A rice mTERF protein V14 sustains photosynthesis establishment and temperature acclimation in early seedling leaves

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Abstract

Background: Plant mitochondrial transcription termination factor (mTERF) family members play important roles in development and stress tolerance through regulation of organellar gene expression. However, their molecular functions have yet to be clearly defined.

Results: Here an mTERF gene V14 was identified by fine mapping using a conditional albino mutant v14 that displayed albinism only in the first two true leaves, which was confirmed by transgenic complementation tests. Subcellular localization and real-time PCR analyses indicated that V14 encodes a chloroplastic protein ubiquitously expressed in leaves while spiking in the second true leaf. Chloroplastic gene expression profiling in the pale leaves of v14 through real-time PCR and Northern blotting analyses showed abnormal accumulation of the unprocessed transcripts covering the rpoB-rpoC1 and/or rpoC1-rpoC2 intercistronic regions accompanied by reduced abundance of the mature rpoC1 and rpoC2 transcripts, which encode two core subunits of the plastid-encoded plastid RNA polymerase (PEP). Subsequent immunoblotting analyses confirmed the reduced accumulation of RpoC1 and RpoC2. A light-inducible photosynthetic gene psbD was also found down-regulated at both the mRNA and protein levels. Interestingly, such stage-specific aberrant posttranscriptional regulation and psbD expression can be reversed by high temperatures (30 ~ 35 °C), although V14 expression lacks thermo-sensitivity. Meanwhile, three V14 homologous genes were found heat-inducible with similar temporal expression patterns, implicating their possible functional redundancy to V14.

Conclusions: These data revealed a critical role of V14 in chloroplast development, which impacts, in a stage-specific and thermo-sensitive way, the appropriate processing of rpoB-rpoC1-rpoC2 precursors and the expression of certain photosynthetic proteins. Our findings thus expand the knowledge of the molecular functions of rice mTERFs and suggest the contributions of plant mTERFs to photosynthesis establishment and temperature acclimation.

Keywords: mTERFs, rpoB-rpoC1-rpoC2 operon, Chloroplast development, Temperature response, Rice

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Background

The mitochondrial transcription termination factor (mTERF) family consists of a group of nucleic acid binding proteins with so-called mTERF repeats of ~31 amino acids forming three helices [1, 2]. Similarity searches and phylogenetic analysis demonstrated that the mTERF family exists only in eukaryotes except for fungi [3]. All these proteins are predicted to localize to mitochondria and/or chloroplasts. Mammal genomes encode only four mTERFs (MTERF1–4) [4], while higher plants harbor approximately 30 members [5]. The mammalian mTERFs regulate mitochondrial gene expression. Human MTERF1, the first identified mTERF, functions in terminating L-strand transcription at the 16S rRNA/leucyl-tRNA boundary [6], transcription activation [7, 8], and DNA replication [9], followed by the discoveries of the roles of MTERF2 in restraining replication fork progression [10], MTERF3 in transcription suppression, replication [11], and ribosomal biogenesis [12], and MTERF4 in transcription activation [13] and ribosomal biogenesis [14]. Plant mTERFs, by contrast, are barely understood for their roles in the regulation of ribosomal biogenesis [14].

Plant mTERFs, by contrast, are barely understood for their roles in the regulation of organelar gene expression. Of the 35 mTERFs in Arabidopsis, barely understood for their roles in the regulation of ribosomal biogenesis [14], there are transcribed by NEP [31], otherwise produce five different overlapping transcripts including psbA, psbK, and psbC, which are precursor transcription, 5′ and 3′ end processing, intercistronic cleavage, 5′ and 3′ end maturation and editing, and intron removal [30]. At least two distinct RNA polymerases, PEP and the nucleus-encoded RNA polymerase (NEP), are responsible for plastid gene transcription during all phases of chloroplast development and in non-green plastid types [31]. The gene encoding the α subunit of PEP, rpoA, is clustered with multiple ribosomal protein-encoding genes in the rpoA operon, while the genes encoding the β, β′ and β′′ subunits of PEP, rpoB, rpoC1, and rpoC2, respectively, form a separate operon. Both of the rpoA and rpoB operons are transcribed by NEP [31–33]. On the other hand, photosynthetic genes, such as psbA, psbD, and psaB, are PEP-dependent. The association between PEP and the promoter regions of most of these genes is significantly increased in the light [34]. For example, a light-responsive promoter was identified between psbl and psbD in the psbK-psbl-psbD-psbC operon, which accounts for the transcription of the dicistronic psbD-psbC. Two other standard PEP promoters residing upstream of psbK and the light-responsive promoter, respectively, otherwise produce five different overlapping transcripts including psbK-psbl-psbD-psbC, psbK-psbl, and psbD-psbC [35]. The light-induced psbD-psbC, which was undetectable in the dark, was abundantly accumulated in green rice seedlings [35].

Here we described the effects of a rice mTERF, V14, on appropriate intercistronic cleavage of the polycistronic

mTERFs. Zm-mTERF4, the BSM ortholog in maize, directly binds the group II introns in certain chloroplastic transcripts and interacts with some of the known chloroplastic splicing factors, thus promoting the splicing of such transcripts, including trnI-GAU, trnA-UGC, and rpl2 [25]. Later, two studies of Arabidopsis mTERF6 demonstrated its DNA-binding activity in vivo, which is required for the transcription termination at a specific site in trnI-GAU and at the 3′-end of rpoA polycistron in chloroplasts [26, 27]. Recently, mitochondrial ZmSmk3 was found involved in the splicing of nad4 intron 1 and nad1 intron 4 in maize [28], and Arabidopsis mTERF9 was shown to promote chloroplast ribosome assembly and translation by interacting with 16S and 23S rRNAs [29]. Despite these advances, the molecular mechanism by which plant mTERFs regulate organellar gene expression is still far from full understanding, and it is not clear if mTERFs involve in processing organellar polycistronic transcripts. Moreover, little information has been provided so far for the impact of mTERFs on chloroplast and mitochondrion development in rice (O. sativa L.), a model crop species.

Derived from a cyanobacterial ancestor, the chloroplast holds many genes organized in gene clusters. Chloroplast mRNA maturation includes multiple steps, which are precursor transcription, 5′ and 3′ end processing, intercistronic cleavage, 5′ and 3′ end maturation and editing, and intron removal [30]. At least two distinct RNA polymerases, PEP and the nucleus-encoded RNA polymerase (NEP), are responsible for plastid gene transcription during all phases of chloroplast development and in non-green plastid types [31]. The gene encoding the α subunit of PEP, rpoA, is clustered with multiple ribosomal protein-encoding genes in the rpoA operon, while the genes encoding the β, β′ and β′′ subunits of PEP, rpoB, rpoC1, and rpoC2, respectively, form a separate operon. Both of the rpoA and rpoB operons are transcribed by NEP [31–33]. On the other hand, photosynthetic genes, such as psbA, psbD, and psaB, are PEP-dependent. The association between PEP and the promoter regions of most of these genes is significantly increased in the light [34]. For example, a light-responsive promoter was identified between psbl and psbD in the psbK-psbl-psbD-psbC operon, which accounts for the transcription of the dicistronic psbD-psbC. Two other standard PEP promoters residing upstream of psbK and the light-responsive promoter, respectively, otherwise produce five different overlapping transcripts including psbK-psbl-psbD-psbC, psbK-psbl, and psbD-psbC [35]. The light-induced psbD-psbC, which was undetectable in the dark, was abundantly accumulated in green rice seedlings [35].

Here we described the effects of a rice mTERF, V14, on appropriate intercistronic cleavage of the polycistronic
*rpoB-rpoC1-rpoC2* precursor in chloroplasts and accumulations of certain photosystem proteins, for example PsbD, during early stage of seedling leaf development. Intriguingly, this regulation pattern is growth stage-specific and temperature-sensitive. We thus suggest a role of V14 in chloroplast development and adaptation to temperature.

**Results**

**V14 is a chloroplastic protein critical to early stage of leaf development**

The V14 locus was previously mapped as a 162-kb region on chromosome 7, using a stage-conditional virescent-14 (*v14*) mutant of Taichung 65 (T65), a *japonica* cultivar [36]. This mutant develops albinism in the first two true leaves at 25 °C and returns green thereafter [30]. Subsequent fine mapping narrowed down the V14 locus to a 30-kb region containing two protein-coding genes (Additional file 1). One of which, Os07g0583200, had a 1283-bp deletion in the promoter and 5′ untranslated/coding regions (−1245 ~ +38) in the mutant [36]. Indeed, deficiencies of Os07g0583200 mRNA [36] and its protein product (Fig. 1A) were observed in *v14*. This gene encodes a putative chloroplastic protein (Refseq, https://www.ncbi.nlm.nih.gov), and its chloroplast localization was verified in rice leaf protoplasts expressing a Os07g0583200-fused eGFP construct (Fig. 1B). These data suggested that Os07g0583200 was a strong candidate for the V14 locus. Transcripts of this gene were observed in all analyzed stages of leaves (Fig. 1C), and their products bear seven consecutive mTERF repeats at the C-terminus [36], which is annotated by Refseq as a rice MTERF9. We further performed transgenic complementation and RNA interference (RNAi) to

![Fig. 1](https://www.ncbi.nlm.nih.gov)

**Fig. 1** V14 is critical to leaf development in the early stages of seedling. A V14 deficiency in the *v14* mutant demonstrated by immunoblotting analysis of the first and third leaves of *v14*. B Chloroplast localization of V14 indicated by the green fluorescence expressed by V14-fused eGFP in chloroplasts. BF, bright field; Chl, autofluorescence of chlorophyll (red); Bar = 10 μM. C Expression of V14 in different organs and at different stages of development. Coty, cotyledon; L1-L5, the first to the fifth true leaf; FL, flag leaf. D Complementation of the *v14* mutant confirmed by phenotypic analysis and V14- and *v14*-specific PCR in *T1* plants. G, green; v, virescent. The un-cropped gel image is provided in Additional file 1 B. Bar = 0.5 cm (E) Reproduction of the *v14*-like phenotype in the *T1* generation of two independent V14-RNAi lines, which is confirmed by phenotypic analyses and the gene expression analysis of V14 by qRT-PCR. Bar = 0.5 cm. The significant difference between T+ (with the transgene) and T− (without the transgene) plants was analyzed by student’s t test (*n* = 3): *, *P* < 0.05; **, *P* < 0.01. (A) and (B). The data presented here are the representative images of three independent experiments. (C) and (E). The relative expression levels shown here are the averages of three independent experiments.
confirm Os07g0583200 represents the V14 locus. Successful complementation of the albinism was achieved in those transgenic v14-T1 plants carrying an Os07g0583200-containing fragment with its native promoter (Fig. 1D). Simultaneously, the v14-like phenotype was reproduced in the RNAi plants manifesting downregulated Os07g0583200 expression (Fig. 1E). We therefore assigned V14 to this gene thereafter.

**V14 sustains functional chloroplasts via posttranscriptional precursor cleavage**

Our prior knowledge of v14 indicates an arrest of chloroplast development in the first two true leaves, as is evident from the absence of mature thylakoids and starch grains [36]. We thus posited transcription deficiency residing in the chloroplasts of the chlorotic leaves. To determine the chloroplastic genes affected, we first used qRT-PCR to assess the difference between v14 and T65 in transcript abundance of all 62 chloroplastic genes in the first true leaves. The results revealed significant gene expression changes (2 fold minimum) in v14 as compared to the wild-type T65, which involved two genes encoding the β′-, and β”-subunits of PEP, rpoC1 and rpoC2, a photosynthetic gene psbD, and a rice-exclusive gene orf56 [37, 38] encoding a truncated NdhH [39] (Fig. 2A). The transcripts rpoC1, rpoC2, and psbD were considerably down-regulated in v14, whilst orf56 was up-regulated (Fig. 2A). Further semi-quantitative RT-PCR analysis of the overlapping region across the coding sequences of psbD and psbC revealed its absence in v14 (Additional file 2A), confirming the significant reduction in psbD mRNA abundance. Given the important roles of the nucleus-encoded sigma factors in PEP activation, the expression of all five sigma factors was also analyzed by qRT-PCR. The results indicated that none of them were affected by V14 deficiency (Additional file 2B). Subsequent immunoblotting analysis confirmed the protein deficiency of RpoC1, RpoC2, and PsbD in v14 (Fig. 2B, Additional file 2C). In addition, the protein levels of some other photosynthetic...
proteins were assessed with commercially available antibodies. We found that two other photosystem proteins PsbA and PsaB also decreased in v14 (Fig. 2B, Additional file 2C), even though their transcript abundance was unchanged (Fig. 2A). In contrast, RpoA and RpoB were more abundant in v14 than in T65 (Fig. 2B, Additional file 2C). This might be attributed to the feedback inhibition of translation that has been observed in bacteria [40].

We next analyzed how V14 influences mRNA levels in chloroplasts. We noted that the V14 target genes are organized in co-transcribed gene clusters with other non-targeted genes. In light of the role of maize Zm-mTERF4 in intron splicing [25], we hypothesized that V14 may intervene in either precursor cleavage or RNA stabilization. To explore the behavior of V14, the abundance of the two intercistronic regions (P1 and P2) from the polycistronic rpoB-rpoC1-rpoC2 precursor (Fig. 2C) were analyzed by semi-quantitative RT-PCR in the first two true leaves of v14 and T65, using the two intercistronic regions (P3 and P4) from the polycistronic psaA-psaB-rps14 precursor (Fig. 2C) as controls. We found that the chlorotic leaves boasted higher amounts of the unprocessed transcripts including the rpoB-rpoC1 and/or rpoC1-rpoC2 regions as compared to the wild-type (Fig. 2C, Additional file 2D). No significant changes were observed for the P3 and P4 regions in v14 (Fig. 2C, Additional file 2D). Using P1 as a probe for Northern hybridization, we also detected a ~9-kb precursor transcript containing rpoB, rpoC1, and rpoC2 only in v14 (Fig. 2D).

Taken together, these data reflect the effects of V14 on the appropriate intercistronic cleavage of polycistronic rpoB-rpoC1-rpoC2 and psbD mRNA, PsbA, and PsaB accumulations. Given that light regulates expression of the PEP components in the first phase of photosynthesis establishment and psbD mRNA, PsbA, and PsaB abundance [41–44], V14 may play a key role in light signaling through chloroplast development.

The albinism phenotype of v14 is temperature-dependent

Similar to the three temperature-conditional rice virescents reported previously [45], v14 developed green leaves at permissive temperatures, 30 °C and 35 °C (Fig. 3A). To ascertain this full recovery at high temperatures, we first evaluated the expression levels of rpoC1,

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**Fig. 3** v14-induced defective posttranscriptional regulation and psbD mRNA reduction is reversed by high temperatures. A The chlorotic leaves of v14 returned green at 30 °C and 35 °C. Bar = 0.5 cm. B qRT-PCR analysis showed the expression recuperation of rpoC1, rpoC2, and psbD in L1 of v14 at 35 °C. C The maximum photosynthetic quantum yields represented as the Fv/Fm ratios recorded in L1, L2, and L3 of v14 confirmed its recovery of chloroplastic function at 35 °C. (B) and (C). The significance compared to T65 grown at 25 °C was analyzed by t test (n = 3). *, P < 0.05; **, P < 0.01. D qRT-PCR showing that V14 is continuously expressed in leaves and unresponsive to high temperature. L1-L3, the first to the third true leaf. The relative expression levels shown here are the averages of three independent experiments.
rpoC2, and psbD in the first true leaves of the v14 plants grown at 25 °C and 35 °C. The RT-PCR analysis showed that the abundance of these transcripts at 35 °C could reach a level comparable to their counterparts in T65 (Fig. 3B). Indeed, the appropriate cleavages of the two intercistronic regions of rpoB-rpoC1-rpoC2 were retrieved in these green-recovered v14 plants (Additional file 3). We further assessed chloroplast function in the v14 plants grown at 35 °C by gauging the Fv/Fm ratio (the maximum photosynthetic quantum yield), a measurement representing Photosystem II efficiency, in the first, second, and third true leaves. In agreement with the expression recuperation of rpoC1, rpoC2, and psbD, the Fv/Fm values recorded in the 35 °C-growing v14 plants showed no significant difference from those in the T65s grown either at 25 °C or 35 °C (Fig. 3C). By contrast, the 25 °C-growing v14 plants still could not fully retrieve the power of photosynthesis in their third leaves even though they returned green (Fig. 3C). Interestingly, V14 mRNA expression in T65 was neither heat-sensitive nor stage-specific, albeit relatively high in the second true leaf (Figs. 1C, 3D).

We thus postulated that there may be unknown V14 parallel factor(s) acting at high temperatures while V14 is inactive. Our similarity searches on NCBI identified 30 other V14-homologs in rice (Additional file 4). Expression analysis of these genes in 25 °C- and 35 °C-grown T65s showed that three of them, Os07g0134700, Os08g0528700, and Os02g0602400, were significantly up-regulated at 35 °C in the second true leaf where V14 expression reaches its peak (Fig. 4A, Additional files 5, 6 and 7). Os07g0134700 and Os02g0602400 are predicted to encode chloroplastic mTERFs (annotated as rice MTERF2 and MTERF5 homologs, respectively, by Refseq), while Os08g0528700 encodes an unannotated mTERF-like protein.

Discussion

The mTERF family earned its name from its founding member, human MTERF1 [6], as a group of transcription termination factors 31 years ago, but more molecular functions have since been linked to it, such as transcription initiation, DNA replication, and intron splicing. Our data presented here extend the understanding of the molecular functions of mTERFs, which may regulate intercistronic cleavage of polycistronic precursors. We showed that V14 is required for the appropriate intercistronic cleavage of rpoB-rpoC1-rpoC2 precursor, thus regulating the abundance of mature rpoC1 and rpoC2 mRNAs that encode two core subunits of PEP. The expression of rpoB, however, is not targeted by V14. Since rpoB is co-transcribed with rpoC1 and rpoC2 by NEP [31–33], this result indicates that V14 specifically regulates precursor processing but not precursor transcription for the rpoB-rpoC1-rpoC2 operon. Despite reduced expression of mature rpoC1 and rpoC2 mRNAs in v14, expression of the PEP-dependent genes is unaffected except for psbD, implicating that low levels of PEP in the proplastids can maintain the expression of most of these genes. This observation is consistent with the developmental and gene-specific regulation of PEP transcription proposed in wheat seedlings [43]. In developing chloroplasts the light-independent PEP functions in the dark as well as in the light for the PEP-dependent genes including psbA, psbC, psbE, and rru16, except for psbD, whilst the light-dependent PEP selectively transcribes psbA and psbD in mature chloroplasts [43]. A light-responsive promoter producing a precursor including psbD and psbC has also been identified in the psbK-psbl-psbD-psbC gene cluster [46]. Considering psbC showed no expression change in v14 while being co-transcribed with psbD, we speculated that V14 may be crucial for psbD mRNA stability in a light-dependent way. We also noted that two other photosystem proteins PsbA and PsbB also decreased because of the V14 deficiency even without alterations in their transcript abundance, which can be explained by the facts that translation and stability of proteins encoded by psbA and psbB are light-dependent during chloroplast development [41]. Furthermore, a light response model established in Arabidopsis indicates that light signals precede plastid signals, where the first phase of photosynthesis establishment relies on light and triggers changes that will initiate chloroplast development, and more importantly initiates expression of the PEP components [47]. This model supports the impaired processing of rpoB-rpoC1-rpoC2 precursor and reduced accumulations of psbD, PsbA, and PsbB observed in the chlorotic leaves of v14, suggesting that V14 is essential for light signaling during chloroplast development. V14 might not act directly on its molecular targets, such as the rpoB-rpoC1-rpoC2 precursor, but interfere in a key step during chloroplast development that introduces these changes, which needs further investigations.

We showed that v14 was rescued by higher temperatures, and that the defective intercistronic cleavage of the rpoB-rpoC1-rpoC2 precursor is temperature-dependent (Fig. 3B, Additional file 3). However, the expression of V14 per se in wild-type is not temperature-sensitive (Fig. 3D). Considering V14 might act via phytohormone-mediated thermosensory pathways [48, 49], we examined the response of v14 seedlings to various phytohormone treatments (Additional file 8). However, none of such treatments could restore the albinoism observed at 25 °C, suggesting that V14 is irreversible to phytohormones. We further identified three mTERF genes showing a similar temporal expression pattern to V14 that were significantly up-regulated by
Fig. 4 A Three rice mTERF genes are heat-inducible, as indicated by qRT-PCR. The significant difference in expression between growth at 35 °C and 25 °C was analyzed by t test (n = 3). *, P < 0.05; **, P < 0.01. B The effects of V14 on chloroplast development are stage-specific and thermo-sensitive.
high temperatures in the second leaf (Fig. 4A), suggesting that these genes may be potential candidates for the unknown V14 parallel(s) whose functions compensate for the V14 deficiency at high temperatures. These data support the notion that plants have evolved functionally redundant members of gene families, by which certain members can be replaced by some other members in a conditional manner.

**Conclusion**

V14 is an essential transcriptional and translational regulator in chloroplasts supporting chloroplast biogenesis in the first two true leaves. While V14 expression is neither stage-specific nor thermo-sensitive, the V14-mediated regulation is stringently modulated by developmental stages and temperature (Fig. 4B). The roles of V14 in chloroplast development are just beginning to emerge. Further studies are needed to dissect the molecular functions of V14 in intercistronic cleavage, mRNA stability, and translational/posttranslational regulation, and to define how such regulations respond to temperature, thus helping to understand the contributions of organellar gene expression to photosynthesis establishment and temperature acclimation.

**Methods**

**Plant materials and treatment**

The seeds of T65 were obtained from Dr. Chuxiong Zhuang’s lab at South China Agricultural University. The seedlings were grown in growth chambers under 16-h light/8-h dark cycles at 25 °C. Details for the positional mapping of the V14 locus were provided in a previous study [36]. For the temperature treatments, the seedlings were grown under 16-h light/8-h dark cycles at 25 °C, 30 °C or 35 °C.

**Intracellular localization of eGFP fusions**

For eGFP visualization, a cDNA fragment of V14 was obtained from T65 using the primers V14-xho5 and V14-spe3 (Additional file 9), and fused with the coding sequence for detecting chloroplastic transcripts were listed in Additional file 10, except for those for amplifying intercistronic regions (P1-P4), the overlapping

file 9, and cloned into a plant expression vector pCAMBIA 1380 with Gibson Assembly® Master Mix (New England Biolabs). The success of the complementation was confirmed by phenotypic analysis and PCR using the V14-specific primers as described in a previous study [36]. For generation of the V14-RNAi plants, a cDNA fragment was obtained from T65 using two primer pairs (Additional file 9), V14i-5-1 and V14i-3-1, and V14i-5-2 and V14i-3-2, and cloned into a plant expression vector pCAMBIA 1301 with Gibson Assembly® Master Mix. The complementation construct was introduced into v14, and the RNAi one was transferred into T65, by Agrobacterium-mediated transformation.

**Chloroplast isolation**

Chloroplasts were prepared as previously published [51]. In brief, 10 g of fresh seedling leaves were frozen in liquid nitrogen and gently ground into fine powder. The powder was then suspended in 100 ml of Medium A (50 mM HEPES-KOH pH 8.0, 330 mM sorbitol, 2 mM EDTA-Na₂, 5 mM ascorbic acid, 5 mM cysteine, 0.05% BSA) and the suspension was filtered through two layers of gauze and then two layers of Miracloth (Merk). This filtrate was subjected to centrifugation (1300xg, 4 °C, 5 min) to collect chloroplast pellets followed by sucrose density gradient centrifugation (30, 40, 55% sucrose density gradient in Medium B, 30000xg, 4 °C, 1 h) using the pellets suspended in 200 μl of Medium B (50 mM HEPES-KOH pH 8.0, 330 mM sorbitol, 2 mM EDTA-Na₂). The green band at the 30 and 40% sucrose interface was collected and rinsed twice with 75 mL of Medium B through centrifugation (2000xg, 4 °C, 15 min). Finally, the pellets were resuspended in 50 μl of TRIzol™ Reagent (ThermoFisher Scientific) for RNA extraction.

**Nucleic acid extraction, qRT-PCR, northern blot and immunoblotting**

Genomic DNA was isolated from leaves with a DNeasy Plant Mini Kit (Qiagen). Total RNA was extracted from leaves or chloroplasts following the instruction of TRIzol™ Reagent. DNase I (Invitrogen) digestion was applied prior to reverse transcription. For assessment of the abundance of nuclear transcripts, Oligo (dT)_20 (50 μM) was used for the synthesis of first-strand cDNA from total RNA extracted from leaves. For assessment of the amounts of all chloroplastic transcripts, Random Hexamers (50 ng/μL) was used for the synthesis of first-strand cDNA from total RNA extracted from chloroplasts. The primers for the nucleus-encoded sigma factors were shown in Additional file 9. All the primer sequences for detecting chloroplastic transcripts were listed in Additional file 10, except for those for amplifying the intercistronic regions (P1-P4), the overlapping
region across \textit{psbD} and \textit{psbC}, and a region downstream of \textit{psbC} (Additional file 2 A), which were given in Additional file 9. Northern blot analysis was performed using total RNA as previously published [52]. The probe P1 was labeled with 0.01 μM digoxigenin (DIG)-deoxyuridine triphosphate by PCR. To extract proteins, seedling leaves were homogenized in 2 x SDS sample buffer (62.5 mm Tris-HCl, pH 6.8, 20% [v/v] glycerol, 4% [w/v] SDS, 100 mm dithiothreitol, and 0.05% [w/v] Bromphenol Blue), incubated at 95 °C for 5 min, and centrifuged at the maximum speed for 20 min. The samples were quantified and subjected to SDS-PAGE (12%) followed by wet transfer to PVDF membranes (Millipore). The membranes were then incubated with the antibodies against V14, RpoA, RpoB, RpoC1, RpoC2, PsbA, PsbD, PsaB, or NdhF. All these antibodies were obtained from BGI, except for the V14 antibody, which was developed by Abmart using a synthetic peptide EGRQPKTRDRCD as the immunogen. All the protein levels were normalized to NdhF by ImageJ, which is shown in Additional file 2C.

**Chlorophyll fluorescence analysis**

The experiments were performed following the protocol published previously [53] with some minor modifications. Six plants for each group were dark-adapted for 20 min before taking measurements with a PAM fluorometer (Walz). All measurements were taken at the same time during the day. A saturating pulse of radiation (2700 μmol m⁻² s⁻¹) was applied to record the maximum fluorescence yield (Fm), and a weak modulating radiation (0.5 μmol m⁻² s⁻¹) was used to measure the minimum fluorescence yield (F₀). The maximum photosynthetic quantum yield was then calculated as Fv (variable fluorescence yield)/Fm = (Fm–F₀)/Fm.

**Abbreviations**

mTERF: Mitochondrial transcription termination factor; PEP: The plastid-encoded plastid RNA polymerase; NEP: The nucleus-encoded RNA polymerase; Fv: Variable fluorescence yield; Fm: The maximum fluorescence yield; F₀: The minimum fluorescence yield; NCB: National Center for Biotechnology Information

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12870-021-03192-2.

**Additional file 1.** (A) Fine mapping of the V14 locus on chromosome 7. The dashed line represents the genomic deletion in the promoter and 5’ untranslated and coding regions (~1245~438) in the v14 mutant. Arrows indicate primers for detecting the endogenous and transgenic fragments of V14 and v14 as shown in Fig. 1D. (B) Complementation of the v14 mutant confirmed by V14- and v14-specific PCR in T1 plants.

**Additional file 2.** Analysis of the \textit{psbD}-containing transcripts by semi-quantitative RT-PCR (31 cycles) (A), expression analysis of the rice sigma factors by qRT-PCR (B), quantification of the immunoblotaling analysis shown in Fig. 2B by ImageJ (C), and analysis of the two intercistronic regions (P1 and P2) of rpoB-rpoC1-rpoC2 in the second true leaves (grown at 25 °C) of v14 by semi-quantitative RT-PCR (31 cycles) (D). (A), The positions of the primers are designated relative to the start codon of the ORFs where they are located. (B), The significance compared to T65 was analyzed by t test (n = 3). (C), All the protein levels were normalized to NdhF. (D), The two intercistronic regions (P3 and P4) of \textit{psaA}-\textit{psaB}-\textit{psaC} were used as the control. (A) and (D), The images presented here are the representatives of three biological repeats.

**Additional file 3.** High temperature (35 °C) rescued the cleavage of the two intercistronic regions (P1 and P2) of \textit{rpoB}-\textit{rpoC1}-\textit{rpoC2} precursor in \textit{L1} of \textit{v14}. The semi-quantitative RT-PCR was carried out by 31 cycles. \textit{L1}, the first leaf. P3 and P4 are the two spacer regions in the \textit{psaA}-\textit{psaB}-\textit{psaC} operon shown in Fig. 2C. The image presented here is the representative of three biological repeats.

**Additional file 4.** Phylogenetic analysis of V14 in rice. The neighbor-joining tree was built on protein sequences using the software PHYLIP (version 3.66) and visualized with the software TreeView and MEGAS. Arrows indicate the three genes with temperature-sensitive expression in the second leaf (Fig. 4).

**Additional file 5.** Gene expression profiles of the other 27 \textit{V14}-homologous genes at different stages of leaf development at 25 °C and 35 °C. The relative expression levels shown here are the averages of three independent experiments.

**Additional file 6.** Gene expression profiles of the other 27 \textit{V14}-homologous genes at different stages of leaf development at 25 °C and 35 °C. The relative expression levels shown here are the averages of three independent experiments.

**Additional file 7.** Gene expression profiles of the other 27 \textit{V14}-homologous genes at different stages of leaf development at 25 °C and 35 °C. The relative expression levels shown here are the averages of three independent experiments.

**Additional file 8.** \textit{v14} seedlings on various phytophormone treatments at 25 °C. Bar = 0.5 cm; L1, the first true leaf; L2, the second true leaf.

**Additional file 9.** Primer sequences for complementation, RNAi, and the assessment of the processing intermediates.

**Additional file 10.** Primer sequences for the mRNA expression profiling of the chloroplastic gene transcripts.

**Additional file 11.** The un-cropped blot images of Figs. 1A, 2B, and D.

**Additional file 12.** The original real-time PCR data for Figs. 2A, 3B, and 3C.

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Not applicable.

**Authors’ contributions**

M. W., F. Z., H. W. and D. X.: Performing experiments and data analyses; Q. Z. and Y. L.: Conceiving the project and Writing the manuscript. All authors reviewed the manuscript. The author(s) read and approved the final manuscript.

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**Availability of data and materials**

The sequences of \textit{v14} and the mutant \textit{v14} have been deposited in NCBI under the submission IDs MZ299153 and MZ299154, respectively.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

Not applicable.
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