



## Review

# An overview of pentatricopeptide repeat proteins and their applications



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## ABSTRACT

Pentatricopeptide repeat (PPR) proteins are a large family of modular RNA-binding proteins which mediate several aspects of gene expression primarily in organelles but also in the nucleus. These proteins facilitate processing, splicing, editing, stability and translation of RNAs. While major advances in PPR research have been achieved with plant PPR proteins, the significance of non-plant PPR proteins is becoming of increasing importance. PPR proteins are classified into different subclasses based on their domain architecture, which is often a reflection of their function. This review provides an overview of the significant findings regarding the functions, evolution and applications of PPR proteins. Horizontal gene transfer appears to have played a major role in the sporadic phylogenetic distribution of different PPR subclasses in both eukaryotes and prokaryotes. Additionally, the use of synthetic biology and protein engineering to create designer PPR proteins to control gene expression *in vivo* is discussed. This review also highlights some of the aspects of PPR research that require more attention particularly in non-plant organisms. This includes the lack of research into the recently discovered PPR-TGM subclass, which is not only the first PPR subclass absent from plants but present in economically and clinically-relevant pathogens. Investigation into the structure and function of PPR-TGM proteins in these pathogens presents a novel opportunity for the exploitation of PPR proteins as drug targets to prevent disease.

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## 1. Pentatricopeptide repeat proteins

Due to the relatively low number of promoters in organelle genomes and the long half-lives of their RNAs, the use of transcriptional regulators to control gene expression is not sufficient. Thus, organelle transcriptomes have a great dependence on RNA-binding proteins to regulate gene expression at the post-transcriptional level [1]. One of the major mediators of organelle post-transcriptional control is the pentatricopeptide repeat (PPR) protein family. The PPR family was simultaneously discovered by two independent research groups during the sequencing of the *Arabidopsis thaliana* genome [2,3]. All PPR proteins contain tandemly repeated sequence motifs (the PPR motifs) which can vary in number [2,3]. These proteins are found in all eukaryotic lineages but appear to have undergone an expansion in terrestrial plants [4]. A small number of PPR-encoding genes have also been reported in

prokaryotes (including pathogenic and symbiotic members of the genera *Rhodobacter*, *Ralstonia*, *Simkania*, *Erwinia*, and *Legionella*), but these genes are proposed to have been acquired via eukaryote-to-prokaryote horizontal gene transfer events [4–9].

Proteins containing PPR motifs are known to have roles in transcription, RNA processing, splicing, stability, editing, and translation (Table 1) [4,10]. As a result, PPR proteins are important for expression of organelle genomes and organelle biogenesis. PPR proteins can be non-catalytic where they act as adaptors by mediating interactions between cognate transcripts and their effectors. Alternatively, increasing evidence is emerging of some PPR proteins that catalyse functions such RNA processing and editing themselves [4,10].

## 2. Structure of PPR proteins

Amino acid alignments between the consensus PPR and the previously characterised tetratricopeptide (TPR) motif revealed that the PPR motif is a degenerate 35 amino acid motif repeated in tandem [3]. The number of PPR motifs within a protein range from 2 to over 26 [11]. The sequence similarity of these helical repeat

Abbreviations: PPR, pentatricopeptide repeat; PRORP, proteinaceous RNase P; SMR, small MutS-related; TGM, tRNA guanine-N7 methyltransferase; TPR, tetratricopeptide repeat.

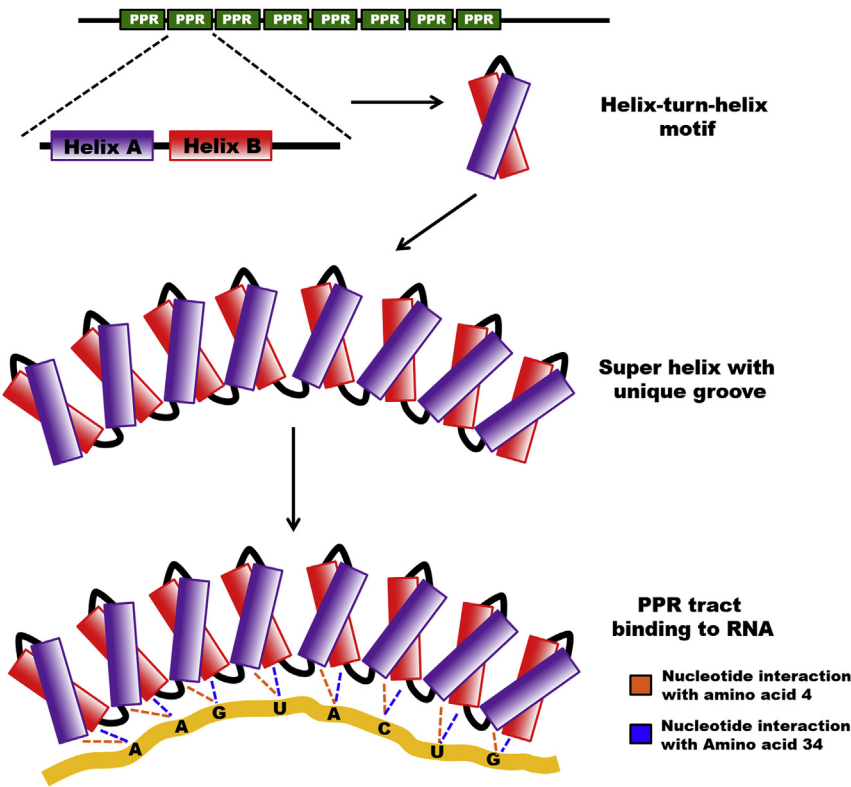
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**Table 1**  
Selected PPR proteins and their respective functions in organelle gene expression.

Organism	Protein	PPR subclass	Localisation	Transcript	Function	Reference
<i>Homo sapiens</i>	POLRMT	mtRNAP	Mitochondria	N/A	Transcription	[18]
<i>Homo sapiens</i>	MRPP3	PRORP	Mitochondria	tRNAs	5' tRNA processing	[53]
<i>Homo sapiens</i>	PTCD1	P	Mitochondria	tRNAs	3' tRNA processing	[76]
<i>Homo sapiens</i>	PTCD2	P	Mitochondria	<i>ND5-Cyt b</i>	Non-tRNA processing	[77]
<i>Homo sapiens</i>	LRPPRC	P	Mitochondria	mRNAs	Polyadenylation, stability	[41,42]
<i>Saccharomyces cerevisiae</i>	Rpo41	mtRNAP	Mitochondria	N/A	Transcription	[61,78]
<i>Saccharomyces cerevisiae</i>	Ccm1p	P	Mitochondria	<i>cob, cox1</i>	Splicing	[79]
<i>Saccharomyces cerevisiae</i>	Pet309	P	Mitochondria	<i>cox1</i>	Stability, translation	[80]
<i>Neurospora crassa</i>	Cya-5	P	Mitochondria	<i>cox1</i>	Translation	[81]
<i>Dictyostelium discoideum</i>	PtcE	PPR-TGM	Mitochondria	tRNAs	tRNA processing	[28]
<i>Trypanosoma brucei</i>	KPAF1/KPAF2	P	Mitochondria	mRNAs	Polyadenylation, polyuridylation, translation	[82]
<i>Trypanosoma brucei</i>	PRORP1	PRORP	Nucleus	tRNAs	5' tRNA processing	[59]
<i>Trypanosoma brucei</i>	PRORP2	PRORP	Mitochondria	tRNAs	5' tRNA processing	[59]
<i>Chlamydomonas reinhardtii</i>	MCA1	P	Chloroplast	<i>petA</i>	Stability	[83]
<i>Arabidopsis thaliana</i>	CRR4	PLS (E)	Chloroplast	<i>ndhD</i>	Editing	[84]
<i>Arabidopsis thaliana</i>	PTAC2	P	Chloroplast	N/A	Transcription	[85]
<i>Arabidopsis thaliana</i>	MTSF1	P	Mitochondria	<i>nad4</i>	Stability	[86]
<i>Arabidopsis thaliana</i>	OTP51	LAGLIDADG	Chloroplast	<i>ycf3</i>	Splicing	[67]
<i>Arabidopsis thaliana</i>	PRORP1	PRORP	Mitochondria chloroplast	tRNAs	5' tRNA processing	[55]
<i>Arabidopsis thaliana</i>	PRORP2/PRORP3	PRORP	Nucleus	tRNAs	5' tRNA processing	[58]
<i>Arabidopsis thaliana</i>	SVR7	PPR-SMR	Chloroplast	<i>atpB, atpE, rbcL</i>	Translation	[70]
<i>Zea mays</i>	Crp1	P	Chloroplast	<i>petA, psaC, petD</i>	Translation processing	[87]
<i>Zea mays</i>	PPR2263	PLS (DYW)	Mitochondria	<i>nad5, cob</i>	Editing	[88]
<i>Physcomitrella patens</i>	PpPPR79	PLS (DYW)	Mitochondria	<i>nad5</i>	Editing	[89]
<i>Physcomitrella patens</i>	PpPPR38	P	Chloroplast	<i>clpP</i>	Splicing	[90]

families, taken together with the greater prevalence of TPR proteins in prokaryotes, led to the hypothesis that the PPR motif emerged from the TPR motif during the early stages of eukaryotic evolution [5]. Similarly to the TPR motif, the PPR motif forms two anti-parallel

$\alpha$ -helices, which interact to produce a helix-turn-helix motif (Fig. 1) [4]. The series of helix-turn-helix motifs formed by PPR motifs throughout the protein produces a superhelix with a central groove that allows the protein to bind RNA [3,4].



**Fig. 1.** PPR protein structure and the mechanism of transcript recognition. Each PPR motif forms two  $\alpha$ -helices, which interact to form a helix turn helix motif. The series of helix turn helix motifs throughout the protein produces a superhelix with an RNA binding groove. Modular recognition of transcripts is mediated by nucleotide interactions with the amino acids at positions 4 and 34 of each PPR motif.

The RNA-binding capabilities of PPR proteins were subsequently confirmed by several studies and supported by RNase protection experiments of the *A. thaliana* chloroplast transcriptome, in which several short (16–28 nucleotides) RNA molecules remained intact following nuclease treatment [12–16]. These RNase footprints correspond to protein target sites, where the binding of the PPR proteins provides the RNAs with protection from nucleases [16].

The insoluble nature of heterologously expressed PPR proteins has impeded studies that attempted to confirm the superhelical structure of these proteins and their mechanism of RNA recognition [17]. Over a decade after the discovery of PPR proteins, the superhelical structure was confirmed. Ringel et al. [18] reported the crystal structure of the mammalian mitochondrial RNA polymerase POLRMT, which contains two PPR motifs towards the N-terminus. The crystal structure of POLRMT confirmed predictions that each PPR motif forms pairs of interacting anti-parallel  $\alpha$ -helices. Following the resolution of the POLRMT structure, subsequent studies have investigated the crystal structure of other PPR proteins. The protein-only mitochondrial RNase P crystal structure from *A. thaliana* (PRORP1) confirmed the role of its PPR motifs in pre-tRNA binding and suggest it has evolved independently from other RNase P proteins that rely on catalytic RNA [19]. Crystal structures of PPR proteins from *Zea mays* (PPR10) and *Brachypodium distachyon* (THA8) in their RNA-free and RNA-bound forms revealed that these proteins undergo conformational changes and form homodimers upon RNA binding [20,21].

### 3. Sequence-specific recognition of RNA by PPR proteins and the use of synthetic biology for *in vivo* manipulation of gene expression

PPR proteins bind their cognate RNA substrates in a sequence-specific manner. The modular recognition and combinatorial amino acid code used by PPR proteins for RNA recognition was confirmed by Barkan et al. [22] who demonstrated positions 6 and 1' in the PROSITE model of the PPR motif (where 1' denotes the first amino acid of the downstream motif) were responsible for RNA binding and nucleotide recognition [22]. It is important to note that some studies use the Pfam model of the PPR motif which designates the beginning and end of the PPR motif two amino acids earlier than the PROSITE model. Therefore, positions 6 and 1' in the PROSITE model are referred to as positions 4 and 34 in studies using the Pfam model [23]. Due to its wide use in NCBI databases, this review will use the Pfam numbering system in reference to the key residues involved in nucleotide recognition from this point forward.

Using positions 4 and 34 within each motif, PPR proteins recognise their cognate transcript in a modular fashion. That is, one PPR motif recognises one nucleotide (Fig. 1). The residues at positions 4 and 34 will determine the nucleotide the motif can recognise. For example a PPR motif with a threonine at position 4, in combination with asparagine at position 34, will recognise adenine, whereas an asparagine and aspartic acid at these respective positions would recognise uracil [22,23]. Crystal structures of PPR proteins have also provided extensive insight into the interaction between PPR motifs and nucleotides, which occurs via van der Waals interactions [21]. Since PPR proteins mediate several different functions in organelle RNA metabolism, elucidation of the recognition code has begun to allow the *in silico* prediction of RNA targets and provide a better indication of the function of specific PPR proteins [22].

Additionally, resolution of the PPR recognition code makes it possible to direct a PPR protein to bind to a specific RNA sequence of interest by manipulating the residues at the aforementioned positions within the PPR motifs [22]. Compared to other helical repeat

RNA-binding proteins, PPR motifs exhibit higher diversity in amino acid composition, suggesting greater versatility for engineering. The creation of designer PPR proteins that recognise an RNA sequence of interest has been achieved using two different approaches. The first strategy involved introducing mutations into the coding sequence of PPR10 from *Z. mays* to change the amino acids encoded at positions 4 and 34 within the sixth and seventh PPR motifs. Using this method, Barkan et al. [22] were able to direct the binding of the protein to an RNA sequence of interest. Rather than mutating existing PPR motifs to influence the RNA-binding preference of the protein, the second strategy involves the creation of an artificial PPR protein scaffold [24]. This was achieved using a consensus design strategy to create synthetic PPR proteins that are stable and programmable to efficiently bind RNA in a sequence-specific manner [24]. These designer PPR proteins constructed via synthetic biology and protein engineering could be used to promote or prevent endogenous gene expression, regulate endogenous RNA metabolism, edit RNA sequences in a site-specific manner or even prevent infection by RNA viruses or viroids [25,26].

### 4. Phenotypes associated with loss of PPR proteins

Most PPR proteins localise either to mitochondria or chloroplasts, where they modulate gene expression at the RNA level. Some of these proteins are even dual targeted to both organelles, suggesting regulated expression between mitochondria and chloroplasts [27]. Since PPR proteins have major roles in organelle gene expression, it is not surprising that defects in their function can yield phenotypes associated with organelle dysfunction. Knock-down of PPR-encoding genes in protists such as *Dictyostelium discoideum* and *Trypanosoma brucei* result in growth defects [28,29]. In plants, mutants of mitochondrial PPR proteins often exhibit defective embryo development and cytoplasmic male sterility [30–35]. Plant mitochondrial genomes often encode factors that induce cytoplasmic male sterility, the inability to produce viable pollen [36]. These factors are normally suppressed by nuclear-encoded restorer of fertility genes, such as the PPR genes [30,33,35–37]. The restorer of fertility PPR proteins appears to function by preventing the translation or accumulation of mitochondrial transcripts, whose gene products induce cytoplasmic male sterility [37]. Plant PPR mutants often have no mechanism to supersede these cytoplasmic male sterility-inducing mitochondrial factors, and therefore cannot produce functional pollen. Mutants of chloroplast PPR proteins in plants also exhibit various phenotypes including defective embryo development, aberrant photosynthetic ability and changes in seed pigmentation [4,13,38]. Rapid methods for the screening and identification of mutations in PPR genes that affect the viability of crops therefore have significant implications in agriculture.

In humans, a point mutation in the gene encoding the leucine-rich pentatricopeptide repeat cassette (LRPPRC) protein is associated with the French-Canadian variant of Leigh syndrome, a neurodegenerative disorder [39]. The mutation results in a cytochrome c oxidase deficiency caused by reduced levels of cytochrome c oxidase mRNAs, suggesting a role for LRPPRC in the stability of these transcripts [39]. The stability of other mitochondrial transcripts is also negatively affected upon knockdown of LRPPRC expression [40]. Ruzzenente et al. [41] demonstrated a role for LRPPRC in polyadenylation, which supports this notion [41]. The role of LRPPRC in facilitating mitochondrial gene expression has also been associated with tumourigenesis, in which the over-expression of LRPPRC in tumours can prevent apoptosis and enhance mitochondrial function [42]. LRPPRC has also been proposed to function in mitochondrial transcription, however this is currently under dispute [43,44]. Thus, further investigation into

LRPPRC is required to elucidate its precise function in mitochondrial RNA metabolism and the molecular pathologies underlying the French-Canadian variant of Leigh syndrome.

## 5. Types of PPR proteins

PPR proteins are classified based on their domain architecture (Fig. 2). P-class PPR proteins possess the canonical 35 amino acid motif and normally lack additional domains [4]. Members of this class have functions in most aspects of organelle gene expression (Table 1). PLS-class PPR proteins have three different types of PPR motifs, which vary in length; P (35 amino acids), L (long, 35–36 amino acids) and S (short, ~31 amino acids), and members of this class are thought to mainly function in RNA editing [4]. Not surprisingly, their presence in particular organisms appears to correlate with the prevalence of RNA editing [45,46]. Subtypes of the PLS class are categorised based on the additional C-terminal domains they possess. In addition to the PLS-type PPR motifs, members of the E subclass contain an E (extended) domain. The E domain is not catalytic, but is predicted to be a protein–protein interaction motif, which acts by recruiting the editing enzyme [47,48]. A second PLS subclass not only contains PPR motifs and an E domain, but also has a DYW domain, named for its highly conserved aspartate–tyrosine–tryptophan (DYW) tripeptide located at the C-terminal end [11]. The DYW domain has been proposed to be an editing domain, as it contains the same conserved catalytic residues found in cytidine deaminase enzymes that mediate C–U conversions [45,46,49]. Similarly to cytidine deaminases, the conserved deaminase residues in the DYW domain possess zinc-binding capabilities and are essential for the editing function of these proteins, providing support for the catalytic role of DYW proteins in editing [50,51]. The PLS-class was originally proposed to be specific to plants, however DYW-containing PPR proteins were subsequently identified in the heterolobosean protist *Naegleria gruberi* and later in several other non-plant organisms [9,52]. Members of the DYW subclass appear to be present in specific eukaryotic lineages but absent in others, suggesting a complex distribution [9]. This punctate phylogenetic distribution indicates that DYW-type PPR proteins in non-plant organisms were acquired by specific lineages from plants via horizontal gene transfer events [9].

While a major group of P-class PPR proteins lack additional domains, multiple subclasses within this group exist that possess catalytic C-terminal domains. In addition to their N-terminal PPR motifs, members of the proteinaceous RNase P (PRORP) subclass

possess a C-terminal metallonuclease domain (Fig. 2) [53,54]. These domains mediate the phosphodiester bond cleavage required to release the 5' end of mitochondrial tRNAs from their polycistronic precursors, and is therefore the catalytic component of the mitochondrial RNase P [53,55,56]. The PPR motifs of PRORP1 in *A. thaliana* are required for recognition of nucleotides C56 and A57 in the T $\Psi$ C loop of precursor tRNAs, which is also likely the case for other PRORP proteins [57]. Unlike most PPR proteins, some members of this subclass do not appear to be confined to organelles, with some PRORP proteins functioning in the nucleus of *A. thaliana* and *T. brucei* [58,59]. Interestingly, *T. brucei* also possesses a mitochondrially targeted PRORP, which can catalyse 5' tRNA processing *in vitro*, even though the *T. brucei* mitochondrial genome does not encode tRNAs [59]. Therefore, investigations into this protein in tRNA and possibly non-tRNA processing will provide further insight into the functions of the PRORP family [54].

PRORP is highly conserved with homologs present in plants, algae, heterokonts and animals [55,56]. However, these proteins are lacking in distinct lineages, such as cellular slime moulds like *D. discoideum*, despite the fact the mitochondrial RNAs of this amoeba undergo tRNA processing [28,56,60]. It will therefore be of interest to investigate why specific eukaryotic lineages appear to have developed non-PRORP mediated mitochondrial tRNA processing mechanisms.

Although not typically considered a PPR subclass, the mitochondrial RNA polymerases of mammals and yeast also contain PPR motifs (Fig. 2) [61–63]. Mitochondrial RNA polymerases possess a unique N-terminal extension, which is absent from their bacteriophage homologs. The N-terminus contains the PPR motifs, which are required for promoter-specific transcription initiation and the coupling of this process to post-transcriptional regulation of mitochondrial RNAs [18,63,64]. Interestingly, PPR motifs appear to be more difficult to detect in mitochondrial RNA polymerases. In fact, it was only after the development of a Markov hidden algorithm for the identification of PPR motifs in yeast that they were identified in yeast mitochondrial RNA polymerases [61]. It is therefore possible that mitochondrial RNA polymerases from other organisms such as *D. discoideum* possess PPR tracts which remain unidentified. This may be the result of the heavy bias toward *in silico* detection of plant PPR motifs due to the significantly greater number of plant PPR sequences available compared to those from non-plant organisms.

LAGLIDADG motifs are sequences required for splicing and are regularly found in maturases and homing endonucleases (Fig. 2)

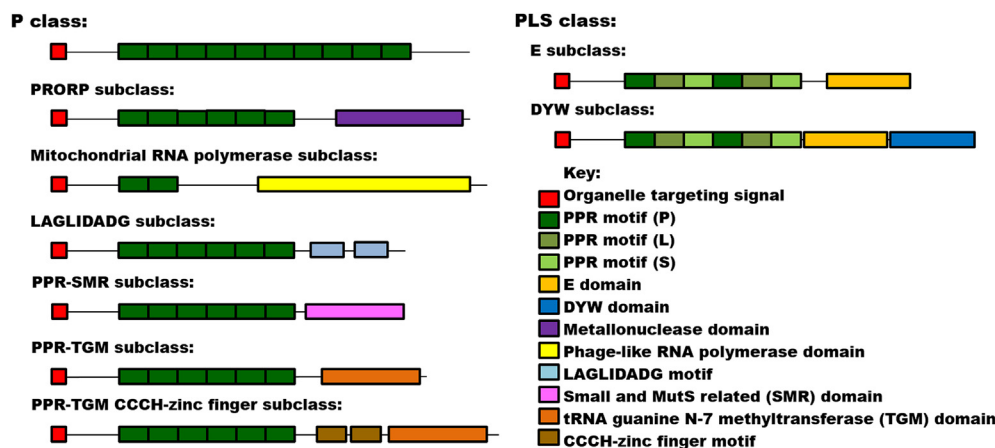


Fig. 2. Basic domain architecture of PPR subfamilies identified to date. Number of PPR motifs displayed in each protein is not characteristic of all members of that respective subfamily and schematics are not to scale.



[65,66]. However, LAGLIDADG motifs are also present in some plant PPR proteins [49,67]. It is therefore not surprising that at least one of these PPR-LAGLIDADG proteins (OTP51) in *A. thaliana* function in transcript splicing [67]. These proteins were predicted to have arisen when an organelle-encoded maturase gene was transferred to the nucleus and fused to a pre-existing PPR-encoding gene [67]. The lack of PPR-LAGLIDADG proteins in other eukaryotic lineages suggests their function in splicing may be catalysed by other proteins in non-plant organisms. PPR-LAGLIDADG proteins remain a largely unexplored subclass of PPR proteins and therefore their significance remains to be investigated.

Only recently discovered was the PPR-SMR group of proteins, whose members contain P-class type PPR motifs with a C-terminal small MutS-related (SMR) domain (Fig. 2) [68–70]. The SMR domain shares homology with bacterial SMR proteins, which have roles in DNA recombination and repair [71]. With the exception of the bacterial pathogen *Legionella longbeachae*, PPR-SMR proteins appear to be restricted to organisms with chloroplasts and have functions in RNA endonucleolytic cleavage and translation [6,68–70]. It is thought that the DNA endonucleolytic activity of the SMR domain, in combination with the RNA-binding ability of the PPR motifs, provides an RNA cleavage function [68]. Interestingly, while the phylogenetic distribution of PPR-SMR proteins would be indicative of functions in chloroplast RNA metabolism, some appear to localise to mitochondria [68,72]. Further research is required to better understand the evolutionary origin, as well as the mechanism of function of PPR-SMR proteins not only in plants but in *L. longbeachae*.

The most recent member of the PPR family identified was the PPR-TGM subfamily. In addition to the PPR tract, these proteins contain a C-terminal tRNA guanine-N7 methyltransferase domain (Fig. 2). The C-terminal domain of the PPR-TGM proteins originated from a chlamydial TGM-encoding gene, acquired via horizontal gene transfer, which evolved PPR motifs following its eukaryotic acquisition [73]. While these proteins are yet to be characterised, their domain architecture suggests they are predicted to function in tRNA metabolism including the S-adenosylmethionine-dependent methylation of tRNA [73]. Individual PPR-TGM proteins were found to contain mitochondrial, chloroplast or no targeting signals, indicating that they function in various locations of the cell. PPR-TGM proteins are only present in eukaryotic microbes including cellular slime moulds, entamoebae, algae and diatoms [28,73]. The absence of PPR-TGM proteins in plants provides a useful tool to investigate the evolution of the PPR family in non-plant lineages. In green algae, the PPR-TGM-encoding gene has undergone a duplication event, resulting not only in one traditional PPR-TGM protein, but also in a second subtype with a CCCH-zinc finger motif, which also possess RNA-binding capabilities. The function of these proteins acquiring a second RNA-binding motif remains unclear [73].

Among the eukaryotic microbes possessing PPR-TGM proteins are the oyster pathogen *Perkinsus marinus* and the clinically-relevant human pathogen *Entamoeba histolytica* [73]. Given *in silico* analyses suggest PPR-TGM proteins play important roles in tRNA function (and consequently translation), as well as the deleterious phenotypes displayed by other PPR mutants, it is likely these proteins are important to these pathogens. Additionally, drugs that inhibit RNA methylation such as sinefungin, an S-adenosylmethionine analogue, have been developed previously [74,75]. Taken together, PPR-TGM proteins represent new putative targets for the development of drugs that can specifically inhibit their activity in pathogenic eukaryotes.

Interestingly, while PPR-TGM proteins have been found in a diverse range of eukaryotic species, they do not seem to be present in organisms that have DYW-type PPR proteins (Table 2) [9,73]. This may imply functional redundancy between these subfamilies of

**Table 2**

Distribution of PPR-TGM and DYW-type PPR proteins in eukaryotes. The presence or absence of a particular PPR subgroup is denoted by '+' or '–', respectively. DYW-type PPR proteins were identified by Schallenberg-Rüdinger et al. [9] PPR-TGM proteins were identified by Manna and Barth [73]. *Arabidopsis thaliana* is representative of all plants.

Organism	DYW-type PPR proteins	PPR-TGM proteins
<b>Amoebozoa</b>		
<i>Dictyostelium</i> spp.	–	+
<i>Polysphondylium pallidum</i>	–	+
<i>Physarum polycephalum</i>	+	–
<i>Acanthamoeba castellanii</i>	+	–
<i>Entamoeba</i> spp.	–	+
<b>Excavata</b>		
<i>Naegleria gruberi</i>	+	–
<i>Malawimonas jakobiformis</i>	+	–
<b>Alveolata</b>		
<i>Perkinsus marinus</i>	–	+
<b>Opisthokonta</b>		
<i>Adineta ricciae</i>	+	–
<i>Philodina roseola</i>	+	–
<b>Chlorophyta</b>		
<i>Ostreococcus</i> spp.	–	+
<i>Micromonas</i> spp.	–	+
<i>Bathycoccus prasinos</i>	–	+
<b>Cryptophyta</b>		
<i>Guillardia theta</i>	–	+
<b>Haptophyta</b>		
<i>Emiliania huxleyi</i>	–	+
<b>Streptophyta</b>		
<i>Nitella</i> spp.	+	–
<b>Embryophyta</b>		
<i>Physcomitrella patens</i>	+	–
<i>Arabidopsis thaliana</i>	+	–
<b>Stramenopila</b>		
<i>Thalassiosira oceanica</i>	–	+
<i>Phaeodactylum tricornutum</i>	–	+

PPR proteins. However, the significance of these opposing phylogenetic distributions remains to be investigated.

## 6. Conclusion

This review has provided an overview of the recent findings in the functional and evolutionary investigations into the PPR family. An emerging feature is the distinct presence and/or absence of specific PPR types in particular lineages. Horizontal gene transfer has likely played an important role in the unique phylogenetic distribution of different PPR subclasses. This includes the rare but nonetheless existence of PPR proteins in prokaryotes, the biological role for which remain to be investigated. It is also clear that non-plant PPR proteins are often overlooked and one major opportunity in non-plant PPR research is the characterisation of PPR-TGM proteins, particularly in pathogenic protists and their potential as putative drug targets. Like the PPR-TGM proteins, investigations into the use of synthetic biology to create designer PPR proteins has several applications in agriculture and medicine which remain to be explored. Future studies providing functional and evolutionary insights into the different PPR subfamilies and the purpose of their phylogenetic distributions will facilitate the understanding of one of the largest eukaryotic protein families identified to date.

## Conflicts of interest

There is no conflict of interest.

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