

ORIGINAL ARTICLE



Mutation of the Mitochondrial Transcription Termination Factor Gene Affects the Expression of Organellar Genes and Nuclear Genes Encoding Alternative Respiratory Pathway Proteins in Arabidopsis

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Received December 22, 2019

Characterization of *mterf13* mutant Arabidopsis plants showed the possible participation of the mTERF13 in the modulation of genetic processes in mitochondria and chloroplasts of Arabidopsis. Moreover, according to data obtained, this gene can encode a potential mitochondrial transcription factor. Mutation of the studied gene through retrograde regulation can also lead to a change in the expression of nuclear genes encoding components of the alternative respiratory pathway.

Key words: mitochondrial transcription termination factors, organellar gene expression, mitochondrial alternative respiratory pathway

Mitochondria, which are energy stations of eukaryotic cells, are one of the main sources of generation of reactive oxygen species (ROS) in plant cells (Møller, 2001). The formation of ROS in plant mitochondria is primarily associated with impaired functioning of the main and alternative respiratory pathways (Møller, 2001; Vanlerberghe, 2013). The protein components of the main and alternative mitochondrial respiratory complexes are predominantly encoded by the nucleus, only certain subunits of the respiratory complexes are encoded by the mitochondrial genome (Gualberto *et al.*, 2014). Expression of a relatively small number of mitochondrial genes in plant mitochondria undergoes complex regulation at various levels (Millar *et al.*, 2004; Holec *et al.*, 2006). Transcription of mitochondrial genes is carried out by 2 phage-type polymerases — RpoTm and RpoTmp, the last of which has double localization — in mitochondria and in chloroplasts (Hedtkke *et al.*, 2000; Kühn *et al.*, 2009). It has been shown that, at least in the case of RpoTmp, the implementation of its function requires the presence of some unknown protein cofactors. (Kuhn *et al.*, 2007). Several potential candidates have been proposed as potential mitochondrial transcription factors, including homologs of the bacterial sigma factor, individual PPR proteins, homologs of mitochondrial transcription factors (mtTFA and mtTFB) found in animals, and others (reviewed by Liere *et al.*, 2011). Reliable data confirming the participation of these factors in the expression of plant mitochondrial genes has not yet been obtained.

In recent years, data have been obtained showing an important role in the implementation of genetic processes in plant organelles of proteins homologous to the mitochondrial transcription termination factor (mTERF), firstly identified in metazoans (reviewed by Klein and Leister, 2015). In *Arabidopsis*, the mTERF gene family consists of 35 representatives, encoding chloroplast, mitochondrial, or double-localized proteins (Kleine *et al.*, 2012). Mutations of *Arabidopsis* mTERF genes may lead to perturbations of plastid and mitochondrial gene expression homeostasis, which in turn, may result in the activation of acclimation and tolerance responses, presumably via retrograde signaling. (Kim *et al.*, 2012; Robles *et al.*, 2015; Leister

et al., 2017; Xu *et al.*, 2017; Robles *et al.*, 2018). The aim of this work was to study the role of the mTERF13 gene of *Arabidopsis* in modulating the expression of organellar genes and nuclear genes encoding the components of the mitochondrial alternative respiration pathways.

MATERIALS AND METHODS

Wild-type *Arabidopsis thaliana* (L.) Heynh plants (Col-0) and the T-DNA insertion SALK_076739 (N658768) line were obtained from the ABRC seed stock center. Seeds were surface sterilized and plated on containing 0.5× Murashige and Skoog salts (Sigma-Aldrich, USA) and 0.8% (w/v) Phytigel (Sigma-Aldrich). Seeds were stratified at 4°C for 3 d. Plants were grown in KBW720 (Binder, Germany) growth chambers at 24/22 °C day/night, 16-h photoperiod and irradiance of 150 μmol m⁻² s⁻¹. Plant for RNA isolation were harvested after 14 d.

GeneJET Plant RNA Purification Kit (ThermoFisher Scientific Baltic, Lithuania) was used for RNA isolation according to the manufacturer's instructions. The RNA was then subjected to two consecutive treatments with DNase (ThermoFisher Scientific Baltic, Lithuania). One microgram of RNA was reverse-transcribed with RevertAid Reverse Transcriptase (ThermoFisher Scientific Baltic, Lithuania) according to the manufacturer's protocol using random hexamers (Evrogen, Russia). qRT-PCR analysis was performed using qPCRMix-HS SYBR (Evrogen, Russia) and the CFX96 Real-Time PCR Detection System (Bio-Rad, USA) according to the manufacturer's instructions. The primer sequences are provided in the Table 1. All reactions were performed in duplicates with the three independently isolated RNA samples. The expression of each gene was normalized to the expression of *gapdh* (At1g13440) and *pp2a* (At1g13320) genes.

RESULTS AND DISCUSSION

In *Arabidopsis*, the mTERF gene family consists of 35 representatives, which are divided by Klein (Kleine, 2012) into several clusters according to co-expression patterns, together with data pertaining to the subcellular localizations of the proteins encoded by the genes co-expressed with each mTERF defined by the Gene

Ontology (GO) Annotations. To date, the role of two mTERF genes from designated by Kleine as a “mitochondrial” cluster, mTERF15 and mTERF18/SHOT1, is best characterized in arabidopsis organelles. The mTERF15 is not, apparently, a transcription factor, but takes part in the maturation of the transcript of the mitochondrial *nad2* gene. Disruption of splicing of intron 3 of the *nad2* gene in the mTERF15 mutant leads to a decrease in the activity of the respiratory complex I in mitochondria of arabidopsis (Hsu Y.-W. *et al.*, 2014).

SHOT1 / mTERF18 is more favorable candidate for the role of mitochondrial transcription modulating factor, since expression of mitochondrial genes is significantly altered in *mterf18/shot1* mutant Arabidopsis plants (Kim *et al.*, 2013). At the same time, a mutation of this gene led to the opposite change in the expression of chloroplast genes (Kim M. *et al.*, 2012). This effect can be determined by experimentally not shown double localization of mTERF18, the possibility of which allows bioinformatic methods of analysis (Kleine, 2012) or can be mediated by complex mechanisms of retrograde/anterograde signaling (Kleine and Leister, 2016). Moreover, a change in the expression of a number of nuclear stress genes and an alternative oxidase *Aox1a* gene was observed in SHOT1/mTERF18 mutant plants as a result of retrograde regulation. A perturbation in organellar gene expression homeostasis and altered expression of stress response genes resulted in enhanced thermotolerance of the *mterf18/shot1* mutant plants (Kim *et al.*, 2012).

In this work we studied the effect of a mutation of another mTERF gene from the “mitochondrial” cluster (Kleine, 2012) – mTERF13 - on the organellar gene expression as well as the expression of nuclear genes encoding the components of the main and alternative mitochondrial respiration pathways. Seeds of T-DNA insertion SALK_076739 line were obtained from the ABRC seed stock center.

The phenotypic characteristics of mutant plants under standard growing conditions did not reveal significant differences in the growth rate of mutant and wild-type plants.

An analysis of the transcript amount of a number of mitochondrial and chloroplast genes in 2-week-old mutant plants showed that the mutation of the mTERF13 gene similar to mutation of the mTERF18 gene leads to a decrease in the expression of chloroplast genes (Fig.1B). But in contrast to the *mterf18/shot1* mutants in the *mterf13* plants the expression of the mitochondrial genes was reduced or unchanged (Fig.1A).

Observed by Kim *et al.* in *mterf18/shot1* mutant plants an increase of abundances of the most of the investigated mitochondrial transcripts can be differently explained and suggests various possible functions of the mTERF18 in the realization of genetic processes in mitochondria of Arabidopsis. In case of the *mterf13* mutant line, the observed decrease of certain mitochondrial gene expression indicates primarily the possible role of this protein as a mitochondrial transcription factor. This assumption will be further verified using other molecular biological approaches.

Table 1. Primers used for quantitative RT-PCR analysis.

Gene	Forward primer, 5'→3'	Reverse primer, 5'→3'
cox1	GACTCTCTATTTTCATTTTCGGTGCCA	TGATCGCCGGGTCGTGCT
matR	TACGATCCCGAGTTTCCAGACACAT	TTCCCCACTCTTCTTTGATCCGTCT
ccmC	CAACCTGGGAGCATTAGCCGA	AGATACGGGTTGAGAAGGGGAAGTTA
nad6	ATGATACTTTCTGTTTTGTCTGAGCCCT	CTGGGATGGGAAACAAAACGGA
cob	CGATGTCCACCCCGCCTCA	GGCTACACCTCCCGCTTTGTCA
orf153a	ACAGACAGTTCCTTTATCACACCTTCACA	GGGAAGATGTCGAGATAGCAGGAGAA
atpI	TCAAGGGGAGTTAGCAGCACCAA	GGGTAAAAGAATTGGGGTTGGTTGA
accD	CCTTGGGGTTATGGATTTTCGGTT	ACTTCCTTCTTGCATTCTGCTCCT
rrn16	GGGTAGCTATATTTCTGGGAGCGA	GTTGCATTACTTATAGCTTCTTCTTCGT
clpP	TCGGAGGAGCAATTACCAAACGT	AATTCTCCCGTTTGTGCCTCATAA
aox1a	CGGCTGGACCACGTTTGTCT	CCAATCGTCGGAGCTCTAGTCCATA
aox1d	GACATCTCATTAGCCACTTGCCCA	TTCCCCACTTACCGGAGATGACGT
ndb2	CTCCAAGGCTCCAATACACATATCTCTC	ATCTCTCCTCGTTACTTGTGGTCATTATTT
gapdh	GGCGAGAGTTTGTGTGTGGTTGA	AAGCAGGGAAACATTAAGAGAAAGCAA
pp2a	AAAAGGTAAAGAAGACAGCAACGAATT	TAGCAGAGAGAAACAATGGTCACGTA

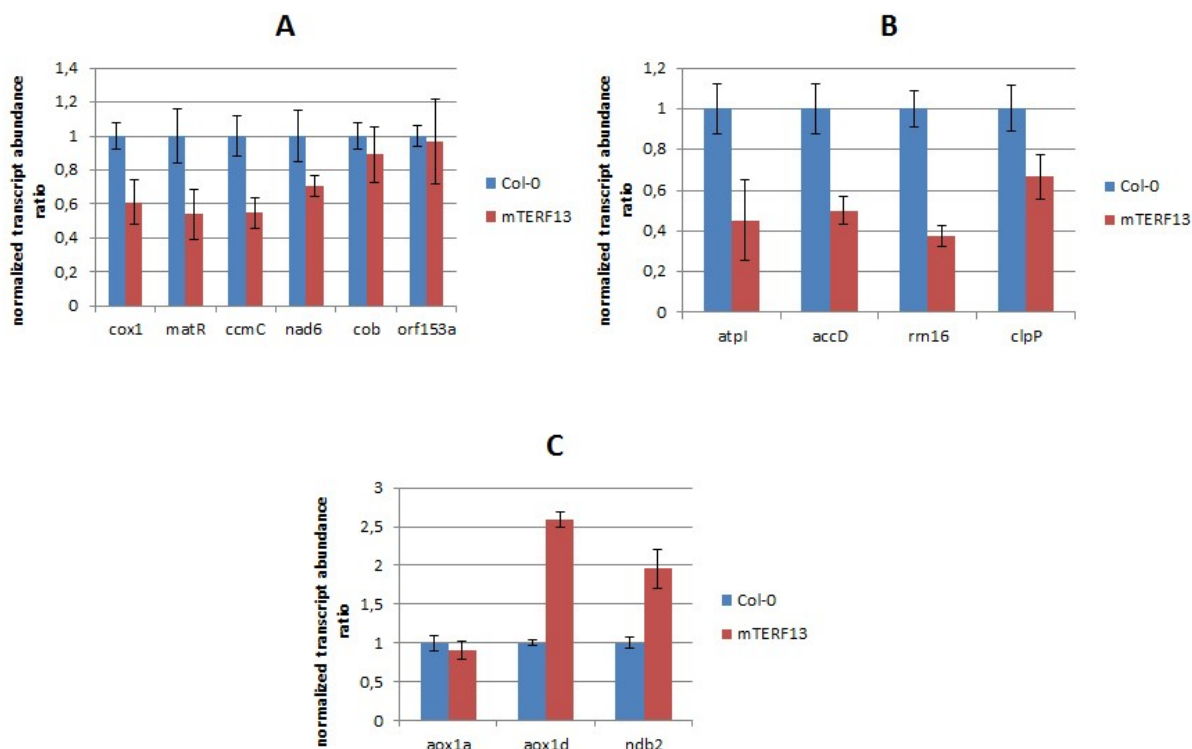


Figure 1. Differential gene expression of organellar and nuclear genes encoding alternative respiration pathway proteins in *mterf13* mutant Arabidopsis plants. (A) Transcript abundance of mitochondrial genes from *mterf13* mutant plants (mTERF13) and wild type (Col-0) plants. (B) Transcript abundance of chloroplast genes from *mterf13* mutant (mTERF13) plants and wild type (Col-0). (C) Transcript abundance of nuclear alternative respiration pathway genes from *mterf13* mutant (mTERF13) plants and wild type (Col-0) plants. Error bars are SD based on three biological replicates.

As a perturbation in organellar gene expression homeostasis may result in change of an expression of nuclear genes encoding alternative mitochondrial pathway proteins (Kim *et al.*, 2013) we also tested the transcript abundances of Arabidopsis alternative oxidase and rotenone-insensitive NADH dehydrogenase genes in *mterf13* mutant plants. The increase in the expression of *aox1d* and *ndb2* genes in mutant plants was observed compared to control plants (Fig.1C). The amount of the *aox1a* gene transcript did not significantly differ in the control and mutant plants. These data together with data on changes in the expression of organellar genes, suggests that, by analogy with other characterized *mterf* mutants (Kim *et al.*, 2013; Robles *et al.*, 2015 and 2018), the *mterf13* plants that are phenotypically not different from control plants when grown under control conditions, may demonstrate altered resistance to abiotic stress factors, what will be interesting to test in the further experiments.

CONCLUSION

Thus, characterization of *mterf13* mutant plants showed the possible participation of the mTERF13 in the modulation of genetic processes in mitochondria and chloroplasts of Arabidopsis. Moreover, according to data obtained, this gene can encode a potential mitochondrial transcription factor. Mutation of the studied gene through retrograde regulation can also lead to a change in the expression of nuclear genes encoding components of the alternative respiratory pathway.

ACKNOWLEDGMENTS

The work was financially supported by RFBR grant No.17-04-01515. We thank the ABRC for providing seed stocks.

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