Biochimica et Biophysica Acta 1847 (2015) 786–797

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

Emerging functions of mammalian and plant mTERFs☆

Tatjana Kleine *, Dario Leister

Plant Molecular Biology (Botany), Department Biology I, Ludwig-Maximilians-Universität München, 82152 Planegg-Martinsried, Germany

ARTICLE INFO

Article history:
Received 3 December 2014
Accepted 21 December 2014
Available online 10 January 2015

Keywords:
Mitochondrial transcription termination factor (mTERF)
mTERF function
Organellar gene expression
Chloroplast
Mitochondrion

ABSTRACT

Organellar gene expression (OGE) is crucial for plant development, respiration and photosynthesis, but the mechanisms that control it are still largely unclear. Thus, OGE requires various nucleus-encoded proteins that promote transcription, splicing, trimming and editing of organellar RNAs, and regulate their translation. In mammals, members of the mitochondrial transcription termination factor (mTERF) family play important roles in OGE. Intriguingly, three of the four mammalian mTERFs do not actually terminate transcription, as their designation suggests, but appear to function in antisense transcription termination and ribosome biogenesis. During the evolution of land plants, the mTERF family has expanded to approximately 30 members, but knowledge of their function in photosynthetic organisms remains sparse. Here, we review recent advances in the characterization of *mterf* mutants in mammals and photosynthetic organisms, focusing particularly on the progress made in elucidating their molecular functions in the last two years. This article is part of a Special Issue entitled: Chloroplast biogenesis.

1. Introduction

In eukaryotes, genetic information is stored not only in the nucleus but in the organellar genomes of mitochondria and, in the case of the plant lineage, also in plastids. Mitochondria and plastids are of endosymbiotic origin, deriving from progenitors that resembled extant α-proteobacteria [1] and cyanobacteria [2], respectively. Owing to gene loss during the early phase of organellar evolution and continuing organelle-to-nucleus gene transfer, organellar genomes are now highly impoverished [3–5]. Thus, although mitochondria and plastids each require more than 1000 proteins to sustain their primarily prokaryotic biochemistry [5,6], their genomes now encode only a small fraction of these, ranging from 3–67 in mitochondria [7] and 15–209 in plastids [8]. Because these genomes encode proteins necessary for essential energy-producing functions, i.e. the light reactions of photosynthesis in chloroplasts (cp) and oxidative phosphorylation (OXPHOS) in mitochondria (mt) [6], both organelles require fully functional gene expression machineries. Indeed, a substantial part of each genome codes for components of organellar gene expression (OGE). However, although mitochondria and plastids preserve features of prokaryotic genome organization, their gene expression systems are far more complex than those of their prokaryotic progenitors [9], and the residual proteins encoded in the organelles are insufficient to regulate their OGE machinery. Thus, the organellar expression system depends on a large number of imported, nucleus-encoded proteins, including additional RNA polymerases and sigma factors, as well as mono- or merospecific RNA maturation factors that promote RNA transcription, splicing, editing, end formation or translation [9–18] (See also other articles in this Special Issue: 1. Chloroplast ribonucleoproteins, Christian Schmitz-Linneweber; 2. Dynamic of plastid transcription, Silva Lerbs-Mache; 3. Chloroplast RNA polymerase Thomas Börner; 6. Plastid intron splicing, Alice Barkan; 7. Organelle RNA editing, Toshiharu Shikanai; 8. Chloroplast ribosome proteins, Ralf Bock).

It has become increasingly clear that families of proteins with similar modular architectures comprising repeated helical motifs play important roles in OGE [14]. These families include proteins with pentatricopeptide repeats (PPRs), half-a-tetratricopeptide (HAT) or octotricopeptide repeats (OPRs), as well as mitochondrial transcription termination factors (mTERFs) [14]. mTERF proteins have been identified in both metazoans and plants (although absent from yeast) [19]. In metazoans, the abbreviation MTERF is often used but, for simplicity, we employ the term mTERF throughout the text. The modular architecture of mTERF proteins is characterized by repeats of a 30-amino-acid motif, the so-called MTERF motif. The number and composition of these motifs, as well as the remaining sequences, vary widely within the family. The functions of mTERF proteins in the regulation of OGE at different levels are of particular interest, and will be outlined in this review by focusing on mTERFs identified in mammals and photosynthetic organisms. For mammalian mTERF proteins, results from 25 years of research are available [20], while the first *Arabidopsis thaliana* mTERF was described only 5 years ago [21]. Consequently, functional roles for plant mTERFs are just beginning to emerge. Furthermore, among metazoan mTERFs, we concentrate here on mammalian

Abbreviations: cp, chloroplast; mt, mitochondrial; mTERF, mitochondrial Transcription tERmination Factor; OGE, organellar gene expression

☆ This article is part of a Special Issue entitled: Chloroplast biogenesis.

* Corresponding author at: Ludwig-Maximilians-Universität München (LMU), Department Biologie I, Botanik; Großhaderner Str. 2, D-82152 Planegg-Martinsried, Germany. Tel.: +49 89/2180 74554; fax: +49 89/2180 74599.

E-mail address: tatjana.kleine@lmu.de (T. Kleine).

http://dx.doi.org/10.1016/j.bbabio.2014.12.009
0005-2728/

mTERFs, and refer the reader to [22] for a detailed review of other metazoan mTERFs.

2. Discovery and history of mTERFs: 1989–2009

Human mTERF1 was identified a quarter of a century ago and recognized as a factor that promotes transcription termination in human mt extracts [20] (Fig. 1A). Many molecular and biochemical characterizations [23–26] of human mTERF1 appeared in the years that followed, with functional studies focusing on in vitro termination and binding assays [26–33]. Concomitantly, mTERFs from other metazoans, including sea urchin [34], Drosophila [35], the green alga *Chlamydomonas reinhardtii* [36] and mouse [37], were described, together with a second mTERF homologue from human [38]. In 2005, Linder et al. [19] published the first phylogeny of mTERFs. In addition to mTERF1, similarity searches have identified three more members in the vertebrate mTERF family (mTERF2-4), all of which are thought to be mt proteins. Meanwhile, a considerably larger number of *MTERF* genes had been identified in plants [19], and in 2009 this first era of mTERF research was insightfully and excellently reviewed in BBA Bioenergetics [22].

3. Mammalian mTERF1: a transcription termination factor?

Although mTERF1 has been extensively studied, its likely in vivo function has only recently emerged [39]. As mTERF1 is the founding member of the family, and its assumed function as a transcription terminator gave the whole family its name, the history of the dissection of its function will be discussed in detail here.

3.1. The mammalian mitochondrial genome

In contrast to the cp genome, the mammalian mt genome contains no introns, and its 37 genes are tightly packed. The two mt DNA strands have different buoyant densities in cesium chloride gradients, and are designated as the heavy (H) and the light (L) strand. The only notable non-coding region is the displacement loop (D-loop), a regulatory region adjacent to *rrn12* that contains the sole promoter (LSP) for L-strand transcription and one H-strand promoter (HSP1) (reviewed in: [40]) (see Fig. 1A). L-strand transcription is initiated from LSP and produces near genome-length transcripts necessary for the expression of the nine L-strand genes—encoding ND6 and eight tRNAs. The H strand

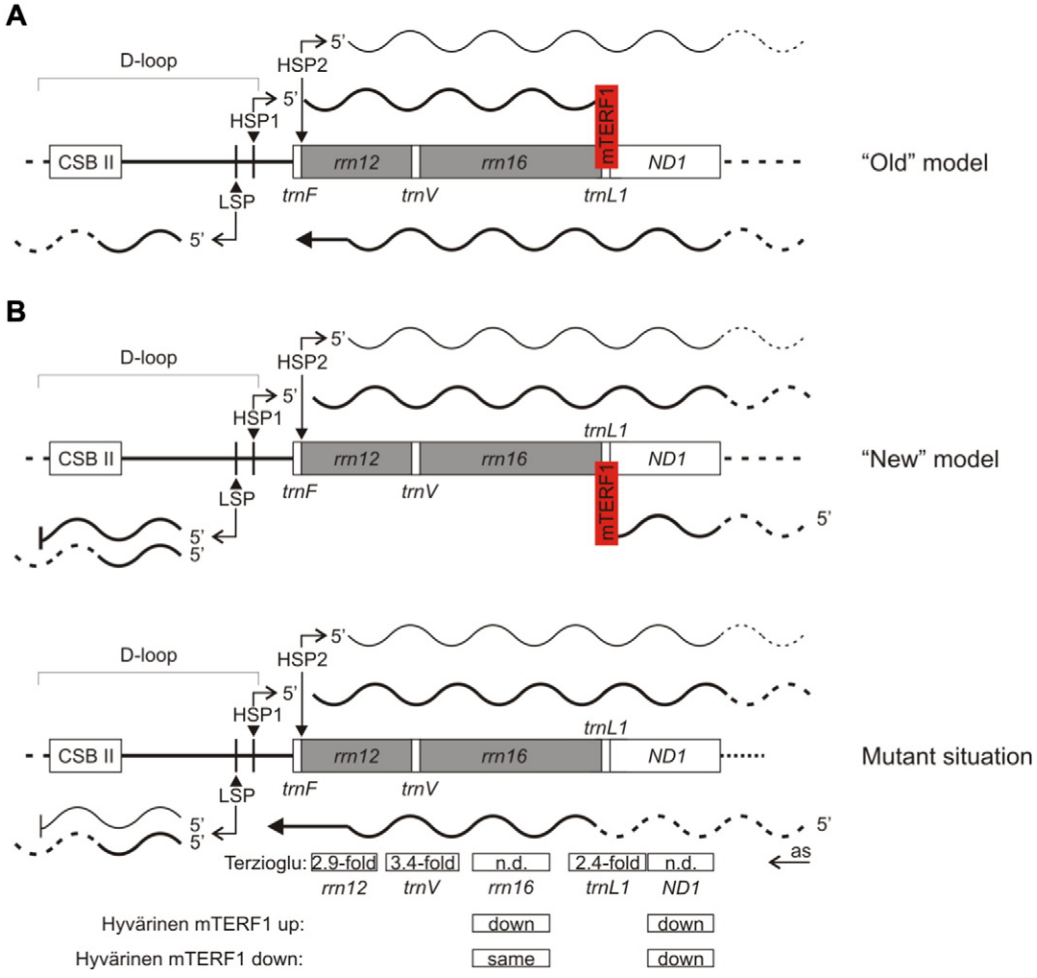

Fig. 1. Proposed models for the molecular function of mammalian mTERF1. The figure depicts the human mitochondrial genome around the target site of mammalian mTERF1 in the gene encoding $tRNA^{Leu}$ (*trnL1*), and the displacement loop (D-loop), the conserved sequence block (CSB), heavy- (HSP) and light-strand promoters (LSP) are shown. Gray boxes indicate rRNA genes, white boxes genes for tRNAs and NADH dehydrogenase subunit 1 (ND1). The wavy lines represent transcripts and their thicknesses refer to different expression levels. (A) According to [20], mTERF1 acts as a "road block" for transcripts originating from the stronger HSP1 promoter, which would explain why steady-state levels of the 12S and 16S rRNA are approximately 50 times higher than those of the mRNAs encoded on the same strand [41,42]. Loss of mTERF1 should then lead to a rise in levels of transcription of sequences downstream of its binding site. However, no evidence for this was found in two independent in vivo studies [39,44]. (B) The currently favored model proposes that the major function of mTERF1 is to prevent L-strand transcription from proceeding around the mtDNA circle, which would otherwise result in transcription interference at the LSP promoter from which they originated [39]. This model is based on the observations that levels of L-strand-derived antisense (as) transcripts covering the region around the mTERF1 binding site (i) vary in human cell lines that overexpress mTERF1 to various extents (Hyvärinen mTERF1 up) or express an RNAi directed against MTERF1 (Hyvärinen mTERF1 down) [44] and (ii) are upregulated downstream of the mTERF1 binding site (Terzioglu [39]). n.d., not determined.

is assumed to be transcribed from two, differentially regulated, promoters, HSP1 and HSP2. Transcription initiated from the weaker HSP2 promoter produces near genome-length primary H-strand transcripts, which are processed to yield the two rRNAs, 14 tRNAs and 12 mRNAs encoded on the H strand [41]. In mouse mitochondria, the level of rRNAs is approximately 50 times higher than that of any of the various mRNAs [42]. Kinetic analyses performed with a human mitochondrial system suggested that this difference in abundance might be attributable to differences in the rates of transcription of the rRNA and mRNA gene regions [43]. Indeed, transcription initiated at the stronger HSP1 promoter appears to start around 25 bp upstream of the $tRNA^{Phe}$ gene and terminates at a specific site within the gene for $tRNA^{Leu}$ located 3′ of the *rrn16* gene (Fig. 1A), and thus accounts for the synthesis of most of the rRNA. Hence, this transcription termination event could explain why the steady-state levels of the 12S and 16S rRNA are approximately 50 times higher than those of the mRNAs encoded on the same strand [41].

3.2. The termination event at the 16S rRNA–$tRNA^{Leu}$ gene boundary

A transcription termination event 3′ of the *rrn16* gene had been noted [41] (see previous section) before mTERF1 was identified [20]. Furthermore, several lines of evidence suggested the presence of a termination site at the 16S rRNA–$tRNA^{Leu}$ gene boundary. The 3′-termini of 12S and 16S rRNAs have been mapped in hamster [45], mouse [46] and human [47] cells, and the 3′-ends of 16S rRNA are much more heterogeneous than those of 12S rRNAs. In mouse cells, the site of the last template-encoded nucleotide can lie anywhere from immediately adjacent to the 5′-end of the $tRNA^{Leu}$ gene to 7 nucleotides downstream, within the $tRNA^{Leu}$ gene sequence [46]. The extent of 16S rRNA 3′-end heterogeneity, as compared to the 12S rRNA ends, suggests that the former may result from imprecise termination of transcription rather than from processing of a primary transcript [45–47]. It is interesting to note that the fraction of 16S rRNA that terminates at the gene boundary and is not adenylated (1.7%) closely matches the approximate proportion of 16S termini expected to be generated by “read-through” transcription, which is estimated to be about 2% [46]. Moreover, a tridecamer sequence found within the gene sequence for $tRNA^{Leu}$ was shown to be necessary for accurate termination immediately upstream of the $tRNA^{Leu}$ gene, and termination at that point was competitively inhibited by DNA containing the tridecamer sequence [48]. Intriguingly, this tridecamer sequence is entirely contained within the region protected by mTERF1 [20]. Furthermore, an A-to-G transition in the middle of the mTERF1-protected region, which has been associated with the MELAS syndrome (mt myopathy, encephalopathy lactic acidosis and stroke-like episodes) in human [49–51], drastically reduces the affinity of mTERF1 for its target sequence [32,33]. Because the MELAS-associated impairment of 16S rRNA transcription termination correlated with reduced affinity of the partially purified mTERF1 protein for the MELAS template, it was suggested that the molecular defect underlying MELAS was the inability to produce the large rRNA in the correct amount relative to other mitochondrial gene products [33]. However, although marked defects in mt protein synthesis and respiratory activity were observed in mtDNA-less cells transformed with MELAS mutant mtDNA, no significant change in the steady-state amounts of the two rRNA species encoded upstream of the termination site, or of the mRNAs encoded downstream of it could be detected [32]. Moreover, a deficit of mTERF1 would be expected to lead to reduced occupancy of its target sites, and thus to increased read-through transcription past these sites (Fig. 1A), but manipulation of human mTERF1 expression levels has minimal effects on steady-state levels of sense-strand transcripts [44]. Instead, abnormal accumulation of *nd1* and *rrn16* antisense transcripts (anti-*nd1*, located upstream of the binding site in the antisense direction, and anti-*rrn16*, located downstream of the binding site in the antisense direction; see Fig. 1B) is observed in cell lines with altered mTERF1 levels: both overexpression and down-regulation of mTERF1 result in less anti-*ND1* RNA, and mTERF1 overexpression in reduces anti-*rrn16* levels, while down-regulation leaves them unchanged [44]. On this basis, Hyvärinen et al. [44] concluded in 2010 that mTERF1 might contribute to the termination of antisense transcription. It must be emphasized here that this study was conducted in cells derived from a cancer cell line, which may not behave in a physiologically normal manner. Moreover, functional redundancy between mTERF1 and other members of the mTERF family in regulating read-through transcription at the 16S rRNA–$tRNA^{Leu}$ gene boundary might mask the primary effects of mTERF1 manipulations. A study from 1997 lends support to this latter notion. mTERF1 synthesized in vitro has the expected specific binding capacity for a double-stranded oligonucleotide containing the tridecamer sequence required for directing termination, and produces a DNase I footprint very similar to that produced by the natural protein [24], but it lacks transcription termination-promoting activity, which suggests that another component might be required for termination [24]. However, recent in vivo footprinting analyses with mouse embryonic fibroblasts derived from *Mterf1* knockout mice and control animals have now excluded the possibility that any other protein can occupy the mTERF1 binding site [39].

3.3. Other functions ascribed to mTERF1

In addition to functioning as a transcription terminator, mTERF1 has also been proposed to act (1) as an activator of HSP1-mediated mt rDNA transcription [20,25,30], and (2) in mtDNA replication [52]. The transcriptional activator function would provide an alternative explanation for the higher abundance of rRNAs relative to the downstream H-strand mRNAs. As a possible mechanism for the function as transcription initiator, simultaneous binding of the monomeric mTERF1 to the termination site and a novel site within the H1 region in vitro was proposed [30]. Such double interaction would result in looping-out of the rDNA, thereby promoting recycling of the transcription machinery through direct delivery of the mtRNAP from the termination site to the H1 initiation site [30]. However, there are difficulties with both of these proposals. First, the presence of such alternative binding sites has been called into question, for subsequent studies have failed to reproduce binding of mTERF1 to the HSP [53]. Furthermore, in vitro and in vivo experiments support high specificity of mTERF1 for the $tRNA^{Leu}$ site [15,39,54,55]. A further argument against an initiation function for mTERF1 is that the whole of the mTERF1 fold should be involved in binding the termination sequence, so that it is not immediately apparent how a single mTERF1 molecule could simultaneously bind both the HSP initiation and termination sites in the transcriptional loop model [40]. Hence, in 2010, over a quarter of a century after the discovery of mTERF1, its function was still elusive—but this has since changed.

3.4. mTERF1 as a terminator of L-strand antisense transcription

Even before human mTERF1 was identified [20], it was observed in an in vitro system derived from human mitochondria that termination at the 16S rRNA–$tRNA^{Leu}$ boundary occurs in a promoter-independent and bidirectional fashion, and with approximately equal efficiency in both directions [56]. Years afterwards it was observed that mTERF1 only partially terminates H-strand transcription, whereas transcription in the L-strand direction is blocked almost completely [26,27]. This polarity may be a result of the preferential recognition of the L-strand by mTERF1 [29] and the fact that the majority of protein–DNA interactions were established with the strand transcribed from the LSP promoter as shown in the crystal structure of mTERF1 bound to dsDNA containing the termination sequence [54]. In 1986, several reasons why it might be useful to terminate L-strand transcription had been suggested [56]. The findings recently obtained using *Mterf1* knockout mice now clearly argue against a role for mTERF1 in the regulation of rRNA synthesis [39]. Strikingly, *Mterf1* knock-out mice do not display any visible phenotype (Table 1). Transcript levels up- and downstream of the binding site, as well as mtDNA levels, are unchanged. But a promoter-proximal LSP transcript and antisense rRNA transcripts downstream of the binding

site accumulate in *Mterf1* knock-out mice (Fig. 1B). In consequence, a model has been suggested in which the major function of mTERF1 is to prevent L-strand transcripts from proceeding around the mtDNA circle and thus avoiding transcriptional interference at the LSP promoter from which they originated [39] (Fig. 1B).

4. Functions of the other mammalian mTERFs (mTERF2–mTERF4)

The atomic structures of mTERF1 [54,57], mTERF3 [58], and a heterodimer between mTERF4 and the 5-methylcytosine rRNA methyltransferase NSUN4 [59,60] have revealed that mTERF proteins adopt a half-doughnut shape and contain a succession of positively charged amino acids on its convex face. In mTERF1, this positively charged patch specifically unwinds the DNA helix and causes base flipping at its binding site in the $tRNA^{Leu}$ gene [54], thus suggesting a mechanism for the termination of antisense transcription of the L-strand discussed above. The fact that mTERF1, mTERF3, and mTERF4 share a common fold and are structurally similar to each other, even though mTERF3 was crystallized in the absence of substrate [58] and mTERF4 in complex with another protein [59], strengthens the conclusion that mTERF proteins have evolved to bind nucleic acids [61]. However, it is becoming increasingly clear that the nucleic acid need not necessarily be dsDNA; RNAs can also serve as targets of mTERFs [62,63].

4.1. Mammalian mTERF2

Mammalian mTERF2 was identified in 2005 in an mRNA differential display screen designed to identify mitochondrial genes the respond to serum starvation [38]. Expression of *MTERF2* is inhibited by the addition of serum to serum-starved cells while, interestingly, *MTERF1* is induced. Expression of the GFP-mTERF2 fusion protein in HeLa cells showed that mTERF2 is localized to mitochondria. Furthermore, *MTERF2* mRNA is highly expressed in heart, liver and the skeletal muscle cells [38]. Four years later it was shown that *Mterf2* knock-out mice behave essentially like wild type, but develop myopathy and memory deficits when fed on a ketogenic (high fat, low carbohydrates) diet [64] (Table 1). Loss of mTERF2 causes a respiratory defect in certain tissues, which is the result of decreased levels of OXPHOS complexes. Levels of transcripts encoding OXPHOS proteins are decreased, the tRNA pool is unbalanced and mtDNA levels are increased in certain tissues [64]. In the absence of mTERF2, in vitro mt transcription is drastically decreased, suggesting that mTERF2 might interfere with mt transcription by interacting with the regulatory elements of mtDNA to stimulate transcription initiation. EMSA and ChIP experiments suggested that mTERF2 binds to the HSP promoter region, and that mTERF2 co-immunoprecipitates with mTERF1 and mTERF3, possibly by binding to the same mtDNA region [64]. In contrast, another report showed that mTERF2 associates with nucleoids, and is therefore located in close proximity to mtDNA, but that its DNA-binding activity is not sequence-specific [65]. Thus, the molecular mechanisms of mTERF2 function remain unclear.

4.2. Mammalian mTERF3

Knock-out *Mterf3* mice are embryo lethal [53] (Table 1). Loss of mTERF3 in the mouse heart perturbs the tRNA pool, as observed in *Mterf2* knock-out mice [64], but leads to higher steady-state levels

Table 1
Functions of mouse mTERFs.

Name	Loc.	Type of mutation	Phenotype	Molecular phenotype	(Proposed) binding site	(Proposed) molecular function	Ref.
mTERF1/mTERF	M	Knock-out	Like wild-type; also when challenged with ketogenic (high fat, low carbohydrate) diet	rRNA transcripts unchanged; mtDNA levels unchanged; decreased levels of a promoter-proximal LSP transcript and increased antisense rRNA transcripts downstream of the mTERF1 binding site	$tRNA^{Leu}$ (UUR)	Termination of L-strand transcription	[1]
mTERF2/mTERFL/mTERF.D3	M	Knock-out	Nearly like wild-type; develop myopathy and memory deficits when challenged with ketogenic diet	Transcription of tRNAs changed; mtDNA levels increased in certain tissues; decrease in transcripts and proteins for OXPHOS	HSP promoter	mTERF1, mTERF2 and mTERF3 regulate transcription initiation by acting on the same site in the HSP promoter.	[2]
					Non sequence-specific DNA binding		[3]
mTERF3/mTERF.D1/CGI-12	M	Knock-out/disruption of *mTERF3* in the heart	Embryo lethal/cardiomyopathy, maximal life span of 17 weeks	Altered tRNA transcription; mtDNA level unchanged; transcription initiation from both LSP and HSP increased; OXPHOS protein subunits reduced	HSP promoter region	mTERF3 represses transcription initiation.	[4]
		Mterf3 heart knock-out [4]		Reduction of 16S rRNA; reduced 39S subunit assembly and concomitantly fewer fully assembled 55S ribosomes, translation impaired	16S rRNA	mTERF3 stabilizes or modifies 16S rRNA and is critical for biogenesis of the 39S ribosomal subunit	[5]
mTERF4/mTERFD.2	M	Knock-out/disruption of *mTERF4* in the heart	Embryo lethal/cardiomyopathy, maximal life span of 21 weeks	Altered transcription of tRNAs; mtDNA level increased; increased steady-state levels of mt transcripts by activation of de novo transcription; greatly increased levels of 28S and 39S subunits, but assembly into functional ribosomes perturbed; translation impaired	16S and 12S rRNA and 7S RNA	mTERF4 forms a heterodimer with the cytosine methyltransferase NSUN4 and targets this enzyme to the large ribosomal subunit [6,7].	[6]

Loc., Localization; M, Mitochondria; OXPHOS, oxidative phosphorylation; Ref., Reference.

of mtDNA transcripts. However, 16S rRNA transcripts are down-regulated, presumably due to incorrect processing. The respiratory-chain deficiency was ascribed to the imbalance between amounts of mtDNA transcripts and rRNAs/tRNAs [53]. Furthermore, mTERF3-deficient mitochondria contain unprocessed precursor mtRNAs; this might be a secondary effect of the respiratory-chain-deficient state or, alternatively, it may suggest an additional function for mTERF3 in RNA processing [53]. ChIP analyses suggested that mTERF3 interacts with the mtDNA promoter region, and could thus inhibit transcription initiation in mammalian mitochondria [53]. Accordingly, it was proposed that mTERF3 does not terminate mt transcription, but regulates transcription initiation events, although the exact mechanism remains to be elucidated. More recently, the function of mTERF3 was revisited by applying a comparative, cross-species approach to further study the in vivo role of mTERF3 in Drosophila and mice [63] (Table 1). It was found that unbalanced mtDNA transcription in knockout and knockdown *Mterf3* Drosophila is not the sole cause of respiratory-chain deficiency, but that 16S rRNA levels are reduced and the assembly of the large (39S) mt ribosomal subunit is impaired, thus impeding translation. These findings motivated the authors to reinvestigate the role of mouse mTERF3. Indeed, in the conditional *Mterf3* heart-knockout—the strain used in the study by Park et al. [53]—a reduction in levels of the 39S mt ribosomal subunit and defective ribosomal assembly were found in the absence of mTERF3 [63]. These findings identify a novel role for mTERF3 in the biogenesis of metazoan mt ribosomes. In light of the results reported in 2007 [64], this would point to crosstalk between transcription initiation and ribosomal biogenesis in the control of mtDNA expression and regulation of OXPHOS capacity [63].

4.3. Mammalian mTERF4

Insights into mTERF4 function have been reported only recently [59,62] (Table 1). Like *Mterf3* knock-out mice [53], *Mterf4* knock-out mice are embryo lethal [62]. Loss of mTERF4 in the mouse heart also perturbs the tRNA pool, but increases steady-state levels of mtDNA transcripts, including 12S and 16S rRNA by activating de novo transcription [62]. *Mterf4* knock-out in the heart leads to striking increases in levels of 28S and 39S subunits, but no corresponding increase in the numbers of fully assembled (55S) ribosomes. Thus, subunit assembly is disturbed, resulting in impaired translation. RNA immunoprecipitation experiments demonstrated that mTERF4 binds 16S and 12S rRNA and also to 7S RNA (an RNA encoded close to the LSP). Thus, it was assumed that mTERF4 not only interacts with the ribosome but also binds proximal to the LSP to activate mt transcription [62]. Moreover, two different immunoprecipitation strategies have identified NSUN4 (NOL1/NOP2/Sun domain family member 4)—a member of a family of RNA 5-methylcytosine (m^5C) methyltransferases [66]—as an interaction partner of mTERF4 [62]. This finding was supported by the 3D crystal structure of the human mTERF4–NSUN4 complex [59]. Many studies have demonstrated that methylation of rRNA is important for ribosome biogenesis. For example, methylation of 12S rRNA is necessary for the stability of the small subunit of the mammalian mt ribosome [67]. Bioinformatics analyses and structural modeling of NSUN4 indicate that NSUN4 lacks an RNA recognition domain, and therefore needs a binding partner to target it to the ribosome [62]. Furthermore, in *Mterf4* knock-out hearts and in HeLa cells expressing *mTERF4* RNAi, absolute levels of NSUN4 are virtually unchanged, but targeting of NSUN4 to the large ribosomal subunit is strongly decreased [62]. The C-terminal domain of mTERF4 binds NSUN4 via conserved polar and hydrophobic interactions. Moreover, a positively charged RNA-binding groove along the concave and convex sides of mTERF4 extends into the active site of NSUN4, close to the pocket where the methyl donor S-adenosyl-L-methionine (SAM) binds to a conserved RNA m^5C methyltransferase site [59]. Thus, mTERF4 probably provides the sequence-specific RNA-binding domain necessary for specific rRNA methylation by NSUN4. In summary, mTERF4 forms a heterodimer with NSUN4 and is required for the recruitment of NSUN4 to the mt large ribosomal subunit to carry out an essential, but still poorly defined, step in ribosomal biogenesis [59,62].

5. Emerging roles for mTERF proteins in photosynthetic organisms

Compared to those in other eukaryotes, the number of mTERF proteins in plants is strikingly high. For example, *A. thaliana* [68,69] and *Zea mays* [70] contain 35 and 31 *MTERF* genes, respectively. Since there are fewer putative mTERF proteins in lower plants—13 in the land moss *Physcomitrella patens*, and six in the green alga *C. reinhardtii*, the mTERF family has apparently expanded during the evolution of land plants [69]. But information relating to their roles in photosynthetic organisms remains scarce. Indeed, so far specific molecular functions have been described only for mTERFs from *C. reinhardtii* [71] and maize [72] respectively. While mutations in six *A. thaliana MTERF* genes have been physiologically characterized in more detail [21,68,73–79] (Table 2), their organellar functions are, for the most part, not yet clearly defined.

5.1. The C. reinhardtii mitochondrial genome

Like its counterpart in humans, the small linear 15.8-kb mt genome of *C. reinhardtii* [80] is compactly organized [81]. It encodes three tRNAs and the small and large mt rRNAs, and eight proteins—including subunits of the respiratory complexes and a reverse transcriptase-like protein. The genes encoding the rRNAs are exceptional in that they are segmented and split into four small (S) and eight large (L) subunit modules, which are scrambled and interspersed with one another or with protein-coding genes [82] (Fig. 2). The two strands of the *C. reinhardtii* mtDNA are transcribed into rightward and leftward transcription units, generating long polycistronic transcripts. Two sequences identified in the intergenic region located between the transcription units may act as a bi-directional promoter [83,84].

5.2. C. reinhardtii MOC1

According to the Subcellular Localization Prediction Tool Dedicated to Green Algae (PredAlgo) [85], of the six MOCs (mTERF-like protein of Chlamydomonas) encoded by *C. reinhardtii*, two (MOC5 and MOC6) are predicted to localize to chloroplasts, and four to mitochondria (MOC1–4). In response to short-term changes in illumination, both plants and green algae optimize absorption of the light energy available by adjusting the antennal cross-sections of the two photosystems by a process referred to as “state transitions” [86]. Among *C. reinhardtii* strains identified in a screen for mutants that are defective in state transitions, the *MOC1* gene was found to be disrupted in *state transition mutant 6* (*stm6*) [36]. The *stm6* mutant is blocked in state 1 and impaired in the phosphorylation of PSII-LHC in the light. In addition, *MOC1* transcription and translation is light-inducible, and loss of MOC1 results in sensitivity to high light. Interestingly, although it is involved in state transitions, MOC1 is targeted to mitochondria. Upon a dark-to-light shift, levels of the mt-encoded *nd1*, *nd6* and *cox1* transcripts are only slightly reduced and *nd6* levels are slightly induced in the wild type, whereas in the *stm6* mutant these responses are far more pronounced [36]. A MOC1-binding site was subsequently identified by means of an EMSA (electromobility shift assay)-based screen using 550- to 600-bp mtDNA fragments and cyclic amplification and selection of targets (CAST) to pinpoint the specific binding sequence. Two identical octanucleotide sequences were identified, which are located in module S3 of the mt gene for the small rRNA [71] (Fig. 2B). In a broader analysis of mt-encoded transcript levels, most transcripts were found to be repressed by 10–30% in *stm6* compared to a *MOC1*-complemented strain, but the *nd1* and *rtl* transcripts are reduced to 54% and 43%, respectively. Strikingly, levels of rRNA-coding modules were less affected (if at all) by the loss of MOC1, which indicates that read-through does not occur at the S3 binding site in the *stm6* mutant. Thus, MOC1 is not a

Table 2
Functions of mTERFs from photosynthetic organisms.

Name	Loc.	Type of mutation	Phenotype	Molecular phenotype	(Proposed) binding site	(Proposed) molecular function	Ref.
Chlamydomonas							
MOC1	M	Knock-out	Perturbed respiration, light sensitivity	Reduced mtDNA levels; transcripts around binding site altered only slightly or not at all; antisense transcripts down-/upstream of the binding site up-/downregulated	DNA sequence within the mt rRNA-coding module S3	Transcription terminator of antisense transcription	[1]
Arabidopsis							
mTERF1/SOLDAT10	C	Amino acid exchange	Paler than wild type; displays mild photo-oxidative stress and enhanced acclimation to more severe stress	*clpP* upregulated, other cp transcripts only slightly affected, but *rrn16S* and *rrn23S* level downregulated, reduced cp protein synthesis			[2]
		Knock-out	Embryo arrest				[2]
mTERF2/EMB2219[a]	O/C, C[b]	Knock-out	Embryo arrest				[3]
mTERF4/BSM/RUG2[a]	C [4,5] and M [5]	*bsm*: line not specified, presumably knock-out	Embryo arrest; supplementation with hormones and plastidic acetyl-CoA carboxylase (ACC2) partially rescues *bsm* mutant cells	*clpP* second group IIa intron, *atpF*, *rpl2*, and *rps12* group IIa introns not spliced; *rbcL*, *atpA* and mature *16S* and *23S* rRNA not detectable	Non-specific cpDNA	Splicing of second group IIa intron of *clpP*	[4]
		rug2-1: amino acid exchange	Small vegetative leaves and rounded and protruding laminae; variegated; heat sensitive	~20% of mt transcripts downregulated; cp *trnC*, *trnS.2*, *16S*, *23S rRNA*, *psbA*, *psbN* downregulated, 23 cp transcripts upregulated			[5]
		rug2-2: SALK_033963: T-DNA insertion in 3′ UTR: knock-down	Milder phenotype than *rug2-1*				[5]
mTERF5/MDA1[a]	C/Y, C[b]	*mda1-1*: SALK_097243, truncated protein lacking 2 mTERF motifs	Pale and small; seedlings increased tolerance to salt and osmotic stresses	*psbA*, *accD*, *rps18* upregulated, *clpP* downregulated; other transcripts not investigated			[6]
		mda1-2: SAIL_425_E03 (see also above), truncated protein lacking 5 mTERF motifs	Pale and small				[6]
mTERF8/PTAC15							[7]
mTERF9/TWIRT1[a]	C [4,8]	*twr-1*: premature stop codon, lacking last mTERF motif	Altered root and shoot meristem function, short roots				[9]
		mterf9: *twr-2*: T-DNA line, lacking the two last mTERF motifs	Pale, stunted growth, reduced mesophyll cell numbers; altered responses to sugars, ABA, salt and osmotic stresses	*psbA* and *psbD* downregulated; *clpP*, *rps18*, *accD*, *rpoB* upregulated; other transcripts not investigated			[10]
mTERF15	M	SALK_134099	Strongly reduced growth, no viable seeds	No mature *nad2* mRNA, but overaccumulation of unspliced transcripts	RNA	Splicing of *nad2* intron 3	[11]
mTERF16[a]	C/N, C[b]	Potential knock-out	Embryo arrest				[4]
mTERF18/SHOT1	M	*shot1-1*: amino acid exchange	Suppressor of the *hot1-4* (a dominant-negative allele of *HSP101*); short hypocotyl in the dark, growth reduction	General upregulation of mt transcripts, cp transcripts downregulated, increased mtDNA copy number			[12]
		shot1-2: premature stop codon, truncated protein	More severe growth reduction than *shot1-1*; suppresses other heat-sensitive mutants, more heat tolerant than wild type				[12]
Maize							
Zm-mTERF4	C	Zm-*mterf4-1*: *Mu* transposon insertion 10 bp upstream of the predicted start codon	Ivory-leaf phenotype, dies after the development of 3–4 leaves	16S and 23S rRNA not detectable, cp ribosomes lost	cp group II introns	Group II intron splicing	[13]
		Zm-*mterf4-2*: *Mu* transposon insertion 18 bp upstream of the predicted start codon	Pale yellow-green, dies after the development of 3–4 leaves				[13]

Note that the numbering of vertebrate [14] and plant mTERFs [15] is not based on homology between vertebrate and *A. thaliana* mTERFs.
C, chloroplasts; M, mitochondria; N, nucleus; Y, cytosol.
[a] Homologue identified in maize nucleoids [16].
[b] Bioinformatic predictions of localizations (Loc.) from TargetP and WoIF PSORT.

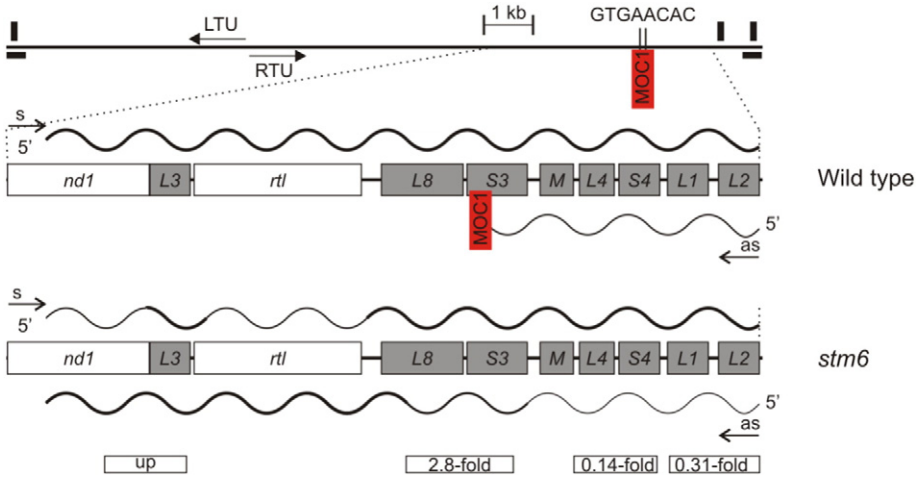

Fig. 2. Proposed model for the molecular function of *C. reinhardtii* MOC1. The figure depicts the region of the *C. reinhardtii* mitochondrial genome around the MOC1 target site in the S3 gene, one of the modules that encode segments of the large (L) and small (S) rRNAs. The leftward and rightward transcription units (LTU and RTU) are indicated, the terminal inverted repeats are represented by horizontal black rectangles at each end of the genome, and the three 86-bp repeats are indicated by vertical black rectangles. Gray boxes indicate gene segments encoding rRNA, white boxes genes for NADH dehydrogenase subunit 1 (*nd1*) and a reverse transcriptase-like protein (*rtl*). The wavy lines represent transcripts and their different thicknesses refer to different expression levels. According to [71], MOC1 acts as a "road block" for antisense transcripts (in the wild type). This model is based on the finding that in the *stm6* mutant levels of antisense (as) transcripts downstream of the MOC1 binding site are increased (up and 2.8-fold in the rectangles), while antisense transcripts derived from sequences upstream of the binding site are reduced (0.14-fold and 0.31-fold).

terminator of sense RNA transcription. However, as in *Mterf1* knock-out mice [39], antisense transcripts are altered in *stm6* cells. Fewer transcripts of sequences upstream of the MOC1-binding site are found, while transcripts derived from regions downstream of the binding site (Fig. 2B) are overrepresented, suggesting that MOC1 acts as a terminator of antisense transcription [71]. The formation of antisense transcripts of the (linear) *C. reinhardtii* mt genome implies the presence of an as yet unidentified (antisense) promoter or the occurrence of circular mt genomes. Indeed, although rare (≤1%), both open and supercoiled circular mtDNA molecules have been observed by electron microscopy [87]. However, although loss of MOC1 results in higher or lower levels of antisense transcripts, their abundance is extremely low in comparison to the sense transcripts, and it therefore seems unlikely that the strong phenotype is caused by detrimental effects of antisense transcripts on mt gene expression. That the reduced amounts of unprocessed transcripts seen in the *stm6* mutant can account for the phenotypic changes also appears very doubtful. Nevertheless, and in contrast to the loss of mTERF1 in mice [39], inactivation of MOC1 in *C. reinhardtii* causes a definite phenotype [71] and impinges on mitochondrial function. But whether MOC1 performs other functions and, if so, what these might be, is currently difficult to decide.

5.3. Plant organellar genomes

Although mitochondrial metabolic functions are basically the same in all organisms, the mt genomes of seed plants exhibit features that are distinct from those of animals and other organisms. First, they are among the largest mt genomes known [88]. The *A. thaliana* mt genome, for example, comprises 366,924 nucleotides and contains 57 genes [88]. Second, they are characterized by large-scale rearrangements, and both mono- and polycistronic transcription units are spaced across the length of the entire genome and are separated by large spacer sequences without any obvious function [89,90]. In consequence, only 10–20% of the "typical" plant mt genome is made up of structural genes, while the rest is taken up by introns, intronic ORFs, unidentified ORFs, pseudogenes, and pieces of foreign (cp and nuclear) DNA [91]. As discussed above, mammals and *C. reinhardtii* use few transcription initiation sites to drive the expression of large mt transcription units, but the mitochondrial genomes of higher plants represent a more complex system with multiple transcription initiation sites [9]. Transcription of mt genes frequently starts from multiple promoters of various types, generating precursor RNAs that undergo various processing steps, such as RNA editing, splicing of group II introns, 3′-end trimming and formation of secondary 5′ termini [90].

The standard cp genome of land plants is a 120- to 160-kb long double-stranded DNA molecule with a quadripartite structure consisting of two inverted repeats (IRa and IRb) that divide the circle into a large (LSC) and small single copy (SSC) region. The IRs are identical in sequence. Hence, the genes encoded within them—the rRNA genes and some other genes—are present in two copies per genome [92]. In general, the cp genome encodes 16S, 23S and 5S rRNA genes and about 30 tRNA genes (sufficient for translation of all amino acids), at least three of the four subunits of a prokaryotic-type RNA polymerase (RpoB, C1, C2) and subunits of the photosynthetic machinery [92]. Strikingly, plant cp and mt genomes have converged to encode a surprisingly constant set of ribosomal proteins [6]. With 100–120 genes residing in a stretch only 120–160 kb long, the plastome is more densely packed than the nuclear and mt genomes of the plant cell [93]. In chloroplasts, genes are still organized into transcriptional units or operons as in bacteria [94]. However, in contrast to the latter and similar to the situation in the plant mt genome, the corresponding poly-cistronic mRNAs in the chloroplasts undergo complex processing events and only after intron splicing, editing, intercistronic cleavage and 5′- and 3′-end definition, are the mature mRNAs ready for translation [11,94]. As a consequence, while the cp genome harbors all the required tRNAs and rRNAs, and cp-encoded RNA polymerase subunits, many additional proteins required for the operation of the complex cp genome expression machinery are encoded by nuclear genes and have to be imported into chloroplasts [9–18].

Importantly, it is generally assumed that many 3′ RNA termini do not arise directly via transcription termination as in the case of Rho-dependent transcription termination in bacteria [95], but are generated by the processing of longer molecules [11]. Recently, PPR proteins together with other classes of RNA-binding proteins have been implicated in the definition of transcript termini. Short non-coding RNA fragments that accumulate in chloroplasts have been identified as footprints of RNA-binding proteins [96,97], most processed mRNA termini are represented by small RNAs whose sequences are highly conserved, and it has been suggested that each such small RNA is the footprint of a PPR-like protein that protects the adjacent RNA from degradation [97]. Hence, the use of a Rho-like mode of transcription termination in plant organelles—especially

in chloroplasts—appears to be very rare or entirely absent. This presents a challenge to efforts to dissect the real functional roles of mTERFs in plant organelles.

5.4. A. thaliana mTERFs

Almost all of the 35 *A. thaliana* mTERF proteins are predicted or have been shown to be targeted to mitochondria and/or chloroplasts [68,69]. The six *A. thaliana MTERF* genes that have been studied in more detail (Table 2) were identified in forward genetic screens for mutations that disrupt abiotic stress responses (*SINGLET OXYGEN-LINKED DEATH ACTIVATOR10*, *SOLDAT10* [21]) and *SUPPRESSOR OF hot1–4 1*, *SHOT1* [73]), meristem function (*TWIRT1, TWR-1* [74]) and leaf morphology (*RUGOSA2*, *RUG2* [75]). An allele of *RUG2* named *BELAYA SMERT* (*BSM*) was also identified in a reverse genetic screen of T-DNA lines with insertions in *MTERF* genes for visible phenotypes [68], as was *MTERF9*, an allele of *TWIRT1* [77]. From screen in which *mterf9* was discovered also yielded the *mda1* (*mterf defective in Arabidopsis1*) mutant, which was subjected to detailed characterization [76]. Recently, *mterf15* was identified in a screen of Arabidopsis T-DNA insertion mutants that are defective in post-embryonic development and/or seed germination [79]. The products of three of these six *MTERF* genes are targeted to chloroplasts, one to both chloroplasts and mitochondria, and two to mitochondria alone (see Table 2).

5.4.1. SOLDAT10

The *soldat10* mutant was the first *mterf* mutant to be characterized in higher plants. The *SOLDAT10* locus was identified by screening for second-site mutations that attenuate the *fluorescent* (*flu*) phenotype [21]. In the *flu* mutant, the photosensitizer protochlorophyllide accumulates in the dark [98]. Consequently, exposure of *flu* mutants to light generates $^1O_2$, which is thought to remain largely restricted to plastids, and *flu* seedlings bleach and die [98]. SOLDAT10 is localized to chloroplasts, and the *soldat10* mutant suffers from mild photo-oxidative stress, while complete inactivation of *SOLDAT10* is apparently lethal [21]. The mild stress experienced by *soldat10* seedlings when grown in low-light conditions leads to a stress acclimation response, which confers enhanced resistance against combined high-light/low-temperature stress. The *soldat10* seedlings look slightly pale and their photosynthetic performance is reduced, but the mutation does not lead to a general impairment of cp transcript accumulation. However, of the 15 cp transcripts tested, 16S and 23S rRNA levels are reduced and the mRNA for ClpP protease is upregulated. As a consequence, protein synthesis in plastids of *soldat10* is reduced. This in turn somehow activates stress-related retrograde signaling to the nucleus [21]. Indeed, specific nuclear genes have previously been shown to be upregulated in *soldat10* seedlings as a response to stress stimuli [99]. The constitutive acclimation to light stress and the disturbance of plastid homeostasis might account for the suppression of the $^1O_2$-mediated death of *soldat10 flu* seedlings [21].

5.4.2. SHOT1

The *shot1-1* mutant was recovered in a screen for suppressors of *hot1-4*, a dominant-negative allele of the heat-shock protein gene *HSP101*, which was expected to identify factors that interact with HSP101 or are involved in thermotolerance [73]. A missense (*shot1-1*) and a T-DNA insertion (*shot1-2*) mutant each suppress the heat-hypersensitivity of *hot1-4* plants. Furthermore, *shot1-2* also suppresses other heat-sensitive mutant phenotypes, and *shot1-2* itself is more heat tolerant than the wild type [73]. SHOT1 is a mitochondrial protein, which excludes any direct interaction with cytosolic/nuclear HSP101. As in the case of *soldat10* seedlings [21], expression patterns of many stress-responsive genes are altered in *shot1-2* seedlings, a finding which indicates that the mutant plants are stressed even under normal growth conditions [73]. However, the increase in thermotolerance associated with impairment of SHOT1 function is not attributable to enhancement of protein quality control. Instead, the *shot1* mutant accumulates lower amounts of reactive oxygen species (ROS) and thus tolerates higher levels of oxidative stress than the wild type, which is reflected in greater tolerance of heat stress. In *shot1-2*, 257 genes are upregulated [73], while 201 genes are upregulated in *soldat10* [21]. Of them only eight genes are in common. It is therefore not surprising that a mutation in the cp mTERF protein SOLDAT10 does not confer thermotolerance [73]. However, also in *shot1*, as assumed for *soldat10* [21], a disturbance in organelle homeostasis could lead to enhanced thermotolerance in *shot1*, because in the *shot1-2* mutant, transcripts of mt genes and cp genes are generally up- and down-regulated, respectively [73].

5.4.3. mTERF9 and MAD1

The fact that the *twr-1* mutant is an *mterf* mutant has often escaped notice, because it primarily served as a proof of principle for a "fast forward genetics" strategy, which combines traditional bulk segregant techniques with targeted genomic enrichment and next-generation sequencing [74]. The *twr-1* mutant shows reduced root growth and delayed shoot meristem activation, which results in a large reduction in the volume of shoot apical meristem in mature mutant embryos [74], but the mutant was not characterized in more detail. More recently, however, a comparative analysis of the *mterf9* mutant (the same T-DNA line as the *twr-2* mutant used in [74]) with the allelic *twr-1* mutant was published [77]. Loss of mTERF9 results in defective chloroplast development, which probably explains the paleness, stunted growth and reduced mesophyll cell numbers in the *mterf9* mutant. The *twr-1* mutant displays a morphological phenotype similar to that of the *mterf9* mutant, although the defects in *twr-1* are more severe, with *twr-1* plants growing more slowly, and being even smaller and paler.

The *mda1* mutant [76] was characterized in basically the same way as the *mterf9* mutant; therefore, those two mutants will be described in parallel. Chloroplast development in the *mda1* mutant is affected in the same way as in the *mterf9* mutant, leading to smaller and paler plants relative to wild type. Interestingly, *mterf9* and *mda1* plants flower earlier than wild type, a phenotypic trait shared by *rug2-1* [75] and *soldat10* [21] plants—the other two mutants that are defective in cp-targeted mTERF proteins.

Mutations in *MTERF9* and *MDA1* both influence cp gene expression. A survey of transcripts of six different plastid genes in *mterf9* shows reduced levels of the plastid-encoded polymerase (PEP)-dependent transcripts *psbA* and *psbD*, and increased levels of the nucleus-encoded polymerase (NEP)-dependent transcripts *rps18*, *accD* and *rpoB*. In addition, the *clpP* transcript, which is dependent on both types of polymerases, is upregulated [77]. In *mda1*, *clpP* transcripts are reduced, while PEP- and NEP-encoded genes are preferentially upregulated or show little change [76].

As discussed above, *soldat10* [21] and *shot1* [73] were both identified in suppressor screens associated with stress responses. Thus, it seems likely that other *mterf* mutants might also be linked to stress responses. Mutant *mda1* and *mterf9* seedlings are indeed less sensitive to salt and osmotic stresses, which might be explained by their reduced sensitivity to ABA [76,77]. Interestingly, older *mda1* plants display reduced tolerance of salt, mild cold and ABA. Furthermore, another *mterf* mutant, the *rug2-1* mutant (which is discussed in more detail below) is sensitive to temperature stress. When grown at 26 °C instead of 20 °C, *rug2-1* growth is arrested, whereas at 16 °C its mutant phenotype is fully suppressed [75]. Independently of the studies with *mterf* mutants, it has been noted before that changes in OGE trigger retrograde signaling, which enables nuclear gene expression to be modified in accordance with the current status of the organelle [100,101]. And more recent findings suggest that disturbances in OGE homeostasis are translated into abiotic acclimation and tolerance responses, presumably via retrograde signaling. For example, as with *soldat10* [21], mutations in a subunit of the plastid-encoded RNA polymerase [102], or the ClpR4 subunit of the cp-localized Clp protease complex [103], or a putative translation elongation factor (At5g13650) [103], not only induce plastid signaling

but also acclimation, which renders seedlings more resistant to simultaneous exposure to low temperature and high light (HL) treatment. In addition, knockdown of cp ribosomal protein S1 (RPS1) expression nearly eliminates the heat stress-activated expression of *HsfA2* and its target genes, resulting in a loss of heat tolerance [104]. Interestingly, it has been noted that early OGE-dependent signaling (triggered by a defective prolyl-tRNA synthetase targeted to both the mt and the cp) and temperature-acclimation and light signaling pathways may target the same transcription factors [105].

5.4.4. BSM/RUG2

In a screen for EMS-induced mutations affecting leaf morphology, mutants with small vegetative leaves and rounded and protruding laminae were identified. These were assigned to a phenotypic class that was named Rugosa (Rug), and they fall into two complementation groups named RUG1 and RUG2 [106]. RUG2 turned out to be an mTERF protein targeted to mitochondria and chloroplasts [75], and a further mutant allele, named *bsm* (*belaya smert*), was isolated in a reverse genetics screen of *mterf* mutants [68]. BSM/RUG2 is essential for normal plant development. The *rug2-1* mutant is unable to germinate in soil, shows the characteristic leaf phenotype, is variegated, and growth and fertility are reduced [75]. In the *bsm* mutant, even embryo development is prematurely arrested, but the authors were able to recover stable shoot cultures on medium supplemented with cytokinin and auxin [68]. BSM/RUG2 is required for the maintenance of the correct levels of transcripts in both mitochondria and chloroplasts [68,75]. Global transcript profiling of cp and mt genes in the *rug2-1* mutant reveals that approximately 22% of the genes are dysregulated in each organelle, with mt genes being preferentially downregulated (except for three encoding proteins of unknown function) while most of the cp genes affected are upregulated (with the exception of genes for two tRNAs, 16S, 23S and 4.5S rRNAs, *psbA* and *psbN*) [75]. In the *bsm* mutant, selected transcript levels were investigated by Northern blot analysis, which permits splicing patterns to be examined. Mature 16S rRNA and 23S rRNA transcripts are undetectable in *bsm* cells. The PEP-dependent genes for RbcL and AtpA were similarly affected, whereas *clpP* transcript levels were boosted and showed a different splicing pattern from that in the wild type [68]. Indeed, RT-PCR revealed that the second *clpP* group IIa intron is not spliced out, and the same holds for group IIa introns of *atpF*, *rpl2*, and *rps12*. The failure to remove the latter three group IIa introns could be mimicked by growing wild-type plants on the antibiotic spectinomycin, an inhibitor of organelle translation, but the second group IIa intron of *clpP* is correctly spliced under these conditions. Furthermore, splicing of this intron is thought to be independent of MatK [107], a maturase assumed to act as a *trans*-acting splicing factor for group IIa introns [108], suggesting a direct role for BSM in splicing out the second *clpP* intron.

5.4.5. mTERF15

The latest *mterf* mutant to be described carries a T-DNA insertion in the gene for mTERF15, a protein that is localized to mitochondria [79]. The *mterf15* mutant grows very slowly, development is retarded and the shriveled seeds are inviable. mTERF15 is crucial for the maintenance of mt biogenesis and mt membrane integrity. The inner membrane systems are aberrant in *mterf15* mitochondria, resulting in a decrease in mt membrane potential. In vitro binding studies with dsDNA-cellulose, and Southwestern and Northwestern analysis on membrane blots bearing the mTERF15 protein, suggest an RNA-binding function for mTERF15. In light of the role of BSM in cp intron splicing [68], Hsu et al. [79] investigated splicing events in mitochondria from wild type, *mterf15* and complemented plants. In *mterf15*, nearly all splicing events occur as in the wild type, except for the splicing of *nad2* intron 3 which is significantly reduced. Moreover, this splicing defect is fully rescued in the complemented plants, which strongly suggests that mTERF15 is involved in splicing of intron 3 in the mt *nad2* transcript.

5.4.6. Double mutants

To identify putative functional relationships between mutated *MTERF* genes, especially between those encoding cp-localized mTERFs, Robles et al. [76,77] examined the phenotypes of double mutants created by crossing mutants homozygous for single, non-allelic mutations with distinct phenotypes and comparing the double mutant phenotype in the F2 with those of the parental single mutants [109]. The double mutant phenotype can be considered as additive if it exhibits a combination of traits present in the single mutant, and as synergistic if the severity of the double mutant phenotype is greater than the sum of their individual effects. Additivity is widely accepted as indicating that the genes under study do not functionally interact [110]. Synergy can be considered as a sign of functional relationship between the genes involved, or may arise when pathways that converge at a node are disrupted or when one mutation enhances sensitivity to the effect of another mutation [109]. Double mutant analyses indicate that MDA1 and SOLDAT10, as well as MDA1 and RUG2, act in different pathways, but the synergistic phenotype of *rug2-1 soldat10* mutants suggests genetic interaction between *rug2-1* and *soldat10* [76] (Fig. 3). In the course of the characterization of the *mterf9* mutant, the mutation was found to interact with *mda1-1*, but *mterf9 rug2-2* and *mterf9 soldat10* display additive phenotypes [77].

Some critical amino acid residues are conserved within mTERF motifs 1–4 of human mTERF3 and *A. thaliana* mTERF9. Furthermore, the two mouse mTERF proteins, mTERF3 and mTERF4, function in the biogenesis of mt ribosomes [62,63]. This prompted Robles et al. [77] to investigate whether mTERF9 and MDA1 might be related to ribosomal functions in the cp. Double mutants were generated between *mda1-1* or *mterf9* and mutations affecting nuclear genes encoding proteins of the large (*rpl24* and *rpl31*) or the small cp ribosomal subunit (*rps5*), respectively. The phenotypes of the *mda1 rpl31*, *mterf9 rpl31* and *mterf9 rps5* double mutants are additive: they display the phenotypic traits of both parental strains. However, the phenotypes of the *mda1-1 rpl24* and *mda1-1 rps5* double mutants are synergistic, implying a functional interaction. Seedling growth undergoes early arrested early. The *mterf9 rpl24* double mutant could not be identified as the two loci lie close together on the same chromosome (MTERF9: AT5G55850; RPL24: AT5G54600).

5.5. Maize mTERF4

The number of *MTERF* genes in maize (31) is comparable to that in *A. thaliana* (35). As in *A. thaliana*, most mTERF proteins in maize are predicted to localize to mitochondria or chloroplasts, and phylogenetic analysis of mTERF proteins in maize, rice, and *A. thaliana* indicates that mt- and cp-targeted mTERF proteins form divergent clades [70]. Levels of *MTERF* transcripts are differentially regulated in maize seedlings

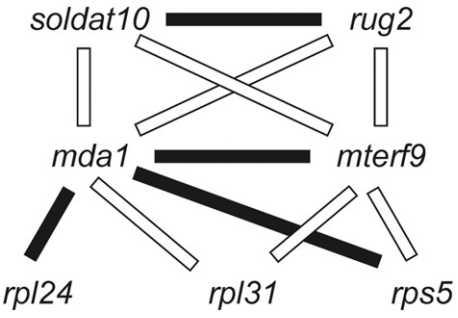

Fig. 3. Summary of the results of double-mutant analyses designed to identify additive (white rectangles) or synergistic (black rectangles) interactions among MTERF genes, and between MDA1 and MTERF9 and genes coding for cp ribosomal proteins. Because the genes for mTERF9 and Rpl24 are located close together on the same chromosome, no mterf9 rpl24 double mutants were recovered.

grown under different light/dark regimes, or exposed to plant hormones and salts [70], which suggests that, like *A. thaliana* mTERFs, mTERFs in maize are involved in abiotic stress responses [21,73,76,77].

One maize *mterf* mutant, Zm-*mterf4*, was identified in the Photosynthetic Mutant Library (PML; http://pml.uoregon.edu/pml_table.php), a collection of Mu transposon-induced non-photosynthetic maize mutants, and was characterized lately [72]. Zm-mTERF4 is an ortholog of the *A. thaliana* mTERF variously named BSM, RUG2 or mTERF4 [68,69,75], and is localized to the cp stroma [72]. The knock-down Zm-*mterf4-1* and Zm-*mterf4-2* alleles harbor insertions 10 and 18 bp upstream of the predicted start codon, and exhibit an ivory or pale-green leaf phenotype, respectively. Plants homozygous for either allele die after the development of three to four leaves upon exhaustion of seed reserves, as is typical for non-photosynthetic maize mutants. Core subunit proteins of photosynthetic complexes are barely detectable in Zm-*mterf4-1*, and, similar to the situation in the *A. thaliana bsm* mutant [68], 16S and 23S rRNAs are undetectable, suggesting that plastid ribosomes are likewise defective in Zm-*mterf4* mutants [72]. To investigate whether Zm-mTERF4 influences cp gene expression at the post-transcriptional level, an RNA coimmunoprecipitation assay was combined with macroarray analyses. Zm-mTERF4 was found to coimmunoprecipitate with RNAs containing group II introns. Together with (i) RT-PCR and Northern blot analyses performed on hypomorphic mutants (and on other cp ribosomal mutants to exclude secondary effects resulting from compromised cp translation), and (ii) sucrose-gradient fractionation of cp stroma followed by immunoprecipitation of different splicing factors with Zm-mTERF4, this result argues that Zm-mTERF4 functions directly in *atpF* and *rpl2* splicing.

Although the *A. thaliana* Zm-mTERF4 ortholog BSM/RUG2/mTERF4 binds non-specifically to cpDNA (RNA binding was not investigated), the function in cp RNA splicing might be conserved in BSM, because BSM is required to splice *clpP* intron 2 [68], a group II intron that is not found in maize.

6. Concluding remarks

The significance of mTERF proteins in mammals and plants is demonstrated by their striking loss-of-function phenotypes. Mouse *Mterf3* [53] and *Mterf4* [62] knock-outs, as well as *A. thaliana* mutants defective in the mTERF proteins SOLDAT10 [21], BSM [68], EMB2219 (mTERF2) [111] and mTERF16 [68], are embryo lethal. In *A. thaliana* and maize [112,113], mTERF proteins have been shown to be associated with the plastid transcriptionally active chromosome (pTAC), a major site of plastid RNA and DNA metabolism [112]. In addition, many mTERF proteins in *A. thaliana* are coexpressed with genes encoding proteins known to be involved in OGE, which argues for a pivotal role for plant mTERFs in OGE [69].

Intriguingly, mammalian mTERF1 is not a terminator of H-strand (sense) transcription, but appears to prevent L-strand transcripts from proceeding all the way around the mtDNA circle and interfering with transcription from the LSP promoter from which they originated [39] (see Fig. 1B). Strikingly, *Mterf1* knock-out mice do not display any apparent phenotype [39], raising the question of the physiological significance of mTERF1 function in mice. MOC1 in *C. reinhardtii* also terminates antisense transcription [71], but it is currently unclear whether MOC1 has other functions. As discussed in Section 5.3, there is very little evidence for a Rho-like mode of transcription termination [95] in plant organelles, especially in chloroplasts, and this raises issues about the functional roles of mTERFs in plant organelles. At this point, the recently defined functions of mammalian mTERF3 [63] and mTERF4 [62] should be considered as case studies for the functional dissection of mTERFs in plants. Mouse mTERF4 is required for the recruitment of NSUN4 to the mt large ribosomal subunit and provides the sequence-specific RNA binding domain necessary for rRNA methylation by NSUN4 to promote ribosomal biogenesis [59,62]. mTERF3 also binds (in vitro) to rRNA and is involved in ribosomal biogenesis [63], although the precise mechanism is still unknown. Thus, mTERF proteins can (i) mediate interactions between nucleic acids and other OGE proteins to implement important steps in OGE, and (ii) are not only capable to bind DNA, but can also interact with RNA. In this context, the accumulation of unprocessed precursor RNAs in mTERF3-deficient mouse mitochondria might be a secondary effect of the respiratory-chain-deficient state or, alternatively, the consequence of an additional mTERF3 function in RNA processing [53]. One future challenge therefore is to elucidate the structural variations of mTERF proteins that condition RNA vs. DNA binding activity. Furthermore, the RNA-binding capacity of mTERF proteins opens up a range of possible functions for mTERF proteins in gene expression in plant organelles, the organization of which is far more complex than that found in mammalian mitochondria. Intriguingly, as a possible conserved function between mammalian and plant mTERFs, maize BSM is responsible for intron splicing [72], and *A. thaliana* BSM/RUG2 [68] and mTERF15 [79] are involved in intron splicing. Further studies on the molecular and physiological functions of the expanded mTERF family in plants will lead to a better understanding of OGE and will help to resolve (i) whether mTERF proteins share conserved molecular functions with their mammalian counterparts or have evolved different functions, and elucidate (ii) the roles of OGE in acclimation responses and the nature of retrograde signal transduction.

Conflict of interest statement

This research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

We are grateful to Paul Hardy for critical reading of the manuscript.

References

[1] S.G. Andersson, O. Karlberg, B. Canback, C.G. Kurland, On the origin of mitochondria: a genomics perspective, Philos. Trans. R. Soc. Lond. B Biol. Sci. 358 (2003) 165–177 (discussion 177–169).
[2] J.A. Raven, J.F. Allen, Genomics and chloroplast evolution: what did cyanobacteria do for plants? Genome Biol. 4 (2003) 209.
[3] T. Kleine, U.G. Maier, D. Leister, DNA transfer from organelles to the nucleus: the idiosyncratic genetics of endosymbiosis, Annu. Rev. Plant Biol. 60 (2009) 115–138.
[4] W. Martin, T. Rujan, E. Richly, A. Hansen, S. Cornelsen, T. Lins, D. Leister, B. Stoebe, M. Hasegawa, D. Penny, Evolutionary analysis of Arabidopsis, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 12246–12251.
[5] J.N. Timmis, M.A. Ayliffe, C.Y. Huang, W. Martin, Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes, Nat. Rev. Genet. 5 (2004) 123–135.
[6] U.G. Maier, S. Zauner, C. Woehle, K. Bolte, F. Hempel, J.F. Allen, W.F. Martin, Massively convergent evolution for ribosomal protein gene content in plastid and mitochondrial genomes, Genome Biol. Evol. 5 (2013) 2318–2329.
[7] R. Szklarczyk, M.A. Huynen, Mosaic origin of the mitochondrial proteome, Proteomics 10 (2010) 4012–4024.
[8] P.J. Keeling, J.D. Palmer, Horizontal gene transfer in eukaryotic evolution, Nat. Rev. Genet. 9 (2008) 605–618.
[9] K. Liere, A. Weihe, T. Borner, The transcription machineries of plant mitochondria and chloroplasts: composition, function, and regulation, J. Plant Physiol. 168 (2011) 1345–1360.
[10] A. Barkan, Expression of plastid genes: organelle-specific elaborations on a prokaryotic scaffold, Plant Physiol. 155 (2011) 1520–1532.
[11] D.B. Stern, M. Goldschmidt-Clermont, M.R. Hanson, Chloroplast RNA metabolism, Annu. Rev. Plant Biol. 61 (2010) 125–155.
[12] R. Stoppel, J. Meurer, The cutting crew–ribonucleases are key players in the control of plastid gene expression, J. Exp. Bot. 63 (2012) 1663–1673.
[13] S. Lerbs-Mache, Function of plastid sigma factors in higher plants: regulation of gene expression or just preservation of constitutive transcription? Plant Mol. Biol. 76 (2011) 235–249.
[14] K. Hammani, G. Bonnard, A. Bouchoucha, A. Gobert, F. Pinker, T. Salinas, P. Giege, Helical repeats modular proteins are major players for organelle gene expression, Biochimie 100 (2014) 141–150.
[15] T.R. Mercer, S. Neph, M.E. Dinger, J. Crawford, M.A. Smith, A.M. Shearwood, E. Haugen, C.P. Bracken, O. Rackham, J.A. Stamatoyannopoulos, A. Filipovska, J.S. Mattick, The human mitochondrial transcriptome, Cell 146 (2011) 645–658.

[16] O. Rackham, T.R. Mercer, A. Filipovska, The human mitochondrial transcriptome and the RNA-binding proteins that regulate its expression, Wiley Interdiscip. Rev. RNA 3 (2012) 675–695.
[17] Q.B. Yu, C. Huang, Z.N. Yang, Nuclear-encoded factors associated with the chloroplast transcription machinery of higher plants, Front. Plant Sci. 5 (2014) 316.
[18] Y. Yagi, T. Shiina, Recent advances in the study of chloroplast gene expression and its evolution, Front. Plant Sci. 5 (2014) 61.
[19] T. Linder, C.B. Park, J. Asin-Cayuela, M. Pellegrini, N.G. Larsson, M. Falkenberg, T. Samuelsson, C.M. Gustafsson, A family of putative transcription termination factors shared amongst metazoans and plants, Curr. Genet. 48 (2005) 265–269.
[20] B. Kruse, N. Narasimhan, G. Attardi, Termination of transcription in human mitochondria: identification and purification of a DNA binding protein factor that promotes termination, Cell 58 (1989) 391–397.
[21] R. Meskauskiene, M. Wursch, C. Laloi, P.A. Vidi, N.S. Coll, F. Kessler, A. Baruah, C. Kim, K. Apel, A mutation in the Arabidopsis mTERF-related plastid protein SOLDAT10 activates retrograde signaling and suppresses (1)O(2)-induced cell death, Plant J. Cell Mol. Biol. 60 (2009) 399–410.
[22] M. Roberti, P.L. Polosa, F. Bruni, C. Manzari, S. Deceglie, M.N. Gadaleta, P. Cantatore, The MTERF family proteins: mitochondrial transcription regulators and beyond, Biochim. Biophys. Acta 1787 (2009) 303–311.
[23] A. Daga, V. Micol, D. Hess, R. Aebersold, G. Attardi, Molecular characterization of the transcription termination factor from human mitochondria, J. Biol. Chem. 268 (1993) 8123–8130.
[24] P. Fernandez-Silva, F. Martinez-Azorin, V. Micol, G. Attardi, The human mitochondrial transcription termination factor (mTERF) is a multizipper protein but binds to DNA as a monomer, with evidence pointing to intramolecular leucine zipper interactions, EMBO J. 16 (1997) 1066–1079.
[25] J. Asin-Cayuela, M. Helm, G. Attardi, A monomer-to-trimer transition of the human mitochondrial transcription termination factor (mTERF) is associated with a loss of in vitro activity, J. Biol. Chem. 279 (2004) 15670–15677.
[26] J. Asin-Cayuela, T. Schwend, G. Farge, C.M. Gustafsson, The human mitochondrial transcription termination factor (mTERF) is fully active in vitro in the non-phosphorylated form, J. Biol. Chem. 280 (2005) 25499–25505.
[27] J. Shang, D.A. Clayton, Human mitochondrial transcription termination exhibits RNA polymerase independence and biased bipolarity in vitro, J. Biol. Chem. 269 (1994) 29112–29120.
[28] A. Prieto-Martin, J. Montoya, F. Martinez-Azorin, New DNA-binding activity of rat mitochondrial transcription termination factor (mTERF), J. Biochem. 136 (2004) 825–830.
[29] S.C. Nam, C. Kang, DNA light-strand preferential recognition of human mitochondria transcription termination factor mTERF, J. Biochem. Mol. Biol. 38 (2005) 690–694.
[30] M. Martin, J. Cho, A.J. Cesare, J.D. Griffith, G. Attardi, Termination factor-mediated DNA loop between termination and initiation sites drives mitochondrial rRNA synthesis, Cell 123 (2005) 1227–1240.
[31] M. Roberti, P. Fernandez-Silva, P.L. Polosa, E. Fernandez-Vizarra, F. Bruni, S. Deceglie, J. Montoya, M.N. Gadaleta, P. Cantatore, In vitro transcription termination activity of the Drosophila mitochondrial DNA-binding protein DmTTF, Biochem. Biophys. Res. Commun. 331 (2005) 357–362.
[32] A. Chomyn, A. Martinuzzi, M. Yoneda, A. Daga, O. Hurko, D. Johns, S.T. Lai, I. Nonaka, C. Angelini, G. Attardi, MELAS mutation in mtDNA binding site for transcription termination factor causes defects in protein synthesis and in respiration but no change in levels of upstream and downstream mature transcripts, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 4221–4225.
[33] J.F. Hess, M.A. Parisi, J.L. Bennett, D.A. Clayton, Impairment of mitochondrial transcription termination by a point mutation associated with the MELAS subgroup of mitochondrial encephalomyopathies, Nature 351 (1991) 236–239.
[34] P. Loguercio Polosa, M. Roberti, C. Musicco, M.N. Gadaleta, E. Quagliariello, P. Cantatore, Cloning and characterisation of mtDBP, a DNA-binding protein which binds two distinct regions of sea urchin mitochondrial DNA, Nucleic Acids Res. 27 (1999) 1890–1899.
[35] M. Roberti, P.L. Polosa, F. Bruni, C. Musicco, M.N. Gadaleta, P. Cantatore, DmTTF, a novel mitochondrial transcription termination factor that recognises two sequences of *Drosophila melanogaster* mitochondrial DNA, Nucleic Acids Res. 31 (2003) 1597–1604.
[36] C. Schönfeld, L. Wobbe, R. Borgstadt, A. Kienast, P.J. Nixon, O. Kruse, The nucleus-encoded protein MOC1 is essential for mitochondrial light acclimation in *Chlamydomonas reinhardtii*, J. Biol. Chem. 279 (2004) 50366–50374.
[37] X. Li, L.S. Zhang, M.X. Guan, Cloning and characterization of mouse mTERF encoding a mitochondrial transcriptional termination factor, Biochem. Biophys. Res. Commun. 326 (2005) 505–510.
[38] Y. Chen, G. Zhou, M. Yu, Y. He, W. Tang, J. Lai, J. He, W. Liu, D. Tan, Cloning and functional analysis of human mTERFL encoding a novel mitochondrial transcription termination factor-like protein, Biochem. Biophys. Res. Commun. 337 (2005) 1112–1118.
[39] M. Terzioglu, B. Ruzzenente, J. Harmel, A. Mourier, E. Jemt, M.D. Lopez, C. Kukat, J.B. Stewart, R. Wibom, C. Meharg, B. Habermann, M. Falkenberg, C.M. Gustafsson, C.B. Park, N.G. Larsson, MTERF1 binds mtDNA to prevent transcriptional interference at the light-strand promoter but is dispensable for rRNA gene transcription regulation, Cell Metab. 17 (2013) 618–626.
[40] K.E. Guja, M. Garcia-Diaz, Hitting the brakes: termination of mitochondrial transcription, Biochim. Biophys. Acta 1819 (2012) 939–947.
[41] J. Montoya, G.L. Gaines, G. Attardi, The pattern of transcription of the human mitochondrial rRNA genes reveals two overlapping transcription units, Cell 34 (1983) 151–159.
[42] J. Battey, D.A. Clayton, The transcription map of mouse mitochondrial DNA, Cell 14 (1978) 143–156.
[43] R. Gelfand, G. Attardi, Synthesis and turnover of mitochondrial ribonucleic acid in HeLa cells: the mature ribosomal and messenger ribonucleic acid species are metabolically unstable, Mol. Cell. Biol. 1 (1981) 497–511.
[44] A.K. Hyvarinen, M.K. Kumanto, S.K. Marjavaara, H.T. Jacobs, Effects on mitochondrial transcription of manipulating mTERF protein levels in cultured human HEK293 cells, BMC Mol. Biol. 11 (2010) 72.
[45] D.T. Dubin, K.D. Timko, R.J. Baer, The 3′ terminus of the large ribosomal subunit ("17S") RNA from hamster mitochondria is ragged and oligoadenylated, Cell 23 (1981) 271–278.
[46] R.A. Van Etten, J.W. Bird, D.A. Clayton, Identification of the 3′-ends of the two mouse mitochondrial ribosomal RNAs. The 3′-end of 16 S ribosomal RNA contains nucleotides encoded by the gene for transfer RNALeuUUR, Int. J. Biol. Chem. 258 (1983) 10104–10110.
[47] D.T. Dubin, J. Montoya, K.D. Timko, G. Attardi, Sequence analysis and precise mapping of the 3′ ends of HeLa cell mitochondrial ribosomal RNAs, J. Mol. Biol. 157 (1982) 1–19.
[48] T.W. Christianson, D.A. Clayton, A tridecamer DNA sequence supports human mitochondrial RNA 3′-end formation in vitro, Mol. Cell. Biol. 8 (1988) 4502–4509.
[49] Y. Goto, I. Nonaka, S. Horai, A mutation in the tRNA(Leu)(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies, Nature 348 (1990) 651–653.
[50] Y. Kobayashi, M.Y. Momoi, K. Tominaga, T. Momoi, K. Nihei, M. Yanagisawa, Y. Kagawa, S. Ohta, A point mutation in the mitochondrial tRNA(Leu)(UUR) gene in MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes), Biochem. Biophys. Res. Commun. 173 (1990) 816–822.
[51] M. Tanaka, H. Ino, K. Ohno, T. Ohbayashi, S. Ikebe, T. Sano, T. Ichiki, M. Kobayashi, Y. Wada, T. Ozawa, Mitochondrial DNA mutations in mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), Biochem. Biophys. Res. Commun. 174 (1991) 861–868.
[52] A.K. Hyvarinen, J.L. Pohjoismaki, A. Reyes, S. Wanrooij, T. Yasukawa, P.J. Karhunen, J.N. Spelbrink, I.J. Holt, H.T. Jacobs, The mitochondrial transcription termination factor mTERF modulates replication pausing in human mitochondrial DNA, Nucleic Acids Res. 35 (2007) 6458–6474.
[53] C.B. Park, J. Asin-Cayuela, Y. Camara, Y. Shi, M. Pellegrini, M. Gaspari, R. Wibom, K. Hultenby, H. Erdjument-Bromage, P. Tempst, M. Falkenberg, C.M. Gustafsson, N.G. Larsson, MTERF3 is a negative regulator of mammalian mtDNA transcription, Cell 130 (2007) 273–285.
[54] E. Yakubovskaya, E. Mejia, J. Byrnes, E. Hambardjieva, M. Garcia-Diaz, Helix unwinding and base flipping enable human MTERF1 to terminate mitochondrial transcription, Cell 141 (2010) 982–993.
[55] A.P. Rebelo, S.L. Williams, C.T. Moraes, In vivo methylation of mtDNA reveals the dynamics of protein–mtDNA interactions, Nucleic Acids Res. 37 (2009) 6701–6715.
[56] T.W. Christianson, D.A. Clayton, In vitro transcription of human mitochondrial DNA: accurate termination requires a region of DNA sequence that can function bidirectionally, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 6277–6281.
[57] N. Jimenez-Menendez, P. Fernandez-Millan, A. Rubio-Cosials, C. Arnan, J. Montoya, H.T. Jacobs, P. Bernado, M. Coll, I. Uson, M. Sola, Human mitochondrial mTERF wraps around DNA through a left-handed superhelical tandem repeat, Nat. Struct. Mol. Biol. 17 (2010) 891–893.
[58] H. Spahr, T. Samuelsson, B.M. Hallberg, C.M. Gustafsson, Structure of mitochondrial transcription termination factor 3 reveals a novel nucleic acid-binding domain, Biochem. Biophys. Res. Commun. 397 (2010) 386–390.
[59] H. Spahr, B. Habermann, C.M. Gustafsson, N.G. Larsson, B.M. Hallberg, Structure of the human MTERF4–NSUN4 protein complex that regulates mitochondrial ribosome biogenesis, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 15253–15258.
[60] E. Yakubovskaya, K.E. Guja, E. Mejia, S. Castano, E. Hambardjieva, W.S. Choi, M. Garcia-Diaz, Structure of the essential MTERF4:NSUN4 protein complex reveals how an MTERF protein collaborates to facilitate rRNA modification, Structure 20 (2012) 1940–1947.
[61] J. Byrnes, M. Garcia-Diaz, Mitochondrial transcription: how does it end? Transcription 2 (2011) 32–36.
[62] Y. Camara, J. Asin-Cayuela, C.B. Park, M.D. Metodiev, Y. Shi, B. Ruzzenente, C. Kukat, B. Habermann, R. Wibom, K. Hultenby, T. Franz, H. Erdjument-Bromage, P. Tempst, B.M. Hallberg, C.M. Gustafsson, N.G. Larsson, MTERF4 regulates translation by targeting the methyltransferase NSUN4 to the mammalian mitochondrial ribosome, Cell Metab. 13 (2011) 527–539.
[63] A. Wredenberg, M. Lagouge, A. Bratic, M.D. Metodiev, H. Spahr, A. Mourier, C. Freyer, B. Ruzzenente, L. Tain, S. Gronke, F. Baggio, C. Kukat, E. Kremmer, R. Wibom, P.L. Polosa, B. Habermann, L. Partridge, C.B. Park, N.G. Larsson, MTERF3 regulates mitochondrial ribosome biogenesis in invertebrates and mammals, PLoS Genet. 9 (2013) e1003178.
[64] T. Wenz, C. Luca, A. Torraco, C.T. Moraes, mTERF2 regulates oxidative phosphorylation by modulating mtDNA transcription, Cell Metab. 9 (2009) 499–511.
[65] M. Pellegrini, J. Asin-Cayuela, H. Erdjument-Bromage, P. Tempst, N.G. Larsson, C.M. Gustafsson, MTERF2 is a nucleoid component in mammalian mitochondria, Biochim. Biophys. Acta 1787 (2009) 296–302.
[66] R. Reid, P.J. Greene, D.V. Santi, Exposition of a family of RNA m(5)C methyltransferases from searching genomic and proteomic sequences, Nucleic Acids Res. 27 (1999) 3138–3145.
[67] M.D. Metodiev, N. Lesko, C.B. Park, Y. Camara, Y. Shi, R. Wibom, K. Hultenby, C.M. Gustafsson, N.G. Larsson, Methylation of 12S rRNA is necessary for in vivo stability of the small subunit of the mammalian mitochondrial ribosome, Cell Metab. 9 (2009) 386–397.

[68] E. Babiychuk, K. Vandepoele, J. Wissing, M. Garcia-Diaz, R. De Rycke, H. Akbari, J. Joubes, T. Beeckman, L. Jansch, M. Frentzen, M.C. Van Montagu, S. Kushnir, Plastid gene expression and plant development require a plastidic protein of the mitochondrial transcription termination factor family, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 6674–6679.
[69] T. Kleine, *Arabidopsis thaliana* mTERF proteins: evolution and functional classification, Front. Plant Sci. 3 (2012) 233.
[70] Y. Zhao, M. Cai, X. Zhang, Y. Li, J. Zhang, H. Zhao, F. Kong, Y. Zheng, F. Qiu, Genome-wide identification, evolution and expression analysis of mTERF gene family in maize, PLoS ONE 9 (2014) e94126.
[71] L. Wobbe, P.J. Nixon, The mTERF protein MOC1 terminates mitochondrial DNA transcription in the unicellular green alga *Chlamydomonas reinhardtii*, Nucleic Acids Res. 41 (2013) 6553–6567.
[72] K. Hammani, A. Barkan, An mTERF domain protein functions in group II intron splicing in maize chloroplasts, Nucleic Acids Res. 42 (2014) 5033–5042.
[73] M. Kim, U. Lee, I. Small, C.C. des Francs-Small, E. Vierling, Mutations in an Arabidopsis mitochondrial transcription termination factor-related protein enhance thermotolerance in the absence of the major molecular chaperone HSP101, Plant Cell 24 (2012) 3349–3365.
[74] M. Mokry, I.J. Nijman, A. van Dijken, R. Benjamins, R. Heidstra, B. Scheres, E. Cuppen, Identification of factors required for meristem function in Arabidopsis using a novel next generation sequencing fast forward genetics approach, BMC Genomics 12 (2011) 256.
[75] V. Quesada, R. Sarmiento-Manus, R. Gonzalez-Bayon, A. Hricova, R. Perez-Marcos, E. Gracia-Martinez, L. Medina-Ruiz, E. Leyva-Diaz, M.R. Ponce, J.L. Micol, Arabidopsis RUGOSA2 encodes an mTERF family member required for mitochondrion, chloroplast and leaf development, Plant J. Cell Mol. Biol. 68 (2011) 738–753.
[76] P. Robles, J.L. Micol, V. Quesada, Arabidopsis MDA1, a nuclear-encoded protein, functions in chloroplast development and abiotic stress responses, PLoS ONE 7 (2012) e42924.
[77] P. Robles, J.L. Micol, V. Quesada, Mutations in the plant-conserved MTERF9 alter chloroplast gene expression, development and tolerance to abiotic stress in *Arabidopsis thaliana*, Physiol. Plant. (2014 Nov 13). http://dx.doi.org/10.1111/ppl.12307 [Epub ahead of print] PubMed PMID: 25393651.
[78] S. Anderson, A.T. Bankier, B.G. Barrell, M.H. de Bruijn, A.R. Coulson, J. Drouin, I.C. Eperon, D.P. Nierlich, B.A. Roe, F. Sanger, P.H. Schreier, A.J. Smith, R. Staden, I.G. Young, Sequence and organization of the human mitochondrial genome, Nature 290 (1981) 457–465.
[79] Y.W. Hsu, H.J. Wang, M.H. Hsieh, H.L. Hsieh, G.Y. Jauh, Arabidopsis mTERF15 is required for mitochondrial nad2 Intron 3 splicing and functional complex I activity, PLoS ONE 9 (2014) e112360.
[80] D. Grant, K.S. Chiang, Physical mapping and characterization of Chlamydomonas mitochondrial DNA molecules: their unique ends, sequence homogeneity, and conservation, Plasmid 4 (1980) 82–96.
[81] M.W. Gray, P.H. Boer, Organization and expression of algal (*Chlamydomonas reinhardtii*) mitochondrial DNA, Philos. Trans. R. Soc. Lond. B Biol. Sci. 319 (1988) 135–147.
[82] P.H. Boer, M.W. Gray, Scrambled ribosomal RNA gene pieces in *Chlamydomonas reinhardtii* mitochondrial DNA, Cell 55 (1988) 399–411.
[83] R.L. Tracy, D.B. Stern, Mitochondrial transcription initiation: promoter structures and RNA polymerases, Curr. Genet. 28 (1995) 205–216.
[84] F. Duby, P. Cardol, R.F. Matagne, C. Remacle, Structure of the telomeric ends of mt DNA, transcriptional analysis and complex I assembly in the dum24 mitochondrial mutant of *Chlamydomonas reinhardtii*, Mol. Gen. Genomics. MGG 266 (2001) 109–114.
[85] M. Tardif, A. Atteia, M. Specht, G. Cogne, N. Rolland, S. Brugiere, M. Hippler, M. Ferro, C. Bruley, G. Peltier, O. Vallon, L. Cournac, PredAlgo: a new subcellular localization prediction tool dedicated to green algae, Mol. Biol. Evol. 29 (2012) 3625–3639.
[86] P. Pesaresi, M. Pribil, T. Wunder, D. Leister, Dynamics of reversible protein phosphorylation in thylakoids of flowering plants: the roles of STN7, STN8 and TAP38, Biochim. Biophys. Acta 1807 (2011) 887–896.
[87] R. Ryan, D. Grant, K.S. Chiang, H. Swift, Isolation and characterization of mitochondrial DNA from *Chlamydomonas reinhardtii*, Proc. Natl. Acad. Sci. U. S. A. 75 (1978) 3268–3272.
[88] M. Unseld, J.R. Marienfeld, P. Brandt, A. Brennicke, The mitochondrial genome of *Arabidopsis thaliana* contains 57 genes in 366,924 nucleotides, Nat. Genet. 15 (1997) 57–61.
[89] J. Forner, B. Weber, S. Thuss, S. Wildum, S. Binder, Mapping of mitochondrial mRNA termini in *Arabidopsis thaliana*: t-elements contribute to 5′ and 3′ end formation, Nucleic Acids Res. 35 (2007) 3676–3692.
[90] S. Holec, H. Lange, J. Canaday, D. Gagliardi, Coping with cryptic and defective transcripts in plant mitochondria, Biochim. Biophys. Acta 1779 (2008) 566–573.
[91] B.F. Lang, M.W. Gray, G. Burger, Mitochondrial genome evolution and the origin of eukaryotes, Annu. Rev. Genet. 33 (1999) 351–397.
[92] B.R. Green, Chloroplast genomes of photosynthetic eukaryotes, Plant J. Cell Mol. Biol. 66 (2011) 34–44.
[93] M. Sugiura, The chloroplast genome, Plant Mol. Biol. 19 (1992) 149–168.
[94] M. Malik Ghulam, F. Courtois, S. Lerbs-Mache, L. Merendino, Complex processing patterns of mRNAs of the large ATP synthase operon in Arabidopsis chloroplasts, PLoS ONE 8 (2013) e78265.
[95] S. Proshkin, A. Mironov, E. Nudler, Riboswitches in regulation of Rho-dependent transcription termination, Biochim. Biophys. Acta 1839 (2014) 974–977.
[96] H. Ruwe, C. Schmitz-Linneweber, Short non-coding RNA fragments accumulating in chloroplasts: footprints of RNA binding proteins? Nucleic Acids Res. 40 (2012) 3106–3116.
[97] P. Zhelyazkova, K. Hammani, M. Rojas, R. Voelker, M. Vargas-Suarez, T. Borner, A. Barkan, Protein-mediated protection as the predominant mechanism for defining processed mRNA termini in land plant chloroplasts, Nucleic Acids Res. 40 (2012) 3092–3105.
[98] R. Meskauskiene, M. Nater, D. Goslings, F. Kessler, R. op den Camp, K. Apel, FLU: a negative regulator of chlorophyll biosynthesis in *Arabidopsis thaliana*, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 12826–12831.
[99] R.G. op den Camp, D. Przybyla, C. Ochsenbein, C. Laloi, C. Kim, A. Danon, D. Wagner, E. Hideg, C. Gobel, I. Feussner, M. Nater, K. Apel, Rapid induction of distinct stress responses after the release of singlet oxygen in Arabidopsis, Plant Cell 15 (2003) 2320–2332.
[100] T. Kleine, C. Voigt, D. Leister, Plastid signalling to the nucleus: messengers still lost in the mists? Trends Genet. TIG 25 (2009) 185–192.
[101] T. Pfannschmidt, Plastidial retrograde signalling—a true "plastid factor" or just metabolite signatures? Trends Plant Sci. 15 (2010) 427–435.
[102] N.S. Coll, A. Danon, J. Meurer, W.K. Cho, K. Apel, Characterization of soldat8, a suppressor of singlet oxygen-induced cell death in Arabidopsis seedlings, Plant Cell Physiol. 50 (2009) 707–718.
[103] G. Saini, R. Meskauskiene, W. Pijacka, P. Roszak, L.L. Sjogren, A.K. Clarke, M. Straus, K. Apel, 'happy on norflurazon' (hon) mutations implicate perturbance of plastid homeostasis with activating stress acclimatization and changing nuclear gene expression in norflurazon-treated seedlings, Plant J. Cell Mol. Biol. 65 (2011) 690–702.
[104] H.D. Yu, X.F. Yang, S.T. Chen, Y.T. Wang, J.K. Li, Q. Shen, X.L. Liu, F.Q. Guo, Downregulation of chloroplast RPS1 negatively modulates nuclear heat-responsive expression of HsfA2 and its target genes in Arabidopsis, PLoS Genet. 8 (2012) e1002669.
[105] D. Leister, I. Romani, L. Mittermayr, F. Paieri, E. Fenino, T. Kleine, Identification of target genes and transcription factors implicated in translation-dependent retrograde signaling in Arabidopsis, Mol. Plant 7 (2014) 1228–1247.
[106] G. Berna, P. Robles, J.L. Micol, A mutational analysis of leaf morphogenesis in *Arabidopsis thaliana*, Genetics 152 (1999) 729–742.
[107] R. Zoschke, M. Nakamura, K. Liere, M. Sugiura, T. Borner, C. Schmitz-Linneweber, An organellar maturase associates with multiple group II introns, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 3245–3250.
[108] J. Vogel, T. Borner, W.R. Hess, Comparative analysis of splicing of the complete set of chloroplast group II introns in three higher plant mutants, Nucleic Acids Res. 27 (1999) 3866–3874.
[109] J.M. Perez-Perez, H. Candela, J.L. Micol, Understanding synergy in genetic interactions, Trends Genet. TIG 25 (2009) 368–376.
[110] R. Martienssen, V. Irish, Copying out our ABCs: the role of gene redundancy in interpreting genetic hierarchies, Trends Genet. TIG 15 (1999) 435–437.
[111] I. Tzafrir, R. Pena-Muralla, A. Dickerman, M. Berg, R. Rogers, S. Hutchens, T.C. Sweeney, J. McElver, G. Aux, D. Patton, D. Meinke, Identification of genes required for embryo development in Arabidopsis, Plant Physiol. 135 (2004) 1206–1220.
[112] W. Majeran, G. Friso, Y. Asakura, X. Qu, M. Huang, L. Ponnala, K.P. Watkins, A. Barkan, K.J. van Wijk, Nucleoid-enriched proteomes in developing plastids and chloroplasts from maize leaves: a new conceptual framework for nucleoid functions, Plant Physiol. 158 (2012) 156–189.
[113] J. Pfalz, K. Liere, A. Kandlbinder, K.J. Dietz, R. Oelmuller, pTAC2, -6, and -12 are components of the transcriptionally active plastid chromosome that are required for plastid gene expression, Plant Cell 18 (2006) 176–197.