoplasts (200 μL), 220 μL of 40% (w/v) PEG4000 solution was added and mixed completely, and then the sampled were incubated at 23 °C for 10–15 min. Afterwards, the protoplasts were washed with W5 or WI solution and cultured for 12–16 h in the dark at 23 °C. The mitochondria in the protoplasm were labeled by a specific probe (MitoTracker Red CMXRos, Thermo Fisher Scientific), and images were acquired with a laser confocal microscope (LSM 880, Zeiss).

Isolation And Analysis Of Mitochondrial Complexes

The plant mitochondrial isolation kit (Biohao, Wuhan; catalog no. P0045) was used to isolate crude mitochondria from WT and *dek55-1* seeds with removed pericarps (on 15 DAP) for analysis of BN-PAGE and complex I activity. The collected mitochondrial precipitate was redissolved in 35 μl of solution buffer and then kept on ice for 30 min. Afterwards, the suspension was centrifuged at 4 °C, the supernatant was collected and loaded on pre-prepared gradient gels (BN1002BOX, Thermo Fisher Scientific), and electrophoresis was performed according to manufacturer's instructions. Next, the gels were placed in 100 mL of fixing solution (methanol/ddH_2O/acetic acid, 4:5:1) for 30 min and then transferred to 0.02% Coomassie R-250 stain (Sigma-Aldrich) for analysis of mitochondrial complex abundance. The gel strips were incubated in assay buffer for 5 min, and the reaction was terminated with the fixing solution for analysis of complex I activity [41].

Abbreviations

AOX
alternative oxidase; atp1:ATP synthase subunit1; BN-PAGE:blue native polyacrylamide gel electrophoresis; C:cytidine; cox:cytochrome c oxidase; DAP:days after pollination; dek:defective kernel; EGFP:enhanced green fluorescent protein; EMP:Empty pericarp; Leu:Leucine; MEF:mitochondrial editing factor; MORFs:Multiple organellar RNA editing factors;MPR25:mitochondrial PPR25; nad:NADH dehydrogenase; ORF:open reading frame; OTP87:organelle transcript processing 87; Phe:Phenylalanine; PPR:Pentatricopeptide repeat; Pro:Proline; qRT-PCR:quantitative reverse transcription polymerase chain reaction; rps13:ribosomal protein S13; RT-PCR:reverse transcription polymerase chain reaction; Ser:Serine; SLO1:slow growth1; SMK:Small kernel; U:uridine; WT:wild type

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded by the National Natural Science Foundation of China (91735301), the National Plant Transgenic Program (2016ZX08003-003), Taishan Scholars Project (ts201712024), Funds of Shandong “Double Tops” Program (SYL2017YSTD03) and the project (dxkt201707) from State Key Laboratory of Crop Biology. The funders had no role in the design of this study, data analysis and interpretation, and the writing of the manuscript or decision to publish.

Authors' contributions

The experiment conceived and supervised by XYZ. RCR, YXW, YJZ, YMW, JZ, JWW, and GMZ performed the experiments. XL isolated the *dek55-1* mutant. The manuscript was drafted by XYZ and RCR, corrected by XHD, and XSZ. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Chen L, Li YX, Li C, Shi Y, Song Y, Zhang D, Li Y, Wang T. Genome-wide analysis of the pentatricopeptide repeat gene family in different maize genomes and its important role in kernel development. BMC Plant Biol. 2018;18(1):366.
2. Lurin C, Andrés C, Aubourg S, Bellaoui M, Bitton F, Bruyère C, Caboche M, Debast C, Gualberto J, Hoffmann B, et al. Genome-wide analysis of Arabidopsis pentatricopeptide repeat proteins reveals their essential role in organelle biogenesis. Plant Cell. 2004;16(8):2089–103.
3. Chen G, Zou Y, Hu J, Ding Y. Genome-wide analysis of the rice PPR gene family and their expression profiles under different stress treatments. BMC Genom. 2018;19(1):720.
4. Schmitz-Linneweber C, Small I. Pentatricopeptide repeat proteins: a socket set for organelle gene expression. Trends Plant Sci. 2008;13(12):663–70.
5. Gutmann B, Royan S, Schallenberg-Rüdinger M, Lenz H, Castleden IR, McDowell R, Vacher MA, Tonti-Filippini J, Bond CS, Knoop V, et al. The expansion and diversification of pentatricopeptide repeat RNA-editing factors in plants. Mol Plant. 2020;13(2):215–30.
6. Small ID, Peeters N. The PPR motif – a TPR-related motif prevalent in plant organellar proteins. Trends Biochem Sci. 2000;25(2):45–7.
7. Yin P, Li Q, Yan C, Liu Y, Liu J, Yu F, Wang Z, Long J, He J, Wang HW, et al. Structural basis for the modular recognition of single-stranded RNA by PPR proteins. Nature. 2013;504(7478):168–71.
8. Zhang Y, Lu C. The enigmatic roles of PPR-SMR proteins in plants. Adv Sci (Weinh). 2019;6(13):1900361.

9. Liu S, Melonek J, Boykin LM, Small I, Howell KA. PPR-SMRs: ancient proteins with enigmatic functions. RNA Biol. 2013;10(9):1501–10.
10. Barkan A, Small I. Pentatricopeptide repeat proteins in plants. Annu Rev Plant Biol. 2014;65:415–42.
11. Zhang L, Zhou W, Che L, Rochaix JD, Lu C, Li W, Peng L. **PPR protein BFA2 is essential for the accumulation of the *atpH/F* transcript in chloroplasts**. Front Plant Sci. 2019;10:446.
12. Dudkina NV, Heinemeyer J, Sunderhaus S, Boekema EJ, Braun HP. Respiratory chain supercomplexes in the plant mitochondrial membrane. Trends Plant Sci. 2006;11(5):232–40.
13. Sun F, Zhang X, Shen Y, Wang H, Liu R, Wang X, Gao D, Yang YZ, Liu Y, Tan BC. The pentatricopeptide repeat protein EMPTY PERICARP8 is required for the splicing of three mitochondrial introns and seed development in maize. Plant J. 2018;95:919–32.
14. Li XJ, Zhang YF, Hou M, Sun F, Shen Y, Xiu ZH, Wang X, Chen ZL, Sun SS, Small I, et al. *Small kernel 1* **encodes a pentatricopeptide repeat protein required for mitochondrial *nad7* transcript editing and seed development in maize (*Zea mays*) and rice (*Oryza sativa*)**. Plant J. 2014;79(5):797–809.
15. Takenaka M, Zehrmann A, Verbitskiy D, Härtel B, Brennicke A. RNA editing in plants and its evolution. Annu Rev Genet. 2013;47:335–52.
16. Ichinose M, Sugita M. RNA editing and its molecular mechanism in plant organelles. Genes. 2017;8(1):5.
17. Xiu Z, Sun F, Shen Y, Zhang X, Jiang R, Bonnard G, Zhang J, Tan BC. **EMPTY PERICARP16 is required for mitochondrial *nad2* intron 4 *cis*-splicing, complex I assembly and seed development in maize**. Plant J. 2016;85(4):507–19.
18. Cai M, Li S, Sun F, Sun Q, Zhao H, Ren X, Zhao Y, Tan BC, Zhang Z, Qiu F. *Emp10* **encodes a mitochondrial PPR protein that affects the *cis*-splicing of *nad2* intron 1 and seed development in maize**. Plant J. 2017;91(1):132–44.
19. Chen X, Feng F, Qi W, Xu L, Yao D, Wang Q, Song R. *Dek35* **encodes a PPR protein that affects *cis*-splicing of mitochondrial *nad4* intron 1 and seed development in maize**. Mol Plant. 2017;10(3):427–41.
20. Ren RC, Wang LL, Zhang L, Zhao YJ, Wu JW, Wei YM, Zhang XS, Zhao XY. **DEK43 is a P-type pentatricopeptide repeat (PPR) protein responsible for the *cis*-splicing of *nad4* in maize mitochondria**. J Integr Plant Biol. 2020;62(3):299–313.
21. Ren RC, Lu X, Zhao YJ, Wei YM, Wang LL, Zhang L, Zhang WT, Zhang C, Zhang XS, Zhao XY. Pentatricopeptide repeat protein DEK45 is required for mitochondrial function and kernel development in maize. J Exp Bot. 2019;70(21):6163–79.
22. 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(3):499–511.
23. Hammani K, des Francs-Small CC, Takenaka M, Tanz SK, Okuda K, Shikanai T, Brennicke A, Small I: **The pentatricopeptide repeat protein OTP87 is essential for RNA editing of** nad7 **and** atp1 **transcripts in Arabidopsis mitochondria**. *J Biol Chem* 2011, **286**(24):21361–21371.

24. Verbitskiy D, Merwe JA, Zehrmann A, Härtel B, Takenaka M. The E-class PPR protein MEF3 of Arabidopsis thaliana can also function in mitochondrial RNA editing with an additional DYW domain. Plant Cell Physiol. 2012;53(2):358–67.
25. Takenaka M. **MEF9, an E-subclass pentatricopeptide repeat protein, is required for an RNA editing event in the *nad7* transcript in mitochondria of Arabidopsis**. Plant Physiol. 2010;152(2):939–47.
26. Härtel B, Zehrmann A, Verbitskiy D, Takenaka M. The longest mitochondrial RNA editing PPR protein MEF12 in Arabidopsis thaliana requires the full-length E domain. RNA Biol. 2013;10(9):1543–8.
27. Toda T, Fujii S, Noguchi K, Kazama T, Toriyama K. **Rice *MPR25* encodes a pentatricopeptide repeat protein and is essential for RNA editing of *nad5* transcripts in mitochondria**. Plant J. 2012;72(3):450–60.
28. Yap A, Kindgren P, Colas des Francs-Small C, Kazama T, Tanz SK, Toriyama K, Small I. **AEF1/MPR25 is implicated in RNA editing of plastid *atpF* and mitochondrial *nad5*, and also promotes *atpF* splicing in Arabidopsis and rice**. Plant J. 2015;81(5):661–9.
29. Weißenberger S, Soll J, Carrie C. **The PPR protein SLOW GROWTH 4 is involved in editing of *nad4* and affects the splicing of *nad2* intron 1**. Plant Mol Biol. 2017;93(4–5):355–68.
30. Wang HC, Sayyed A, Liu XY, Yang YZ, Sun F, Wang Y, Wang M, Tan BC. **SMALL KERNEL4 is required for mitochondrial *cox1* transcript editing and seed development in maize**. J Integr Plant Biol. 2019. doi:10.1111/jipb.12856.
31. Sun F, Wang X, Bonnard G, Shen Y, Xiu Z, Li X, Gao D, Zhang Z, Tan BC. *Empty pericarp7* **encodes a mitochondrial E-subgroup pentatricopeptide repeat protein that is required for *ccmFN* editing, mitochondrial function and seed development in maize**. Plant J. 2015;84(2):283–95.
32. Li X, Gu W, Sun S, Chen Z, Chen J, Song W, Zhao H, Lai J. *Defective Kernel 39* **encodes a PPR protein required for seed development in maize**. J Integr Plant Biol. 2018;60(1):45–64.
33. Qi W, Tian Z, Lu L, Chen X, Chen X, Zhang W, Song R. **Editing of mitochondrial transcripts *nad3* and *cox2* by Dek10 is essential for mitochondrial function and maize plant development**. Genetics. 2017;205(4):1489–501.
34. Lu X, Liu J, Ren W, Yang Q, Chai Z, Chen R, Wang L, Zhao J, Lang Z, Wang H, et al. Gene-indexed mutations in maize. Mol Plant. 2018;11(3):496–504.
35. Clifton SW, Minx P, Fauron CM, Gibson M, Allen JO, Sun H, Thompson M, Barbazuk WB, Kanuganti S, Tayloe C, et al. Sequence and comparative analysis of the maize NB mitochondrial genome. Plant Physiol. 2004;136(3):3486–503.
36. Xiao H, Zhang Q, Qin X, Xu Y, Ni C, Huang J, Zhu L, Zhong F, Liu W, Yao G, et al. **Rice *PPS1* encodes a DYW motif-containing pentatricopeptide repeat protein required for five consecutive RNA-editing sites of *nad3* in mitochondria**. New Phytol. 2018;220(3):878–92.
37. Xie T, Chen D, Wu J, Huang X, Wang Y, Tang K, Li J, Sun M, Peng X. *Growing Slowly 1* **locus encodes a PLS-type PPR protein required for RNA editing and plant development in Arabidopsis**. J Exp Bot. 2016;67(19):5687–98.

38. Li XL, Huang WL, Yang HH, Jiang RC, Sun F, Wang HC, Zhao J, Xu CH, Tan BC. **EMP18 functions in mitochondrial *atp6* and *cox2* transcript editing and is essential to seed development in maize**. New Phytol. 2019;221(2):896–907.
39. Vanlerberghe GC. Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. Int J Mol Sci. 2013;14(4):6805–47.
40. Kühn K, Yin G, Duncan O, Law SR, Kubiszewski-Jakubiak S, Kaur P, Meyer E, Wang Y, Small CC, Giraud E, et al. Decreasing electron flux through the cytochrome and/or alternative respiratory pathways triggers common and distinct cellular responses dependent on growth conditions. Plant Physiol. 2015;167(1):228–50.
41. Ren X, Pan Z, Zhao H, Zhao J, Cai M, Li J, Zhang Z, Qiu F. **EMPTY PERICARP11 serves as a factor for splicing of mitochondrial *nad1* intron and is required to ensure proper seed development in maize**. J Exp Bot. 2017;68(16):4571–81.
42. Qi W, Yang Y, Feng X, Zhang M, Song R. **Mitochondrial function and maize kernel development requires Dek2, a pentatricopeptide repeat protein involved in *nad1* mRNA splicing**. Genetics. 2017;205(1):239–49.
43. Okuda K, Myouga F, Motohashi R, Shinozaki K, Shikanai T. Conserved domain structure of pentatricopeptide repeat proteins involved in chloroplast RNA editing. Proc Natl Acad Sci U S A. 2007;104(19):8178–83.
44. Yang YZ, Ding S, Wang HC, Sun F, Huang WL, Song S, Xu C, Tan BC. **The pentatricopeptide repeat protein EMP9 is required for mitochondrial *ccmB* and *rps4* transcript editing, mitochondrial complex biogenesis and seed development in maize**. New Phytol. 2017;214(2):782–95.
45. Andrés-Colás N, Zhu Q, Takenaka M, De Rybel B, Weijers D, Van Der Straeten D. Multiple PPR protein interactions are involved in the RNA editing system in Arabidopsis mitochondria and plastids. Proc Natl Acad Sci U S A. 2017;114(33):8883–8.
46. Diaz MF, Bentolila S, Hayes ML, Hanson MR, Mulligan RM. A protein with an unusually short PPR domain, MEF8, affects editing at over 60 Arabidopsis mitochondrial C targets of RNA editing. Plant J. 2017;92(4):638–49.
47. Guillaumot D, Lopez-Obando M, Baudry K, Avon A, Rigaill G, Falcon de Longevialle A, Broche B, Takenaka M, Berthomé R, De Jaeger G, et al. Two interacting PPR proteins are major Arabidopsis editing factors in plastid and mitochondria. Proc Natl Acad Sci U S A. 2017;114(33):8877–82.
48. Wang Y, Liu XY, Yang YZ, Huang J, Sun F, Lin J, Gu ZQ, Sayyed A, Xu C, Tan BC. *Empty Pericarp21* **encodes a novel PPR-DYW protein that is required for mitochondrial RNA editing at multiple sites, complexes I and V biogenesis, and seed development in maize**. PLoS Genet. 2019;15(8):e1008305.
49. 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 U S A. 2012;109(13):5104–9.

50. Glass F, Härtel B, Zehrmann A, Verbitskiy D, Takenaka M. **MEF13 requires MORF3 and MORF8 for RNA editing at eight targets in mitochondrial mRNAs in** *Arabidopsis thaliana*. Mol Plant. 2015;8(10):1466–77.
51. Bentolila S, Oh J, Hanson MR, Bukowski R. Comprehensive high-resolution analysis of the role of an Arabidopsis gene family in RNA editing. PLoS Genet. 2013;9(6):e1003584.
52. Chateigner-Boutin AL, des Francs-Small CC, Delannoy E, Kahlau S, Tanz SK, de Longevialle AF, Fujii S, Small I: **OTP70 is a pentatricopeptide repeat protein of the E subgroup involved in splicing of the plastid transcript** rpoC1. *Plant J* 2011, **65**(4):532–542.
53. Castandet B, Choury D, Begu D, Jordana X, Araya A. Intron RNA editing is essential for splicing in plant mitochondria. Nucleic Acids Res. 2010;38(20):7112–21.
54. Farré JC, Aknin C, Araya A, Castandet B. **RNA editing in mitochondrial** *trans***-introns is required for splicing**. PLoS One. 2012;7(12):e52644.
55. Keren I, Tal L, des Francs-Small CC, Araujo WL, Shevtsov S, Shaya F, Fernie AR, Small I, Ostersetzer-Biran O. **nMAT1, a nuclear-encoded maturase involved in the** *trans***-splicing of** *nad1* **intron 1, is essential for mitochondrial complex I assembly and function**. Plant J. 2012;71(3):413–26.
56. Brangeon J, Sabar M, Gutierres S, Combettes B, Bove J, Gendy C, Chétrit P, Des Francs-Small CC, Pla M, Vedel F, et al. **Defective splicing of the first** *nad4* **intron is associated with lack of several complex I subunits in the** *Nicotiana sylvestris* **NMS1 nuclear mutant**. Plant J. 2000;21(3):269–80.
57. Ren Z, Fan K, Fang T, Zhang J, Yang L, Wang J, Wang G, Liu Y. **Maize empty** *Pericarp602* **encodes a P-Type PPR protein that is essential for seed development**. Plant Cell Physiol. 2019;60(8):1734–46.
58. Pan Z, Ren X, Zhao H, Liu L, Tan Z, Qiu F. **A mitochondrial transcription termination factor, ZmSmk3, is required for** *nad1* **intron4 and** *nad4* **intron1 splicing and kernel development in maize**. G3 (Bethesda). 2019;9(8):2677–86.
59. Chen Z, Wang HC, Shen J, Sun F, Wang M, Xu C, Tan BC. PPR-SMR1 is required for the splicing of multiple mitochondrial introns, interacts with Zm-mCSF1, and is essential for seed development in maize. J Exp Bot. 2019;70(19):5245–58.
60. Jiao Y, Peluso P, Shi J, Liang T, Stitzer MC, Wang B, Campbell MS, Stein JC, Wei X, Chin CS, et al. Improved maize reference genome with single-molecule technologies. Nature. 2017;546(7659):524–7.
61. Liu YJ, Xiu ZH, Meeley R, Tan BC. *Empty pericarp5* **encodes a pentatricopeptide repeat protein that is required for mitochondrial RNA editing and seed development in maize**. Plant Cell. 2013;25(3):868–83.
62. Yoo SD, Cho YH, Sheen J. Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nat Protoc. 2007;2(7):1565–72.

Figures

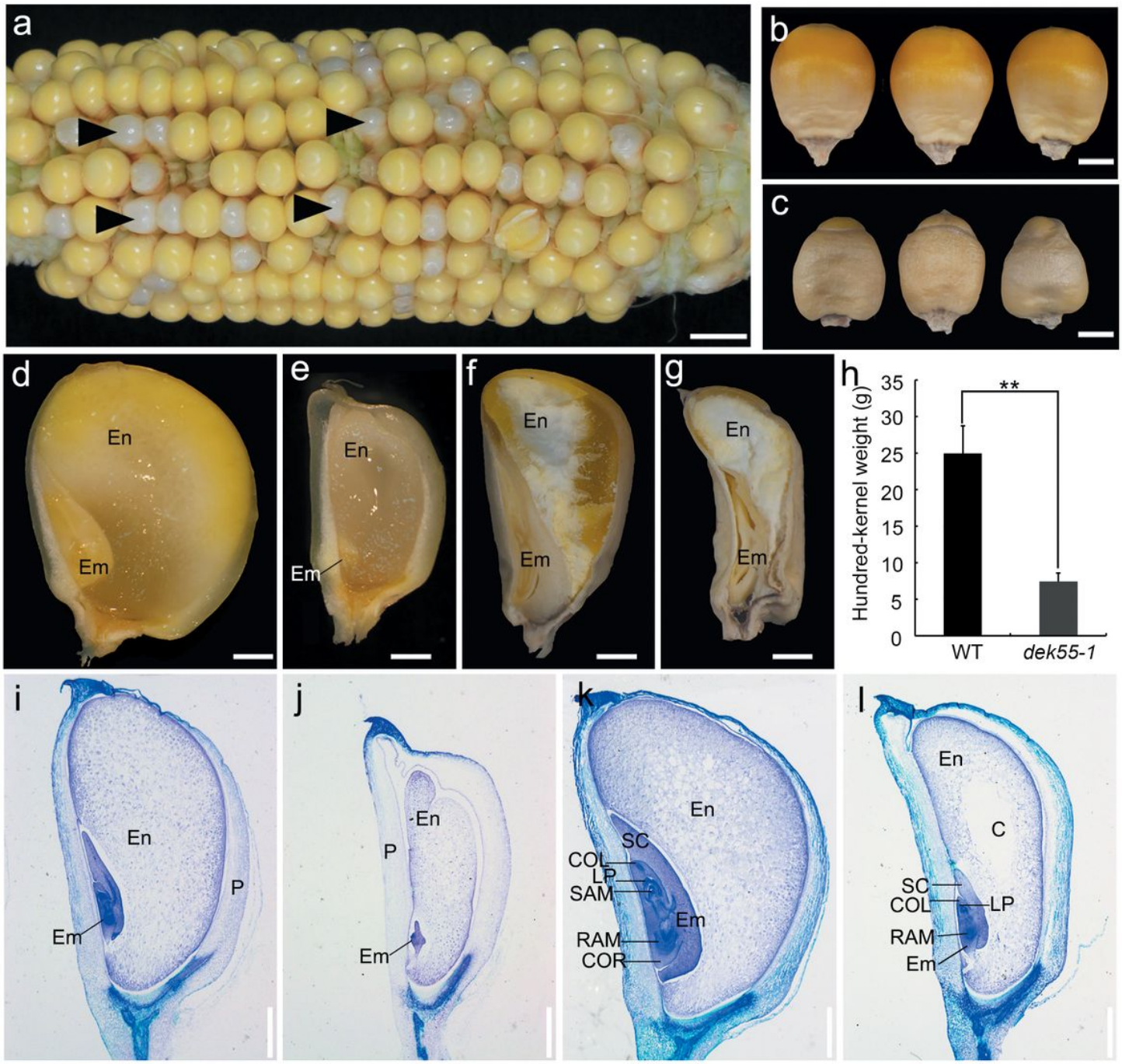

Figure 1

Phenotypic characterization of dek55-1 kernels. (a) The self-pollinated dek55-1 heterozygotes ears at 15 DAP. Some mutant kernels are indicated with arrowheads . Scale bars=1 cm. (b-c). The mature kernels of wild type (WT) and dek55-1. (b), WT; (c), dek55-1. Scale bars=2 mm. (d-g) Comparative anatomy of WT and dek55-1 kernels at 15 DAP and mature. (d and f), WT kernels. (e and g) dek55-1 kernels. Scale bars=1 mm. (h) Hundred-kernel weight of WT and dek55-1 kernels at maturity. (Asterisks indicate significantly different, **P < 0.05, Student's t-test) (i-l) Histological analysis of WT and dek55-1 kernels at 12 and 18 DAP. (i and k), WT at 12 and 18 DAP. (j and l) dek55-1 kernels at 12 and 18 DAP. Scale bars=1 mm. En,

endosperm; Em, embryo; P, pericarp; LP, leaf primordia; RAM, root apical meristem; SAM, shoot apical meristem; SC, scutellum; COL, coleoptile; COR, coleorhiza; C, cavity.

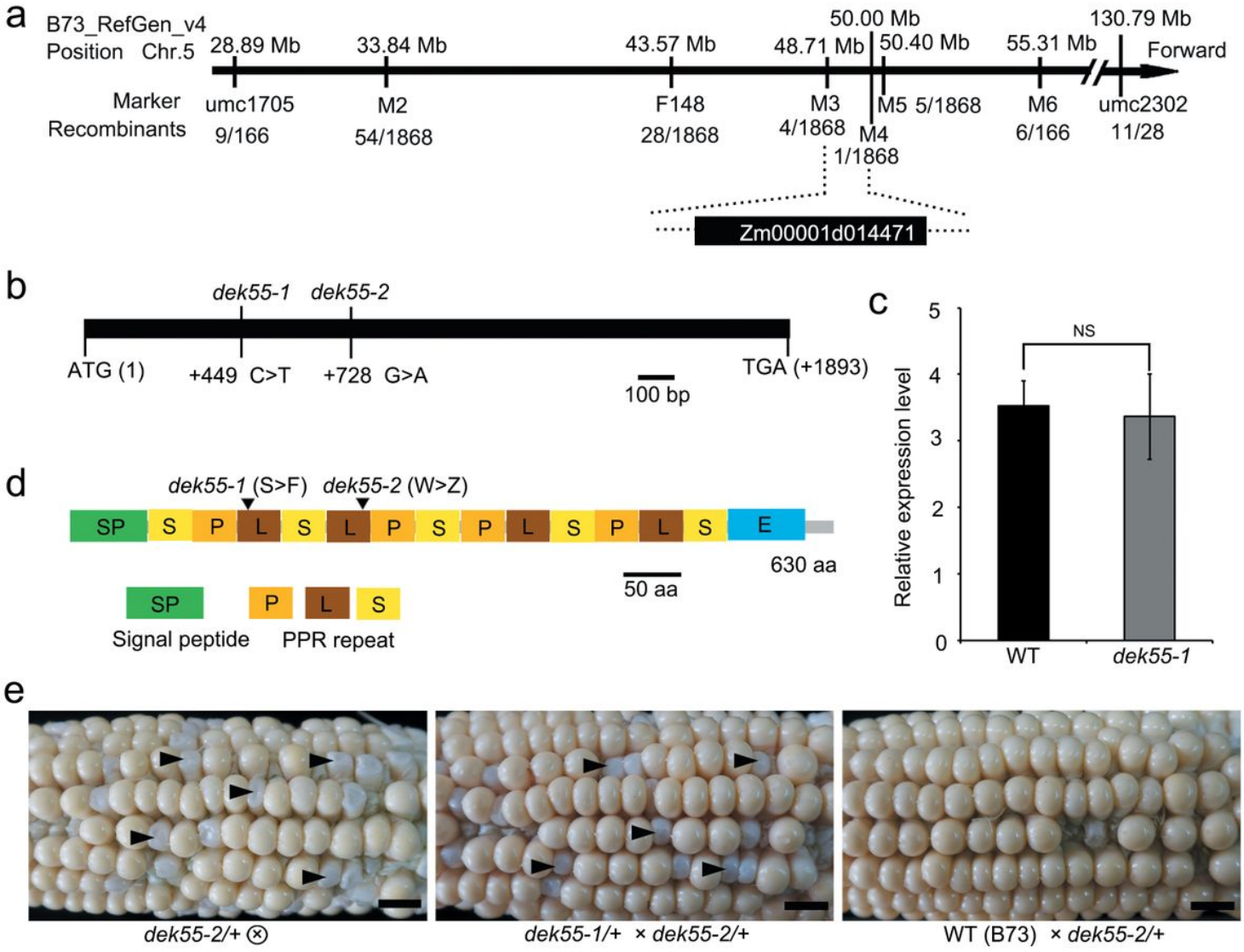

Figure 2

Map-based cloning and identification of DEK55. (a) Fine mapping of the DEK55 locus. The DEK55 locus was mapped to a 1.29 Mb region between marker 3 (M3) and M4 on chromosome 5, in which there are 25 candidate genes. The physical location of polymorphic molecular markers and number of recombinants are shown in the schematic diagram. (b) Schematic structure of dek55 gene. The mutation sites of dek55-1 and dek55-2 are shown. (c) Relative expression level of DEK55 in WT and dek55-1. Values are means of three biological replicates. Error bars represent the standard deviation (SD). (No significantly (NS), $P > 0.05$, Student's t-test). (d) Schematic diagram of DEK55 protein containing total 13 PPR domains (P, L and S) and E domain. The amino acid changes in dek55-1 and dek55-2 are indicated. (e) The self-pollinated dek55-2/+ (heterozygote) at 15DAP, dek55-1/+ and dek55-2/+ were used in an allelism test of dek55. The dek55-2/+ cross to B73 (WT) as control. Some mutant kernels are indicated by black arrowheads. Scale bars=1 cm.

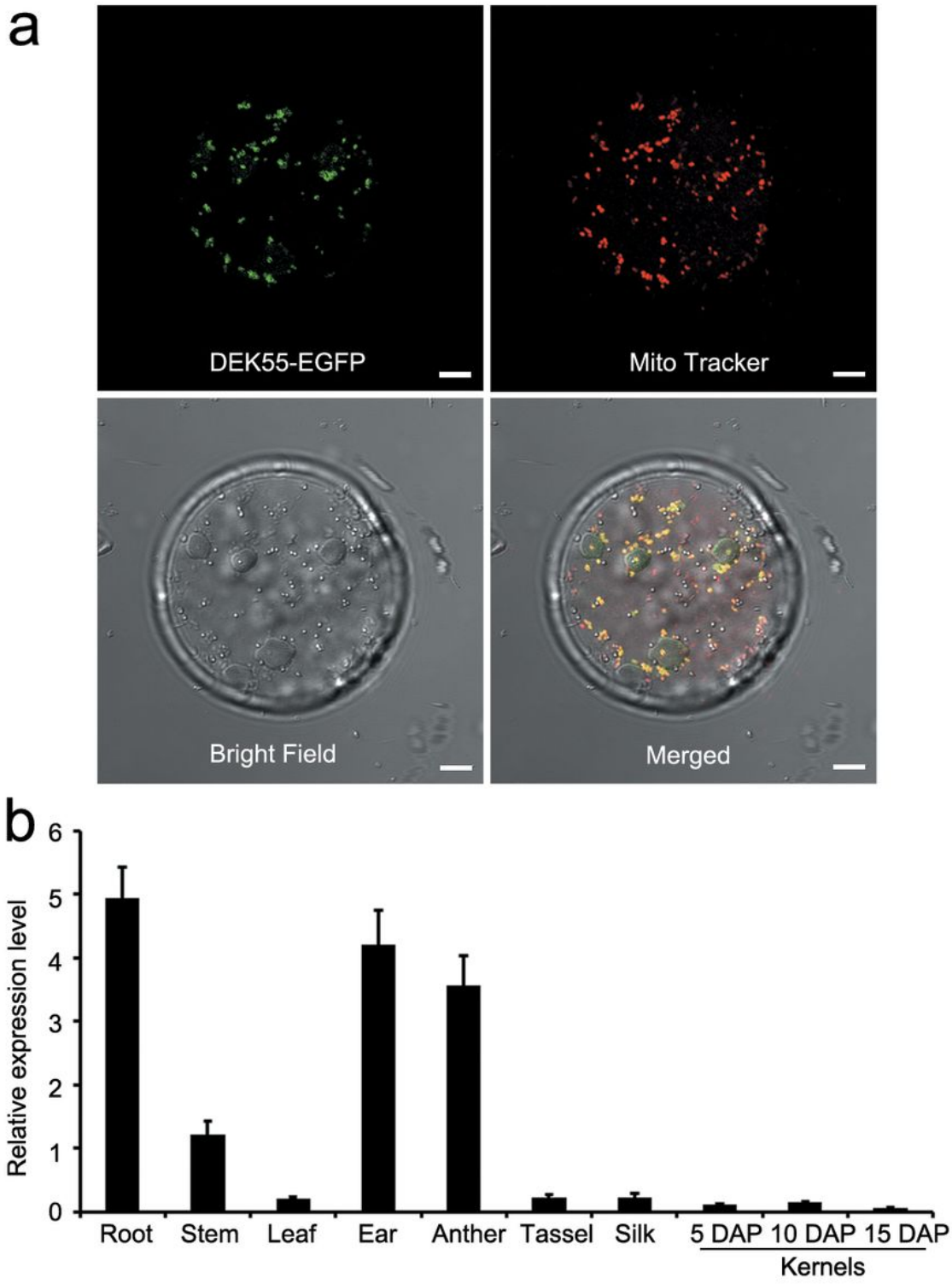

Figure 3

Subcellular localization of DEK55 and expression pattern of DEK55. (a) The subcellular localization of DEK55 was determined by transient expression of DEK55-EGFP fusion protein in maize protoplast. Mitochondria were marked by Mito Tracker (red). Scale bars=5 μm. (b) Analysis of the relative expression level of DEK55 in a various tissues and kernels at 5, 10, and 15 DAP. ZmActin gene (GRMZM2G126010)

was used as an internal control. Values are means of three replicates. Error bars represent the standard deviation (SD).

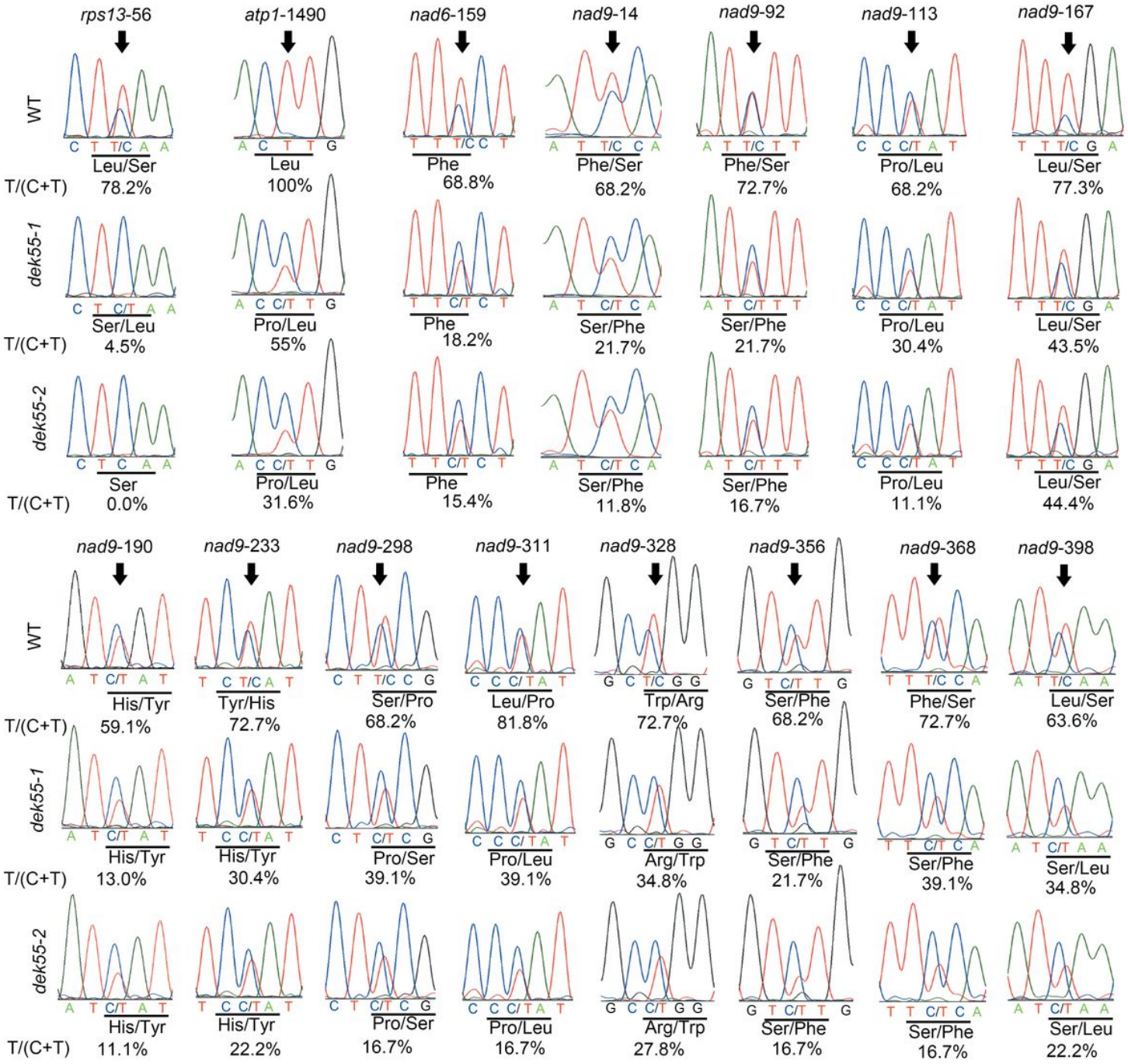

Figure 4

RNA C to U editing of rps13-56, atp1-1490, nad6-159 and nad9 transcripts at multiple sites in maize mitochondria The sequence chromatograms containing editing sites are shown. Arrows mark the editing sites. The amino acid in editing site is indicated on the bottom. The editing ratio is presented under each target site, which by single clone sequenced to count.

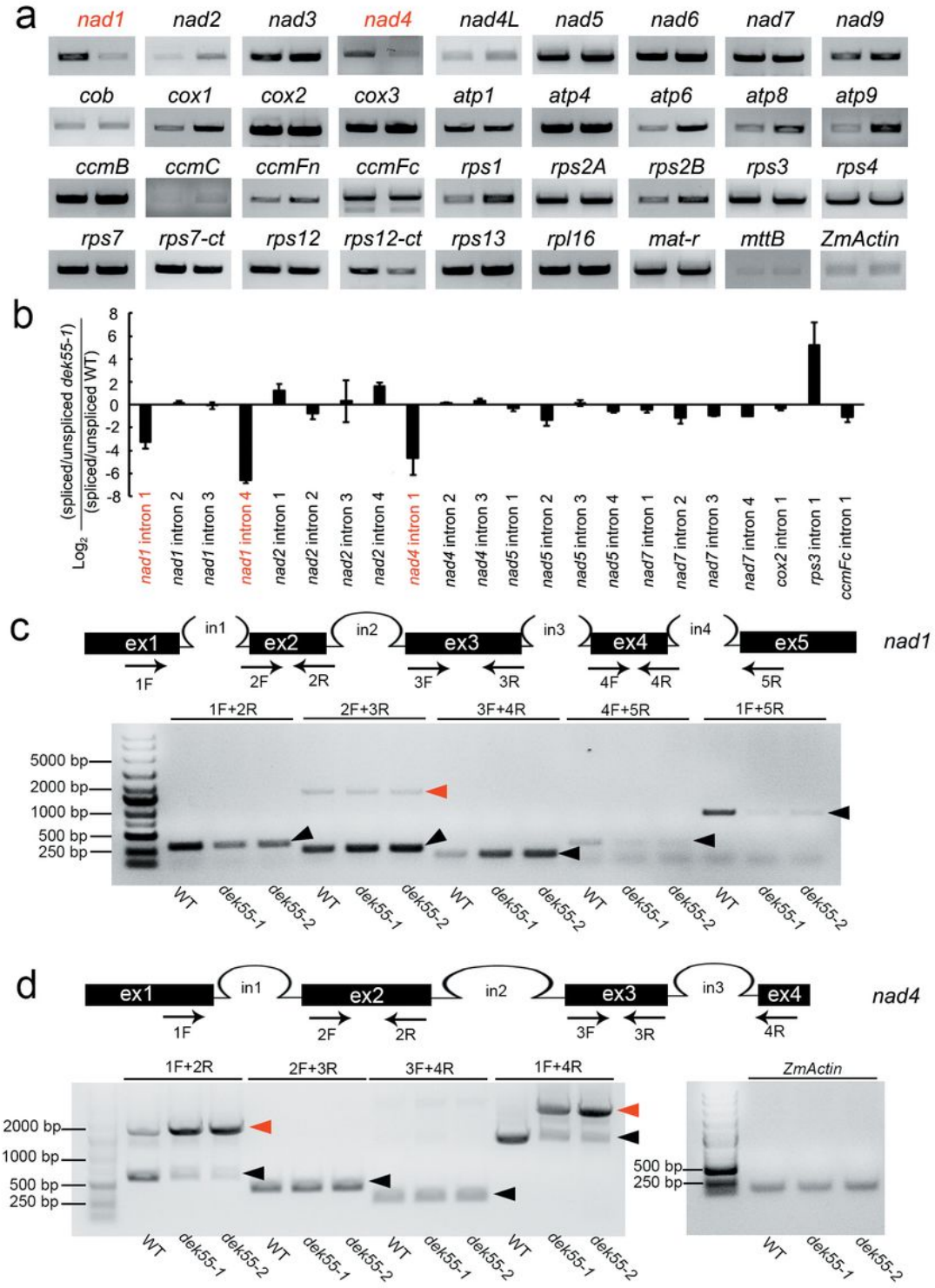

Figure 5

Mutation of dek55 decreased the amount of mature nad1 and nad4 transcripts of dek55, and impaired in nad4 intron 1 cis-splicing and nad1 intron1 and 4 trans-splicing. (a) The expression of 35 mitochondrion-encoded genes in WT (left) and dek55-1 (right) were detected by RT-PCR. ZmActin gene (GRMZM2G126010) was used as an internal control. Both nad1 and nad4 were marked in red because their transcript abundant were significantly decreased. (b) The splicing efficiency of all 22 group II introns

in maize mitochondrial-encoded genes was determined in dek55-1 and WT kernels by qRT-PCR. Values shown are calculated from the mean of three biological replicates, and error bars represent the standard deviation (SD). (c-d) Schematic structure of nad1 gene (c) and nad4 gene (d). The primers used for amplification are indicated. RT-PCR analysis of intron-splicing efficiency of nad1 in WT, dek55-1 and dek55-2 mutant kernels at 15 DAP. All PCR products were confirmed by sequencing. ZmActin gene (GRMZM2G126010) was used as an internal control. The unspliced and spliced fragments were indicated by red and black arrowheads. Exon was indicated as “ex”, and intron was indicated as “in”. The gel images in (a, c, d) were cropped and original gel images were shown in the Additional file 1: Figs. S2-S3.

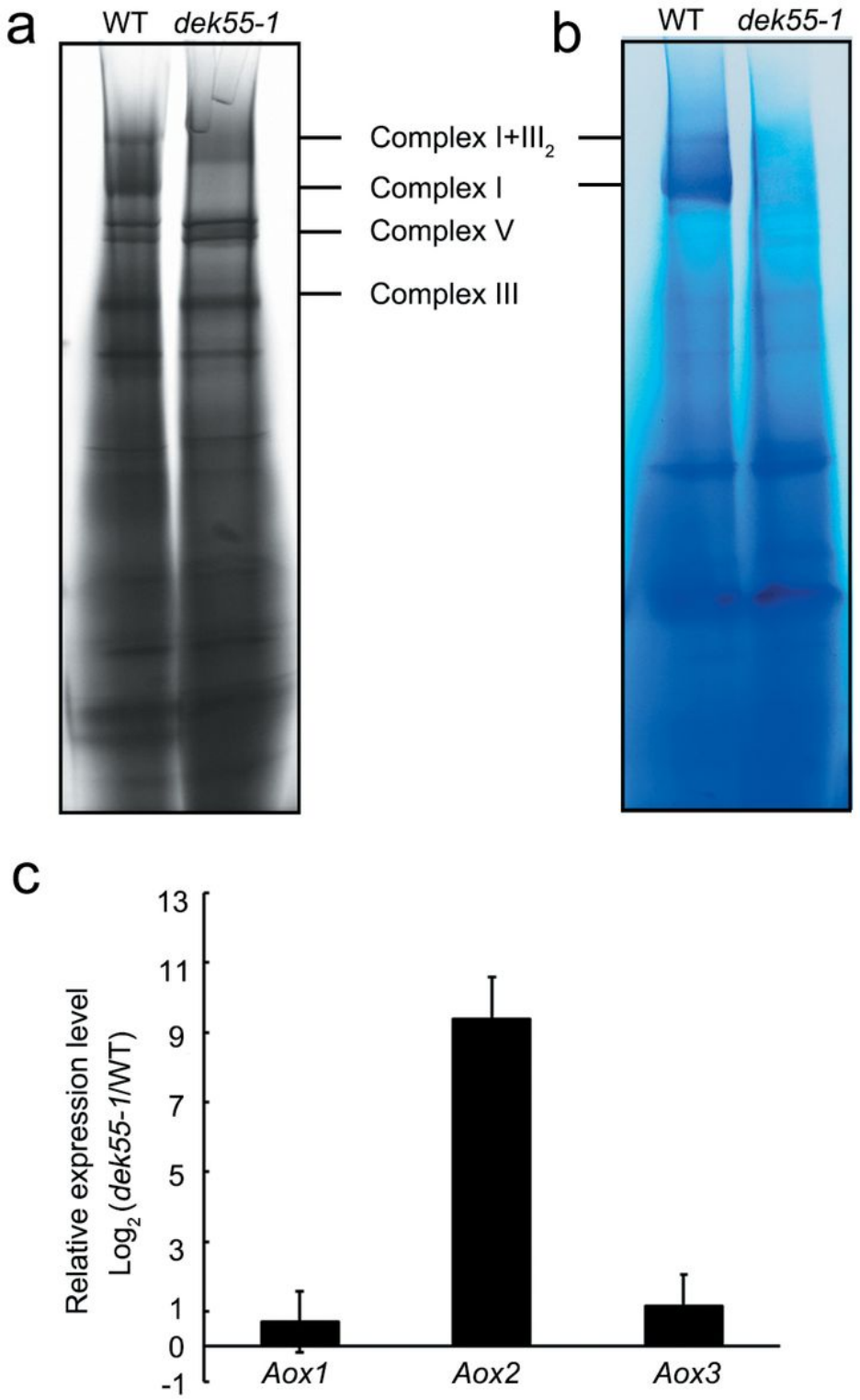

Figure 6

The mitochondrial function was impaired in dek55-1 mutant. (a) BN-PAGE analysis of mitochondrial complexes those were isolated from WT and dek55-1 kernels at 15 DAP, respectively. The gels were stained with Coomassie Brilliant Blue. The position of the mitochondrial complexes are marked. (b) In-gel NADH dehydrogenase activity analysis of complex I. The positions of complex I and super complex I+III2 were indicated. (c) qRT-PCR analysis of Aox genes (Aox1, Aox2, and Aox3) expression in WT and dek55-1

kernels at 15 DAP. ZmActin gene (GRMZM2G126010) was used as an internal control. Values shown are calculated from the mean of three biological replicates, and error bars represent the standard deviation (SD). The gel images in (a-b) were cropped and original gel images were shown in the Additional file 1: Fig. S4.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1revisiontrackedcopy.docx
- Additionalfile1revision.docx