Mitochondrial ATP synthase activity affects plastid retrograde signaling in Arabidopsis

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Abstract

Plastid retrograde signaling plays a key role in coordinating the expression of plastid genes and photosynthesis-associated nuclear genes (*PhANGs*). Although plastid retrograde signaling can be substantially compromised by mitochondrial dysfunction, it is not yet clear whether specific mitochondrial factors are required to regulate plastid retrograde signaling. Here, we show that mitochondrial ATP synthase β-subunit mutants with decreased ATP synthase activity are impaired in plastid retrograde signaling. Transcriptome analysis revealed that the expression levels of *PhANGs* were significantly higher in seedlings affected in *AT5G08670,* the gene of the β-subunit of mitochondrial ATP synthase than in wild-type (WT) seedlings upon treatment with lincomycin (LIN) and norflurazon (NF). Further studies showed that the expression of nuclear genes involved in chloroplast and mitochondrial retrograde signaling was affected in *AT5G08670* mutant seedlings treated with LIN. These changes might be associated with the repression of some transcriptional factors (TFs), such as *ARF3/ETTIN* (*ETT*), *PLASTID TRANSCRIPTION FACTOR 1* (*PTF1*), *CYCLOIDEA AND PCF TRANSCRIPTION FACTOR 2* (*TCP2*), and *KOW DOMAIN-CONTAINING TRANSCRIPTION FACTOR 1* (*KTF1*). These findings indicate that the activity of mitochondrial ATP synthase affects plastid retrograde signaling.

INTRODUCTION

The chloroplast is a complex cellular organelle that not only performs photosynthesis but also synthesizes various macromolecules and metabolites including fatty acids, vitamins, tetrapyrroles, and amino acids required for plant growth (Allen, Paula, Puthiyaveetil, & Nield, 2011; Jarvis & Lopez-Juez, 2014). Chloroplasts contain their own DNA and protein-synthesizing apparatus. Many key components of the photosynthetic machinery are encoded by the chloroplast genome (Waters & Langdale, 2009). Mitochondria, which are present in all eukaryotes, are the site of cellular respiration that provides energy in the form of ATP via oxidative phosphorylation for driving cellular metabolism. Endo-symbiotic evolution within higher plants resulted in the emergence of semi-autonomous organelles such as chloroplasts and mitochondria (Rigas, Daras, Tsitsekian, Alatzas, & Hatzopoulos, 2014). Most mitochondrial and chloroplast proteins are encoded by the nuclear genome, expressed in the cytosol, and then imported into the organelles (Yusuke et al., 2014). Although the nucleus regulates the development and function of organelles, signals are also sent to the nucleus by these organelles to deliver information regarding their growth and developmental status to adjust the expression of nuclear genes (Nott, Jung, Koussevitzky, & Chory, 2006). For example, *COE1* and*COE2* , which play a role in plastid retrograde signaling, have a significant impact on chloroplast biogenesis and plant growth (Sun, Xu, Liu, Kleine, & Leister, 2016; Wu et al., 2022).

Retrograde signaling modulates nuclear gene expression in response to changes in organellar status. Mitochondria and chloroplasts, the two energy-converting organelles of plants, are closely coordinated to balance their activity (Wang et al., 2020). Likewise, anterograde signaling pathways control the expression of nuclear genes encoding factors involved in plastid gene expression and tetrapyrrole biosynthesis (Woodson & Chory, 2008). Plastids produce retrograde signals to alter the expression of nuclear genes in response to stress-related damage (Inaba, Yazu, Ito-Inaba, Kakizaki, & Nakayama, 2011). Developing chloroplasts in *Arabidopsis thaliana* are vulnerable to photo-oxidative damage because they lack protective carotenoids. This type of damage perturbs the tetrapyrrole biosynthetic pathway resulting in the accumulation of several tetrapyrrole intermediates and the down-regulation of *PhANGs* (Strand, Asami, Alonso, Ecker, & Chory, 2003). Various *genome-uncoupled*(*gun*) mutants in which this type of retrograde signaling is impaired (e.g., *gun1 – gun6*) have been identified and characterized in previous studies (Koussevitzky, 2007; Larkin, Alonso, Ecker, & Chory, 2003; Mochizuki, Brusslan, Larkin, Nagatani, & Chory, 2001; Strand et al., 2003; Susek, Ausubel, & Chory, 1993; Woodson, Perez-Ruiz, & Chory, 2011).

In plants, mitochondrial retrograde signaling is associated with reactive oxygen species (ROS) signaling, pathogen sensing, and programmed cell death (Rhoads & Subbaiah, 2007; Woodson & Chory, 2008). Some types of cytoplasmic male sterility in flowering plants are induced by retrograde signaling in response to mitochondrial dysfunction (Sota & Kinya, 2008). Calcium ion signaling, protein kinases, nuclear TFs, and rare protein subunits have been shown to be involved in the mitochondrial retrograde signaling pathway (Guha & Avadhani, 2013; Guha, Srinivasan, Koenigstein, Zaidi, & Avadhani, 2016; Thierry, Sébastien, & Patricia, 2015).

Chloroplasts and mitochondria are metabolically interdependent in the photosynthetic cells of plants (Raghavendra & Padmasree, 2003). Carbon dioxide (CO_2) and ATP produced by the mitochondria are used during photosynthesis, and the photosynthetic byproducts oxygen (O_2) and malate are used by the mitochondria. Mitochondria also consume the redox products of chloroplasts and protect them from damage caused by over-reduction of the photosynthetic electron transport chain and photoinhibition (Raghavendra & Padmasree, 2003). During photorespiration exchange of serine and glycine between mitochondria and chloroplasts is mediated by peroxidases (Peter et al., 2004).

In plant cells, ATP synthases are present in chloroplasts and mitochondria. The mitochondrial ATP synthase, also known as F1Fo-ATP synthase, catalyzes oxidative phosphorylation and uses the transmembrane proton gradient to synthesize ATP (Stock, Leslie, & Walker, 1999). Mitochondrial ATP synthase consists of two separate parts: F1 and Fo. The F1 portion protrudes into the mitochondrial matrix and consists of the *a, b, g* and *d* -subunits. The β -subunit carries the catalytic site for ATP synthesis, and catalysis occurs through highly coordinated conformational changes in the α andβ -subunits (Xu, Pagadala, & Mueller, 2015). Fo acts as a turbine driven by proton flow and is inserted in the inner membrane and linked to the F1 rotor.

The C-terminal domain of the β -subunit consists of a highly conserved helix-turn-helix motif termed "DELSEED-loop," likely to be involved in the coupling between catalysis and rotation (Mnatsakanyan, Krishnakumar, Suzuki, & Weber, 2009). Loss of the 10 residues of the DELSEED-loop abolishes ATP synthesis (Mnatsakanyan, Kemboi, Salas, & Weber, 2011).

Low levels of ATP are sensed by SnRK1 which phosphorylates the SOG1 protein, thereby increasing the expression of cell cycle-related genes (*cyclin-dependent protein kinase 3* ;2, *CYCA3* ;2 or*cyclin D-type protein 3;3* , *CYCD3;3*) to inhibit plant cell growth (Hamasaki et al., 2019).

Here, we studied a mutant affected in *AT5G08670* encoding the mitochondrial ATP synthaseβ- subunit which leads to a decrease in ATP synthase level and activity. Transcriptome analysis revealed that the transcriptome profile of this mutant is significantly altered compared to the wild type. The expression of chloroplast and mitochondrial retrograde signaling-related genes and some TFs are affected in the mutant treated with LIN, an inhibitor of chloroplast protein synthesis. These results indicate that mitochondrial ATP levels affect plastid retrograde signaling.

RESULTS

Ισολατιον, σεχυενσε αναλψσις, ανδ λοσαλιζατιον οφ μιτοσηονδριαλ ΑΤΠ σψντηασε β-συβυνιτ

The mitochondrial ATP synthase β- subunit (ATPB) is encoded by three genes (*AT5G08670, AT5G08680, AT5G08690*) with highly similar amino acid sequences (98% sequence identity). The mature protein consists of 556 amino acids and has a molecular mass of 59'630 Da and an isoelectric point of 6.53. Multiple sequence alignment analysis and the phylogenetic tree show that mitochondrial and chloroplast ATPB genes cluster in two branches (Figure 1a). We further examined the expression of these genes and found that *AT5G08680* is a pseudogene that is not expressed. Thus, in this study, we only focus on the*AT5G08670* and *AT5G08690* genes. They are spaced approximately 4,400 bp apart, and their precursor proteins consist of a total of 566 amino acids (Figure 1b, c). The location and presequence size of these proteins were determined using several online protein-peptide prediction programs which predicted a mitochondrial presequence with a cleavage site localized specifically between amino acids numbers 51 and 52 (Figure S1). A comparison of the precursor proteins for *AT5G08690* (NP 568204) and *AT5G08670* (NP 568203) revealed that there are only two residues that differ between the two proteins (Figure 1c) within the presequence. To analyze the expression patterns of *AT5G08670* and *AT5G08690* , we generated transgenic plants expressing*AT5G08670* p:β -glucuronidase (GUS) and*AT5G08690* p:GUS. These two lines showed a similar spatiotemporal expression pattern of *AT5G08670* and *AT5G08690* in the early developmental stages of seedlings. However, at the later developmental stages, *AT5G08670* expression declined, and by 21-days, it was no longer expressed (Figure 1d). These findings indicate that these two proteins are highly similar although their spatiotemporal expression patterns are different, which might contribute to functional differences in subsequent developmental stages.

The *AT5G08670* mutant is a *genomes uncoupled (gun)*mutant

Retrograde signaling is triggered when plastid gene expression (PGE) is disrupted at the transcriptional or translational levels (Woodson & Chory, 2008). Inhibition of PGE causes changes in the expression of*PhANG* and the *LIGHT-HARVESTING CHLOROPHYLL A/B-BINDING*(*LHCB*) genes (Zhang et al., 2011). When WT seedlings are exposed to the plastid development inhibitors norflurazon (NF) and lincomycin (LIN), plastid retrograde signaling is induced (Strand et al., 2003). However, in plastid-to-nucleus signaling mutants, retrograde signaling is impaired and LIN or NF treatment no longer represses the expression of *LHCB* genes to the same extent as in WT (Strand et al., 2003). To determine whether*AT5G08670* is involved in plastid retrograde signaling, T-DNA insertion mutants of*AT5G08670* (*SALK_047877, SALK_083115*) were obtained (Figure 2a), and the homozygous T-DNA insertion was confirmed (Figure S1). To further check the impairment of retrograde signaling, the lines*SALK_047877* , *SALK_083115* , and *genomes uncoupled1* (*gun1*) (as an experimental control) were treated with LIN and NF. Expression of *LHCB1.2* was found by Real-Time Quantitative Reverse Transcription PCR (qRT-PCR) to be significantly higher in *gun1* , *SALK_047877* and *SALK_083115* than in WT seedlings treated with LIN and NF treatment (Figure 2b, c), thus revealing a conspicuous *gun* phenotype (Figure 2b, c). These results suggest that *AT5G08670* may play a role in plastid retrograde signaling.

AT5G08670 protein is localized in mitochondria

Fluorescence microscope analysis of protoplasts of *Arabidopsis* from a transgenic line expressing a*35S::AT5G08670-GFP* fusion protein indicated that*AT5G08670-GFP* is targeted to mitochondria (Figure 3) thus confirming its mitochondrial localization, In public databases (The Arabidopsis Information Resource, TAIR; www.arabidopsis.org),*AT5G08670* is also annotated as a mitochondrial protein.

Loss of expression of *AT5G08670* leads to a decrease in mitochondrial ATP synthase level and activity

Because *AT5G08670* and *AT5G08690* encode an identical mature ATP synthase *b* -subunit, it was important to determine whether the level of mitochondrial ATP synthase was diminished and whether its activity was affected in the *AT5G08670* mutants. Immunoblotting with ATPB antibody revealed that the level of ATP *b* -subunit dropped to 25% of WT level in the *AT5G08670* mutants, and total ATP synthase activity was

found to be significantly lower than in WT (Figure 4a-b). Taken together, these results indicate that changes in mitochondrial ATP synthase activity affect plastid retrograde signaling.

Detection of differentially expressed genes (DEGs) of*ATG08670* mutants by transcriptome analysis.

RNA-seq analysis was performed to study the effects of the T-DNA insertions in *AT5G08670* on the transcriptome. Principal component analysis (PCA) was performed using the expression of genes to examine the distribution of samples and explore relationships between samples. Samples in the same group were more concentrated in spatial distribution. In the control group, the samples clustered in the same region. After LIN treatment, the distributions of mutant and WT differed (Figure 5a). The number of counts for each gene was normalized using DESeq software. The negative binomial distribution test was performed to determine the multiplicity of differences in data, estimate expression, and evaluate the significance of reading differences using the base mean values. The results of the difference in ploidy and significance tests were used to screen for differential expression of protein-coding genes. These differences are shown in the volcano plot, with non-significant differences in gene expression in grey, and significantly down- and up-regulated genes in red and green, respectively (Figure 5b). In WT,*gun1* , *SALK_047877* , and *SALK_083115* , DEGs were found to be, respectively, 11,192 (42 percent up-regulated, 58 percent down-regulated), 6842 (35 percent up-regulated, 65 percent down-regulated), 6242 (35 percent up-regulated, 65 percent down-regulated), and 5837 (38 percent up-regulated, 62 percent down-regulated) (Figure 4c). Unsupervised hierarchical clustering of DEGs was performed. The results show that after LIN treatment, WT, the two *SALK* lines, and the*gun1* mutant cluster each in separate branches (Figure 5d). These results indicate that the transcriptional patterns of the mutants and WT differ considerably after LIN treatment.

GO enrichment analysis of DEGs

Up-regulated genes and down-regulated genes were divided into four classes of DEGs. For each of the four groups of down-regulated genes, a Venn diagram was created. The results showed that there were 1617 down-regulated genes in WT, *gun1* , *SALK_047877* ,*SALK_083115,* and 322 down-regulated genes in the mutants only (Figure 6a). GO enrichment analysis of the 322 down-regulated genes in the mutants revealed that they were mainly enriched in the response to fatty acids, in nucleobase transport, toxin metabolic processes, regulation of defense response, and plant hormone signal transduction, (Figure 6b). For each of the four groupings of up-regulated genes, Venn diagrams were created. The analysis indicated 373 up-regulated genes in WT, *gun1* , *SALK_047877* , and *SALK_083115* , 185 up-regulated genes in the mutants only, and 2688 up-regulated genes in WT only (Figure 6c). GO enrichment analysis of the 185 up-regulated genes in the mutants showed that they were mainly enriched in tetrapyrrole metabolism, ribosome components, cytokinin signaling, embryo development, and photosynthesis (Figure 6d). GO enrichment analysis of the 2688 up-regulated genes in WT revealed that they were mainly enriched in the response to salicylic acid, vesicle-mediated transport, autophagy, autophagosome organization, and salicylic acid-mediated signaling pathway (Figure 6e).

GO enrichment analysis of plastid-related DEGs

A comparison of chloroplast and mitochondrial DEGs for the LIN versus (vs) control comparison revealed differences. GO enrichment analysis revealed that most down-regulated mitochondria-related DEGs in*gun1* , *SALK_047877* , and *SALK_083115* were enriched in the generation of precursor metabolites and energy, carbon metabolism, NADH metabolic process, and amino acid metabolic processes (Supplementary Figure 3a). Up-regulated mitochondria-related DEGs in*gun1* , *SALK_047877* , and *SALK_083115* were mainly enriched in valine, leucine, and isoleucine degradation, amino acid catabolism, mitochondrial transmembrane transport, and reactive oxygen species (Supplementary Figure 3b). Down-regulated and up-regulated chloroplast-related DEGs were enriched in similar GO terms in*gun1* , *SALK_047877* ,*SALK_083115,* and WT seedlings, such as the response to cold, biosynthesis of amino acids, starch metabolic processes, photosynthesis, carbon metabolism, biosynthesis of amino acids (Supplementary Figure 3c-d). However, protein import into chloroplast stroma was only enriched in WT (Figure 6c). These findings indicate that the effect

of LIN treatment on the expression of chloroplast-related genes is similar in both mutant and WT seedlings, although the magnitude of the effect of LIN on mutants and WT seedlings differs to some extent.

Expression of nuclear genes of mitochondrial proteins is affected by the loss of *AT5G08670*

RNA-seq analysis revealed that the expression of *AT5G08670* was significantly lower in*gun1* , *SALK_047877* , and*SALK_083115* than in WT seedlings (Figure 7a). In contrast, expression of *AT5G08690* was significantly higher in *gun1* ,*SALK_047877* , and *SALK_083115* than in WT seedlings suggesting some compensatory mechanism (Figure 7b). After LIN treatment, the expression of both *AT5G08670* and *AT5G08690* decreased (Figure 7a-b). The expression of *AT5G08670* was decreased and that of *AT5G08690* was raised in the *gun1* mutant compared with WT seedlings (Figure 7a-b). Similar to *gun1* , the expression of*AT5G08690* was higher in the *SALK* lines than in WT seedlings treated with LIN. These results indicate that*GUN1* -mediated signals influence the expression of nuclear genes of mitochondrial proteins upon LIN treatment. We next analyzed the regulatory effects of *AT5G08670* on some key genes involved in plastid and mitochondrial signaling. *GUN1* , *GUN4* , and*GUN5* are important regulators of plastid signaling. After LIN treatment, the expression of *GUN4* and *GUN5* was decreased to different degrees in the T-DNA insertion mutants, in which the expression of*GUN1* increased relative to the control (Figure 8a). We also analyzed the expression of important regulators of nuclear genes of mitochondrial proteins such as *ALTERNATIVE OXIDASE 1D*(*AOX1D*), *AOX1A* , *AOX1C* , and *AOX2.* The expression of *AOX1D* and *AOX1A* increased in WT seedlings treated with LIN, and the expression of these genes was decreased in*SALK_047877* and *SALK_083115* (Figure 8b). The expression of *AOX2* was suppressed in all mutants treated with LIN (Figure 8b). Further, the analysis of the expression of carbon metabolism genes revealed that *1-Aminocyclopropane-1-Carboxylate Oxidase 1*(*ACO1*) was decreased in all groups, except in WT seedlings following LIN treatment; the expression of *ACO2* , *ACO3* ,*hexokinase 1* (*HXK1*), and *HXK2* was decreased to different degrees in all samples (Figure 8c-d), and the expression of*mitochondrial Malate Dehydrogenase 1* (*mMDH1*) was significantly inhibited in WT seedlings (Figure 8d). The transcript levels of LIN-repressed genes, such as *GUN4* , *GUN5* ,*AOX1C* , *HXK1* , *HXK2* , and *mMDH1* , were higher in*gun1* , *SALK_047877,* and *SALK_083115* seedlings than in WT seedlings treated with LIN. In contrast, the transcript levels of the LIN-induced genes *AOX2* , *AOX1D* , *AOX1A* ,*ACO1* , and *ACO2* were lower in *gun1* ,*SALK_047877,* and *SALK_083115* , seedlings than in WT seedlings in the presence of LIN. These findings indicate that*GUN1* and *AT5G08670* dependent signaling pathways play important roles in regulating the expression of nuclear genes of both chloroplast and mitochondrial proteins in response to LIN (Figure 10).

Identification of important TFs downstream of*AT5G08670*-dependent signaling

Differentially expressed genes of transcription factors were ordered into up-regulated and down-regulated groups. Venn diagrams were plotted for the 3 groups of up-regulated transcription factors. The results showed that there were 5 up-regulated genes in WT, *SALK_047877* ,*SALK_083115,* and 56 up-regulated genes in the WT only (Figure 9a, Table S1). Venn diagrams were performed for the three groups of down-regulated genes. The results showed that there were 16 down-regulated genes in WT, *SALK_047877* , *SALK_083115* , and 71 down-regulated genes in the WT only (Figure 9b, Table S2). Based on differently expressed TFs, we constructed a regulatory network of TFs to characterize the downstream network associated with*AT5G08670* -dependent signaling. Analysis of the protein-protein interaction network revealed that *ETT* , *PTF1* , *TCP2* , and *KTF1* play key regulatory roles in *AT5G08670* -dependent signaling (Figure 9c). *ETT1* is located in mitochondria and plays a role in protecting mitochondrial components such as DNA from oxidative injury resulting from the generation of superoxide by the mitochondria (Paul & Snyder, 2010). Furthermore, plastid transcription 1 (*PTF1*) is involved in plastid protein synthesis and acts as a TF of *PSBD* that encodes protein D2 of PSII (Baba, Nakano, & Yoshida, 2001; Kim M, 1995). *TCP2* positively regulates mRNA expression of *HY5-HOMOLOG* (*HYH*) (He, Zhao, Kong, Zuo, & Liu, 2016), which is a photomorphogenesis-mediated transcription factor (Holm, Ma, Qu, & Deng, 2002). These findings indicate that*AT5G08670* -dependent signaling might involve specific TFs in mitochondria, chloroplasts, and nuclei to regulate plastid retrograde signaling.

DISCUSSION

AT5G08670 affects the expression of *PhANGs*

Mitochondria and chloroplasts are essential for plant growth and development. Plastid development is regulated by the nucleus but also affects nuclear transcriptional activity through plastid feedback by retrograde signaling. Mitochondrial development is also affected by chloroplasts. In rice, a single chloroplast mutation causes inhibition of mitochondrial development (Chen et al., 2018). Therefore, it is possible that mitochondrial gene defects might also affect chloroplast development. *AT5G08670* encodes an ATP synthase *?* -subunit in mitochondria. The mitochondrial *?* -subunit is encoded by three genes: *AT5G08670* , *AT5G08680* , and *AT5G08690* (Figure 1a). Only *AT5G08670* and *AT5G08690* are expressed and they encode an identical mature protein (Figure 1c), implying functional redundancy between these two genes.

The plastid retrograde signaling pathway has been extensively studied, and several mutants with impaired retrograde signaling have been identified in *Arabidopsis* , such as *gun1* , *gun4* ,*coe1* , and *coe2* (Koussevitzky, 2007; Larkin et al., 2003; Sun et al., 2016; Wu et al., 2022). In these mutants *PhANGs* are significantly expressed in chloroplasts under biotic or abiotic stress, and the normal connection between the chloroplast and nuclear genome is compromised in these mutants (Susek et al., 1993). Expression of*LHCB* was higher in the mutant seedlings with a T-DNA insertion in*AT5G08670* than in WT seedlings upon LIN and NF treatment (Figure 2a-b), suggesting that *AT5G08670* is involved in plastid retrograde signaling. Transcriptome analysis of the *AT5G08670*mutant revealed that LIN affects the expression of nuclear genes encoding both mitochondrial and chloroplast proteins (Figure 8) raising the possibility that *AT5G08670* might be involved in regulating both plastid and mitochondrial retrograde signaling although the mechanisms of mitochondrial regulation of chloroplast development are unknown. However, in maize mutants with defective mitochondrial genes (Gu, Miles, & Newton, 1993; Roussell, Thompson, Pallardy, Miles, & Newton, 1991), the leaves show yellow or pale green stripes indicating that mitochondria affect the development of chloroplasts.

Mitochondrial proteins contribute to plastid retrograde signaling

The *gun* mutants are the best-characterized regulators of plastid retrograde signaling. Whereas *GUN1* encodes a chloroplast nucleoid pentatricopeptide repeat protein (Koussevitzky, 2007), the other GUN proteins are involved in the tetrapyrrole biosynthetic pathway.*GUN2* encodes a heme oxygenase (Strand et al., 2003), *GUN3*encodes a photosensitive pigment chromophore synthase (Susek et al., 1993), *GUN4* encodes a regulatory protein involved in chlorophyll synthesis (Larkin et al., 2003), and *GUN5* encodes the H subunit of magnesium chelatase (Mochizuki et al., 2001). GUN1 might also play a role in the tetrapyrrole pathway (Cheng et al., 2011). Because these GUN proteins are located in the chloroplast, they play a direct role in the retrograde chloroplast signaling pathway (Surpin, Larkin, & Chory, 2002). However, little is known regarding the roles of mitochondrial proteins in regulating plastid retrograde signaling.

The nucleus controls the majority of processes in chloroplasts, including organelle gene expression (OGE) via 'anterograde signaling'. The nucleus, in turn, depends on the signals originating from the chloroplasts that convey information to the nucleus via 'retrograde signaling'. This system allows for changes in nuclear gene expression (NGE) in response to the status of the chloroplast. We propose a model from our study in which environmental cues affect the chloroplast and mitochondrial state which in turn gives rise to retrograde signals that alter nuclear gene expression from the transcriptional to the post-translational level and ultimately feedback to plastid function (Figure 10). Initially, the environmental stimulus is perceived by the chloroplast. In this study, environmental factors include LIN, NF, and high light (shown by the yellow arrows) (Figure 10). After LIN treatment, the expression of*GUN4* and *GUN5* was decreased to different degrees in the mutants, in which the expression of *GUN1* increased relative to the control (Figure 8a). We also analyzed the expression of important mitochondria-related genes like *ALTERNATIVE OXIDASE 1D*(*AOX1D*), *AOX1A, AOX1C,* and *AOX2* in the mitochondria. The expression of *AOX1D* and *AOX1A* increased in WT seedlings treated with LIN, and the expression of these genes was decreased in *SALK_047877* and *SALK_083115* (Figure 8b). The expression of AOX2 was suppressed in all mutants treated with LIN (Figure 8b). Further, analysis of the expression of carbon metabolism genes revealed that *1-Aminocyclopropane-1-Carboxylate Oxidase 1*(*ACO1*) was decreased in all groups, except in WT seedlings following LIN treatment; the expression of *ACO2* , *ACO3* ,*hexokinase 1* (*HXK1*), and *HXK2* was decreased to different degrees in all samples (Figure 8c-d),

and the expression of*mitochondrial Malate Dehydrogenase 1* (*mMDH1*) was significantly inhibited in WT seedlings (Figure 9d). The transcript levels of *GUN4* , *GUN5* , *AOX1C* , *HXK1* ,*HXK2* , and *mMDH1* , were higher in *gun1* ,*SALK_047877* , and *SALK_083115* seedlings than in WT seedlings treated with LIN. In contrast, the transcript levels of the LIN-induced genes *AOX2* , *AOX1D* , *AOX1A* , *ACO1* , and *ACO2* were lower in *gun1* , *SALK_047877* , and*SALK_083115* , seedlings than in WT seedlings in the presence of LIN. In summary, these findings indicate that *GUN1* and*AT5G08670* dependent signaling pathways play important roles in regulating the expression of nuclear genes of both chloroplast and mitochondrial proteins in response to LIN (Figure 10).

The level of ATP synthase*?* -subunit and the total ATP synthase activity in the mutants were significantly lower than in WT (Figure 4a and b). It is thus likely that the effects observed are due to changes in cellular ATP indicating that perturbation of ATP homeostasis in mitochondria affects not only mitochondrial but also chloroplast metabolism and retrograde signaling. Interactions between chloroplast and mitochondrial ATP metabolism have also been observed in Chlamydomonas in a suppressed strain of a chloroplast mutant lacking the*atpB* gene. This suppressed strain was able to grow photoautotrophically in the absence of chloroplast ATP synthase (Lemaire, Wollman, & Bennoun, 1988). In this strain, photosynthesis was sensitive to specific inhibitors of mitochondrial electron transport suggesting that photosynthesis was restored through an unusual interaction between mitochondria and chloroplast involving the export of reduced compounds from the chloroplast to mitochondria to stimulate the synthesis of mitochondrial ATP which in turn would be exported from the mitochondria to the chloroplast. It remains to be determined how ATP levels are sensed in mitochondria and chloroplasts and how perturbations in ATP homeostasis in either organelle are compensated.

EXPERIMENTAL PROCEDURES

Plant materials and growth conditions

All *Arabidopsis thaliana* used in this study were in the Columbia (Col-0) ecotype background and were obtained from the Arabidopsis Biological Resource Center. Homozygote mutants were identified by PCR with corresponding primers. All mutants and WT *Arabidopsis* were grown in an artificial climate chamber with the following growth conditions: 21–23, 100 μmol photons $m^{-2}s^{-1}$, 18-h light/8-h dark cycle, and 60–70% humidity. For the NF and LIN treatments, surface-sterilized mutant and WT seeds were planted on 1/2 Murashige and Skoog (1/2 MS) medium (PhytoTechnology Laboratories, LLC, USA) containing 1% sucrose and 0.8% agar supplemented with either 5 μM NF (Sandoz Pharmaceuticals; Vienna, Austria) or 220 μg/mL LIN (Sigma; St. Louis, MO).

DNA extraction

Fresh seedling (0.1 g) samples were cut, wrapped in tinfoil, and quick-frozen in liquid nitrogen barrels. The quick-frozen samples were then placed into a mortar and pestle and ground quickly and thoroughly. Next, 650 μL of preheated cetyltrimethylammonium bromide buffer was added and mixed with the ground samples; the same amount of chloroform was then added, and the contents were mixed slowly. After centrifugation at 12,000 rpm for 15 min, the supernatant of the liquid was transferred to a 1.5 mL Eppendorf (EP) tube, the same amount of pre-cooled isopropyl alcohol on ice was added, and the mixture was mixed by slowly inverting the EP tube. The supernatant was removed after centrifugation at 12,000 rpm and 4ºC for 10 min. One mL of 70 % ethanol was added to the EP tube to remove the liquid supernatant, and DNA was recovered by immersing the EP tube in ethanol solution. Centrifugation was performed at 12,000 rpm and 4ºC for 10 min, the supernatant was discarded, and this procedure was repeated (i.e., a total of two rounds of centrifugation). The EP tube was left open, and the ethanol was dried at room temperature until it had completely volatilized; 50 μL of sterile water was then added to dissolve the DNA. After dissolution, the DNA was stored at –20ºC.

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from 80–100 mg of frozen, homogenized*Arabidopsis* tissue using the MagMAX

Plant RNA Isolation Kit (Applied Biosystems, Foster City, CA, United States) following the manufacturer's instructions. cDNA was synthesized using the NovoScript Plus All-in-one 1st Stand cDNA Synthesis, SuperMix Kit. Quantitative RT-PCR was performed using the NovoStart SYBR qPCR SuperMix Kit in a QuantStudio TM 12K Flex Real-Time PCR system (Applied Biosystems, Foster City, CA, United States). The thermal cycling conditions were as follows: 95°C for 2 min; 40 cycles of 95°C for 20 sec; and 60°C for 30 sec. The primers were shown in Table S3. Data were analyzed using QuantStudio TM 12K Flex software (Applied Biosystems, Foster City, CA, United States). Significant differences were evaluated using Student's t -test, and asterisks indicated significant

P-values.

RNA sequencing analyses

Total RNA was extracted using the mirVana miRNA Isolation Kit (Ambion) following the manufacturer's protocol. The integrity of RNA was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Samples with an RNA integrity index greater than 7 were used in subsequent analyses. Libraries were constructed using the TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, San Diego, CA, USA) following the manufacturer's protocol. Sequencing of these libraries was then conducted on an Illumina sequencing platform (HiSeqTM 2500 or Illumina HiSeq X Ten), and 125 bp/150 bp paired-end reads were generated.

GUS staining and histological analysis

Histochemical GUS staining was performed with a GUS Staining Kit (G3061, Solarbio Co., Beijing, China) following the manufacturer's instructions. Samples were fixed in 90% acetone at –20°C, rinsed four times with 0.1 M sodium phosphate buffer (pH 7.4), and then incubated in X-Gluc solution (0.1 M sodium phosphate (pH 7.4), 3 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, and 0.5 g L^{-1}5-bromo-4-chloro-3-indolyl-β -d-glucuronide cyclohexilammonium salt) at 37°C. After staining, chlorophyll was removed from the samples by incubating them in methanol; they were then mounted in a clearing solution (a mixture of chloral hydrate, water, and glycerol in a ratio of 8:2:1). Observations were made using a stereomicroscope (MZ16F, Leica Microsystems, Germany) or a microscope equipped with Nomarski optics (BX51, Olympus Co., Tokyo, Japan). To characterize vascular patterns, cotyledons were fixed in a mixture of ethanol and acetic acid in a ratio of 9:1, dehydrated through a graded series of ethanol, and then mounted with a clearing solution (Konishi & Sugiyama, 2003).

Total protein extraction and immunoblot analysis

The leaves of seedlings were harvested, and total protein was prepared following the methods of Sun et al. (2016). For immunoblot analyses, the proteins were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (15% acrylamide) (Schägger and von Jagow, 1987). Proteins were then transferred to polyvinylidene difluoride membranes (Ihnatowicz et al., 2004) and probed with their corresponding antibodies. Enhanced chemiluminescence was used for signal detection (GE Healthcare Biosciences).

Mitochondrial ATP synthase activity assay

Mitochondrial ATP synthase activity was measured by a colorimetric assay using the ATPase activity assay kit (D799641-0050, Shenggong, China) following the manufacturer's instructions. The seedlings were cultured on 1/2 MS medium for 2 weeks and leaves were collected for determination of ATP synthase activity. To approximately 0.1g of tissue, 1mL of reagent I was added, and the mixture was homogenized in an ice bath, centrifuged at 8000g for 10min at 4°C and the supernatant was used as the ATP synthase extract. Sample processing was according to the manufacturer's protocol. Finally, ATP synthase activity was determined by measuring the OD at 660 nm. Each experiment was performed with three biological samples, each with three technical replicates.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPORTING INFORMATION

This article contains supporting information.

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AUTHOR CONTRIBUTIONS

Author Contributions: Conceptualization of the project: X.S. and Z.L. Experimental design: X.S. and Z.L. Performance of some specific experiments: H.L., Y.Z. (Yaping Zhou), X.Y., Y.Z. (Yixin Zhang), Z.Z., S.S., M.H., A.Q., Y.L., J.Y., and G.B. Data analysis: H.L., Z.L., and X.S. Manuscript drafting: H.L. and X.S. Contribution to the editing and proofreading of the manuscript draft: M.J. and J-D.R. All authors have read and agreed with the published version of the manuscript.

FIGURE LEGENDS

Figure 1. Sequence analysis and tissue-specific localization of mitochondrial ATP synthase Beta subunit.

(a) Phylogenetic analysis of beta subunits of mitochondrial and chloroplast ATP synthase. (b) Gene structure diagram of *AT5G08670*and *AT5G08690* . (c) Alignment of amino acid sequences (NP_5683203 corresponds to *AT5G08670* , NP_5683204 corresponds to*AT5G08690* , and Helix corresponds to conserved domains). (d) GUS activity in *AT5G08670* p:GUS and *AT5G08690* p:GUS transgenic plants at different developmental stages. Scale bar, 1 mm.

Figure 2. Characterization of the luciferase activity and phenotypes of complemented plants.

(a) The T-DNA insertion lines, SALK_047887 and *SALK_083115*contain insertions within the 3' UTR and exon 1 of the *AT5G08670* , respectively. Transcription proceeds from left to right. (b) Relative expression level of *LHCB1.2* in mutants and WT plants after LIN treatment. (c) Relative expression level of *LHCB1.2* in WT and mutant plants after NF treatment.

Figure 3. Subcellular localization of AT5G08670 and phenotypic analysis of complemented plants. Subcellular localization of AT5G08670-GFP fusion proteins in *Arabidopsis* protoplasts. Scale bar, 10 μm.

Figure 4. ATP synthase *b*-subunit and total ATP synthase activity are decreased in the *AT5G08670* mutant. (a) Immunoblotting of mitochondrial ATP synthase *?* **-** subunit in WT and *AT5G08670* mutant. (b) Activity of ATP synthase in WT and *AT5G08670* mutant.

Figure 5. Analysis of DEGs after LIN treatment.

(a) Principal component analysis of gene expression in the experimental and control groups. (b) Volcano plot of DEGs (non-significantly different gene expression in grey, significantly different up-regulated and down-regulated genes in red and green; log2 FoldChange (FC)>1, P -value<0.05). (c) Statistics on the number of DEGs after LIN treatment. (d) Heatmap showing the expression patterns of DEGs in different comparison groups (P-value<0.05 and |log2FC|>1).

Figure 6. GO enrichment analysis after LIN treatment.

(a) Venn diagram analysis of significantly down-regulated genes after LIN treatment. (b) GO enrichment analysis of down-regulated genes in mutants after LIN treatment. (c) Venn diagram analysis of significantly up-regulated genes after LIN treatment. (d) GO enrichment analysis of up-regulated genes in mutants after LIN treatment. (e) GO enrichment analysis of significantly up-regulated genes in WT after LIN treatment.

Figure 7. Analysis of the expression of *AT5G08670* and*AT5G08690*.

(a) Analysis of *AT5G08670* expression in WT and mutants in the control and with LIN treatment, respectively. (b) Analysis of*AT5G08690* expression in WT and mutants in the control and with LIN treatment, respectively. Significant differences are indicated by asterisks (one–way ANOVA with Tukey's multiple comparisons test of mutants vs. WT, and * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n=3).

Figure 8. Expression analysis of mitochondria and chloroplast representative genes.

(a) Analysis of the expression of chloroplast-related genes. (b) Analysis of the expression of mitochondrial-related genes. (c-d) Analysis of the expression of carbon metabolism-related genes. Significant differences are indicated by asterisks (one–way ANOVA with Tukey's multiple comparisons test, * $P < 0.05$, ** $P < 0.01$, and ***$P < 0.001$, n=3).

Figure 9. Analysis of the TF network of DEGs for the T-DNA insertion mutants vs. WT comparison under LIN treatment.

(a) Venn diagram analysis of significantly up-regulated TFs after LIN treatment. (b) Venn diagram analysis of significantly down-regulated TFs after LIN treatment. (c) Interaction network diagram of differentially expressed transcription factors. The network was created cytoscape. The color key indicates low to high interaction strength.

Figure 10. A schematic presentation of retrograde signaling in plant cells.

Environmental factors in this study are the chloroplast development inhibitors LIN and NF which act in the chloroplast (shown by the yellow arrows) and generate photooxidative damage under high light. The signal generated by the chloroplast is transduced to the nucleus (shown by a red line) causing the necessary changes in NGE (plastid retrograde signaling). The transcriptome analysis shows that the expression of chloroplast and mitochondrial retrograde signaling-related genes is affected in *AT5G08670* seedlings treated with LIN and associated with the repression of some TFs, such as *ETT, PTF1, TCP2* , and*KTF1* .

Supporting information

The following supplemental materials are available.

Figure S1. Location of *AT5G08670* and amino acid sequence analysis.

Figure S2. Identification of homozygous T-DNA insertion mutants.

Table S1. The genes list of significantly up-regulated TFs after LIN treatment.

Table S2. The genes list of significantly down-regulated TFs after LIN treatment.

Table S3. The primers list of qRT-PCR.

REFERENCES

Allen, J. F., Paula, W., Puthiyaveetil, S., & Nield, J. (2011). A structural phylogenetic map for chloroplast photosynthesis. *Trends in Plant Science, 16* , 645-655.

Anders, S., & Huber, W. (2012). Differential expression of RNA-Seq data at the gene level–the DESeq package. *Heidelberg, Germany: European Molecular Biology Laboratory (EMBL), 10* , f1000research.

Baba, K., Nakano, T., & Yoshida, Y. S. (2001). Involvement of a Nuclear-Encoded Basic Helix-Loop-Helix Protein in Transcription of the Light-Responsive Promoter ofpsbD. *Plant Physiology, 125* , 595-603.

Bai, M. Y., Shang, J. X., Oh, E., Fan, M., Bai, Y., Zentella, R., . . . Wang, Z. Y. (2012). Brassinosteroid, gibberellin and phytochrome impinge on a common transcription module in Arabidopsis. *Nature Cell Biology, 14* , 810-817.

Chen, N., Wang, P., Li, C., Wang, Q., Pan, J., Xiao, F., . . . Deng, X. (2018). A Single Nucleotide Mutation of the IspE Gene Participating in the MEP Pathway for Isoprenoid Biosynthesis Causes a Green-Revertible Yellow Leaf Phenotype in Rice. *Plant Cell Physiol, 59* , 1905-1917.

Cheng, J., He, C. X., Zhang, Z. W., Xu, F., Zhang, D. W., Wang, X., . . . Lin, H. H. (2011). Plastid signals confer Arabidopsis tolerance to water stress. *Zeitschrift fur Naturforschung C: Journal of Biosciences, 66* , 47-54.

Gu, J., Miles, D., & Newton, K. J. (1993). Analysis of Leaf Sectors in the NCS6 Mitochondrial Mutant of Maize. *Plant Cell, 5* , 963-971.

Guha, M., & Avadhani, N. G. (2013). Mitochondrial retrograde signaling at the crossroads of tumor bioenergetics, genetics and epigenetics.*Mitochondrion, 13* , 577-591.

Guha, M., Srinivasan, S., Koenigstein, A., Zaidi, M., & Avadhani, N. G. (2016). Enhanced osteoclastogenesis by mitochondrial retrograde signaling through transcriptional activation of the cathepsin K gene.*Annals of the New York Academy of Sciences, 1364* , 52-61.

Hamasaki, H., Kurihara, Y., Kuromori, T., Kusano, H., Nagata, N., Yamamoto, Y. Y., . . . Matsui, M. (2019). SnRK1 Kinase and the NAC Transcription Factor SOG1 Are Components of a Novel Signaling Pathway Mediating the Low Energy Response Triggered by ATP Depletion.*Frontiers in plant science, 10* , 503.

He, Z., Zhao, X., Kong, F., Zuo, Z., & Liu, X. (2016). TCP2 positively regulates HY5/HYH and photomorphogenesis in Arabidopsis. *Journal of Experimental Botany 67* , 775-785.

Heineke, D., Bykova, N., Gardeström, P., & Bauwe, H. (2001). Metabolic response of potato plants to an antisense reduction of the P-protein of glycine decarboxylase. *Planta, 212* , 880-887.

Holm, M., Ma, L. G., Qu, L. J., & Deng, X. W. (2002). Two interacting bZIP proteins are direct targets of COP1-mediated control of light-dependent gene expression in Arabidopsis. *Genes & development, 16* , 1247-1259.

Inaba, T., Yazu, F., Ito-Inaba, Y., Kakizaki, T., & Nakayama, K. (2011). Retrograde signaling pathway from plastid to nucleus.*International Review of Cell and Molecular Biology, 290* , 167-204.

Jarvis, P., & Lopez-Juez, E. (2014). Biogenesis and homeostasis of chloroplasts and other plastids *Nature Reviews Molecular Cell Biology 15* , 147-147.

Kim M, M. J. (1995). Identification of a Sequence-Specific DNA Binding Factor Required for Transcription of the Barley Chloroplast Blue Light-Responsive psbD-psbC Promoter. *The Plant Cell, 7* , 1445-1457.

Konishi, M., & Sugiyama, M. (2003). Genetic analysis of adventitious root formation with a novel series of temperature-sensitive mutants of Arabidopsis thaliana. *Development, 130* , 5637-5647.

Koussevitzky, S. (2007). Signals from Chloroplasts Converge to Regulate Nuclear Gene Expression. *Science, 316* , 1698-1698.

Larkin, R. M., Alonso, J. M., Ecker, J. R., & Chory, J. (2003). GUN4, a regulator of chlorophyll synthesis and intracellular signaling.*Science, 299* , 902-906.

Lemaire, C., Wollman, F. A., & Bennoun, P. (1988). Restoration of phototrophic growth in a mutant of Chlamydomonas reinhardtii in which the chloroplast atpB gene of the ATP synthase has a deletion: an example of mitochondria-dependent photosynthesis. *Proceedings of the National Academy of Sciences, 85* , 1344-1348.

Mnatsakanyan, N., Kemboi, S. K., Salas, J., & Weber, J. (2011). The beta subunit loop that couples catalysis and rotation in ATP synthase has a critical length. *Journal of Biological Chemistry, 286* , 29788-29796.

Mnatsakanyan, N., Krishnakumar, A. M., Suzuki, T., & Weber, J. (2009). The role of the betaDELSEED-loop of ATP synthase. *Journal of Biological Chemistry, 284* , 11336-11345.

Mochizuki, N., Brusslan, J. A., Larkin, R., Nagatani, A., & Chory, J. (2001). Arabidopsis genomes uncoupled 5 (GUN5) mutant reveals the involvement of Mg-chelatase H subunit in plastid-to-nucleus signal transduction. *Proceedings of the National Academy of Sciences of the United States of America, 98* , 2053-2058.

Nott, A., Jung, H. S., Koussevitzky, S., & Chory, J. (2006). Plastid-to-nucleus retrograde signaling. *Annual Review of Plant Biology, 57* , 739-759.

Paul, B. D., & Snyder, S. H. (2010). The unusual amino acid L-ergothioneine is a physiologic cytoprotectant. *Cell Death and Differentiation, 17* , 1134-1140.

Peter, L., Antje, K., Babett, D., Gerd, H., Bernhard, G., & Vassilios, P. (2004). A Novel Arabidopsis thaliana Protein is a Functional Peripheral-Type Benzodiazepine Receptor. *Plant and Cell Physiology* , 723.

Raghavendra, A. S., & Padmasree, K. (2003). Beneficial interactions of mitochondrial metabolism with photosynthetic carbon assimilation. *Trends in Plant Science, 8* , 546-553.

Rhoads, D. M., & Subbaiah, C. (2007). Mitochondrial retrograde regulation in plants. *Mitochondrion, 7* , 177-194.

Rigas, S., Daras, G., Tsitsekian, D., Alatzas, A., & Hatzopoulos, P. (2014). Evolution and significance of the Lon gene family in Arabidopsis organelle biogenesis and energy metabolism. *Frontiers in plant science, 5* , 145.

Roussell, D. L., Thompson, D. L., Pallardy, S. G., Miles, D., & Newton, K. J. (1991). Chloroplast Structure and Function Is Altered in the NCS2 Maize Mitochondrial Mutant. *Plant Physiology, 96* , 232-238.

Sota, F., & Kinya, T. (2008). Genome Barriers between Nuclei and Mitochondria Exemplified by Cytoplasmic Male Sterility. *Plant and Cell Physiology, 49* , 1484-1494.

Stock, D., Leslie, A. G., & Walker, J. E. (1999). Molecular architecture of the rotary motor in ATP synthase. *Science, 286* , 1700-1705.

Strand, Å., Asami, T., Alonso, J., Ecker, J. R., & Chory, J. (2003). Chloroplast to nucleus communication triggered by accumulation of Mg-protoporphyrinIX. *Nature, 421* , 79-83.

Sun, X., Xu, D., Liu, Z., Kleine, T., & Leister, D. (2016). Functional relationship between mTERF4 and GUN1 in retrograde signaling. *Journal of Experimental Botany, 67* , 3909-3924.

Surpin, M., Larkin, R. M., & Chory, J. (2002). Signal transduction between the chloroplast and the nucleus. *The Plant Cell, 14* , 327-338.

Susek, R. E., Ausubel, F. M., & Chory, J. (1993). Signal transduction mutants of Arabidopsis uncouple nuclear CAB and RBCS gene expression from chloroplast development. *Cell, 74* , 787-799.

Thierry, A., Sébastien, M., & Patricia, R. (2015). Mitochondria Retrograde Signaling and the UPRmt: Where Are We in Mammals? *International Journal of Molecular Sciences, 16* , 18224-18251.

Wang, Y., Selinski, J., Mao, C., Zhu, Y., Berkowitz, O., & Whelan, J. (2020). Linking mitochondrial and chloroplast retrograde signalling in plants. *Philosophical Transactions of the Royal Society B, 375* , 20190410.

Waters, M. T., & Langdale, J. A. (2009). The making of a chloroplast. *The EMBO journal, 28* , 2861-2873.

Woodson, J. D., & Chory, J. (2008). Coordination of gene expression between organellar and nuclear genomes. *Nature Reviews Genetics, 9* , 383-395.

Woodson, J. D., Perez-Ruiz, J. M., & Chory, J. (2011). Heme synthesis by plastid ferrochelatase I regulates nuclear gene expression in plants. *Current Biology, 21* , 897-903.

Wu, R., Liu, Z., Wang, J., Guo, C., Zhou, Y., Bawa, G., . . . Sun, X. (2022). COE2 Is Required for the Root Foraging Response to Nitrogen Limitation. *International Journal of Molecular Sciences, 23* , 861.

Xu, T., Pagadala, V., & Mueller, D. M. (2015). Understanding structure, function, and mutations in the mitochondrial ATP synthase.*Microbial cell, 2* , 105-125.

Yusuke, K., Naomi, H., Yoshiki, N., Takafumi, S., Mami, N., Takayuki, F., . . . Osami, M. (2014). Algae Sense Exact Temperatures: Small Heat Shock Proteins Are Expressed at the Survival Threshold Temperature in Cyanidioschyzon merolae and Chlamydomonas reinhardtii. *Genome Biology and Evolution, 6* , 2731-2740.

Zhang, Z. W., Yuan, S., Feng, H., Xu, F., Cheng, J., Shang, J., . . . Lin, H. H. (2011). Transient accumulation of Mg-protoporphyrin IX regulates expression of PhANGs - New evidence for the signaling role of tetrapyrroles in mature Arabidopsis plants. *Journal of plant physiology, 168* , 714-721.