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# 5′ and 3′ ends of chloroplast transcripts can both be stabilised by protein 'caps': a new model for polycistronic RNA maturation

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**Chloroplast gene expression relies on nucleus-encoded factors acting posttranscriptionally in a gene-specific manner. Among those, RNA stability factors bind to the 5′UTR of their target RNAs to protect them from 5′→3′ exonucleases. By contrast, little was know, up to now, on the molecular events involved in the complex processing of chloroplast polycistronic transcripts. In this issue of *The EMBO Journal*, Pfalz *et al* convincingly demonstrate that PPR10, a maize PPR protein, binds a conserved sequence in the intergenic regions of two distinct polycistronic transcripts. Once bound, PPR10 defines the termini of the processed RNAs issued from these polycistronic precursors by impeding the progression of exonucleases acting from both the 5′ and 3′ directions. Other PPR proteins likely acting similarly, Barkan and co-workers (Pfalz *et al*, 2009) propose a new and stimulating model for the maturation of chloroplast transcripts that would involve poorly specific endonucleases and secondary structures or bound proteins that protect transcripts from 5′→3′ or 3′→5′ exoribonucleases.**

Chloroplasts evolved from free-living cyanobacteria captured by a primitive eukaryotic cell. They have retained from their ancestor a prokaryotic-like gene expression machinery and polycistronic transcription units. These latter, however, do not merely correspond to bacterial operons as their expression is not controlled by specific transcriptional repressors/activators. Furthermore, most polycistronic transcripts comprise genes contributing different functions and are often trimmed to monocistronic RNAs.

After endosymbiosis, most genes of the endosymbiont, including a subset of those encoding subunits of the photosynthetic apparatus, were transferred to the nucleus of the host. This massive gene transfer, together with the differentiation in plants of various types of plastids, necessitated new strategies to coordinate the expression of the nuclear and organelle genomes. As a result, the regulation of organelle genes expression now differs widely from that prevailing in cyanobacteria: transcriptional regulations only play a limited role in chloroplasts, where gene expression is mainly controlled at the posttranscriptional level. Posttranscriptional steps of organelle genes expression include *cis*- and *trans*-splicing, editing, cleavage between the coding regions by endonucleases, processing of RNA 5′- and 3′-ends by exonucleases and translational activation. These latter RNA maturation events generate for a given polycistronic unit a complex pattern of mono- and oligo-cistronic RNAs. Each of these posttranscriptional steps is tightly controlled by trans-acting factors of nuclear origin (reviewed in Barkan and Goldschmidt-Clermont, 2000). Strikingly, most of these factors are gene specific, one factor being required for the expression of one, or a few, organelle mRNA(s). Altogether, several hundred nucleus-encoded factors should be required for the proper expression of the organelle genome.

The PPR protein family, named from the repetition of a 35 residue degenerate motif (Small and Peeters, 2000), is highly represented among these trans-acting factors. PPR proteins are found in all eukaryotes but this family is greatly expanded in land plants, with >450 members in *Arabidopsis* or rice. Most PPR proteins are targeted to organelles, where they interact specifically with one or a few target mRNA(s) to assist the posttranscriptional steps of gene expression (reviewed in Schmitz-Linneweber and Small, 2008). Although several PPR proteins have been characterised, their mode of action is still poorly understood.

Up to now, chloroplast RNA metabolism was thought to result from the interplay between distinct 5′→3′ and 3′→5′ exonucleases and sequence-specific endonucleases (reviewed in Bollenbach *et al*, 2004). Sequence-specific endonucleases would cleave the polycistronic transcripts within intergenic regions. Gene-specific trans-acting factors encoded in the nucleus would bind the 5′ UTR of their chloroplast mRNA targets and protect them against 5′→3′ exonucleases, whose role in chloroplast mRNA decay pathways is well established (Drager *et al*, 1998). Chloroplast transcripts would be further stabilised by stable stem-loops structures at their 3′ ends, protecting them, in a rather unspecific way, from 3′→5′ exonucleotidic degradation. This model, however, failed to account for several puzzling observations: (1) some chloroplast transcripts lack stable stem-loop structures at their 3′ends; (2) in several instances, as described in this issue by Barkan and co-workers for the maize transcription units *atpI-atpH*, *psaJ-rpl33*, *psbH-petB* and *petB-petD*, the 5′ end of the downstream transcript in a polycistronic unit overlaps by about 20–30 nts the 3′ end of the upstream transcript, in a

manner that cannot be explained by a single endonucleotidic cleavage event and (3) the existence of sequence-specific endonucleases is only poorly supported by experimental data.

The functional characterisation of a maize PPR protein, PPR10, published in this issue of *The EMBO Journal*, helps clarifying these issues by providing important new insights into chloroplast RNA processing and setting the grounds for a revised model of chloroplast mRNA metabolism. Maize mutant lines lacking expression of PPR10 cannot grow photosynthetically. In an elegant series of technically demanding experiments, including 'RIP-chip' assays originally set up by the Barkan's group (Schmitz-Linneweber *et al*, 2005), Pfalz *et al* attributed this phenotype to a specific reduction in the abundance of transcripts derived from two independent transcription units, *atpI-atpH-atpF-atpA* and *petL-petG-psaJ-rpl33-rps18*. The authors show that PPR10 is required for the stable accumulation of two sets of transcripts whose 5′ or 3′ ends map within the *atpI-atpH* and *psaJ-rpl33* intergenic regions. The two intergenic regions share a conserved ~25 nt sequence that corresponds to the short overlap between the 3′ ends of *atpI* (or *psaJ*) mRNAs and the 5′ ends of *atpH* (or *rpl33*) transcripts. They convincingly demonstrate that PPR10 specifically binds this conserved sequence, both *in vivo* and *in vitro*. Thus, PPR10 defines the termini of chloroplast transcripts by acting as a barrier against exonucleases operating from both the 5′ and 3′ directions. Although the protective function of PPR proteins against 5′→3′ exonucleases was already documented (Loiselay *et al*, 2008), the finding that PPR proteins can substitute 3′ stem-loops structures to protect RNAs from 3′→5′ degradation is unprecedented. However, as discussed by Pfalz *et al*, this mechanism likely prevails for other PPR proteins characterised earlier, such as CRP1 or HCF152 (Barkan *et al*, 1994; Meierhoff *et al*, 2003), suggesting a general role for protein binding to intergenic regions in RNA maturation.

On the basis of this discovery and on recent advances on bacterial mRNA metabolism, Barkan and co-workers propose a new and stimulating model for chloroplast RNA maturation. Processing of polycistronic transcripts would be initiated by endonucleases showing little sequence specificity, such as the chloroplast homologs of bacterial RNAses E and J, that would preferentially target those intergenic regions that are not protected by ribosomes or gene-specific factors. The resulting cleavage products would then be trimmed by 5′→3′ and 3′→5′ exoribonucleases untill those latter encounter secondary structures or bound proteins that will stop their progression. The 5′→3′ exonucleolytic activity is probably carried out by the chloroplast ortholog of RNAse J, which, in addition to its endonucleotidic activity, behave as an 5′→3′ exonuclease in *Bacillus subtilis* (Mathy *et al*, 2007). Furthermore, the differential stability of the transcripts derived from a same polycistronic precursor RNA would be inversely correlated to the length of their unprotected UTRs, more accessible to endonucleases.

This brand-new picture of chloroplast mRNA metabolism will undoubtedly stimulate future research, aimed to test and bring experimental support to the attractive and thought–provoking model put forward in this article.

## Conflict of interest

The authors declare that they have no conflict of interest.

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