

REVIEW

The multifarious roles of PPR proteins in plant mitochondrial gene expression

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With a few rare exceptions, genes encoding pentatricopeptide (PPR) proteins are present in all sequenced eukaryotic genomes but absent from prokaryotic and archaeal genomes. The family has greatly expanded in plants, to more than 400 genes in each species. So far, the evidence indicates that PPR proteins are generally involved in regulation of organelle genome expression, in other words they are eukaryotic proteins selected for the control of genomes of prokaryotic origin. PPR proteins are localised in both plastids and mitochondria, and appear to have similar roles in both cases. They have been implicated in almost all stages of gene expression, including messenger RNA (mRNA) transcription, splicing, processing, editing, translation and stability. The most probable hypothesis for explaining these diverse roles is that PPR proteins are sequence-specific RNA-binding adaptors capable of directing effector enzymes to defined sites on mRNAs. Much of the recent interest in the role of PPR proteins in mitochondria has been driven by the discovery that most cytoplasmic male sterility systems comprise fertility restorer genes that are members of this fascinating family.

Introduction

The plant mitochondrial proteome can be estimated to be 2000–3000 gene products (Millar et al. 2005). However, the mitochondrial genome itself only codes for a tiny fraction of these proteins (30–40 in plants, Adams et al. 2002), including subunits of complexes involved in oxidative phosphorylation, cytochrome *c* biogenesis and ribosomes. All other mitochondrial proteins are nuclearly encoded, in particular most of the components of the transcription and translation apparatus. Mitochondrial gene expression is reliant on multiple posttranscriptional steps and any regulation of expression is likely to occur at this level (for review see Binder and Brennicke 2003). Very few of the proteins involved in these posttranscriptional processes are known, but in recent years pentatricopeptide (PPR) proteins have emerged as likely candidates in many

facets of organelle gene expression. The PPR family is a eukaryote-specific protein family particularly prevalent in higher plants. Mutants defective for individual PPR proteins have been known since 1995 with the analysis of the yeast *Pet309* gene, but the extent and coherence of the family was only recognised after the completion of the *Arabidopsis* genome sequencing project (Aubourg et al. 2000, Small and Peeters 2000). This review concentrates on what is known about PPR proteins in plant mitochondria, but will discuss results obtained on chloroplasts or mitochondria from other organisms when these suggest ways to fill in gaps in our knowledge (Table 1).

The PPR family

PPR proteins are named after their eponymous 35 amino acid motifs (Small and Peeters 2000). PPR motifs are

Abbreviations – BSF, Bicoid mRNA stability factor; GFP, green fluorescent protein; LSFC, Leigh syndrome, French Canadian variant; mRNA, messenger RNA; NAD(P)H, nicotinamide adenine dinucleotide phosphate reduced; ORF, open reading frame; PPR, pentatricopeptide; QTL, quantitative trait locus; Rf factors, fertility restorers; TPR, tetratricopeptide; UTR, untranslated region.

Table 1. A table summarising the pentatricopeptide (PPR) proteins described in this review. PPR proteins are involved in an impressively diverse array of molecular and physiological processes. Cp, chloroplast; Mt, mitochondria; CMS, cytoplasmic male sterility.

Gene	Organism	Physiological process	Molecular process	Target	Subclass	Localisation	Reference
<i>PGR3</i>	<i>Arabidopsis</i>	Photosynthesis	RNA stabilisation, translation	<i>petL operon</i>	P	Cp	Yamazaki et al. 2004
<i>CRR2</i>	<i>Arabidopsis</i>	Photosynthesis	RNA cleavage	<i>ndhB</i>	DYW	Cp	Hashimoto et al. 2003
<i>Hcf152</i>	<i>Arabidopsis</i>	Photosynthesis	Splicing, processing	<i>petB</i>	P	Cp	Meierhoff et al. 2003
<i>CRR4</i>	<i>Arabidopsis</i>	Photosynthesis	Editing	<i>ndhD</i>	E+	Cp	Kotera et al. 2005
<i>PTAC2</i>	<i>Arabidopsis</i>	Plastid biogenesis	Transcription		P	Cp	Pfalz et al. 2005
<i>MCA1</i>	<i>Chlamydomonas</i>	Photosynthesis	RNA stabilisation	<i>petA</i>	P	Cp	Lown et al. 2001
<i>PPR2</i>	Maize	Plastid biogenesis	Translation?		P	Cp	Williams and Barkan 2003
<i>CRP1</i>	Maize	Photosynthesis	Translation	<i>petA, psaC</i>	P	Cp	Fisk et al. 1999, Schmitz-Linneweber et al. 2005
<i>P67</i>	Radish	Unknown	RNA binding		P	Cp	Ikeda and Gray, 1999
<i>OsPPR1</i>	Rice	Plastid biogenesis	Unknown		E	Cp	Gothandam et al. 2005
<i>Bsf</i>	<i>Drosophila</i>	Unknown	RNA stabilisation	<i>bicoid 3'</i>	P	Cytoplasm	Mancebo et al. 2001
<i>LRPPRC, hLRP130</i>	Human	Respiration	RNA stabilisation	<i>coxI, coxIII</i>	P	Mt	Mili and Pinol-Roma 2003, Mootha et al. 2003, Xu et al. 2004
<i>Cya5</i>	<i>Neurospora</i>	Respiration	Translation	<i>coxI</i>	P	Mt	Coffin et al. 1997
<i>Rf</i>	Petunia	CMS	RNA degradation		P	Mt	Bentolila et al. 2002
<i>Rfo</i>	Radish	CMS	Translation	<i>orf138</i>	P	Mt	Brown et al. 2003, Desloire et al. 2003
<i>Rfk1</i>	Radish	CMS	Translation	<i>orf125</i>	P	Mt	Koizuka et al. 2003
<i>Rf-1, Rf1a</i>	Rice	CMS	RNA cleavage	<i>atp6-orf79</i>	P	Mt	Akagi et al. 2004, Komori et al. 2004, Wang et al. 2006
<i>Rf1b</i>	Rice	CMS	RNA degradation	<i>atp6-orf79</i>	P	Mt	Wang et al. 2006
<i>Rf1</i>	Sorghum	CMS	Unknown		E	Mt	Klein et al. 2005
<i>P63</i>	Wheat	Unknown	Transcription	<i>coxII</i>	P	Mt	Lahmy et al. 2000
<i>Aep3</i>	Yeast	Respiration	RNA stabilisation, processing	<i>atp6, atp8</i>	P	Mt	Ellis et al. 2004
<i>Pet309</i>	Yeast	Respiration	RNA stabilisation, translation	<i>coxI</i>	P	Mt	Krause et al. 2004, Manthey and McEwen 1995, Manthey et al. 1998
<i>GRP23</i>	<i>Arabidopsis</i>		Transcription		P	Nuclear	Ding et al. 2006

characteristically found as tandem arrays, from two to as many as 26 motifs per protein, with an average in plants of 12 (Lurin et al. 2004). The PPR motif is not one, but in fact three closely related motifs: the canonical PPR motif (P motif), common to all eukaryotes, and two variants specific to plants, the PPR-like S motif (for short) and the PPR-like L motif (for long). *Arabidopsis* has at least 442 PPR proteins, 242 of them with only P motifs, whereas the others are predominantly comprised of repeats of P-L-S triplets PLS subfamily (Lurin et al. 2004). The latter subfamily was previously termed the *Arabidopsis* plant combinatorial and modular protein family (Aubourg et al. 2000). The plant-specific PPR proteins usually contain additional C-terminal domains, respectively, E, E+ and DYW motifs found in 191, 145 and 87 *Arabidopsis* proteins (Lurin et al. 2004). These domains are like

‘Russian dolls’; an E motif always follows the final PPR motif, an E+ motif always follows an E motif and a DYW motif always follows an E+ motif (Fig. 1). Whereas PPR, E and E+ motifs are highly degenerate and can be difficult to recognise, DYW motifs contain highly conserved regions including some invariant amino acids. It has been suggested that this indicates that this domain may have catalytic activity (Aubourg et al. 2000, Lurin et al. 2004).

Almost half of the plant PPR proteins are predicted to be targeted to mitochondria and one-quarter to plastids (Lurin et al. 2004), and given the uncertainties in gene models and targeting predictions, these numbers could be underestimated. Exclusively organellar targeting has been confirmed by the limited experimental testing done so far (Lurin et al. 2004). Only one plant PPR protein has been shown so far to localise outside mitochondria or plastids

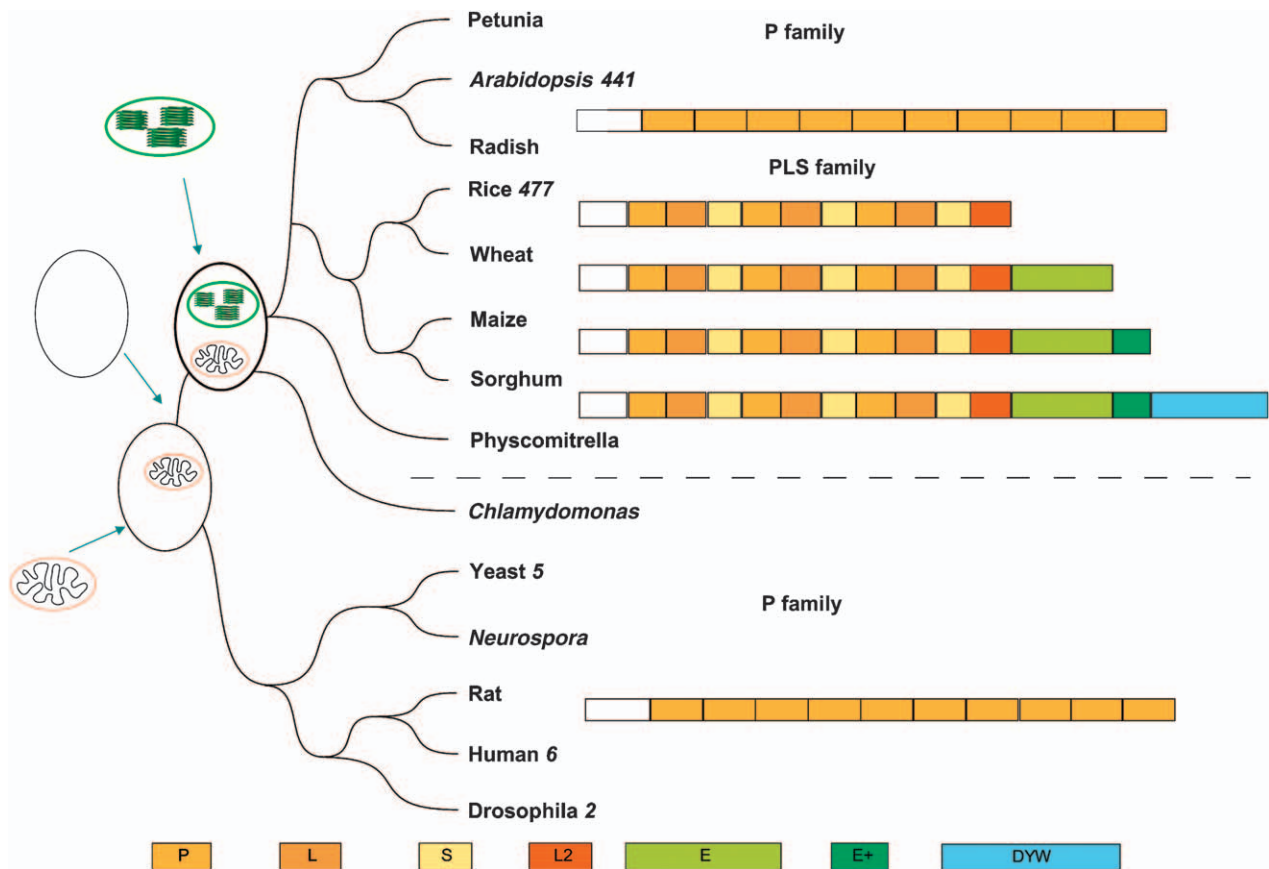


Fig. 1. The pentatricopeptide (PPR) family. Typical structures of proteins from each of the principal subfamilies and subgroups are shown. The structures are purely indicative, and the number and even order of repeats can vary in individual proteins. P family PPR proteins are found in all eukaryotes with mitochondrial genomes. PLS family PPR proteins are confined to land plants. The numbers of different PPR proteins encoded in each species are given when known (i.e. when the genome has been fully or almost fully sequenced).

(Ding et al. 2006). It is too early to say whether this recent example is the forerunner of a sizeable group of non-organellar PPR proteins or whether it will prove to be an extremely rare exception, but the bioinformatics predictions leave open the possibility of tens and maybe as many as a 100 PPR proteins with primary roles in the nucleus or cytosol. PPR proteins from other organisms are without exception mitochondrial proteins, although some may also have roles in other compartments (Mili and Pinol-Roma 2003). *Giardia*, which lacks true mitochondria and a mitochondrial genome, also lacks PPR proteins (McArthur et al. 2000).

PPR motifs appear to be related to tetratricopeptide (TPR) motifs (Small and Peeters 2000) known to mediate protein–protein interactions (Blatch and Lassle 1999). Like TPR motifs, PPR motifs are predicted to consist of a pair of antiparallel α helices, and tandem PPR motifs probably form a helical binding surface. Characteristic differences between TPR and PPR motifs suggested that

PPR proteins might bind to RNA sequences (Small and Peeters 2000), a prediction that has been confirmed in several cases (Lahmy et al. 2000, Lurin et al. 2004, Mancebo et al. 2001, Meierhoff et al. 2003, Mili and Pinol-Roma 2003, Nakamura et al. 2003). PPR–RNA complexes can be highly specific, especially in vivo (e.g. the maize chloroplast). Chloroplast RNA processing 1 (CRP1) protein binds specifically to *petA* and *psaC* transcripts (Schmitz-Linneweber et al. 2005). It is not clear whether other RNA-binding proteins contribute to the specificity and strength of these interactions in vivo, as although PPR proteins are generally in complexes of relatively large size, their partner proteins have yet to be identified (Nakamura et al. 2004).

Much of our knowledge of the roles of PPR proteins comes from genetic evidence in a variety of organisms. Mutant phenotypes have revealed a wide range of defects in all stages of RNA metabolism, indicating surprisingly diverse functions for even very similar PPR proteins.

Transcription

In most mitochondria, transcription is effected by phage T7-type RNA polymerases imported from the cytosol. Transcription initiates at more or less well-defined promoters and often gives rise to long polycistronic transcripts. In plants, multiple degenerate promoters are used and the specificity of transcription initiation appears to be highly relaxed (Holec et al. 2006, Kühn et al. 2005). The necessity for accessory transcription factors to achieve efficient and specific initiation on promoters is controversial and may vary between organisms. A PPR protein has been identified as implicated in transcription in wheat mitochondria (Ikeda and Gray 1999); p63 copurified with transcriptionally active fractions of mitochondrial proteins, improved yield of *coxII* transcripts in in vitro assays and was characterised as a DNA-binding protein, with affinity for the core promoter of *coxII*. A related PPR protein (pTAC2) has been shown to be associated with transcriptionally active chromosomes from plastids (Pfalz et al. 2005). More recently, the *Arabidopsis* protein GRP23 has been described as a nuclear PPR protein, which interacts with the nuclear RNA polymerase II (Ding et al. 2006). Again, the proposition was made that the PPR protein could be implicated in recognition of DNA *cis*-regulatory elements. At this point, it is worth highlighting that the mammalian mitochondrial RNA polymerases contain PPR motifs and that yeast mitochondrial RNA polymerase associates with protein complexes, including PPR proteins (Shadel 2004). Clearly an association between RNA polymerases (and thus transcription) and PPR proteins is a widespread and recurring theme. However, given the data on other PPR proteins, it is more likely that polymerase-associated PPRs are binding and stabilizing nascent RNA chains rather than the DNA substrate. As Shadel (2004) suggests, PPR proteins are likely to be involved in coupling transcription to downstream processing and translation.

RNA stabilisation, degradation, processing

One of the simplest ways to control gene expression in mitochondria is by acting on the quantity of particular messenger RNAs (mRNAs), either by promoting RNA stability or by activating RNA degradation. Many PPR proteins have been described to be involved in these processes. In *Drosophila melanogaster*, Bicoid mRNA stability factor (BSF) is a PPR protein that binds to the 3' untranslated region of *bicoid* mRNA, and promotes the stabilisation of this mRNA (Mancebo et al. 2001). Given that BSF contains a strongly predicted mitochondrial targeting sequence and BSF tagged with GFP localizes to what look like mitochondria (Mancebo et al. 2001), it is likely that this PPR protein also has a mitochondrial

function. A human homologue of BSF, leucine-rich PPR-motif containing, LRPPRC (also known as LRP130) has been shown to bind to both nuclear and mitochondrial mRNA in vivo (Mili and Pinol-Roma 2003). Mutations in LRPPRC give rise to Leigh syndrome, French Canadian variant (LSFC) (Mootha et al. 2003). In LSFC patients, synthesis of COXI and COXIII subunits are specifically decreased, and the level of *cox1* and *cox3* transcripts is very much lower than usual. This indicates that LRPPRC acts as an mRNA stabilisation factor (Xu et al. 2004). In *Chlamydomonas*, MCA1 is a PPR protein essential for the accumulation of *petA* transcript in chloroplasts (Lown et al. 2001). It is not clear to what extent the effects of mutations described above are direct effects on mRNA stabilisation. Often a lack of processing or translation can lead to similar destabilisation of mRNAs. A typical example is the *Saccharomyces cerevisiae* Aep3 protein, a PPR protein involved in the processing of polycistronic mRNAs containing *atp6* and *atp8* (Ellis et al. 2004). In the absence of Aep3, the precursor RNA is not processed correctly and the level of *atp8/6* mRNAs drastically reduced. Similar entwined effects on processing and RNA accumulation have been seen in chloroplast PPR mutants; chlororespiratory reduction 2 (CRR2) is essential for the cleavage of *rps7-ndhB* RNAs (Hashimoto et al. 2003), proton gradient regulation 3, PGR3 is essential for the accumulation of *petL-petG-psaI* tricistronic transcripts (Yamazaki et al. 2004), and high chlorophyll fluorescence (HCF152) is required for the processing and accumulation of *psbB-psbT-psbH-petB-petD* RNAs (Meierhoff et al. 2003).

The case of cytoplasmic male sterility (CMS) and fertility restoration

Cytoplasmic male sterility is a maternally inherited trait leading to pollen abortion and that thus converts normally hermaphroditic plants into females (reviewed in Budar et al. 2003). It can be selected for in some natural populations, which tend towards an equilibrium of male-fertile and male-sterile plants. In the vast majority of cases, the sterility-inducing factor is a novel open reading frame (ORF) in the mitochondrial genome that codes for a hydrophobic protein capable in some way of altering mitochondrial function leading to pollen abortion. Fertility can be restored by nuclearly encoded factors (known as fertility restorers or Rf factors) that suppress the effects of the mitochondrial ORF or its expression. Mitochondrial CMS ORFs and nuclear Rfs appear to be species-specific, but for virtually all the CMS/Rf systems studied so far, the Rf factor is a PPR gene. The single exception is the *Rf2* gene from maize, encoding an aldehyde dehydrogenase (Cui et al. 1996) and which probably restores fertility by an entirely different mechanism.

The first of the PPR restorers to be cloned was *Rf* from petunia, a gene needed to suppress expression of the mitochondrial CMS gene *pcf* (Bentolila et al. 2002). As in most of the CMS systems studied to date, the exact mode of action of *Rf* is not clear. The Petunia CMS-associated fused gene (PCF) protein is much reduced in *Rf* plants and *pcf* transcripts show differences in processing patterns and abundance. As discussed in the previous section, this could result from *Rf* action in RNA processing, RNA stability or translation. Whatever the mode of action of *Rf*, it appears to be highly specific to the *pcf* transcript whereas very similar petunia PPR genes have no effect. Almost exactly comparable CMS/*Rf* systems have been subsequently characterised in several plant species. Radish *Rfo* is essential to suppress the expression of mitochondrial *orf138* (Brown et al. 2003, Desloire et al. 2003), and the almost identical radish *Rfk1* suppresses expression of *orf125* (Koizuka et al. 2003).

The most intensively studied CMS/restorer systems in recent times are undoubtedly those found in rice, given the considerable potential agricultural interest for CMS in this species. Rice *Rf* genes have been cloned by four different groups (Akagi et al. 2004, Kazama and Toriyama 2003, Komori et al. 2004, Wang et al. 2006). The male sterility in this case is the because of the product of *orf79*, just downstream of one of the two copies of the *atp6* gene. The product of this ORF is predicted to be a transmembrane protein, and causes male sterility when expressed in transgenic rice plants (Wang et al. 2006). In fertility-restored plants, mRNAs containing *atp6* and *orf79* are drastically reduced. The four groups have shown that the *Rf-1* locus contains a cluster of related PPR genes. Depending on the plant material being studied, only one or two of these related genes encodes the fertility restoration factor. *Rf-1a* promotes cleavage of the *atp6-orf79* transcript, whereas *Rf-1b* promotes its degradation. Either can restore fertility, and when both are present, *Rf-1a* is epistatic over *Rf-1b*.

All of these *Rf* genes encode classical PPR proteins containing between 11 and 17 P motifs, but a recently cloned restorer gene from sorghum shows a quite different structure (Klein et al. 2005). The sorghum *Rf1* gene encodes an E-class PPR with no relation to the *Rf* genes described above. The mode of action of sorghum *Rf1* is unknown.

Editing

Amongst the different posttranscriptional processes, editing is one of the least understood. In plant mitochondria, editing occurs with high frequencies, as there are 441 sites in *Arabidopsis* (Giege and Brennicke 1999) and 491 in rice (Notsu et al. 2002). It consists of the replacement of a cytosine originally encoded at the

DNA level by a uracil in the mature RNA. Although the numbers of editing sites in plastids and mitochondria are very different (plastids contain at least 10 times fewer sites), most of the evidence implies that the mechanisms in the two organelles are extremely similar. In vitro editing systems suggest that the process proceeds by deamination of the target cytosine (Hirose and Sugiura 2001, Yu and Schuster 1995), and have permitted the identification of the region of mRNA essential for efficient edition (Takenaka et al. 2004). In plant organelles, this corresponds to the 20 nucleotides before the editing site, but often less than 10 after the editing site. As little obvious conservation of sequence is observed in these regions, it is likely that numerous *trans*-factors are necessary to specify editing sites. Recently, a PPR protein was identified as a good candidate specificity factor. CRR4 is essential for the edition of the cytosine present in the ACG initiator codon of the plastid *ndhD* transcript (Kotera et al. 2005). CRR4 is an E-class protein, with 12 PPR-related motifs. CRR4 is the first editing factor ever identified in plants, but is unlikely to encode the enzymatic activity responsible for the base alteration (Kotera et al. 2005, Shikanai 2006). RNA editing of the type found in plant organelles appears to be lacking from green algae and in fact the appearance of editing in the plant lineage approximately corresponds to the expansion of the PPR family in plants, which predates the appearance of mosses (Hattori et al. 2004). The fact that the first editing factor to be identified in plants is a member of the plant-specific PLS subfamily makes it attractive to imagine that many of the other members of this subfamily may also be involved in editing. Bentolila et al. (2005) have recently mapped a quantitative trait locus (QTL) implicated in RNA editing in *Arabidopsis* mitochondria. The region spanning the QTL contains two PPR genes belonging to the DYW class, but more proof is needed before concluding that they encode editing factors.

Translation

The final posttranscriptional step in the process of gene expression is translation, and once more PPR proteins have been found to be involved. Historically, the first two PPR proteins to be described, Pet309p from yeast and its orthologue from *Neurospora* CYA5 (Coffin et al. 1997, Manthey and McEwen 1995, Manthey et al. 1998), have been since shown to be factors essential for translation of COX1 in mitochondria. Pet309p has been shown genetically to associate with the 5' untranslated leader of *cox1* mRNA (although biochemical evidence of this is lacking) and in addition is part of a protein complex attached to the mitochondrial inner membrane (Naithani et al. 2003). The same complex includes translational activators of other mRNAs (Krause et al. 2004, Naithani et al. 2003).

PPR proteins have been found to be associated with the small subunit of the mitochondrial ribosome in both yeast and mammals (Gavin et al. 2002, Koc and Spremulli 2003). Pet309p also associates with Nam1p, which in turn is known to bind to the mitochondrial RNA polymerase (Naithani et al. 2003). Thus is created a link between transcription and translation, and the general belief now is that transcription, translation and assembly into respiratory complexes are all linked at the inner mitochondrial membrane (Krause et al. 2004, Shadel 2004). PPR proteins such as Pet309p and Aep3p undoubtedly play a big role in creating and maintaining these links (Fig. 2).

In plants, our knowledge is much less advanced, but it seems highly likely that many PPR proteins have similar functions in plant organelles. Maize *ppr2* mutants are defective for plastid translation (Williams and Barkan 2003), but the molecular role of PPR2 is unknown and it may be required for expression of a component of the translation apparatus rather than play a role in translation

itself. More complete evidence is available for another maize PPR protein, CRP1. *Crp1* mutants are unable to translate *petA* and *psaC* mRNAs leading to defects in cytochrome *b₆f* and the photosystem I core complexes (Fisk et al. 1999). CRP1 is associated with the 5' UTRs of *psaC* and *petA* mRNAs (Schmitz-Linneweber et al. 2005) and thus resembles Pet309p in many respects. Many of the PPR proteins described earlier in this review probably play similar roles but the data are lacking to show that they directly affect translation or bind to regions of their target mRNAs likely to be implicated in translational control.

Concluding remarks and perspectives

PPR proteins are involved in many (at first sight) unrelated aspects of RNA processing within organelles. They can also be divided into multiple different subfamilies and subclasses depending on their motif structure. However, one would be mistaken to believe that there is a rigid

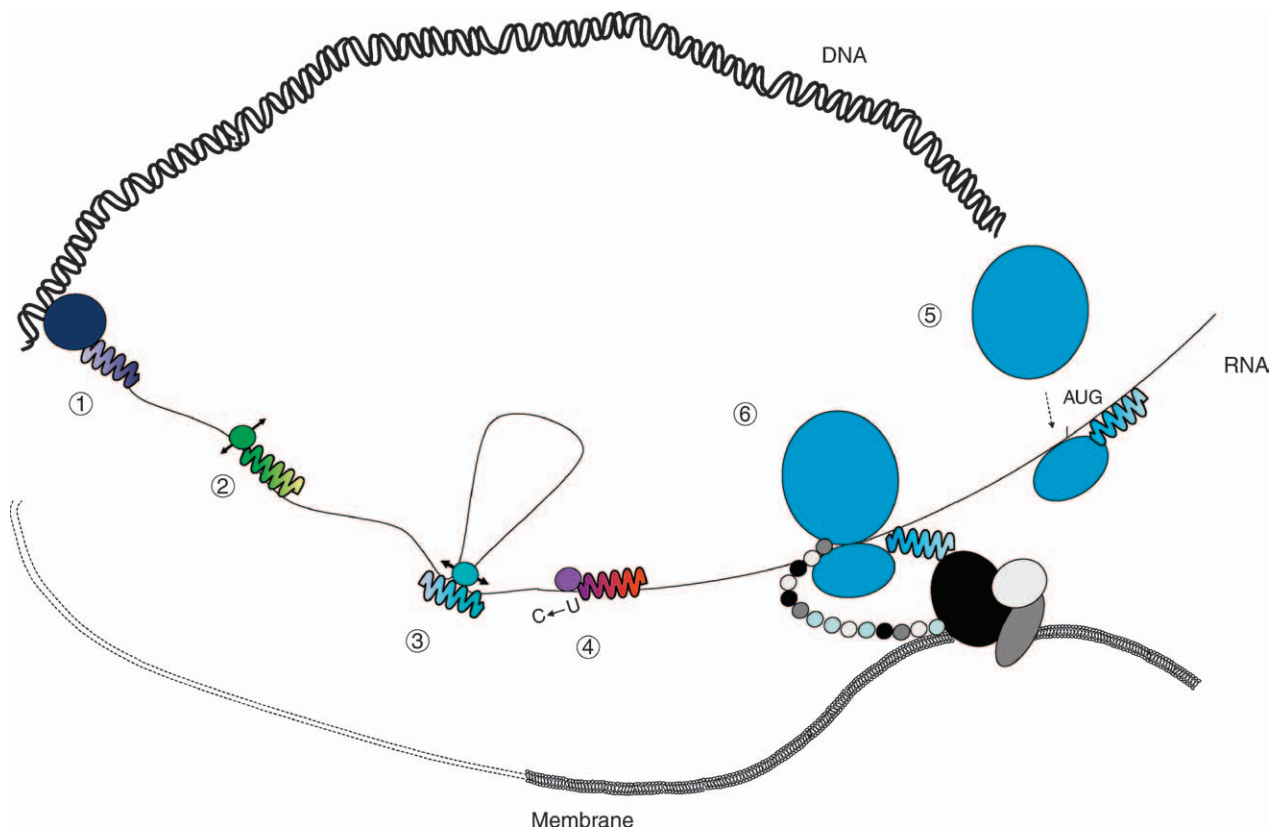


Fig. 2. Multifarious roles for pentatricopeptide (PPR) proteins in mitochondrial gene expression. PPR proteins have been found associated with every stage of gene expression imaginable between transcription and translation. 1: PPR proteins have been found associated with the mitochondrial RNA polymerase; 2: PPR proteins have been implicated in RNA cleavage; 3: PPR proteins have been implicated in splicing; 4: PPR proteins have been implicated in editing; 5: PPR proteins are strongly thought to play a role in translation initiation; 6: PPR proteins are thought to be associated with ribosomes and to in some cases to tether the translation machinery to the mitochondrial inner membrane to facilitate insertion of newly synthesized polypeptides into the correct complex.

relation between the structure of the PPR protein and the process it is involved in; for the moment the evidence suggests that closely similar PPR proteins often appear to have quite different physiological and even molecular functions. The easiest way to explain this seems to be that they share a single primary function—recognition of specific RNA sites. Binding of a PPR protein to its RNA target may have widely different and even antagonistic effects in different cases depending on the position of the target site in the RNA and the nature of other proteins bound to, or competing for, the same site. For example CRP1 is required for translation of its target transcripts, whereas the very similar PPR proteins Rfo and Rfk probably block translation of their target transcripts. In addition, as depicted in Fig. 2, transcription, posttranscriptional processing and translation are undoubtedly linked to some extent within organelles and thus multiple ‘knock-on’ effects are to be expected. The physiological role of each PPR is almost entirely dependent on the nature of the gene product encoded by its target transcript and thus entirely unpredictable at the current time.

Over the next few years, research is likely to concentrate on two areas in particular; (1) the identification of the RNA targets of PPR proteins and (2) the identification of the other proteins present in these RNA–protein complexes. The former will be facilitated by the new RIP-chip (RNA immunoprecipitation and chip hybridisation) approach pioneered by Schmitz-Linne-weber (2005), which shows considerable promise for mapping PPR binding sites. Similar immunoprecipitation techniques coupled with proteomics should go some way towards solving the second problem too. Identifying the other proteins complexed with PPR proteins should help characterise the effector enzymes (nucleases, deaminases) involved in different stages of posttranscriptional processing. Finally, an open question that needs to be solved is whether any of these PPR proteins actually regulate the expression of their target genes in response to cellular requirements, or are they just an unavoidable requirement? Many PPR proteins are absolutely essential for plant embryogenesis and development (Cushing et al. 2005, Lurin et al. 2004) so cannot be lost, but it is tempting to imagine that they play more subtle roles in optimising expression of organellar genes, for example by being implicated in control by epistasy of synthesis (Wostrikoff et al. 2004). Genetic approaches are probably best for detecting and unravelling functions in signalling and feedback control, but they will be difficult to carry out successfully when the processes affected in the mutants are so important at the whole plant level. The best hope will be to concentrate on non-essential components of the organellar photosynthetic or respiratory machinery, for example the plastid nicotinamide adenine dinucleotide

phosphate reduced (NAD(P)H) dehydrogenase (Hashimoto et al. 2003, Kotera et al. 2005).

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