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Review

Regulated chloroplast transcription termination

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ABSTRACT

Transcription termination by the RNA polymerase (RNAP) is a fundamental step of gene expression that involves the release of the nascent transcript and dissociation of the RNAP from the DNA template. However, the functional importance of termination extends beyond the mere definition of the gene borders. Chloroplasts originate from cyanobacteria and possess their own gene expression system. Plastids have a unique hybrid transcription system consisting of two different types of RNAPs of dissimilar phylogenetic origin together with several additional nuclear encoded components. Although the basic components involved in chloroplast transcription have been identified, little attention has been paid to the chloroplast transcription termination. Recent identification and functional characterization of novel factors in regulating transcription termination in *Arabidopsis* chloroplasts *via* genetic and biochemical approaches have provided insights into the mechanisms and significance of transcription termination in chloroplast gene expression. This review provides an overview of the current knowledge of the transcription termination in chloroplasts.

1. Introduction

Viridiplantae possess two endosymbiotic organelles, chloroplasts and mitochondria, in contrast to metazoa and fungi harboring only the latter. These organelles originated from cyanobacteria and alpha-proteobacteria, respectively [1,2]. Although both organelles contain their own genomes, most endosymbiotic genes have been transferred to the nucleus or got lost over the course of genome-organelle co-evolution [3,4]. This has resulted in highly reduced organellar genomes that retained only a small number of the original genes. Most of the organellar proteins are encoded by nuclear genomes, synthesized in the cytosol and subsequently imported into the organelles. Thus, the proper expression of genes requires the tight coordination between the organellar and nuclear genomes [5–7].

Chloroplast gene expression is rather complex. It combines both eubacterial and eukaryotic features derived from the cyanobacteria ancestor or the host cell [6]. However, many features especially of the organellar gene expression system also evolved *de novo* [8]. One example is the chloroplast transcription machinery. The transcription of chloroplast genes depends on two RNA polymerases (RNAPs): a phage-type nucleus-encoded plastid RNA polymerase (NEP) and a prokaryotic type, plastid-encoded RNA polymerase (PEP) [9]. The PEP complex comprises four core subunits α, β, β′, and β″, which display high similarities to counterparts in cyanobacteria. Beside the “eubacterial” subunits, a number of additional nucleus-encoded proteins of eukaryotic origin involved in the chloroplast transcription have been identified in the PEP complex, the plastid TRANSCRIPTIONALLY ACTIVE CHROMOSOME (TAC) or the chloroplast nucleoid *via* proteomic approaches [10–15]. The genetic data have shown that many of those play important roles in the accumulation of PEP-depended mRNAs [15–17] and tRNAs [18]. It is assumed that they might provide additional regulatory functions that adapt chloroplast transcription in response to environmental signals and developmental cues, however, the molecular mechanisms still remain largely unknown.

Termination is the last important step of transcriptional processes [19]. The role of transcription termination is not restricted to the release of the RNAP from the DNA template. The growing evidence indicates that it is also important to avoid interference with expression of downstream genes, to prevent formation of antisense RNAs and to ensure a pool of RNAPs available for reinitiation or new transcription

Abbreviations: RNAP, RNA polymerase; NEP, nucleus-encoded plastid RNA polymerase; PEP, plastid-encoded RNA polymerase; *rut*, Rho using termination; RNE, RNase E

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[20–22]. Termination mechanisms vary considerably in different organisms, ranging from relatively simple to exceptionally complex processes. In bacteria such as *Escherichia coli*, there are at least two conserved mechanisms of transcription termination: Rho-dependent termination and intrinsic termination (dissociation of the transcription complexes is accomplished without the assistance of Rho factors) [23–25]. In yeast and mammal cells, three different strategies for transcription termination have been developed depending on distinct RNAPs [26]: termination by RNAP II is coupled with the processing of the pre-mRNA [27–29], RNAP III termination is an autonomous process occurring at T-rich sequences located at the 3′-end of the genes [30,31], and RNAP I relies on a specific terminator composed of an oligo-dT stretch and associated factors [32,33].

Intriguing issues regarding the chloroplast transcription system are the mechanisms and significance of transcription termination. Due to the cyanobacterial origin, one might expect that the termination machinery in chloroplasts is similar to that of bacteria. However, no homologs of known bacterial proteins involved in transcription termination have been identified in chloroplasts so far. In addition, it is generally accepted that it is the RNA maturation rather than the transcription termination that creates defined 3′ termini [34,35]. Thus, the degree to which the transcription termination affects chloroplast gene expression remains an open question. For a long time, the termination of transcription in chloroplasts has received relatively little attention until two publications appeared that provided insights into the regulation of chloroplast transcription termination in *Arabidopsis* [36,37]. In this short review, we will summarize the progress that has been made in terms of the mechanism of chloroplast transcription termination, focusing especially on the functions of RHON1 and mTERF6, and provide perspectives in this area. Although some knowledge of chloroplast transcription termination arises from that of bacteria, mechanisms of transcription termination in bacteria will not be presented in details due to the space limitation. Those, who wish to obtain further details about transcription termination in bacteria, are referred to the review articles of Peters et al. [25] and Mitra et al. [38].

2. Transcription termination in chloroplasts

2.1. Does the 3′ stem-loop structure present in some chloroplast genes function as transcription terminator?

The intrinsic transcription termination in bacteria depends on a hairpin structure in the nascent RNA which leads to the release of the transcript and the RNAP from the DNA template without the assistance of additional factors [25,39]. DNA sequence analyses have revealed that inverted repeat sequences are present in the 3′ end of many chloroplast genes, including *rbcL*, *petD*, *psbA*, *psbC*, and *rpoA* [40,41]. The inverted repeat sequences can fold into stem-loop structures similar to the intrinsic terminators in *E. coli*. Thus, it seems reasonable to speculate that the inverted repeat sequences in plastid DNA might function as intrinsic transcription terminators for chloroplast RNAP [42].

Whether the inverted repeat sequences of 3′ ends of chloroplast genes can actually act as transcription terminators was firstly addressed by Stern and Gruissem [43] by means of a homologous *in vitro* transcription system from spinaches. They found that of inverted repeat sequences in the 3′ end of *psbA*, *rbcL*, *petD*, and *rpoA*, only the *petD* inverted repeat was partially effective as a transcription terminator in this *in vitro* assay. Based on this study, it was proposed that the role of chloroplast inverted repeats is not to terminate transcription but to stabilize transcripts by either preventing 3′-5′ exonucleolytic activity and/or by serving as a platform for RNA-binding proteins that protect the transcripts from degradation [44].

This notion was further supported by *in vivo* evidence from *Chlamydomonas reinhardtii* chloroplasts [45–47]. It was found that less than 50% of 3′ ends of *atpB* transcripts in *Chlamydomonas* chloroplasts resulted from transcription termination. The other half of the *atpB* 3′ termini were produced by posttranscriptional processing events: in the first step the long *atpB* precursor transcript is endonucleolytically cleaved and then further processed by 3′-5′ exonucleolytic digestion to produce the mature transcript [45,46]. In addition, when the native 3′ end of *petD* was replaced by a variety of 3′ ends from other chloroplast genes in *Chlamydomonas*, none of the 3′ ends in either sense or antisense orientation prevented read-through, indicating that the 3′ end sequences of chloroplast genes are not efficient transcription terminators [47]. Therefore, it has been proposed that most of the RNA stem loop structures formed at the 3′ ends of mature RNAs in *Chlamydomonas* chloroplasts might not be related to transcription termination.

Interestingly, it was found that two tRNA genes, *trnH1* and *trnS*, can efficiently terminate transcription in a spinach *in vitro* transcription system [43,48]. Furthermore, tRNA genes are often found downstream of several transcription units in the chloroplast genome of plants (*e.g.*, *atpBE*, *rrnV1*; *psaAB*; *tmfM*; *atpHFA*, *tmS3*; *psbA*, *tmH1*) [49]. Thus, it seems as if different vascular plant chloroplast tRNA genes might have a potential role in both termination and punctuation [50], which was originally established in vertebrate mitochondrial. The vertebrates mitochondrial genome is transcribed symmetrically as polycistronic precursors spanning the entire heavy and light strands [51], however, the 22 tRNAs interspersed throughout the mitochondrial genome serve as punctuation marks that are recognized and cleaved at 5′ and 3′ ends by the mitochondrial nucleases to be process later on into mature species [50,52]. However, this concept does not hold true for *Chlamydomonas* chloroplasts [45,46].

Despite these considerations, it is generally thought that the 3′ inverted repeat sequences of chloroplast genes do not represent efficient termination signals [45–47]. However, their role in termination activity could not be ruled out completely based on current evidences in spinach or *Chlamydomonas*, showing that for some genes (*e.g. petD* and *atpB*) the inverted repeats can still terminate transcription with a considerable efficiency [43,46]. In addition, chloroplast RNAPs can efficiently recognize bacterial threonine, histidine, and T7 early Rho-independent terminators [53–56], suggesting that a specific unknown RNA structure and/or sequence that might not be related to inverted repeats can indeed terminate the transcription of chloroplast RNAPs. The termination activity at 3′ ends of *petD* and *atpB* might result from this unknown RNA structure and/or sequence. In addition, termination of transcription by spinach chloroplast RNAP was also modulated by downstream DNA sequences in a sequence-specific manner [57]. In this context, the structure/sequence-dependent termination still holds true in chloroplasts of vascular plants but might be more complicated than expected.

A canonical intrinsic terminator of *Escherichia coli* is an RNA signal composed of a GC-rich RNA hairpin followed by a run of U residues. Termination occurs in two steps: RNAP pausing within the U track, followed by RNA release [39]. However, most of the hairpin structures formed at the 3′ UTRs of chloroplast mRNAs do not fit with this feature. In fact, the role of the termination hairpins appears to be indirect as it can be replaced by oligonucleotides that pair to the nascent RNA to mimic the hairpins [58,59], suggesting the structural flexibility of 3′ UTR is involved in transcription termination. The intrinsic terminator signals can only be frequently identified in *E. coli* but many other bacterial and archaeal genomes lack such intrinsic terminator signals [60], implying the existence of a different type of signal or dependence on a termination factor in these species. These findings further support the assumption that structure/sequence correlations not related to hairpin structures accomplish chloroplast transcription termination.

It has been suggested that PEP predominantly mediates the transcription of photosynthesis-related genes while NEP rather mediates the transcription of the house keeping genes [61–64]. Nevertheless, this division of labor between PEP and NEP is also a challenge because NEP is in fact able to transcribe the complete plastid genome [65]. In addition, most plastid genes (including genes coding for photosynthesis

proteins) have both PEP and NEP promoters [63,66–69]. So far, almost all of the studies for the chloroplast transcription termination have focused on PEP-dependent genes whereas mechanisms of NEP-dependent genes remain elusive. The NEP is similar to single-subunit phage RNAPs which are found to terminate at both class I and class II termination signals. The former is similar to intrinsic termination signals of bacterial RNAP, however, the latter consists of a conserved sequence, HATCTGTT (H designating A, C, or T) [70–72]. No class II-like termination signal has been identified in chloroplast transcripts yet and it is still unknown whether the NEP relies on the class I or class II termination signals. If it is the latter, the NEP and PEP seem to employ distinct intrinsic mechanisms to terminate transcription in chloroplasts. Thus, it is of interest to address whether the distinct termination mechanisms of NEP and PEP relate to the division of labor between PEP and NEP if this division really exists in *planta* [67–69].

2.2. Is there a Rho-dependent termination in chloroplasts?

The Rho-dependent termination is the major way to terminate the transcription in eubacteria and the transcription termination of a large fraction of genes relies on this mechanism in *E. coli* (reviewed by [73], the process is depicted in Fig. 1). The Rho factor is a ring-shaped, homohexameric protein that utilizes its RNA-dependent ATPase activity to translocate along the mRNA and to eventually dislodge the RNAP [23,38]. In *E. coli*, Rho is a 419-amino acid protein containing several domains. The N-terminal domain of Rho contains the primary RNA-binding site. Its C-terminal domain (CTD) contains an ATP-binding and hydrolysis signature motif, called the P-loop, which shows significant sequence homology to F_1 ATPases [74–76].

No homologs of the Rho factor are found in chloroplasts of vascular plants or *Chlamydomonas*, suggesting that the Rho-dependent termination might not exist in chloroplasts. However, the BLAST analysis of the *Arabidopsis* genome showed that several chloroplast proteins contain RNA-binding domains, which are similar to primary RNA-binding domains located at the N-terminus of Rho factors (Table 1). These proteins were referred to as RHON proteins. Besides its role in supporting RNase E (RNE) activity [77], the study of Chi et al. [36] showed that the chloroplast RHON1 protein is also involved in the transcription termination of *rbcL*.

RHON1 was originally identified in a screen for interaction partners of the endonuclease RNE. It was shown that RHON1 is involved in RNE-mediated plastid RNA processing by conferring sequence specificity to the RNE through its RNA-binding activity [77]. Similar to *rne* mutants, several plastid RNA precursors accumulated in *rhon1* mutant, one of which is a large RNA precursor spanning the *rbcL*, *accD*, *psaI*, *ycf4*, *cemA*, and *petA* genes [77]. A PEP promoter (PrbcL-179) is responsible for the generation of monocistronic *rbcL* [78] whereas two NEP promoters (PaccD-172, 252) have been found to drive transcription of the *Arabidopsis accD* gene [79]. In this context, the transcription of *rbcL* and *accD* seem to be independent. However, a large polycistronic precursor transcript of *accD-psaI-ycf4-cemA-petA* was also reported [80]. The RNA gel blot analyses showed that levels of both the precursor and mature forms of *accD*, *psaI*, *ycf4*, *cemA*, and *petA* mRNAs were increased, whereas levels of monocistronic *rbcL* remained unchanged in *rhon1*. If RHON1 is involved in the cleavage of this precursor, the transcription rates of *accD*, *psaI*, *ycf4*, *cemA*, and *petA* should not be changed. However, a run-on assay showed that the transcription rates of *accD*, *psaI*, *ycf4*, *cemA*, and *petA* were increased in *rhon1* plants but the transcription rates of *rbcL* were not affected. Considering this, the increase of *accD*, *psaI*, *ycf4*, *cemA*, and *petA* transcription rate might result from the reading-through of *rbcL* [36]. Therefore, this genetic evidence pointed to the possibility that RHON1 is involved in *rbcL* transcription termination rather than in the post-transcriptional processing of the *rbcL-accD-psaI-ycf4-cemA-petA* precursor [36].

Further biochemical analyses showed that RHON1 might be able to terminate *rbcL* transcription similar to Rho factors. Firstly, RHON1 can

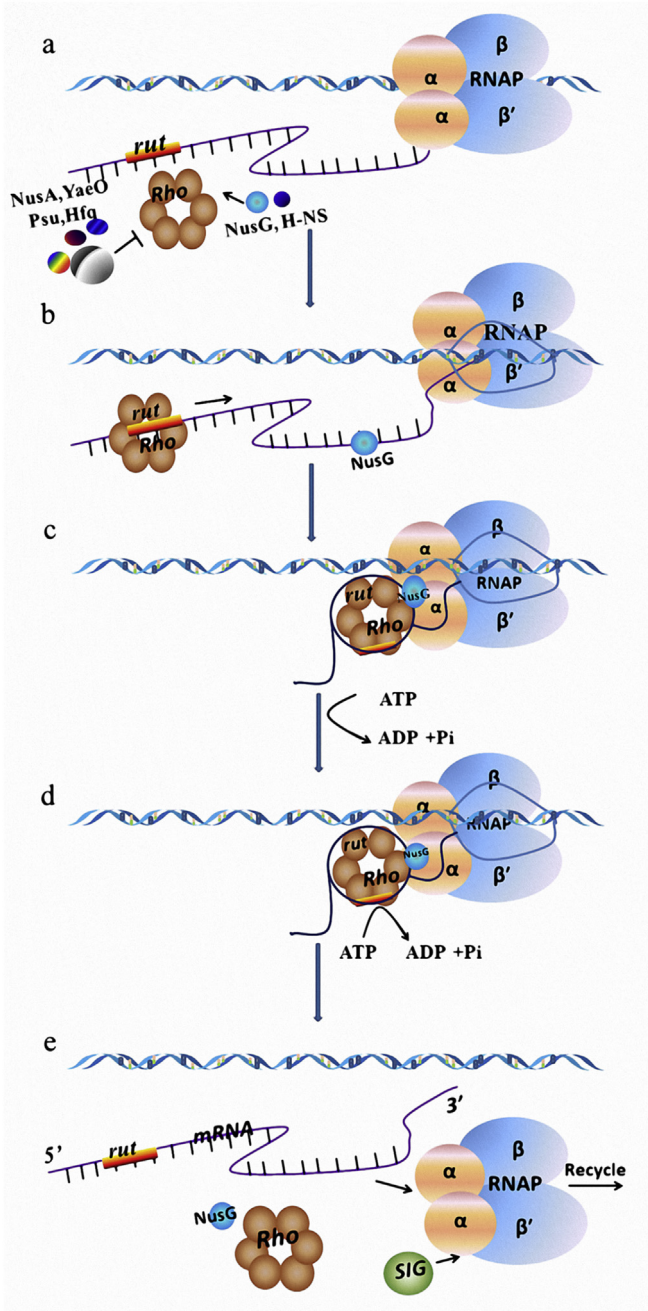

Fig. 1. Model of Rho-dependent termination.
(a) Hexameric Rho loaded onto the RNA transcript at *rut* sites at the time of termination. Cofactors of Rho (NusG, NusA, H-NS, YaeO, Psu, Hfq) are indicated; (b) Rho catches up with the RNAP; (c) *rut* RNA remains bound to the Rho-NTD during translocation, forming a loop between the primary and secondary RNA-binding sites; (d) Rho contacts the elongation complex (EC). The presence of NusG and ATP accelerates the conformational change of RNAP; (e) dissociation of the EC.

specifically bind to the mRNA as well as to single-stranded DNA of the *rbcL* 3′ UTR. Secondly, RHON1 displays ATPase activity depending on its RNA-binding ability. These two features are similar to those of bacterial Rho factors. More direct evidence came from an *in vitro* transcription termination assay displaying that RHON1 could actually terminate transcription of *rbcL* depending on ATPase activity [36]. Thus, RHON1 seems to terminate *rbcL* transcription in a similar way to that of the Rho factor in *Escherichia coli*. However, the mechanism of termination between chloroplast RHON1 and eubacterial Rho factors

Table 1
Homologs of the Rho protein and cofactors in *Arabidopsis*.

Group	Name	Gene	Location	Function	References
Group I: The Rho Cluster	RHON1	AT1G06190	**C**	Rho termination factor	[36]
	–	AT4G18740	**M/C/Y**	Rho termination factor	–
	–	AT2G41550	**N/O**	Rho termination factor	–
Group II: The NusG Cluster	PTAC13	AT3G09210	**C/M**	Plastid transcriptionally active 13	[11]
	Spt5	AT2G34210	**N/O**	Putative transcription elongation factor SPT5	[92]
	KTF1	AT5G04290	**N**	SPT5-LIKE, transcription regulation	[93,94]
	GTA2	AT4G08350	**N**	SPT5-LIKE, transcription regulation	[92]
Group III: The NusA Cluster	RAD51	AT5G20850	**N/M**	DNA repair (Rad51) family protein; unwinds duplex DNA by DNA-dependent ATPase activity	[95,96]
	DMC1	AT3G22880	**N**	DNA repair/DNA metabolic process, DNA-dependent ATPase activity.	[97–99]

Homologs of the Rho protein and Rho cofactors identified in the *Arabidopsis* genome. The subcellular localization was predicted by TargetP. Bold lettering indicates the cellular compartment to which the gene products are targeted. C, chloroplast; M, mitochondria; N, nucleus; O, others; Y, cytosol.

might be different. The mRNA region bound by RHON1 (between 88 and 133 nucleotides downstream of the *rbcL* termination codon) does not show any similarity to *rut* (Rho using termination) signal sequences which is recognized by Rho factors [38]. The RHON1-binding sequence contains only few C residues compared to that of Rho. In addition, the ATPase domain of RHON1 belongs to P-type ATPases rather than to the F-type in Rho. Therefore, it stands to reason that the mechanism of action of RHON1 is not identical to that of Rho factors.

The Rho-dependent mechanism seems not to be restricted to eubacteria and chloroplasts of vascular plants, as similar ATP-driven termination machineries have also been discovered in eukaryotic cells [81]. For instance, Sen1p is the key enzyme of the termination reaction in yeast cells. Like the bacterial termination factor Rho, Sen1p recognizes the nascent RNA and hydrolyzes ATP to dissociate the elongation complex [82,83].

The function of Rho requires several cofactors in bacterial systems, including NusG [84,85], NusA [86,87], H-NS [88], YaeO [89], Psu [90], and the RNA chaperone Hfq [91]. Interestingly, homologs of NusG and NusA are also found in vascular plants (Table 1) [92–99]. There are five NusG homologs in *Arabidopsis*. One is called pTAC13 [11]. The function of pTAC13 still remains unknown and it is of interest to address the question whether its function is related to chloroplast transcription termination. In *E. coli*, Rho acts on a naked nascent transcript that is not engaged in translation or bound to RNA-binding proteins. However, RHON1 exists as a large RNA-protein complex *in vivo* and directly binds to RNE [77]. This difference between RHON1 and Rho factors suggests a possible link between chloroplast transcription termination and RNA processing.

Termination of *rbcL* transcription was originally inferred from the transformation of the plastid genome of *Nicotiana tabacum* [100–102]. When plastid vectors were targeted to the *rbcL-accD* intergenic region of the tobacco plastid genome [103], *rbcL* read-through transcripts were detectable [101–104]. However, when the insertion site was moved 170 nucleotides further downstream of *rbcL*, the *rbcL* read-through was eliminated [103]. Therefore, the presence of a termination or processing site in the intergenic region of *rbcL* and *accD* has been proposed. However, whether a termination or processing site has been disrupted in the resulted tobacco transformants has not been addressed further. In the context of RHON1 function, it is very likely that there is a termination site between *rbcL* and *accD*. However, this does not exclude the presence of a possible processing site as well.

2.3. Significance of RHON1-dependent transcription termination

The antisense RNAs play important roles in posttranscriptional regulation and numbers of antisense RNAs have been detected in chloroplasts [66,105–109]. Some antisense RNAs of chloroplasts might inhibit the translation of sense RNAs encoded on the opposite strand [108,109] or protect unstable transcripts from 3′ → 5′ exonuclease activity by the formation of double-stranded RNA/RNA hybrids [105,107], suggesting the regulatory function of antisense RNAs in chloroplast gene expression. Given that one consequence of inefficient transcriptional termination is the production of antisense RNAs [110], one might argue that transcriptional termination affects chloroplast gene output by modulating the generation of antisense RNAs. However, this possibility has not been addressed experimentally yet. The study of Chi et al. [36] showed that the inefficient termination of *rbcL* actually affects the expression of the downstream *accD* gene presumably by a transcription interference mechanism [36].

Transcriptional interference is defined as the suppressive influence of one transcriptional process on a second downstream located transcriptional process due to the read-through [111]. Transcriptional interference can occur between convergent (face-to-face), tandem (co-directional), or overlapping arrangements of promoters, where the association and elongation of RNAPs from one promoter disrupts RNAPs and/or transcription factors at a second promoter [112–115]. It is potentially widespread in organisms and has been addressed in yeast and bacterial cells [114,115]. However, whether or not it also exists in chloroplasts still remains an open question.

In the *rhon1* mutant, an intriguing finding is the fact that the transcription initiation of *accD* is altered significantly (Fig. 2). In WT plants, there are two NEP-dependent transcription start sites for *accD*: P*accD*-252 and P*accD*-172 [79]. However, in *rhon1* multiple transcription initiation sites of *accD* scattered all over the *rbcL-accD* intergenic region were identified. Some transcription initiation sites were even mapped within the 5′-end of the *accD* coding region. In addition, these transcript initiation sites disappeared when *rhon1* seedlings were treated with spectinomycin, suggesting that they depend on upstream *rbcL* transcription [36]. It is likely that this is a consequence of inefficient transcription termination of *rbcL*. Thus, one of the functions of RHON1 in efficient termination of transcription may be to avoid aberrant transcription initiation in the *rbcL-accD* intergenic region.

If the aberrant transcription initiation in the *rbcL-accD* intergenic region can be regarded as the consequence of transcription interference and how this transcription interference occurs is still an open question. A previous study has shown that chloroplast transcription initiation sites in vascular plants are not very stringent [79,116]. The elongating RNAP complex across the termination site of *rbcL* might interfere with the transcription initiation complex of the *accD* transcriptional unit and reduce the transcription accuracy. Alternatively, PEP might not be released from the template due to the lack of RHON1; it might slide on the template and finally reinitiate transcription nonspecifically at different positions in the *rbcL-accD* intergenic region. Nevertheless, as mentioned above, *accD* is exclusively transcribed by NEP while *rbcL* is mainly transcribed by PEP. These two distinct RNAPs recognize different promoters *via* different mechanisms but the competition between both enzymes has not been described yet. In addition, unspecific initiation by PEP can occur only at a nick in the DNA template, one of the reasons why sonicated DNA is used for unspecific transcription activity assays of PEP preparations [117]. It is unlikely that such DNA template defects

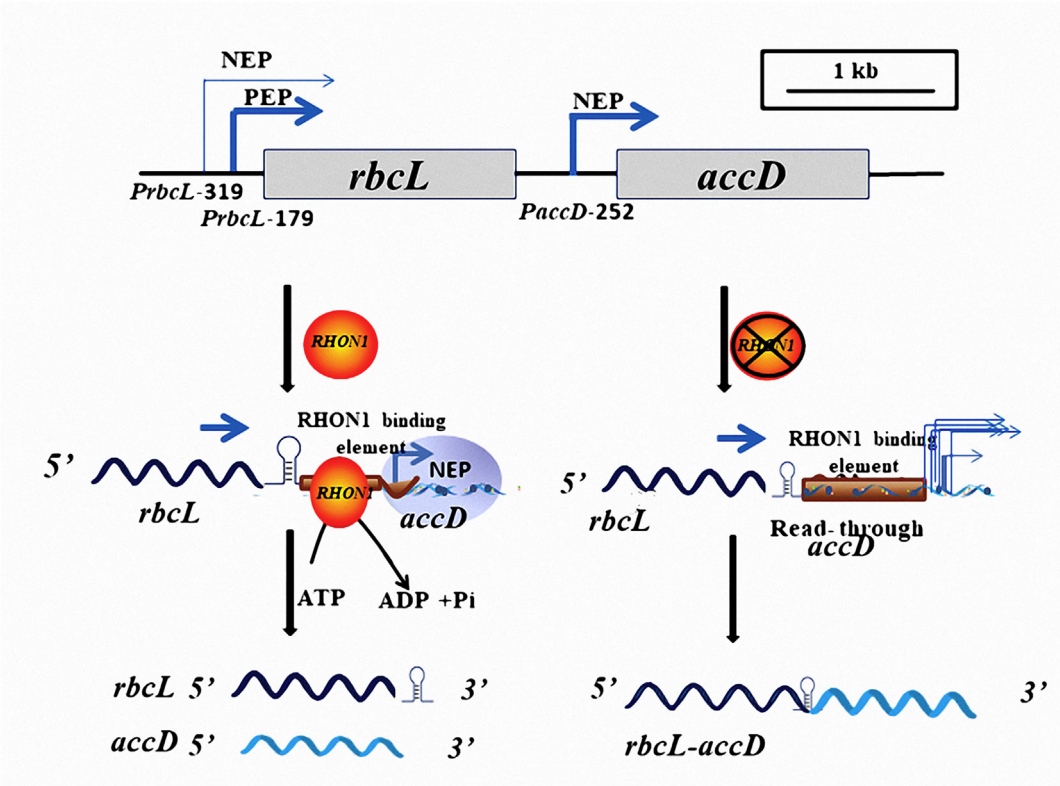

Fig. 2. Model of RHON1 action in *rbcL* transcription termination.
Left: RHON1 binds to the elements adjacent to the stem-loop structure of the *rbcL* 3′ UTR *via* its RNA binding domain (RD). After binding to its target, RHON1 catalyzes the dissociation of *rbcL* mRNA from genomic DNA and RNAP *via* its ATPase domain (AD).
Right: When RHON1 is absent, read-through products of *rbcL* are produced. In addition, aberrant transcription initiation of *accD* occurs, which might result from the transcription interference in the *rbcL-accD* intergenic region.

occur in *rhon1* mutant. Considering these conflicts, it is an enormous challenge to dissect the mechanism of the transcription interference between NEP and PEP in this transcription unit, if it really exists. In yeast cells, the transcription interference usually reduces the promoter activity of downstream genes [114] and such aberrant transcription initiation has never been reported for other chloroplast transcription units but might be worth studying in the future.

The interplay between *rbcL* and *accD* expression mediated by RHON1 also contributes to the developmental profile of *accD* mRNA accumulation. The expression of plastid genes generally varies in response to developmental signals depending on the RNAP and promoter usage [70,118–121]. As preferential NEP-dependent gene, *accD* transcript accumulation is subjected to a dynamic pattern during chloroplast development [67,122]. However, the inefficient transcription termination of *rbcL* impaired the expression profile of *accD*, resulting in the constitutive transcription of *accD* during chloroplast development [123]. In addition, the accumulation of *accD* transcripts accordingly resulted in an increase in AccD protein and fatty-acid contents, suggesting that inefficient *rbcL* transcription termination might lead to the physiological changes in the chloroplast [123]. The elaioplasts have been reported to possess crucial biosynthetic functions, one of which is the biosynthesis of long-chain fatty-acid in developing seeds [124]. Thus it is of interest to study the effects of such interplay between *rbcL* and *accD* expression on the elaioplast biogenesis and function.

2.4. The possible role of mTERF in chloroplast transcription termination

The mTERF protein family is proposed to be the result of the expansion and functional diversification of existing gene families. Proteins of this family contain variable repeats of a 30-amino-acid motif, the so-called MTERF motif [116,125–127]. Vertebrates have four MTERF proteins: MTERF1-MTERF4. Human mTERF1 was the first identified mTERF factor that promotes transcription termination in mitochondria and thus the whole family was named accordingly [128–130]. MTERF1 is a DNA-binding protein of 39 kDa that interacts with a 28 bp region within the $tRNA^{Leu}$ gene, located immediately downstream of the rRNA genes in the mitochondrial genome [131]. The association of MTERF1 with its target site has been reconstituted in a pure recombinant *in vitro* system [132], suggesting that MTERF1 might function in H-strand transcription. However, further studies showed that MTERF1 only partially terminates H-strand transcription, whereas transcription in the opposite direction (L-strand transcription) is almost completely blocked [133]. The *in vivo* study in *mTERF1* knockout mice showed that the main function of MTERF1 is indeed to prevent L-strand transcripts from proceeding around the mtDNA circle and causing transcription interference at the L-strand promoter from which they originated [133]. Nevertheless, the human MTERF1 protein might possess multiple functions. It is also supposed to function as an activator of mitochondrial rRNA transcription and modulator of mtDNA replication [134,135].

Plant genomes harbor a considerably larger number of MTERFs than animal genomes. *Arabidopsis* and rice contain at least 35 and 48 MTERF proteins, respectively [126,136], and even more have been proposed for other plant species. Nonetheless, only nine MTERFs have been characterized in land plants and *Chlamydomonas*. Among them, MOC1 [137], SHOT1 [138] and MTERF15 [139] are located in mitochondria; BSM/RUG2 [140,141] and MTERF6 [37] are located in both chloroplast and mitochondria, while MDA1, MTERF9 [142,143], SOLDT10 [144], and zmMTERF4 [145] are exclusively located in chloroplast. The wide distribution of MTERF proteins in organelles along with their proposed RNA/DNA-binding properties suggests that they might have a role in organellar gene expression. Actually, at least three members of

plant MTERFs (BSM/RUG2, MTERF15, zmMTERF4) are found to function in organellar intron splicing [139–141,145].

MOC1 is the first characterized mTERF protein that terminates mitochondrial DNA transcription in *Chlamydomonas* [137]. MOC1 binds specifically to the mitochondrial rRNA-coding module S3. However, levels of rRNA-coding modules were only mildly affected, indicating that the read-through does not occur at the S3 binding site in *moc1* mutants. Instead, as in *mterf1* knock-out mice, the level of certain antisense RNA species was increased in *moc1* mutant, suggesting that MOC1 acts as a transcription terminator of antisense RNA. The mTERF-mediated transcription termination seems to be an evolutionary-conserved mechanism occurring in phototrophic protists and metazoa.

The study of Romani et al. [37] raises the possible function of *Arabidopsis* MTERF6 (AtMTERF6) in the transcription termination of chloroplast $tRNA^{Ile}$. AtMTERF6 is localized in both chloroplast and mitochondria [136]. In the leaky *mterf6-1* mutant, the maturation of chloroplast ribosomal RNAs (rRNAs) is perturbed in *mterf6-1* mutants. *In vitro* and *in vivo* analyses proved that mTERF6 can bind to the chloroplast *trnI.2* [$tRNA^{Ile}$(GAU)] located downstream of the *rrn16* in the *rrn* gene cluster. This binding site of MTERF6 is similar to that of mTERF1 in their relative positions in the organelle genomes [133]. Recombinant AtMTERF6 can bind to its chloroplast DNA target site and terminates transcription *in vitro*. In addition, an intact mTERF6 target site is necessary for correct transcription termination and mutations in this target site resulted in abnormal transcription termination. Considering the sequence similarity between human MTERF1 and AtMTERF6, it seems that mTERF6 protein is able to promote transcription termination *in vitro* [37]. In *atmterf6* mutants, the levels of downstream *trnA.1* transcripts were increased; however, whether this resulted from the read-through of *trnI.2* and the impact of AtMTERF6 on the transcription on the opposite strand of chloroplast DNA have not been investigated yet. Whether AtMTERF6 functions in the transcription termination of antisense RNA like MOC1 still awaits further investigations.

3. Conclusion and perspective

In theory, the proper transcription termination is necessary and important for the production of functional RNA species. However, it has received little attention to date and most aspects of chloroplast transcription termination still remain elusive. Because of the intensive post-transcriptional processing steps, for some RNA species, it is difficult to discriminate whether the native 3′ terminus of chloroplast RNAs resulted from transcription termination or post-transcriptional processing because both transcription termination and processing results in an 3′-OH. This is quite different from the 5′ end of chloroplast mRNA: the transcription initiation and 5′ processing sites can be distinguished easily [66,146,147]. In addition, the transcription termination and RNA processing steps might be coupled in chloroplasts as it is the case in yeast cells [27]. Although this hypothesis has not been established experimentally yet, the proteomics data showed that the proteins involved in DNA transcription and RNA processing are both enriched in the nucleoids of chloroplasts [14], suggesting a possible association of these two steps. In this context, the defects in transcription termination might be masked by the processing defects. Elucidation of the relationship between transcription termination and post-transcription processing will be useful to understand the mechanism of chloroplast transcription termination.

It is generally accepted that most 3′ends are generated by processing, rather than directly by transcription termination [6,34,35]. However, the growing evidence showed that specific transcription termination is also required for some specific chloroplast genes. It is still an open question why some genes require a strict transcription termination, while others do not. In addition, the termination mechanisms of different genes seem to be distinct. For *rbcL* termination, the RHON1-dependent mechanism is even species-specific and it does not exist in rice chloroplasts [36]. Whether this relates to the gene structure and/or its location on chloroplast genome among different species is still an open question. Further studies on the chloroplast transcription termination in an evolutionary context will be necessary to shed light on these processes.

One striking feature of chloroplasts is the existence of pentatricopeptide repeat (PPR) proteins which constitute one of the largest protein families in land plants [148]. Some PPR proteins have been proposed to be protective factors for 3′-ends of chloroplast RNAs [148]. If transcription and RNA processing are inherently linked in chloroplasts, the possible direct (or indirect) role of PPR proteins in transcription termination could not be overlooked. Preliminary experiments have identified a leucine-rich pentatricopeptide repeat-containing protein that appears to bind at the mouse HSP distal termination region [149], suggesting the possible role of PPR proteins in mitochondrial transcription termination. The further elucidation of the role of PPR proteins in chloroplast transcription termination will provide insight into this area.

Transparency document

The Transparency document associated with this article can be found, in online version.

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