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REVIEW

RNA methylation in chloroplasts or mitochondria in plants

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ABSTRACT

Recent advances in our understanding of epitranscriptomic RNA methylation have expanded the complexity of gene expression regulation beyond epigenetic regulation involving DNA methylation and histone modifications. The instalment, removal, and interpretation of methylation marks on RNAs are carried out by writers (methyltransferases), erasers (demethylases), and readers (RNA-binding proteins), respectively. Contrary to an emerging body of evidence demonstrating the importance of RNA methylation in the diverse fates of RNA molecules, including splicing, export, translation, and decay in the nucleus and cytoplasm, their roles in plant organelles remain largely unclear and are only now being discovered. In particular, extremely high levels of methylation marks in chloroplast and mitochondrial RNAs suggest that RNA methylation plays essential roles in organellar biogenesis and functions in plants that are crucial for plant development and responses to environmental stimuli. Thus, unveiling the cellular components involved in RNA methylation in cell organelles is essential to better understand plant biology.

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Introduction

RNA modifications have recently emerged as a ubiquitous additional regulatory layer in the flow of genetic information from DNA through RNA to protein. Approximately 160 chemical modifications in RNAs have been identified to date [1,2], among which methylation is of particular interest for its implications in almost every step of RNA metabolism, including RNA processing, maturation, transport, translation, and degradation [3–7]. Several recent studies demonstrated the importance of RNA methylation in plants, influencing flowering, shoot and root development, organ formation, and abiotic or biotic stress responses [8–11]. Methyltransferases (referred to as 'writers'), demethylases (referred to as 'erasers'), and RNA-binding proteins (RBPs; referred to as 'readers'), which instal, remove, and interpret methylation marks, respectively, have been identified and characterized in animals [12–15]. By contrast, the identity and functions of their plant counterparts are just beginning to be uncovered. Moreover, most of these studies have focused on the nuclear-cytoplasmic RNA modifications, i.e. the cellular components responsible for RNA methylation and interpretation in the nucleus and cytoplasm as well as their roles in plant development have been extensively reviewed [8–11,16,17]. Although methylation levels in chloroplast and mitochondrial RNAs are particularly high [18], only few writers in plant chloroplasts and mitochondria have been characterized to date (Fig. 1). Given its importance in animal mitochondria [19], RNA methylation may have similar essential roles in plant organelles. In this review, we present the latest discoveries about chloroplast and mitochondrial RNA methylation in plants. Moreover, we summarize the current knowledge regarding RNA methylation in animal mitochondria as well as in bacteria and suggest a comparative 'endosymbiotic' approach to increase our understanding of the roles of RNA methylation in plant organelles.

Methylation of mRNA, tRNA, and rRNA in chloroplasts and mitochondria in plants

RNA metabolism, including processing, splicing, editing, and decay, is essential for chloroplast and mitochondrial gene regulation [20,21], which is crucial for plant survival and fitness in response to dynamically changing environmental conditions. Given that RNA methylation affects transcript fates and translation in the nucleus and cytoplasm [22–24], it also likely plays an essential role in plant organelles. In animals and plants, N^6-methyladenosine (m^6A) is the most abundant methylation mark in mRNAs [25,26]. Chloroplast and mitochondrial RNAs are highly m^6A-methylated (98–100% of transcripts in chloroplasts and 86–90% in mitochondria) with approximately 4–6 m^6A sites per transcript [18,27]. Majority of highly m^6A-methylated nuclear transcripts encode proteins that target chloroplasts [27,28], and a negative correlation between m^6A methylation levels and gene expression has been demonstrated in both organelles [18,27]. Unlike nuclear mRNAs showing the enrichment of m^6A primarily near the stop codon and in the 3′UTR, m^6A sites in the organellar mRNAs appear to be distributed throughout the transcripts, with the exception of introns, suggesting a role of RNA methylation in splicing [18]. Furthermore, the position of methylation sites might be an important signal to regulate mRNA translation in plant mitochondria [29].

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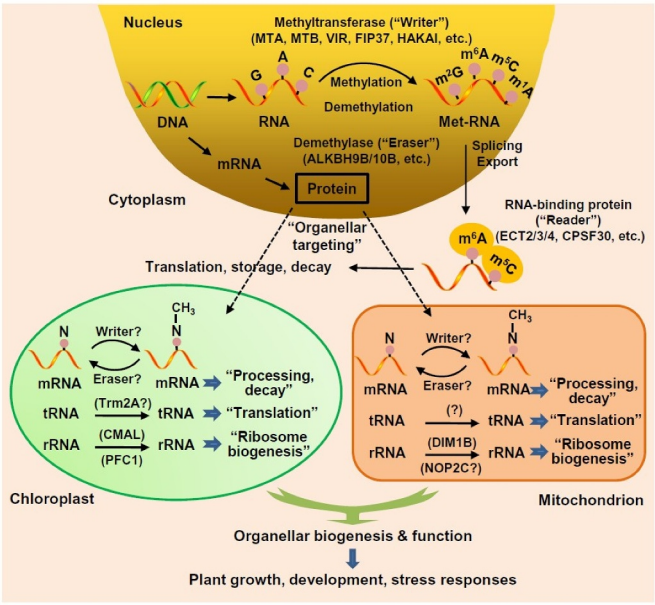

Figure 1. Cellular factors involved in RNA methylation and their roles in plant growth and stress responses. The proteins that instal, remove, and recognize methylation marks are methyltransferases ('writers'), demethylases ('erasers'), and RNA-binding proteins ('readers'), respectively. Several of these factors in the nucleus and cytoplasm have been identified, which affect splicing, RNA export, translation, and RNA storage and decay. The nucleus-encoded potential writer, eraser, and reader proteins are transported to chloroplasts or mitochondria, which are responsible for the methylation of mRNAs, tRNAs, and rRNAs. These cellular factors in organelles are yet to be identified. RNA methylation plays a vital role in organellar biogenesis and function, which is essential for plant growth, development, and stress responses.

Compared to those in animals, the variety of methylated nucleotides and their density in tRNAs and rRNAs are high in plant organelles [18,29,30], occurring at structurally and functionally important positions [31–33]. Methylated residues in tRNA and rRNA appear to be evolutionarily conserved from unicellular algae up to dicotyledons and monocotyledons [34]. In particular, organelle-encoded rRNAs show 5-methylcytosine (m^5C) methylation at several structurally and functionally important positions, whereas organelle-encoded tRNAs are mostly depleted of this specific modification, in contrast to those of nuclear origin [34]. These results might reflect divergent mechanisms of post-transcriptional regulation of tRNAs in chloroplasts and mitochondria, which retain the bacteria-derived features.

Confirmed RNA methyltransferases in chloroplasts and mitochondria in plants

The identity and functions of writers, readers, and erasers in chloroplasts and mitochondria in plants remain largely elusive (Fig. 1). To date, only a few methyltransferases responsible for rRNA methylation in chloroplasts and mitochondria in plants have been identified and characterized. Two m^6A rRNA dimethylases of the ksgA/DIM family have been characterized in *Arabidopsis thaliana*. One of them, PFC1 (paleface1), is responsible for the dimethylation of two adenines in 16S rRNA, which is important for chloroplast development and chlorophyll biosynthesis under cold stress [35]. Similarly, in mitochondria, DIM1B modifies 18S rRNA at positions A1914 and A1915 [36]. Recently, the S-adenosyl-methionine (SAM)-dependent methyltransferase, Chloroplast MraW-like (CMAL), was identified as the writer responsible for installing N^4-methylcytosine 1352 (m^4C_{1352}) in chloroplast 16S rRNA. Loss of this modification causes severe growth defects due to impaired plastid development and translation [37,38]. Moreover, *cmal* mutants showed an altered response to gibberellic acid, auxin, and abscisic acid [37]. Given that the nature of writers, erasers, and readers in chloroplasts and mitochondria remains unclear, it is imperative to identify these cellular factors in plant organelles via systematic comparative analysis of the methyltransferases that are evolutionarily conserved among different organisms, which will be the main topic of this review (see below).

Potential mRNA writers in plant chloroplasts

Several mRNA writers responsible for m^6A, m^1A, m^3C, m^5C, and m^7G have been identified in the nucleus in animals and plants [39–40] (Table 1). In contrast, mRNA writers in chloroplasts and mitochondria in plants are yet to be discovered.

Considering the high levels of m^6A methylation in chloroplasts and mitochondria [18], it is highly likely that plant organelles contain writer proteins that are responsible for m^6A modification. LC-MS/MS and prediction analysis using the SUBA server showed that the m^6A writer components (methyltransferase A (MTA), MTB, and FIP37) found in plant nuclei are also possibly localized in chloroplasts and mitochondria [29,41,42] (Table 1). However, whether these m^6A writer proteins are indeed responsible for m^6A methylation in organelles remains to be determined. Additionally, as more than 3,000 and 2,000 nucleus-encoded proteins are transported to chloroplasts and mitochondria, respectively [43], it is probable that writer, eraser, and reader proteins are among them (Fig. 1). In particular, several putative SAM-dependent methyltransferase proteins, including At1g78140, At2g41040, At4g29590, Ag5g44590, At5g44600, and At5g63100, which are targeted to chloroplasts in *Arabidopsis* (The Plant Proteome Database, http://ppdb.tc.cornell.edu) (Table 1), might be potential m^6A writers in chloroplasts. Further biochemical analysis, such as confirmation of *in vitro* methyltransferase activity of the purified recombinant proteins, as well as molecular studies of the mutants of putative SAM-dependent methyltransferases, will shed light on the nature of writer proteins in plant organelles.

Lessons from the mitochondrial RNA methylation in animals

mRNA methylation

The role of mRNA methylation has been extensively studied in animals due to its various roles in RNA processing, cell metabolism regulation, developmental processes, and diseases [26,58,59]. Although the roles of mRNA methylation in mitochondria are similar to those in other cellular compartments, specific functions are determined by the position of the modified residue along the transcript. For instance, nucleus-

Table 1. List of mRNA methyltransferases in animals, yeast, and plants alongside their putative orthologs in *Arabidopsis* chloroplasts.

Animal nucleus	Yeast	Plant nucleus	References	*Arabidopsis* chloroplasts[+]
METTL3 (m^6A)	Ime4 (m^6A)	MTA (m^6A)	[39,44,45]	MTA (MS, *)
METTL14 (m^6A)		MTB (m^6A)	[46,47]	MTB (MS, *)
VIRMA (m^6A)		Virilizer (m^6A)	[47,48]	
WTAP (m^6A)	Mum2 (m^6A)	FIP37 (m^6A)	[49–51]	FIP37 (MS, *)
HAKAI (m^6A)		HAKAI (m^6A)	[47]	
ZC3H13 (m^6A)			[52]	
NSUN2 (m^5C)		NSUN2 (m^5C) TRM4B (m^5C)	[53–55]	
TRMT6/61A (m^1A) TRMT61B (m^1A)[#]		TRMT61A (m^1A)	[56,60]	
METTL8 (m^3C)			[57]	
METTL1 (m^7G)			[40]	
		The Plant Proteome Database (http://ppdb.tc.cornell.edu)		At1g78140 (*), At2g41040 (*) At4g29590 (*), Ag5g44590 (*) At5g44600 (*), At5g63100 (*)

[+]The putative mRNA methyltransferases in *Arabidopsis* chloroplasts are indicated with confirmed or predicted subcellular localization. MS, mass spectroscopy analysis; *, predicted. [#]TRMT61B is a mitochondria-localized methyltransferase in humans.

encoded transcripts show N^1-methyladenosine (m^1A) enrichment predominantly in the 5′ UTR, whereas mitochondrial mRNAs (mt-mRNAs) are modified primarily in the CDS and 3′ UTR [60–62]. Unlike some other methylation marks, m^1A interferes with the normal Watson-Crick base pairing via the addition of a positive charge to the modified nucleoside, giving rise to alternative conformations or destabilizing transcript structures [63,64]. This difference in m^1A topology between the nucleus-cytoplasm and mitochondria is also correlated with an enhancement or impairment of translation in the two compartments, respectively. Both phenomena are suggested to be caused by structural destabilization upon m^1A incorporation, which is correlated with inefficient transcript-ribosome association in mitochondria [60,61]. Despite the essential role of mRNA methylation in animal mitochondria, TRMT61B responsible for m^1A modification is the only mt-mRNA writer identified to date [60] (Table 1).

tRNA methylation

All 22 human mitochondrial tRNAs (mt-tRNAs) are modified at position 9, and five of them also have m^1A at position 58 [60,65], which is essential for folding and stabilizing the correct three-dimensional structures of mt-tRNAs through Hoogsteen pairing [63,64,66,67], affecting polysome association and translation initiation [68]. Several writer proteins responsible for tRNA methylation in animal mitochondria, yeast, and bacteria have been identified (Table 2). The cytoplasmic m^1A58 is generated by a yeast-like heterodimeric methyltransferase complex (Trmt61A/Trmt6), whereas the m^1A58 in human mt-tRNA is installed by bacteria-derived Trmt61B, an ortholog of bacterial TrmI [69–70], which is also responsible for the methylation of m^1A947 in mitochondrial 16S rRNA [71]. In contrast, methylation of m^1A and 1-methylguanosine (m^1G) at position 9 of mt-tRNA is carried out by the RNase P protein complex TRMT10C/SDR5C1, the paralog of *Saccharomyces cerevisiae* Trm10 [72–73]. m^1A marks on tRNA in both cytoplasm and mitochondria are reverted by the α-ketoglutarate-Fe(II)-dependent dioxygenase ALKBH1 [68,74]. The lack of these proteins causes mitochondrial dysfunction, abnormal development, and severe diseases [72,75–77].

Together with m^1G and pseudouridine (Ψ), m^5C is the most abundant methylation mark in tRNA, and its presence is particularly higher in mitochondria than that of nucleus-encoded transcripts, suggesting additional roles of this modification in the regulation of organellar RNA metabolism [78–80]. For instance, the combined functions of ALKBH1 and the methyltransferase NSUN3 are essential for 5-formilcytidin (f^5C) biogenesis at position 34 of mt-$tRNA^{Met}$ via m^5C formation [74,81–83]. These secondary modifications at the anticodon wobble position are required for recognizing non-canonical codons during mitochondrial translation [84,85]. Another NSUN family protein, NSUN2, is responsible for the methylation of cytosine 48, 49, and 50 in most mammalian mt-tRNAs in addition to several cytoplasmic tRNAs, mRNAs, and non-coding RNAs [86,87]. Recently, the human methyltransferase TRMT2B, an ortholog of yeast Trm2 and bacterial TrmA, has been identified as a writer for the installation of highly conserved m^5U54 in mt-tRNA and m^5U429 in 12S rRNA in human mitochondria [88–89]. In addition, several writer proteins responsible for tRNA methylation in yeast and bacteria have been identified (Table 2): for instance, yeast Trm8 and bacterial YggH responsible for m^7G [90,91], and yeast Trm1 and Trm13 responsible for $m^2{}_2G$ and Am, respectively [92,93]. However, the animal and plant counterparts of these enzymes are still unknown.

rRNA methylation

Contrary to other RNA molecules and their cytoplasmic or bacterial counterparts, mammalian mitochondrial rRNAs (mt-rRNAs) are less heavily methylated. In fact, only 10 modifications in mt-rRNA have been identified to date, with a prevalence of Ψ and 2′-*O*-methylation (Gm) [96]. Nevertheless, these modifications are conserved between bacteria and humans and are indispensable for 55S ribosome biogenesis [97]. Only five modified

Table 2. List of tRNA methyltransferases in animal mitochondria, yeast, and bacteria alongside their putative orthologs in *Arabidopsis* organelles.

Animal mitochondria	Yeast	Bacteria	References	*Arabidopsis* organelles[+]
Trmt61B (m^1A58)	TRM61 TRM6	TrmI	[69,70,94]	At5g14600 (N) At2g45730 (N)
Trmt10C (m^1A9; m^1G9)	Trm10	–	[72,73,75]	At5g47680 (N*)
NSUN2 (m^5C48-49-50)	Nop2p	RsmB/Nop2p	[86,87]	–
NSUN3 (m^5C34)	Nop2p	RsmB/Nop2p	[81–83]	–
Trmt2B (m^5U54)	Trm2	TrmA	[88,89,95]	At3g21300 (C*/M*) At2g28450 (N*)
–	Trm8 (m^7G)	YggH (m^7G)	[90,91]	At5g24840 (N*/M*) At5g17660 (C*)
–	Trm1 ($m^2{}_2G$)	–	[92]	At5g15810 (M*) At3g56330 (C*)
–	Trm13 (Am)	–	[93]	At4g01880 (C*)

[+]The putative tRNA methyltransferases in *Arabidopsis* organelles are indicated with their subcellular localization. N, nucleus; C, chloroplast; M, mitochondria; *, predicted. En dash indicates the absence of specific modifications.

nucleosides have been identified in the large subunit of mammalian mitochondrial ribosome [96]. Among these, Gm is known to greatly influence RNA structures due to its stabilizing property and the ability of modulating polarity, steric hindrance, and base pairing [64]. In particular, human Gm1145 and Gm1370 are localized in the evolutionarily conserved A-loop and peptidyl-site of 16S rRNA, which is important for the recognition of tRNA. The absence of methyltransferases responsible for these modifications results in the reduction of translation and respiratory activity and defective mitochondrial biogenesis [98–99].

Several methyltransferases responsible for methylation of mt-rRNA in small ribosomal subunit and their implications in mitochondrial biogenesis and translation in animals have been characterized (Table 3). Human METTL15, an ortholog of bacterial RsmH/MraW, catalyzes m^4C839 and m^5C841 modifications in mitochondrial 12S rRNA and is required for mitoribosome biogenesis [100–101]. The lack of m^5C methylation in 12S mt-rRNA at position 841, installed by NSUN4, a mammalian ortholog of bacterial YebU, results in severe impairments in ribosome biogenesis and translation [102–103]. TFB1M in mouse mitochondria and DIM1 in yeast are methyltransferases responsible for N^6,N^6-dimethyladenosine at the conserved stem-loop structure of 12S mitochondrial rRNA, which is essential for the assembly of functional ribosomes [104,105]. The lack of these two modifications causes a reduction in electron transport chain components, which results in abnormal mitochondria development and human diseases [105–107]. In addition, a few methyltransferases responsible for methylation of mt-rRNA in large ribosomal subunit have been identified (Table 3). MRM1, an ortholog of yeast Pet56 and bacterial RlmB, and MRM3 are responsible for Gm1145 and Gm1370, respectively, in human mitochondria [98,108–110]. Human MRM2, an ortholog of *E. coli* RlmE that catalyzes 2′-*O*-methylation of uridine residue, is involved in the biogenesis of the large subunit of the mitochondrial ribosome [98,99,111].

Table 3. List of rRNA methyltransferases in animal mitochondria, yeast, and bacteria alongside their putative orthologs in *Arabidopsis* organelles.

Ribosomal subunit	Animal mitochondria	Yeast	Bacteria	References	*Arabidopsis* organelles[+]
Small subunit	METTL15 (m^4C839)		RsmH/MraW ($m^4Cm1402$)	[100,112,113,101]	CMAL (m^4C1352) (C)
	NSUN4 (m^5C841)	–	YebU (m^5C1407)	[102,103,114]	At4g40000 (M*)
	TFBIM ($m_2{}^6A1583$-1584)	DIM1 ($m_2{}^6A1781$-1782	KsgA/RsmA ($m_2{}^6A1518$-1519)	[35,104,105]	DIM1B ($m_2{}^6A1914$-1915) (M) PFC1 ($m_2{}^6A$) (C)
	Trmt2B (m^5U429)	–	–	[72]	At3g21300 (C*)
	–	snR70 (Cm1639)	yraL ($m^4Cm1402$)	[101,124]	At1g45110 (C*)
	–	–	RsmB (m^5C967)	[125]	At1g06560 (C*/M*) At3g13180 (C*) At5g26180 (C*)
	–	–	RsmD (m^2G966)	[126]	At3g28460 (C*)
	–	–	RsmE (m^3U1498)	[127]	At1g50000 (C*)
	–	–	RsmG (m^7G527)	[128]	At5g50110 (C*/M*)
	MRM1 (Gm1145)	Pet56 (Gm2270)	RlmB (Gm2251)	[98,108–110]	At2g19870 (C*/M*)
	MRM2 (Um1369)	MRM2 (Um2791 21S mt-rRNA)	RlmE (Um2552)	[98,99,111]	At4g25730 (N*) At5g13830 (N*)
	MRM3 (Gm1370)	–	–	[98,99]	At5g15390 (C*) At4g38020 (C*)
	–	–	RlmA (m^1G745)	[129]	At1g69520 (M*) At1g69523 (C*) At1g69526 (C*) At2g41040 (C*)
Large subunit	–	–	RlmC (m^5U747)	[130]	At3g21300 (C*)
	–	–	RlmD (m^5U1939)	[130]	At3g21300 (C*)
	–	–	RlmH ($m^3\Psi1915$)	[131]	At5g10620 (C*)
	–	–	RlmN (m^2A2503)	[132]	At1g60230 (C*) At2g39670 (C*)

[+]The putative rRNA methyltransferases in *Arabidopsis* organelles are indicated with their subcellular localization. N, nucleus; C, chloroplast; M, mitochondria; *, predicted. En dash indicates the absence of specific modifications.

Potential tRNA and rRNA writers in plant organelles: Evolutionary perspective

Although mitochondrial and plastid genomes have shrunk during the endosymbiotic evolution [115,116], plants still retain a large number of nucleus-encoded proteins of α-proteobacterial and cyanobacterial origin [117,118]. Moreover, their organellar RNA metabolism, albeit with some differences between mitochondria and chloroplasts, exhibits both eukaryotic features, such as introns and splicing, and typical prokaryotic characteristics, including polycistronic transcripts, polyadenylation-mediated RNA decay, 70S ribosomes, and Shine-Dalgarno sequence-mediated translation initiation [20,21,119–121]. Therefore, RNA methylation in chloroplasts and mitochondria might play different roles compared with that in the nucleus and cytoplasm. High levels of RNA methylation [18] and the importance of post-transcriptional regulation in chloroplasts and mitochondria [20,21] point firmly towards the centrality of this chemical modification in organellar metabolism in plants. However, the limited knowledge regarding the chloroplast- or mitochondria-localized writers, readers, and erasers hinders a deeper understanding of epitranscriptomics in these cellular compartments. Reverse genetic approach searching for the plant orthologs of known cellular factors in other organisms, such as bacteria, yeast, and human mitochondria, will be helpful for this purpose. Similarities in RNA methylation between animal mitochondria and bacteria have been documented in recent reviews [19,97].

Systematic comprehensive bioinformatics analyses show that *Arabidopsis* possesses putative orthologs of bacterial, yeast, or animal mitochondrial tRNA methyltransferases (Table 2). Combined LC-MS/MS data [41,42], The Plant Proteome Database (http://ppdb.tc.cornell.edu), protein sequence homology and conserved domain analysis using PROSITE (https://prosite.expasy.org), and subcellular localization prediction analysis using PSORT server (https://psort.hgc.jp) and TargetP server (http://www.cbs.dtu.dk/services/TargetP) reveal that chloroplasts and mitochondria in *Arabidopsis* contain several potential methyltransferases responsible for tRNA methylation. These include At3g21300 (ortholog of TrmA for m^5U), At5g17660 and At5g24840 (ortholog of YggH for m^7G), At3g56330 and At5g15810 (ortholog of Trm1 for $m^2{}_2G$), and At4g01880 (ortholog of Trm13 for Am) (Table 2). Interestingly, in contrast to animals, chloroplast- and mitochondria-encoded tRNAs in *Arabidopsis* and across *Plantae* are devoid of or rare m^5C [34], suggesting independent evolution of organelle methylation in these two domains. Given that tRNA methyltransferases target specific substrates and display diverse functions [122] and might be associated with plant development and stress responses [123], it will be of great interest to characterize above-mentioned nucleus-encoded organellar proteins as the potential writers of tRNA methylation. In particular, considering that four methylated nucleosides in tRNA, m^1A, m^7G, Am, and Cm, were found to be associated with stress response in rice (*Oryza sativa*) and *Arabidopsis* [123], identification of tRNA methyltransferases responsible for these modifications will be beneficial to develop stress-tolerant crops via engineering RNA methylation.

Notably, rRNA methylation is localized mainly in the highly conserved peptidyl-site region of the ribosomal subunit across all domains of life and plays a vital role in translation [97,101], which raises the possibility that methyltransferases responsible for rRNA methylation are evolutionarily conserved among different organisms. Systematic bioinformatics analyses mentioned above reveal that chloroplasts and mitochondria in *Arabidopsis* contain orthologs of several methyltransferases responsible for rRNA methylation in *E. coli* (Table 3). These include At1g45110 (ortholog of yraL for m^4Cm [101,124]), At1g06560, At3g13180 and At5g26180 (ortholog of RsmB for m^5C [125]), At3g28460 (ortholog of RsmD for m^2G [126]), At1g50000 (ortholog of RsmE for m^3U [127]), At5g50110 (ortholog of RsmG for m^7G [128]), At1g69520, At1g69523, At1g69526 and At2g41040 (ortholog of RlmA for m^1G [129]), At3g21300 (ortholog of RlmC and RlmD for m^5U [130]), At5g10620 (ortholog of RlmH for $m^3\Psi$ [131]), and At1g60230 and At2g39670 (ortholog of RlmN for m^2A [132]). Given that information on mapping of all modified rRNA residues and enzymes responsible for rRNA methylation are now available for model species, such as *E. coli* and *S. cerevisiae* [97], it is feasible to identify and determine rRNA methyltransferases in plant organelles via a comparative approach. Biochemical analysis of the *in vitro* methyltransferase activity of the purified recombinant proteins and genetic studies of the mutants of potential rRNA methyltransferases mentioned above will greatly enhance our understanding of the nature of rRNA writer proteins in plant organelles and their physiological significance in plant growth, development, and response to environmental cues.

Conclusions and future prospects

Recent technical advances in methylome analysis have revealed the transcriptome-wide mapping of several RNA methylation marks, including m^6A and m^5C, in plant chloroplasts and mitochondria [18,34]. However, the significance of these widespread modifications and their role in organellar RNA metabolism are largely unknown. In particular, only a few writer components of RNA methylation have been identified in plastids and mitochondria, limiting our understanding of the role of organellar RNA methylation. Although our knowledge regarding RNA methylation in plant organelles is limited, rapid progress is possible via comparative analysis of chloroplast and mitochondrial proteomes with microorganism counterparts. Notably, the evolutionary conservation of several methylation marks and the prokaryotic origin of organelle-localized proteins have been demonstrated [34,117]. Our systematic analysis on RNA methylation in bacteria, yeast, animal mitochondria, and their counterparts in plant organelles could stimulate further researches aiming at identifying cellular factors responsible for RNA methylation in plant organelles. Focused biochemical assays to confirm the methyltransferase activity of putative writer proteins and the molecular analysis of the SAM-dependent methyltransferases will greatly accelerate the discovery of the roles of RNA methylomes in plant organelles. Considering that photosynthesis in chloroplasts and energy metabolism in mitochondria are

essential for plant survival and adaptation to changing environmental stimuli, engineering RNA methylation in these organelles via A-to-G or C-to-T base editing using CRISPR/Cas9 technology [133,134] can be a powerful means for crop improvement. Many unsolved questions and challenging hypotheses await further investigation to obtain deeper insights into the roles and significance of RNA methylation in plant organelles.

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Author Contributions

Conceived and designed the concept: H.K. Compiled and analyzed data: S.M. Wrote the paper: S.M., H.K.

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