

# Regulation of chloroplast translation: interactions of RNA elements, RNA-binding proteins and the plastid ribosome

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## Abstract

Chloroplast gene expression is primarily controlled during the translation of plastid mRNAs into proteins, and genetic studies have identified *cis*-acting RNA elements and *trans*-acting protein factors required for chloroplast translation. Biochemical analysis has identified both general and specific mRNA-binding proteins as components of the regulation of chloroplast translation, and has revealed that chloroplast translation is related to bacterial translation but is more complex. Utilizing proteomic and bioinformatic analyses, we have identified the proteins that function in chloroplast translation, including a complete set of chloroplast ribosomal proteins, and homologues of the 70 S initiation, elongation and termination factors. These analyses show that the translational apparatus of chloroplasts is related to that of bacteria, but has adopted a number of eukaryotic mechanisms to facilitate and regulate chloroplast translation.

## Introduction

Chloroplasts contain all the components necessary for the transcription and translation of the approx. 100 genes encoded on the circular chloroplast genome. The vast majority of chloroplast-encoded genes are involved in gene expression and photosynthesis [1], and are primarily regulated post-transcriptionally, through mRNA processing and stability, and during translation of plastid mRNAs into proteins (reviewed in [2]). Although the processes involved in translation and mRNA stability have not yet been fully characterized, it is clear that they are tightly intertwined and altering one of these processes affects the other. To understand fully the molecular mechanisms of regulated translation in the chloroplast, it is necessary to characterize the regulatory mRNA elements, *trans*-acting factors that recognize these elements and the translational apparatus itself, including the ribosome and general translation factors. In the present study, we have summarized the status of our knowledge on the components and mechanisms of translation in the chloroplast of the green alga, *Chlamydomonas reinhardtii*.

Genetic studies have identified a number of RNA elements that play a regulatory role in chloroplast translation, as well as nuclear gene products that are required for the translation of various chloroplast messages (reviewed in [3]). Proteomic analysis of chloroplast translation has revealed that it shares a high degree of homology with bacterial translation [4,5]. *In silico* analysis of the completed *Chlamydomonas* nuclear

and plastid genome sequences has allowed the identification of homologues for all the requisite translation factors found in bacteria [6]. Taken together, these findings reveal that chloroplast translation, despite being primarily bacterial in nature, shares many aspects of eukaryotic translational regulation. The translational apparatus and mRNAs of plastids are similar to those in bacteria, whereas the presence of message-dependent *trans*-acting factors is reminiscent of eukaryotic systems.

## *Cis*-acting RNA elements involved in chloroplast translation

Genetic analysis had identified earlier that the 5'-UTRs (5'-untranslated regions) of plastid mRNAs contained specific elements essential for translation [7]. Using chimaeric reporter genes, a number of these elements have been mapped, and shown to interact with nuclear factors as a prerequisite for translation [8–11]. In prokaryotic systems, translation initiation is largely dependent on base-pairing between the 16 S rRNA and the 5'-UTR of mRNAs. This Shine–Dalgarno interaction has also been observed in chloroplast translation [11–16], although not all plastid mRNAs appear to use this mechanism for translation initiation [17–19]. The binding of specific translational activators to 5'- and 3'-UTRs of chloroplast mRNAs has also been studied in both higher plants and algae [20–23]. The importance of RNA secondary structures in UTR function has been investigated as well [11,14,24–27]. Although each of these studies has identified RNA elements or *trans*-acting protein factors that affect translation, a comprehensive model of chloroplast translation integrating these elements is yet to emerge.

**Key words:** bioinformatics, chloroplast, initiation, proteomics, ribosome, translation.

**Abbreviations used:** EF, elongation factor; IF, initiation factor; PSRP, plastid-specific ribosomal protein; RAP38, ribosome-associated protein of 38 kDa; RF, release/recycling factor; RRF, ribosome-recycling factor; UTR, untranslated region.

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## mRNA-interacting proteins

A number of biochemical and genetic studies have identified nuclear-encoded proteins, which are required for the translation of specific chloroplast mRNAs [21,23,28–33]. Most of these factors are found to interact with mRNAs in their 5'-UTRs. These factors have been studied genetically through investigation of mutants repressed in the expression of single chloroplast messages and through biochemical analysis of specific binding of proteins to the 5'-UTR of mRNAs [8,10,34]. Many of these regulatory factors appear to be message-specific, whereas the rest of the nuclear factors are probably involved in translation of classes of messages, such as those that are light-regulated or are members of the same photosystem complex. Similar requirements for nuclear factors have been noted in higher plant systems as well [35,36].

Proteins binding to the 5'-UTR of the *psbA* message, whose rate of translation is greatly enhanced in response to exposure to light, include a chloroplast PAB, a 38 kDa RNA-binding protein and a protein disulphide-isomerase homologue [37]. The formation of a protein complex on the 5'-UTR of the *psbA* message and the subsequent translation of the message have been shown to be responsive to the redox status of the chloroplast, and molecular mechanisms for the interactions of these proteins have been proposed [38]. There are other examples of mRNA-binding proteins involved in plastid translation, and some of these may participate in translation in response to a variety of environmental cues.

The *trans*-acting factors can also be considered as a form of signalling between the chloroplast and the nucleus. The proteins coded in the plastid only function when they are in a complex made up of both nuclear- and chloroplast-encoded subunits, such as the photosystem complexes [39]. Hence, there is a great benefit to photosynthetic organisms if they are capable of co-ordinating the rate of synthesis of proteins, so as not to waste valuable resources synthesizing excess subunits, which will be degraded if they are not incorporated into functional complexes.

## Proteome of the chloroplast ribosome

### The small subunit

LC-MS/MS analysis has been used to identify the protein components of the *Chlamydomonas* chloroplast ribosome [4,5]. From the small subunit, 22 proteins were identified, including homologues of 20 of the 21 *Escherichia coli* proteins. A homologue of S11 was not identified on the plastid ribosome, but was identified from the *C. reinhardtii* EST database. Two additional plastid-specific ribosomal proteins (PSRP-3 and -7) were identified [4]. PSRP-3 shares homology with a previously identified spinach chloroplast ribosomal protein and with *ycf65*, a hypothetical protein found in the genome of many algae and other photosynthetic organisms [40,41]. The newly identified PSRP, denoted PSRP-7, shares a small degree of homology with the ribosomal protein S1. The *E. coli* S1 protein contains six S1 domains, whereas the plastid S1 proteins contain only the first three S1 domains. PSRP-7

has domains homologous with the first *E. coli* S1 domain and has weak homology to the second S1 domain, but otherwise has no similarity to S1 [4]. The limited S1 domain homology suggests that PSRP-7 may have some limited functions in common with the ribosomal protein S1, such as mRNA binding.

The proteins S2, S3, S5 and PSRP-7 are uncharacteristically large for ribosomal proteins (Table 1, shown in boldface). Table 1 summarizes the PSRPs and translation factors identified, along with their mass and similarity to *E. coli*. These larger proteins, along with small changes in mass of many other ribosomal proteins, make the protein components of the small subunit of the *Chlamydomonas* chloroplast ribosome almost double that of the *E. coli* 30 S (approx. 600 kDa versus 350 kDa). The S2 protein has an additional large N-terminal domain that doubles the mass of the S2 protein in plastids when compared with its bacterial homologue. The S5 protein has an N-terminal addition that nearly quadruples the mass of S5 in plastids when compared with bacteria. S3 houses an internal insertion that nearly triples its mass relative to bacterial S3. None of these plastid unique domains are homologous with any of the biochemically characterized ribosomal or translation factor proteins. The proteins S2, S3 and S5 are located on the solvent side of the small subunit of the ribosome (Figure 1). The additional domains found in these proteins would connect to the 30 S subunits at the locations of the marked residues in Figure 1. These additional domains, probably in conjunction with the two PSRPs, are hypothesized to form a functional domain on the surface of the 30 S subunit close to where mRNA interacts with the ribosome. This implicates these additional domains in aspects of the regulation of chloroplast translation, perhaps in the selection and correct positioning of mRNAs on the ribosome for translation initiation.

### The large subunit

From the large subunit of the *Chlamydomonas* chloroplast ribosome, 28 proteins were identified, including homologues of 27 of the 33 *E. coli* proteins [5]. One additional PSRP was identified having homology to PSRP-6, identified previously from the spinach chloroplast ribosome [42]. Homologues of the *E. coli* proteins L33, L34 and L36 were identified by *C. reinhardtii* genome searches but not by proteomic analysis of the ribosome, probably because their small mass complicated their recognition by MS after enzymic digestion. Homologues of the *E. coli* proteins L25, L29 and L30 were not identified in the genome or the proteome. L25 and L30 appear to be missing in the chloroplast of higher plants as well [6], and the L29 protein is non-essential for ribosome function in *E. coli* [43]. It thus appears that the composition of the large subunit of the ribosome is more highly conserved compared with the small subunit, consistent with the conserved catalytic role that the large subunit plays.

### Proteins that are 70 S-specific

Two additional proteins were identified in the whole chloroplast ribosome, which were not found in either of the subunits

**Table 1** | Proteomic and *in silico* identification and characterization of *C. reinhardtii* chloroplast ribosomal proteins

Protein	Molecular mass (kDa)*	Similarity to <i>E. coli</i> (%)	Protein	Molecular mass (kDa)*	Similarity to <i>E. coli</i> (%)
General translation factors			50S subunit proteins		
IF-1	11 245.9	81†	L1	28 512.8	63
IF-2	N.A.‡	68†	L2	30 807.5	70
IF-3	N.A.‡	63†	L3	25 371.4	63
EF-Tu	45 612.1	82†	L4	23 203.6	54
EF-Ts	N.A.‡	73†	L5	23 101.0	70
EF-G	79 739.4	73†	L6	21 004.6	62
EF-P	20 925.6	74†	L9	20 211.4	52
RF-1	36 566	79†	L10	22 173.8	44
RF-2	N.A.‡	89†	L11	14 735.2	73
RF-3	N.A.‡	84†	L12	13 355.4	59
RRF	22 022.3	72†	L13	21 281.6	66
30 S subunit proteins			L14	13 445.7	75
S1	43 958.3	50	L15	23 317.8	53
<b>S2</b>	<b>63 159.3</b>	58§	L16	15 468.3	73
<b>S3</b>	<b>81 795.6</b>	63§	L17	14 170.6	57
S4	29 886.7	55	L18	12 938.7	62
<b>S5</b>	<b>61 876.0</b>	63§	L19	13 196.3	57
S6	13 288.2	48	L20	13 416.7	75
S7	18 995.5	61	L21	14 205.7	58
S8	17 078.0	69	L22	14 451.9	61
S9	20 899.9	44	L23	11 186.1	54
S10	14 679.9	69	L24	14 935.6	56
S11	14 095.5	N.A.	L25	N.A.‡	
S12	14 472.1	83	L27	14 129.0	69
S13	15 441.8	69	L28	18 434.3	57
S14	11 616.8	60	L29	N.A.‡	
S15	12 393.0	61	L30	N.A.‡	
S16	11 479.5	58	L31	11 550.2	55
S17	9 023.4	56	L32	6 879.9	39
S18	16 274.6	59	L33	8 581.9	60
S19	10 306.1	80	L34	6 527.6	80
S20	14 222.6	50	L35	7 902.1	47
S21	17 733.6	59	L36	4 260.2	81
PSRP-1	25 401.9	N.D.¶	PSRP-5	N.A.‡	
PSRP-2	N.A.‡		PSRP-6	5 864.7	N.D.¶
PSRP-3	29 329.5	N.D.¶	70 S-specific proteins		
PSRP-4	8 529.4	N.D.¶	RAP38	40 222.7	N.D.¶
<b>PSRP-7</b>	N.A.‡		RAP41	44 320.1	N.D.¶

\* Predicted on the basis of sequence.

† Similarity to *Synechocystis* sp. PCC 6803 homologue, over compared residues.

‡ Not available, full-length clone was not identified.

§ Similarity over homologous regions only.

|| Similarity score not available.

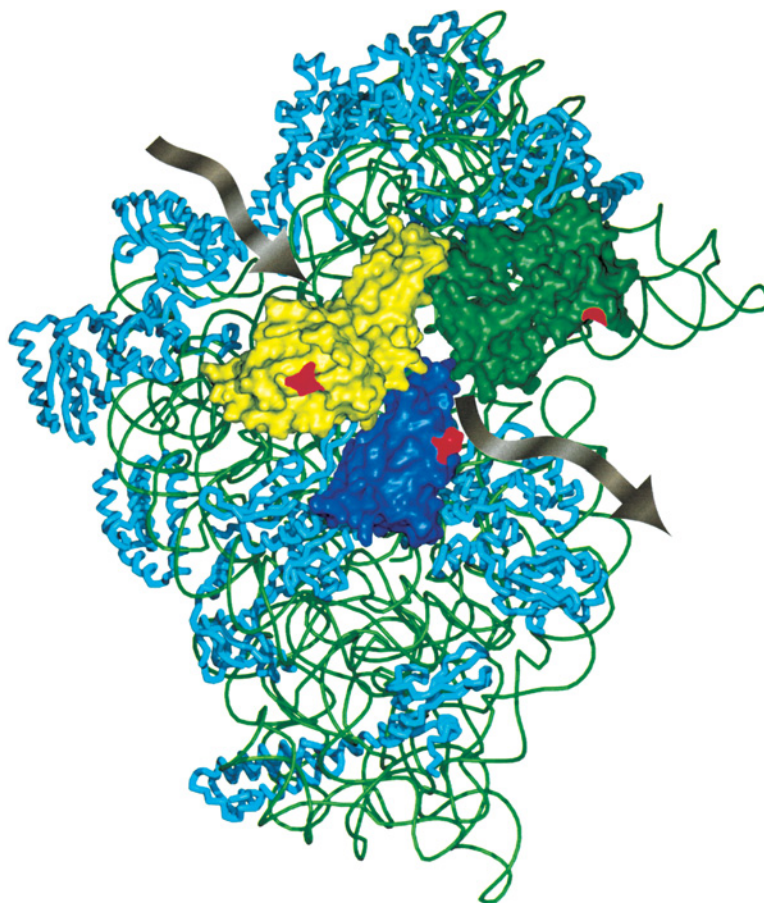
¶ *E. coli* homologue was not detected in database searches.

and they were designated RAP38 and RAP41 (ribosome-associated protein of 38 and 41 kDa respectively) [5]. These proteins were identified by SDS/PAGE analysis to be in stoichiometric amounts with ribosomal proteins. Although these two proteins share no homology with *E. coli* RRF (ribosome-recycling factor), they may share some recycling

and/or maintenance-regulatory function. These two proteins, which share a high degree of homology with each other, are homologous with spinach CSP41, a chloroplast-localized RNA-binding protein/endo-RNase [44]. Although CSP41 is not found associated with the ribosome in higher plants, homologous mRNA processing activities could make RAP38

**Figure 1 | Ribosomal small-subunit proteins containing chloroplast unique domains**

The bacterial 30 S ribosomal subunit (PDB 1J5E; [48]) is shown from the solvent side. rRNA (light green) and most of the proteins (light blue) are shown as backbone trace. Ribosomal proteins S2 (yellow), S3 (green) and S5 (blue) are shown as surfaces. Marked in red on each of these subunits is the residue where the unique chloroplast protein domain would be connected. For perspective, the additional domains from these three proteins would equal an additional S2, two additional S3s and three additional S5s. Arrows mimic the travel of mRNA around the back of the neck of the 30 S subunit, with S3 and S5 proteins flanking the site where the mRNA leaves the ribosome. GRASP [49], Molscript and POV-ray were used for creating this Figure.



and RAP41 important regulatory additions to the plastid ribosome.

**General translation factors**

Release of the nearly complete *C. reinhardtii* nuclear genome sequence by the JGI (Joint Genome Institute; <http://genome/jgi-psf.org/chlre1/chlre1.home.html>), combined with the recent completion of the chloroplast genome sequence and annotation [1], has allowed the *in silico* identification of general translation factors functioning in *C. reinhardtii* chloroplasts [6]. There are three IFs (initiation factors) required for translation in *E. coli*, namely IF1, IF2 and IF3 (reviewed in [45]). Homologues for each of these initiation factors are identified in the *Chlamydomonas* nuclear genome. There are also four EFs (elongation factors) required for translation in *E. coli*, namely EF-tu, EF-ts, EF-G and EF-P (reviewed in [46,47]), and homologues for each of these have also been identified in the *Chlamydomonas* nuclear

genome. There are four RFs (release/recycling factors) in *E. coli*, namely RF-1, RF-2, RF-3 and RRF (or RF-4) [46]. Homologues for each of these have also been identified in *Chlamydomonas*. These results show the conservation of general translation factors between *Chlamydomonas* chloroplast and *E. coli*, indicating that the basic processes of translation are very similar in both the chloroplast and bacteria.

**Discussion**

Chloroplasts are responsible for the transcription and translation of the genes encoded in their genome. The machinery used to accomplish this shares a high degree of homology with that found in bacteria. Plastid genes contain consensus prokaryotic promoters and bacterial-like sigma factors and polymerases [7]. Ribosome-binding sequences are found in many chloroplast mRNAs as a requirement for translation, presumably through a similar binding between mRNA and

the ribosome as found in bacterial translation initiation. The spacing of these ribosome-binding sequences is farther from the start codon when compared with bacteria, suggesting that, in chloroplasts, an additional positioning of the mRNA after initial recognition is required to bring the start codon into the correct register. Unique proteins present on the plastid ribosome, perhaps together with *trans*-acting factors necessary for the translation of certain RNAs, may play a role in the positioning of the start site for translation initiation. Message-specific RNA elements have also been identified in plastid mRNAs as essential for translation. How these RNA elements facilitate translation is unknown, but it seems logical that they interact with either the ribosome or *trans*-acting factors to facilitate translation initiation.

The chloroplast ribosome itself is quite well conserved with the eubacterial ribosome, although there are several unique proteins and protein domains found on plastid ribosomes. These additional domains are primarily confined to the small subunit of the ribosome, consistent with the regulatory role of this subunit in selection and recruitment of mRNAs for translation. A high degree of homology between the chloroplast and bacteria with regard to general translation factors and a majority of the ribosomal proteins implies that many of the basic processes of translation are conserved between bacteria and the chloroplast. The presence of two additional proteins in whole plastid ribosomes not found in either of the subunits suggests that there are also some unique regulatory or maintenance functions at work in chloroplast ribosomes that are not present in bacterial ribosomes. Whether plastid unique protein domains are involved in interactions with mRNA or nuclear *trans*-acting factors is still not clear, and further studies in this area will help to define the roles of these factors in the translation of chloroplast mRNAs.

Nuclear-encoded *trans*-acting factors required for the translation of chloroplast mRNAs afford photosynthetic organisms a degree of co-ordination between the nuclear and chloroplast genomes. These factors are also important for the response of the chloroplast to stimuli such as light and biotic and abiotic stresses. How these proteins affect the translation initiation of their bound mRNAs, through interactions with RNA elements in the 5'-UTR of the message or perhaps with the small subunit of the ribosome, remains unsolved. Ultimately, it seems probable that the RNA elements and the corresponding protein factors of a number of chloroplast mRNAs have to be examined before a complete understanding of translation initiation in the chloroplast can be achieved. All these studies would greatly benefit from an elucidation of the detailed structures of the translational apparatus.

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